ASSAYS FOR IDENTIFYING COMPOUNDS THAT MODULATE BITTER TASTE

Cross-Reference to Related Application

[0001] This application claims priority to U.S. Provisional Application 61/549,693, filed October 20, 2011, which application is incorporated herein by reference in its entirety.

Field of the Invention

[0002] The present invention relates to assays for identifying bitter taste modulators.

Background of the Invention

[0003] The sense of taste, *e.g.*, in human, can detect at least five traditional tastes: sweet, sour, salty, bitter, and umami (savory). Many nutritious substances including vegetables, foods, food ingredients and nutrients comprise bitter tastants and/or have a bitter taste. In addition, many pharmaceutical substances important to maintain or improve health comprise bitter tastants and/or have a bitter taste. While certain food products and consumer products have desirable bitter tastes, including coffee, beer and dark chocolate, in many contexts, consumers dislike such bitter tastes. For example, many consumers dislike the perception of certain bitter tastants and/or bitter taste and will avoid food or pharmaceutical products with an undesirable bitter tastant or bitter taste in favor of food and pharmaceutical products that have reduced levels of undesirable bitter tastants or that have reduced or that completely lack bitter taste. This aversion to products containing undesirable bitter tastants and/or having undesirable bitter taste may be caused by perception of bitter tastants and/or bitter taste mediated by activation of bitter taste receptors present in the oral cavity and/or in the gastrointestinal tract. In many cases, consumer dislike of bitter tastants and/or bitter taste prevents or hampers improvement of the nutritive quality and safety of foods as desired levels of nutrients or preservatives comprising bitter tastants and/or having bitter taste cannot be used. Also, dislike of or aversion to the bitter tastants or bitter taste of some pharmaceutical agents negatively impacts compliance with prescribed regimens for their use.

[0004] For instance, several additives, preservatives, emulsifiers and foodstuffs used in the production of food products comprise bitter tastants and/or have a bitter taste. While these additives, preservatives, emulsifiers and foodstuffs may affect the taste of a food product, they may also be important for improving the shelf life, nutritive quality, or texture of the food product. For example, the increasing trend of hypertension and cardiovascular disease has been attributed, in part, to the high sodium intake of the Western diet. Accordingly, substitution of sodium chloride with another salty tasting compound is desirable. The most common sodium chloride substitute is potassium chloride, which, to a portion of the population, is perceived as possessing a bitter taste in addition to its salty taste. The bitter taste of potassium chloride limits the extent to which it may be used to replace sodium chloride in foods without causing undesired bitter taste for the portion of the population sensitive to it.

[0005] Another common food additive, sodium lactate, has a broad antimicrobial action, is effective at inhibiting spoilage, and growth of pathogenic bacteria, and is commonly used in food products (e.g., meat and poultry products) to extend shelf life and increase food safety. Due to its sodium content, however, sodium lactate, can be undesirable as a preservative. Potassium lactate, which has similar antimicrobial properties, has been used in lieu of sodium lactate. However, potassium lactate is also associated with a bitter taste which limits the extent to which it may be used to replace sodium lactate in foods without causing undesired bitter taste.

[0006] In addition, the increasing incidence of obesity and diabetes has been attributed, in part, to the high sugar intake of many diets. Accordingly, substitution of sugar with another sweet tasting compound is desirable. Artificial and natural sugar substitutes that may be used to reduce sugar in foods are often associated with bitter taste which again limit the extent to which these may be used to replace sugar in foods without causing adverse bitter taste. For example, a common sugar substitute is Acesulfame K, which also has a bitter taste in addition to its sweet taste.

[0007] Without being limited by theory, bitter, sweet, and umami tastants and compounds typically elicit a taste response via G-protein coupled receptors, while salty and sour tastants and compounds are typically hypothesized to elicit a taste response via ion channels. Bitter taste receptors belong to the TAS2R (also referred to as T2R) family of G-protein coupled receptors that induce intracellular calcium concentration changes in response to a bitter tastant. TAS2R receptors act via gustducin, a taste-specific G-protein. There are at least twenty-five different members of the TAS2R family, suggesting that the perception of bitter taste is complex, involving several different tastant-receptor interactions. Some of the TAS2R members, e.g., TAS2R60, are orphan receptors, which have not had a ligand identified. Compounds capable of modulating the activation and/or signaling of bitter taste receptors in the oral cavity and/or the gastrointestinal tract could be effective to allow desired usage levels of bitter tastants or bitter tasting substances in food and pharmaceutical products without resulting in consumer dislike of such products due to perception of the increased levels of bitter tastants or bitter tastes. In some instances, blockers or modulators of bitter taste receptors and bitter taste may reduce the perception of bitter tastants and/or bitter taste

via the bitter taste receptors and/or taste transduction signaling machinery present in the oral cavity and/or the gastrointestinal tract.

[0008] Traditionally in food preparation and pharmaceuticals, bitter taste was masked using sweeteners and other tastants, including salt. In some cases, however, this is undesirable or insufficient because it can alter, mask, or interfere with other tastes/flavors/impressions (e.g., non bitter tastes or desired bitter tastes) in the food product. Additionally, this approach has rarely been able to completely mask the bitter taste present in such food products or pharmaceuticals. For that reason, compounds which reduce bitter taste instead of, or in addition to, masking agents are preferred.

[0009] It is, therefore, desirable to provide assays to identify compounds that may be added to food products, consumer products and pharmaceuticals comprising bitter tastants or having a bitter taste to eliminate, modulate or reduce the perception of the bitter tastants or bitter taste or to reduce the corresponding activation of the bitter taste receptors in the oral cavity and/or the gastrointestinal tract. Similarly, it is desirable to identify compounds that do not activate other bitter taste receptors (i.e., compounds having off-target affects).

Summary of the Invention

[0010] The present invention is based on applicants' discovery, disclosed herein, of agonists for the TAS2R receptors TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, TAS2R44, TAS2R46, and TAS2R60. The assignment of agonists to these receptors makes assays for identifying compounds that modulate bitter taste possible. For example, the present invention provides methods of identifying compounds that inhibit the bitter taste due to these agonists. The present invention also provides methods of identifying compounds that selectively inhibit the bitter taste due to these agonists. The present invention further provides methods of identifying compounds that mimic the bitter taste due these agonists. The present invention also provides methods of identifying compounds that enhance the bitter taste due to these agonists.

Methods of Identifying Compounds That Inhibit Bitter Taste

[0011] One aspect of the present invention provides methods for identifying compounds that inhibit the bitter taste due to KCl. In some embodiments, the method comprises providing a first cell and a second cell, wherein the first and second cell each express one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, wherein the first and second cell express the same one or more bitter taste receptors; contacting the first cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the second cell with a test compound and the tastant; assaying the first and second cells for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first cell to the bitter taste receptor activation of the second

cell, wherein the test compound is an inhibitor of bitter taste due to KCl if the bitter taste receptor activity of the second cell is less than the bitter taste receptor activity of the first cell.

[0012] In some embodiments, the method comprises providing a cell that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; performing a first assay for bitter taste receptor activation; washing the cell; contacting the cell with a test compound and the tastant; performing a second assay for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first assay to the bitter taste receptor activation of the second assay, wherein the test compound is an inhibitor of bitter taste due to KCl if the bitter taste receptor activity of the second assay is less than the bitter taste receptor activity of the first assay.

[0013] Another aspect of the present invention provides methods for identifying compounds that inhibit the bitter taste due to potassium lactate. In some embodiments, the method comprises providing a first cell and a second cell, wherein the first and second cell each express one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, wherein the first and second cell express the same one or more bitter taste receptors; contacting the first cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the second cell with a test compound and the tastant; assaying the first and second cells for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first cell to the bitter taste receptor activation of the second cell, wherein the test compound is an inhibitor of bitter taste due to potassium lactate if the bitter taste receptor activity of the second cell is less than the bitter taste receptor activity of the first cell.

[0014] In some embodiments, the method comprises providing a cell that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; performing a first assay for bitter taste receptor activation; washing the cell; contacting the cell with a test compound and the tastant; performing a second assay for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first assay to the bitter taste receptor activation of the second assay, wherein the test compound is an inhibitor of bitter taste due to potassium lactate if the bitter taste receptor activity of the second assay is less than the bitter taste receptor activity of the first assay.

[0015] Another aspect of the present invention provides methods for identifying compounds that inhibit the bitter taste due to Acesulfame K. In some embodiments, the method comprises providing a first cell and a second cell, wherein the first and second cell each express one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, wherein the first and second cell express the same one or more bitter taste receptors; contacting the first cell with a tastant that activates one or more

of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the second cell with a test compound and the tastant; assaying the first and second cells for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first cell to the bitter taste receptor activation of the second cell, wherein the test compound is an inhibitor of bitter taste due to Acesulfame K if the bitter taste receptor activity of the second cell is less than the bitter taste receptor activity of the first cell.

[0016] In some embodiments, the method comprises providing a cell that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the cell with a tastant that activates one or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; performing a first assay for bitter taste receptor activation; washing the cell; contacting the cell with a test compound and the tastant; performing a second assay for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first assay to the bitter taste receptor activation of the second assay, wherein the test compound is an inhibitor of bitter taste due to Acesulfame K if the bitter taste receptor activity of the second assay is less than the bitter taste receptor activity of the first assay.

[0017] In some embodiments, the tastant is KCl, potassium lactate, Acesulfame K or a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide.

[0018] In some embodiments, the bitter taste receptor is complexed to a G protein. In some embodiments, the G protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0019] In some embodiments, bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is determined using a calcium-sensitive fluorescent dye, such as Fluo-4 or Calcium-3 dye.

[0020] In some embodiments, the cells of the method are present in *in vitro* cell lines. In some embodiments, the cells are present in panels of *in vitro* cell lines.

Methods of Identifying Compounds That Selectively Inhibit Bitter Taste

[0021] One aspect of the present invention provides methods for identifying compounds that selectively inhibit the bitter taste due to KCl. In some embodiments, the method comprises providing a first and a second panel of cell lines in which each panel comprises cell lines that express one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a tastant that activates at least two of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting each cell line in the second panel with a test compound and the tastant; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste

receptor activation of the corresponding cell line in the second panel, wherein the test compound is an selective inhibitor of bitter taste due to KCl if the bitter taste receptor activity of at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the second panel compared to the first panel. In some embodiments, if the bitter taste receptor activity is less in at least three of the cell lines of the second panel, wherein the cell lines are selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is less in at least four of the cell lines of the second panel, wherein the cell lines are selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is less in at least five of the cell lines of the second panel, wherein the cell lines are selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is less in the second panel for each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. In some embodiments, the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines.

[0022] Another aspect of the present invention provides methods for identifying compounds that selectively inhibit the bitter taste due to potassium lactate. In some embodiments, the method comprises providing a first and a second panel of cell lines in which panel comprises cell lines that express one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a tastant that activates at least two of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting each cell line in the second panel with a test compound and the tastant; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein the test compound is an selective inhibitor of bitter taste due to potassium lactate if the bitter taste receptor activity of at least two of the group selected from TAS2R4, TAS2R9,

TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the second panel compared to the first panel. In some embodiments, if the bitter taste receptor activity is less in at least three of the cell lines of the second panel, wherein the cell lines are selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity is less in at least four of the cell lines of the second panel, wherein the cell lines are selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity is less in at least five of the cell lines of the second panel, wherein the cell lines are selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity is less in at least six of the cell lines of the second panel, wherein the cell lines are selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity is less in the second panel for each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. In some embodiments, the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines.

[0023] Another aspect of the present invention provides methods for identifying compounds that selectively inhibit the bitter taste due to Acesulfame K. In some embodiments, the method comprises providing a first and a second panel of cell lines in which panel comprises cell lines that express one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a tastant that activates at least two of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting each cell line in the second panel with a test compound and the tastant; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein the test compound is

an selective inhibitor of bitter taste due to Acesulfame K if the bitter taste receptor activity of at least two of the group selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less in the second panel compared to the first panel. In some embodiments, if the bitter taste receptor activity is less in at least three of the cell lines of the second panel, wherein the cell lines are selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity is less in at least four of the cell lines of the second panel, wherein the cell lines are selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity is less in at least five of the cell lines of the second panel, wherein the cell lines are selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity is less in at least six of the cell lines of the second panel, wherein the cell lines are selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity is less in the second panel for each of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. In some embodiments, the test compound does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines.

[0024] In some embodiments, the tastant is KCl, potassium lactate, Acesulfame K or a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide.

[0025] In some embodiments, the bitter taste receptor is complexed to a G protein. In some embodiments, the G protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0026] In some embodiments, bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is determined using a calcium-sensitive fluorescent dye, such as Fluo-4 or Calcium-3 dye.

[0027] In some embodiments, the cells of the method are present in *in vitro* cell lines. In some embodiments, the cells are present in panels of *in vitro* cell lines.

Methods of Identifying Compounds That Mimic Bitter Taste

[0028] One aspect of the present invention provides methods for identifying compounds that mimic bitter taste due to KCl. In some embodiments, the method comprises providing a first and a second panel of cell lines in which panel comprises cell lines that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a negative control; contacting each cell line in the second panel with a test compound; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein the test compound mimics bitter taste due to KCl if the test compound induces TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. In some embodiments, the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines. In some embodiments, the negative control is the assay buffer before addition of the test compound.

[0029] Another aspect of the present invention provides methods for identifying compounds that mimic bitter taste due to potassium lactate. In some embodiments, the method comprises providing a first and a second panel of cell lines in which panel comprises cell lines that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a negative control; contacting each cell line in the second panel with a test compound; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein the test compound mimics bitter taste due to potassium lactate if the test compound induces TAS2R4, TAS2R9, TAS2R13, TAS2R14,

TAS2R44, TAS2R46 and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. In some embodiments, the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines. In some embodiments, the negative control is the assay buffer before addition of the test compound.

[0030] Another aspect of the present invention provides methods for identifying compounds that mimic bitter taste due to Acesulfame K. In some embodiments, the method comprises providing a first and a second panel of cell lines in which panel comprises cell lines that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a negative control; contacting each cell line in the second panel with a test compound; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein the test compound mimics bitter taste due to Acesulfame K if the test compound induces TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. In some embodiments, the test compound does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines. In some embodiments, the negative control is the assay buffer before addition of the test compound.

[0031] In some embodiments, the bitter taste receptor is complexed to a G protein. In some embodiments, the G protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0032] In some embodiments, bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is determined using a calcium-sensitive fluorescent dye, such as Fluo-4 or Calcium-3 dye.

[0033] In some embodiments, the cells of the method are present in *in vitro* cell lines. In some embodiments, the cells are present in panels of *in vitro* cell lines.

Methods of Identifying Compounds That Enhance Bitter Taste

[0034] One aspect of the present invention provides methods for identifying compounds that enhance the bitter taste due to KCl. In some embodiments, the method comprises providing a first cell and a second cell that each express one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the first cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the second cell with a test compound and the tastant; assaying the first and second cells for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first cell to the bitter taste receptor activation of the second cell, wherein the test compound is an enhancer of bitter taste due to KCl if the bitter taste receptor activity of the second cell is more than the bitter taste receptor activity of the first cell. In some embodiments, the method comprises providing a cell that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; performing a first assay for bitter taste receptor activation; washing the cell; contacting the cell with a test compound and the tastant; performing a second assay for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first assay to the bitter taste receptor activation of the second assay, wherein the test compound is an enhancer of bitter taste due to KCl if the bitter taste receptor activity of the second assay is more than the bitter taste receptor activity of the first assay.

[0035] Another aspect of the present invention provides methods for identifying compounds that enhance the bitter taste due to potassium lactate. In some embodiments, the method comprises providing a first cell and a second cell that each express one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the first cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the second cell with a test compound and the tastant; assaying the first and second cells for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first cell to the bitter taste receptor activation of the second cell, wherein the test compound is an enhancer of bitter taste due to potassium lactate if the bitter taste receptor activity of the second cell is more than the bitter taste receptor activity of

the first cell. In some embodiments, the method comprises providing a cell that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the cell with a tastant that activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; performing a first assay for bitter taste receptor activation; washing the cell; contacting the cell with a test compound and the tastant; performing a second assay for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first assay to the bitter taste receptor activation of the second assay, wherein the test compound is an enhancer of bitter taste due to potassium lactate if the bitter taste receptor activity of the second assay is more than the bitter taste receptor activity of the first assay.

[0036] Another aspect of the present invention provides methods for identifying compounds that enhance the bitter taste due to Acesulfame K. In some embodiments, the method comprises providing a first cell and a second cell that each express one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the first cell with a tastant that activates one or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the second cell with a test compound and the tastant; assaying the first and second cells for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first cell to the bitter taste receptor activation of the second cell, wherein the test compound is an enhancer of bitter taste due to Acesulfame K if the bitter taste receptor activity of the second cell is more than the bitter taste receptor activity of the first cell. In some embodiments, the method comprises providing a cell that expresses one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the cell with a tastant that activates one or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; performing a first assay for bitter taste receptor activation; washing the cell; contacting the cell with a test compound and the tastant; performing a second assay for bitter taste receptor activation; and comparing the bitter taste receptor activation of the first assay to the bitter taste receptor activation of the second assay, wherein the test compound is an enhancer of bitter taste due to Acesulfame K if the bitter taste receptor activity of the second assay is more than the bitter taste receptor activity of the first assay.

[0037] In some embodiments, the tastant is KCl, potassium lactate, Acesulfame K or a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide.

[0038] In some embodiments, the bitter taste receptor is complexed to a G protein. In some embodiments, the G protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0039] In some embodiments, bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is determined using a calcium-sensitive fluorescent dye, such as Fluo-4 or Calcium-3 dye.

[0040] In some embodiments, the cells of the method are present in *in vitro* cell lines. In some embodiments, the cells are present in panels of *in vitro* cell lines.

Methods of Determining Whether Bitter Tastants Are Present In A Composition

[0041] One aspect of the present invention provides methods for determining if KCl is present in a composition. In some embodiments, the method comprises providing a first and a second panel of cell lines in which each cell line expresses one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a negative control; contacting each cell line in the second panel with a composition; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein KCl is present in the composition if the composition induces TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel and does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines. In some embodiments, the negative control is the assay buffer before addition of the composition.

[0042] Another aspect of the present invention provides methods for determining if potassium lactate is present in a composition. In some embodiments, the method comprises providing a first and a second panel of cell lines in which each cell line expresses one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a negative control; contacting each cell line in the second panel with a composition; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein potassium lactate is present in the composition if the composition induces TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel and does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38,

TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines. In some embodiments, the negative control is the assay buffer before addition of the composition.

[0043] Another aspect of the present invention provides methods for determining if Acesulfame K is present in a composition. In some embodiments, the method comprises providing a first and a second panel of cell lines in which each cell line expresses one or more bitter taste receptors selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 and each receptor is expressed in at least one cell line and each panel contains the same cell lines; contacting each cell line in the first panel with a negative control; contacting each cell line in the second panel with a composition; assaying each cell line in the first and second panels for bitter taste receptor activation; and comparing the bitter taste receptor activation of each cell line in the first panel to the bitter taste receptor activation of the corresponding cell line in the second panel, wherein Acesulfame K is present in the composition if the composition induces TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptor activity in the second panel compared to the first panel and does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, each cell line of the first panel is washed after the assay for bitter taste receptor activation to provide the second panel of cell lines. In some embodiments, the negative control is the assay buffer before addition of the composition.

[0044] In some embodiments, the composition is an extract from a food product. In some embodiments, the composition comprises a pharmaceutically active ingredient.

[0045] In some embodiments, the bitter taste receptor is complexed to a G protein. In some embodiments, the G protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0046] In some embodiments, bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is determined using a calcium-sensitive fluorescent dye, such as Fluo-4 or Calcium-3 dye.

[0047] In some embodiments, the cells of the method are present in *in vitro* cell lines. In some embodiments, the cells are present in panels of *in vitro* cell lines.

[0048] Particular embodiments of the invention are set forth in the following numbered paragraphs:

1. A method for identifying a compound that inhibits the bitter taste due to KCl comprising:

a) providing a first and a second cell,

wherein each cell expresses one or more bitter taste receptors independently selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors and

wherein each cell expresses the same one or more bitter taste receptors;

b) contacting said first cell with a tastant,

wherein the tastant activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors;

c) contacting said second cell with a test compound and the tastant;

d) assaying said first and second cells for bitter taste receptor activation; and

e) comparing the bitter taste receptor activation of said first cell to the bitter taste receptor activation of said second cell,

wherein the test compound is an inhibitor of bitter taste due to KCl if bitter taste receptor activity of said second cell is less than the bitter taste receptor activity of said first cell.

2. A method for identifying a compound that inhibits the bitter taste due to potassium lactate comprising:

a) providing a first and a second cell,

wherein each cell expresses one or more bitter taste receptors independently selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors and

wherein each cell expresses the same one or more bitter taste receptors;

b) contacting said first cell with a tastant,

wherein the tastant activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors;

c) contacting said second cell with a test compound and the tastant;

d) assaying said first and second cells for bitter taste receptor activation; and

e) comparing the bitter taste receptor activation of said first cell to the bitter taste receptor activation of said second cell,

wherein the test compound is an inhibitor of bitter taste due to potassium lactate if bitter taste receptor activity of said second cell is less than the bitter taste receptor activity of said first cell.

3. The method of any one of paragraphs 1-2, wherein the tastant is KCl, potassium lactate, or a universal bitter compound.

4. The method of any one of paragraphs 1-3, wherein the bitter taste receptor is complexed to a G protein.

5. The method according to paragraph 4, wherein said G protein is a G_q protein, an alpha transducin or an alpha gustducin.

6. The method according to paragraph 5, wherein the G_q protein is a $G_{\alpha 15}$ protein.

7. The method according to any one of paragraphs 1-6, wherein bitter taste receptor activity is determined by measuring intracellular calcium concentration.

8. The method according to paragraph 7, wherein the concentration of intracellular calcium is determined using a calcium-sensitive fluorescent dye.

9. The method according to paragraph 8, wherein the calcium-sensitive fluorescent dye is Fluo-4 or Calcium-3 dye.

10. The method according to any one of paragraphs 1-9, wherein said first and second cells are present in *in vitro* cell lines.

11. The method according to any one of paragraphs 1-9, wherein said first and second cells are present in panels of *in vitro* cell lines.

12. The method according to any one of paragraphs 1-11, wherein the universal bitter compound is denatonium benzoate or denatonium saccharide.

13. A method for identifying a compound that selectively inhibits the bitter taste due to KCl comprising:

a) providing a first and second panel of cell lines,

wherein each cell line comprises cells that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptor,

wherein each receptor is expressed in at least one cell line, and

wherein the first and second panels comprise the same cell lines;

b) contacting each cell line in the first panel with a tastant,

wherein the tastant activates at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60;

c) contacting each cell line in the second panel with a test compound and the tastant;

d) assaying each cell line for bitter taste receptor activation;

wherein, the test compound is an selective inhibitor of bitter taste due to KCl if the bitter taste receptor activity of at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the second panel compared to the first panel.

14. The method of paragraph 13, wherein the bitter taste receptor activity of at least three of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the second panel compared to the first panel.

15. The method of paragraph 13, wherein the bitter taste receptor activity of at least four of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the second panel compared to the first panel.

16. The method of paragraph 13, wherein the bitter taste receptor activity of at least five of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the second panel compared to the first panel.

17. The method of paragraph 13, wherein the bitter taste receptor of activity of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the second panel compared to the first panel.

18. The method according to any one of paragraphs 13-17, wherein each panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor,

wherein each receptor is expressed in at least one cell line, and

wherein the first and second panels comprise the same cell lines.

19. The method according to paragraph 18, wherein the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel.

20. A method for identifying a compound that selectively inhibits the bitter taste due to potassium lactate comprising:

a) providing a first and second panel of cell lines,

wherein each cell line comprises cells that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptor,

wherein each receptor is expressed in at least one cell line, and

wherein the first and second panels comprise the same cell lines;

b) contacting each cell line in the first panel with a tastant,

wherein the tastant activates at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60;

c) contacting each cell line in the second panel with a test compound and the tastant;

d) assaying each cell line for bitter taste receptor activation;

wherein, the test compound is an selective inhibitor of bitter taste due to potassium lactate if the bitter taste receptor activity of at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the second panel compared to the first panel.

21. The method of paragraph 20, wherein the bitter taste receptor activity of at least three of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the second panel compared to the first panel.

22. The method of paragraph 20, wherein the bitter taste receptor activity of at least four of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the second panel compared to the first panel.

23. The method of paragraph 20, wherein the bitter taste receptor activity of at least five of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the second panel compared to the first panel.

24. The method of paragraph 20, wherein the bitter taste receptor activity of at least six of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the second panel compared to the first panel.

25. The method of paragraph 13, wherein the bitter taste receptor of activity of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the second panel compared to the first panel.

26. The method according to any one of paragraphs 20-25, wherein each panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor,

wherein each receptor is expressed in at least one cell line, and

wherein the first and second panels comprise the same cell lines.

27. The method according to paragraph 26, wherein the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel.

28. The method of any one of paragraphs 13-27, wherein the tastant is KCl, potassium lactate, or a universal bitter compound.

29. The method of any one of paragraphs 13-28, wherein the bitter taste receptors are complexed to a G protein.

30. The method according to paragraph 29, wherein the G protein is a G_q protein, an alpha transducin or an alpha gustducin.

31. The method according to paragraph 30, wherein the G_q protein is a $G_{\alpha 15}$ protein.

32. The method according to any one of paragraphs 28-31, wherein bitter taste receptor activity is determined by measuring intracellular calcium concentration.

33. The method according to paragraph 32, wherein the concentration of intracellular calcium is determined using a calcium-sensitive fluorescent dye.

34. The method according to paragraph 33, wherein the calcium-sensitive fluorescent dye is Fluo-4 or Calcium-3 dye.

35. The method according to any one of paragraphs 28-34, wherein the universal bitter compound is denatonium benzoate or denatonium saccharide.

36. The method according to any one of paragraphs 13-34, wherein the cell lines from the first panel are washed after the bitter taste receptor activation assay to provide the second panel of cell lines.

37. A method for identifying a compound that enhances the bitter taste due to KCl comprising:

a) providing a first and a second cell,

wherein each cell expresses one or more bitter taste receptors independently selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors;

b) contacting said first cell with a tastant,

wherein the tastant activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors;

c) contacting said second cell with a test compound and the tastant;

d) assaying said first and second cells for bitter taste receptor activation; and

e) comparing the bitter taste receptor activation of said first cell to the bitter taste receptor activation of said second cell,

wherein the test compound enhances bitter taste due to KCl if bitter taste receptor activity of said second cell is more than the bitter taste receptor activity of said first cell.

38. A method for identifying a compound that enhances the bitter taste due to potassium lactate comprising:

a) providing a first and a second cell,

wherein each cell expresses one or more bitter taste receptors independently selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptor;

b) contacting said first cell with a tastant,

wherein the tastant activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors;

c) contacting said second cell with a test compound and the tastant;

d) assaying said first and second cells for bitter taste receptor activation; and

e) comparing the bitter taste receptor activation of said first cell to the bitter taste receptor activation of said second cell,

wherein the test compound enhances bitter taste due to potassium lactate if bitter taste receptor activity of said second cell is more than the bitter taste receptor activity of said first cell.

39. The method of any one of paragraphs 37-38, wherein the tastant is KCl, potassium lactate, or a universal bitter compound.

40. The method of any one of paragraphs 37-39, wherein the bitter taste receptor is complexed to a G protein.

41. The method according to paragraph 40, wherein the G protein is a G_q protein, an alpha transducin or an alpha gustducin.

42. The method according to paragraph 41, wherein the G_q protein is a $G_{\alpha 15}$ protein.

43. The method according to any one of paragraphs 37-42, wherein bitter taste receptor activity is determined by measuring intracellular calcium concentration.

44. The method according to paragraph 43, wherein the concentration of intracellular calcium is determined using a calcium-sensitive fluorescent dye.

45. The method according to paragraph 44, wherein the calcium-sensitive fluorescent dye is Fluo-4 or Calcium-3 dye.

46. The method according to any one of paragraphs 37-45, wherein the cell is present in an *in vitro* cell line.

47. The method according to any one of paragraphs 37-45, wherein the cell is present in a panel of *in vitro* cell lines.

48. The method according to any one of paragraphs 39-47, wherein the universal bitter compound is denatonium benzoate or denatonium saccharide.

49. The method according to any one of paragraphs 37-48, wherein the first cell is washed after the bitter taste receptor activation assay to provide the second cell.

50. A method for identifying a compound that inhibits the bitter taste due to KCl comprising:

a) providing a cell,

wherein the cell expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors;

b) contacting said cell with a tastant,

wherein the tastant activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors;

c) assaying said cell for bitter taste receptor activation;

d) washing said cell;

e) contacting said cell with a test compound and the tastant;

f) assaying said cell for bitter taste receptor activation; and

g) comparing the bitter taste receptor activation of said cell in step (c) to the bitter taste receptor activation of said cell in step (f),

wherein the test compound is an inhibitor of bitter taste due to KCl if bitter taste receptor activity in step (f) is less than the bitter taste receptor activity in step (c).

51. A method for identifying a compound that inhibits the bitter taste due to potassium lactate comprising:

a) providing a cell,

wherein the cell expresses one or more bitter taste receptors selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors;

b) contacting said cell with a tastant,

wherein the tastant activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors;

c) assaying said cell for bitter taste receptor activation;

d) washing said cell;

e) contacting said cell with a test compound and the tastant;

f) assaying said cell for bitter taste receptor activation; and

g) comparing the bitter taste receptor activation of said cell in step (c) to the bitter taste receptor activation of said cell in step (f),

wherein the test compound is an inhibitor of bitter taste due to potassium lactate if bitter taste receptor activity in step (f) is less than the bitter taste receptor activity in step (c).

52. The method of any one of paragraphs 50-51, wherein the tastant is KCl, potassium lactate, or a universal bitter compound.

53. The method of any one of paragraphs 50-52, wherein the bitter taste receptor is complexed to a G protein.

54. The method according to paragraph 53, wherein said G protein is a G_q protein, an alpha transducin or an alpha gustducin.

55. The method according to paragraph 54, wherein the G_q protein is a $G_{\alpha 15}$ protein.

56. The method according to any one of paragraphs 50-55, wherein bitter taste receptor activity is determined by measuring intracellular calcium concentration.

57. The method according to paragraph 56, wherein the concentration of intracellular calcium is determined using a calcium-sensitive fluorescent dye.

58. The method according to paragraph 57, wherein the calcium-sensitive fluorescent dye is Fluo-4 or Calcium-3 dye.

59. The method according to any one of paragraphs 50-58, wherein the cell is present in an *in vitro* cell line.

60. The method according to any one of paragraphs 50-58, wherein the cell is present in a panel of *in vitro* cell lines.

61. The method according to any one of paragraphs 52-60, wherein the universal bitter compound is denatonium benzoate or denatonium saccharide.

Brief Description of the Drawings

[0049] **Figure 1** demonstrates that the response of all twenty-five bitter cell lines to the endogenous receptor agonist isoproterenol is highly uniform across all lines. The percent response (Y-axis) was plotted as a function of isoproterenol concentration (X-axis), and the results were curve-fitted to calculate an EC_{50} value (concentration of half-maximal receptor activation). For all twenty-five cell lines, the EC_{50} value was approximately 4.9 ± 0.41 nM.

[0050] **Figure 2** demonstrates that six receptors showed a robust functional response to KCl (20mM), indicating that these six receptors are specifically tuned to detect KCl. None of the remaining nineteen receptors provided a functional response above threshold (40%).

[0051] **Figure 3** provides (A) the dose-response curve of KCl signaling in response to addition of KCl in cell lines expressing KCl-specific receptors, and (B) the dose-response curve of KCl signaling in response to addition of buffer in cell lines expressing KCl-specific receptors. Y-axis represents fluorescence (RFU). The X-axis represents log KCl Concentration (M) in (A). The X-axis in (B), which had no KCl added, reflects the log KCl Concentration (M) in (A).

[0052] **Figure 4** demonstrates that seven receptors showed a robust functional response to potassium lactate (20mM), indicating that these seven receptors are specifically tuned to detect potassium lactate. None of the remaining eighteen receptors provided a functional response above threshold (40%).

[0053] **Figure 5** demonstrates that seven receptors showed a robust functional response to Acesulfame K (20mM), indicating that these seven receptors are specifically tuned to detect Acesulfame K. None of the remaining eighteen receptors provided a functional response above threshold (40%).

[0054] **Figure 6** demonstrates the functional reactivity of all twenty-five bitter cell lines to KCl, potassium lactate, and Acesulfame K.

Detailed Description of the Invention

[0055] In order that the invention described herein may be fully understood, the following detailed description is set forth.

[0056] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are illustrative only, and are not intended to be limiting. All publications, patents and other documents mentioned herein are incorporated by reference in their entirety for all purposes.

[0057] Throughout this specification, the word "comprise" or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or groups of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The terms "or

more" and "at least" are used interchangeably, herein. For example, "two or more" and "at least two" may be used interchangeably. Similarly, the terms "is less" or "is greater" are used interchangeably, herein. For example, "A is less than B" and "B is greater than A" may be used interchangeably.

[0058] The terms "agonist," "potentiator" or "activator" refer to a compound or substance that increases bitter taste receptor activity, resulting in a change in the amount or distribution of an intracellular molecule or the activity of an enzyme which is part of the intracellular signaling pathway for the bitter taste receptor. Examples of the intracellular molecule include, but are not limited to, free calcium, cyclic adenosine monophosphate (cAMP), inositol mono-, di- or tri-phosphate. Examples of the enzyme include, but are not limited to, adenylate cyclase, phospholipase-C, G-protein coupled receptor kinase.

[0059] The terms "antagonist," "inhibitor" or "blocker" refer to a compound or substance that decreases bitter taste receptor activity, resulting in a change in the amount or distribution of an intracellular molecule or the activity of an enzyme which is part of the intracellular signaling pathway for the bitter taste receptor. Examples of the intracellular molecule include, but are not limited to, free calcium, cyclic adenosine monophosphate (cAMP), inositol mono-, di- or tri-phosphate. Examples of the enzyme include, but are not limited to, adenylate cyclase, phospholipase-C, G-protein coupled receptor kinase. As used herein, an inhibitor, antagonist or blocker may act upon all or upon a specific subset of bitter taste receptors. The inhibitor, antagonist or blocker may decrease the activity of a TAS2R receptor by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100%.

[0060] The terms "artificial sweetener" and "sugar substitute" refer to a food additive that confers a sweet taste but has less caloric energy than sugar. In some instances, the caloric energy of the "artificial sweetener" or "sugar substitute" is negligible.

[0061] The term "bitter" or "bitter taste" as used herein refers to the perception or gustatory sensation resulting following the detection of a bitter tastant. The following attributes may contribute to bitter taste: astringent, bitter-astringent, metallic, bitter-metallic, as well as off-tastes, aftertastes and undesirable tastes including but not limited to freezer-burn and card-board taste, and/or any combinations of these. It is noted that, in the art, the term "off-taste" is often synonymous with "bitter taste." Without being limited by theory, the diversity of bitter tastes may reflect the large number of bitter taste receptors and the differential detection of bitter tastants by these receptors. Bitter taste as used herein includes activation of a bitter taste receptor by a bitter tastant. Bitter taste as used herein also includes activation of a bitter taste receptor by a bitter tastant followed by downstream signaling. Bitter taste as used herein also includes activation of a signaling pathway after stimulation by a bitter tastant. Bitter taste as used herein further includes perception resulting from signaling following the detection of a bitter tastant by a bitter taste

receptor. Bitter taste as used herein further includes perception resulting from signaling following contacting a bitter taste receptor with a bitter tastant. Bitter taste can be perceived in the brain.

[0062] The term "bitter taste receptor" refers to a receptor, typically a cell surface receptor, to which a bitter tastant can bind. Bitter taste receptors may be present in the oral cavity, and/or extra-oral tissues, *e.g.*, in taste-like, hormone producing cells throughout the gastrointestinal tract, including the stomach, intestines, and colon. Bitter receptors can also be present in vitro, such as in an assay, including but not limited to a cell based assay or a binding assay.

[0063] The term "bitter tastant," "bitter ligand," or "bitter compound" refers to a compound that activates or that can be detected by a bitter taste receptor and/or confers the perception of a bitter taste in a subject. A "bitter tastant" also refers to a multiplicity of compounds that combine to activate or be detected by a bitter taste receptor and/or confer the perception of a bitter taste in a subject. A "bitter tastant" further refers to a compound that is enzymatically modified upon ingestion by a subject to activate or be detected by a bitter taste receptor and/or confer the perception of a bitter taste in a subject. Because the perception of bitter taste may vary from individual to individual, some individuals may describe a "bitter tastant" as a compound which confers a different kind of bitter taste compared to the kind of bitter taste perceived for the same compound by other individuals. The term bitter tastant also refers to a compound which confers a bitter taste.

[0064] The term "cell line" or "clonal cell line" refers to a population of cells that are all progeny of a single original cell. As used herein, cell lines are maintained *in vitro* in cell culture and may be frozen in aliquots to establish banks of clonal cells.

[0065] The term "consumer product" refers to health and beauty products for the personal use and/or consumption by a subject. Consumer products may be present in any form including, but not limited to, liquids, solids, semi-solids, tablets, capsules, lozenges, strips, powders, gels, gums, pastes, slurries, syrups, aerosols and sprays. Non-limiting examples of consumer products include nutriceuticals, nutritional supplements, lipsticks, lip balms, soaps, shampoos, gums, adhesives (e.g., dental adhesives), toothpastes, oral analgesics, breath fresheners, mouthwashes, tooth whiteners, and other dentifrices.

[0066] The term "contacting" refers to any interaction between an antagonist, an agonist, a modulator, a tastant or a test compound with a polypeptide (e.g., a TAS2R receptor) or a host cell expressing a polypeptide, whereby any of the at least two components can be independently of each other in a liquid phase, for example in solution, or in suspension or can be bound to a solid phase, for example, in the form of an essentially planar surface or in the form of particles, pearls or the like. The polypeptide may be TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, or TAS2R60. Similarly, the host cell may express TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16,

TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, TAS2R60, or a combination thereof.

[0067] The term "diet" collectively refers to the food products and/or beverages consumed by a subject. A subject's "diet" also includes any consumer products or pharmaceutical compositions the subject ingests.

[0068] The term "edible composition" refers to a composition suitable for consumption, typically via the oral cavity (although consumption may occur via non-oral means such as inhalation). Edible compositions may be present in any form including, but not limited to, liquids, solids, semi-solids, tablets, lozenges, powders, gels, gums, pastes, slurries, syrups, aerosols and sprays. As used herein, edible compositions include food products, pharmaceutical compositions, and consumer products. The term edible compositions also refers to, for example, dietary and nutritional supplements. As used herein, edible compositions also include compositions that are placed within the oral cavity but not swallowed, including professional dental products, such as dental treatments, fillings, packing materials, molds and polishes. The term "comestible" refers to similar compositions and is generally used as a synonym to the term "edible."

[0069] The term "effective amount" refers to an amount sufficient to produce a desired property or result. For example, an effective amount of a compound used in an assay of the present invention is an amount capable of reducing the perception of bitter taste associated with a bitter tastant. Typically, an effective amount of a compound used in an assay of the present invention is an amount capable of inhibiting the activation of a bitter taste receptor by a bitter tastant. Alternatively, an effective amount of a compound used in an assay of the present invention is an amount capable of activating a bitter taste receptor in the absence of another bitter tastant.

[0070] The term "flavor modifier" refers to a compound or a mixture of compounds that, when added to an edible composition, such as a food product, modifies (e.g., masks, eliminates, decreases, reduces, or enhances the perception of) a flavor (e.g., sweet, salty, umami, sour, or bitter taste) present in the edible composition.

[0071] The phrase "functional bitter taste receptor" refers to a bitter taste receptor that responds to a known activator or a known inhibitor in substantially the same way as the bitter taste receptor in a cell that normally expresses the bitter taste receptor without engineering. Bitter taste receptor behavior can be determined by, for example, physiological activities and pharmacological responses. Physiological activities include, but are not limited to, the sense of bitter taste. Pharmacological responses include, but are not limited to, a change in the amount or distribution of an intracellular molecule or the activity of an enzyme which is part of the intracellular signaling pathway for the bitter taste receptor when a bitter taste receptor is contacted with a modulator. For example, a pharmacological response may include an increase in intracellular free calcium when the bitter taste receptor is activated, or a decrease in intracellular free calcium when the bitter taste receptor is blocked.

[0072] The term "modulator" refers to a compound or substance that alters the structure, conformation, biochemical or biophysical properties or functionality of a bitter taste receptor, either

positively or negatively. The modulator can be a bitter taste receptor agonist (potentiator or activator) or antagonist (inhibitor or blocker), including partial agonists or antagonists, selective agonists or antagonists and inverse agonists, and can be an allosteric modulator. A substance or compound is a modulator even if its modulating activity changes under different conditions or concentrations or with respect to different forms of bitter taste receptors, e.g., naturally occurring form vs. mutant form, and different naturally-occurring allelic variants of a bitter taste receptor (e.g., due to polymorphism). As used herein, a modulator may affect the activity of a bitter taste receptor, the response of a bitter taste receptor to another regulatory compound or the selectivity of a bitter taste receptor. A modulator may also change the ability of another modulator to affect the function of a bitter taste receptor. A modulator may act upon all or upon a specific subset of bitter taste receptors. Modulators include, but are not limited to, potentiators, activators, inhibitors, agonists, antagonists and blockers.

[0073] As used herein, the term "native" protein (e.g., bitter taste receptor) refers to a protein that does not have a heterologous amino acid sequence appended or inserted to it. For example, "native bitter taste receptor" used herein includes bitter taste receptors that do not have a tag sequence that is expressed on the polypeptide level. By referring to bitter taste receptors as native, applicants do not intend to exclude bitter taste receptor variants that comprise an amino acid substitution, mutation or deletion, or variants that are fragments or spliced forms of naturally occurring, or previously known receptor proteins.

[0074] The term "off-target effects" refers to the unintended modulation, activation or inhibition of an untargeted taste receptor. For example, a bitter taste modulator exhibits off-target effects if it is intended to modulate the activity of a particular subset of bitter taste receptors, and it also modulates other bitter taste receptors or the activity of other taste receptors, such as sweet taste receptors and umami taste receptors. Similarly, if a taste modulator is intended to modulate the bitter taste due to a bitter tastant, the taste modulator exhibits off-target effects if it modulates the taste due to another tastant or if it confers a taste on its own. Off-target effects of bitter taste modulators can result in the activation or inhibition of salty, sweet, sour, umami and/or other bitter tastes.

[0075] The terms "parts per million" and "ppm" are used in the food industry to refer to a low concentration of a solution. For example, one gram of solute in 1000 ml of solvent has a concentration of 1000 ppm and one thousandth of a gram (0.001g) of solute in 1000 ml of solvent has a concentration of one ppm. Accordingly, a concentration of one milligram per liter (i.e. 1 mg/L) is equal to 1 ppm.

[0076] The phrase "percent identical" or "percent identity" in connection with amino acid and/or nucleic acid sequences refers to the similarity between at least two different sequences. This percent identity can be determined by standard alignment algorithms, for example, the Basic Local Alignment Tool (BLAST) described by Altshul et al. ((1990) J. Mol. Biol., 215: 403 410); the algorithm of Needleman et al. ((1970) J. Mol. Biol., 48: 444 453); or the algorithm of Meyers et al. ((1988) Comput. Appl. Biosci., 4: 11 17). A set of parameters may be the Blosum 62 scoring

matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller ((1989) CABIOS, 4:11-17) that has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity is usually calculated by comparing sequences of similar length. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, the GCG Wisconsin Package (Accelrys, Inc.) contains programs such as "Gap" and "Bestfit" that can be used with default parameters to determine sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutation thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters. A program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, Methods Enzymol. 183:63-98 (1990); Pearson, Methods Mol. Biol. 132:185-219 (2000)). The length of polypeptide sequences compared for identity will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. The length of a DNA sequence compared for identity will generally be at least about 48 nucleic acid residues, usually at least about 60 nucleic acid residues, more usually at least about 72 nucleic acid residues, typically at least about 84 nucleic acid residues, and preferably more than about 105 nucleic acid residues.

[0077] The terms "perception of a bitter taste," "perception of saltiness," "perception of a flavor" and similar terms, refer to the awareness of a subject of a particular taste or flavor.

[0078] The term "selective bitter taste modulator" refers to a compound that modulates the bitter taste due to a specific bitter tastant without modulating any other tastants or conferring its own taste. For example, a compound that selectively inhibits bitter taste due to KCl decreases bitter taste due to KCl without increasing or decreasing the taste due to another tastant, including other bitter tastants. In some embodiments selective bitter taste modulators are agonists or antagonists for a particular bitter taste receptor or a particular subset of bitter taste receptors. For example, a compound that selectively inhibits bitter taste due to KCl antagonizes the bitter taste receptor activity of one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 without activating or inhibiting any other taste receptor, including other bitter taste receptors.

[0079] The term "stable" or "stably expressing" is meant to distinguish the cells and cell lines of the invention from cells with transient expression as the terms "stable expression" and "transient expression" would be understood by a person of skill in the art.

[0080] The term "stringent conditions" or "stringent hybridization conditions" describe temperature and salt conditions for hybridizing one or more nucleic acid probes to a nucleic acid sample and washing off probes that have not bound specifically to target nucleic acids in the

sample. Stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. An example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 60°C. A further example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 65°C. Stringent conditions include hybridization in 0.5M sodium phosphate, 7% SDS at 65°C, followed by at least one wash at 0.2X SSC, 1% SDS at 65°C.

[0081] The term "subject" refers to a mammal. In preferred embodiments, the subject is human. In some embodiments, a subject is a domestic or laboratory animal, including but not limited to, household pets, such as dogs, cats, pigs, rabbits, rats, mice, gerbils, hamsters, guinea pigs, and ferrets. In some embodiments, a subject is a livestock animal. Non-limiting examples of livestock animals include: alpaca, bison, camel, cattle, deer, pigs, horses, llamas, mules, donkeys, sheep, goats, rabbits, reindeer, and yak.

[0082] The term "sweet flavor" refers to the taste elicited by, for example, sugars. Non-limiting examples of compositions eliciting a sweet flavor include glucose, sucrose, fructose, saccharin, cyclamate, aspartame, Acesulfame potassium, sucralose, alitame, and neotame. The amount of sweet flavor or the sweetness of a composition can be determined by, *e.g.*, taste testing.

[0083] The term "universal bitter compound" refers to a compound that activates all twenty-five bitter taste receptors. Non-limiting examples of universal bitter compounds include denatonium benzoate and denatonium saccharide.

[0084] The present invention provides assays for identifying compounds that modulate bitter taste. For example, the present invention provides methods of identifying compounds that inhibit the bitter taste due to KCl, potassium lactate or Acesulfame K. The present invention also provides methods of identifying compounds that selectively inhibit the bitter taste due to KCl, potassium lactate, or Acesulfame K. The present invention further provides methods of identifying compounds that mimic the bitter taste due to KCl, potassium lactate, or Acesulfame K. The present invention also provides methods of identifying compounds that enhance the bitter taste due to KCl, potassium lactate or Acesulfame K. The present invention additionally provides methods for determining if KCl, potassium lactate or Acesulfame K are present in a composition.

Cells and cell lines expressing bitter taste receptors

[0085] The present invention relates to *in vitro* assays utilizing cells and cell lines that express or have been engineered to express one or more bitter taste receptors. In some embodiments, the cells or cell lines of the invention express one or more functional bitter taste receptors.

[0086] According to one embodiment of the invention, the cells and cell lines are transfected with a nucleic acid encoding a bitter taste receptor. In other embodiments, the cells and cell lines endogenously express a bitter taste receptor. In some embodiments the cells and cell lines are transfected with a nucleic acid encoding an allelic variant (i.e., a polymorphism) of a bitter taste

receptor, or a mutant bitter taste receptor. The cell lines of the invention may stably express the introduced bitter taste receptor. In another embodiment, the cells and cell lines have a bitter taste receptor activated for expression by gene activation. In some embodiments, the bitter taste receptor is a native bitter taste receptor.

[0087] In a particular embodiment, the cells and cell lines express an endogenous bitter taste receptor as a result of engineered gene activation, *i.e.*, activation of the expression of an endogenous gene, wherein the activation does not naturally occur in a cell without proper treatment. Engineered gene activation may turn on the expression of an endogenous bitter taste receptor, for example, where the endogenous bitter taste receptor is not expressed in the cell line without the proper treatment. Alternatively, engineered gene activation may result in increased expression level of the endogenous bitter taste receptor, for example, where the expression level of the endogenous gene in the cell line is undesirably low without the proper treatment, for example, not sufficient for functional assay of the bitter taste receptor in the cell line. Alternatively, engineered gene activation may be used to over-express an endogenous bitter taste receptor, for example, for isolating the endogenous bitter taste receptor from the cell line. Engineered gene activation can be achieved by a number of means known to those skilled in the art. For example, one or more transcription factors or transactivators of transcription of a gene can be over-expressed or induced to express by, e.g., introducing nucleic acids expressing the transcription factors or transactivators into a cell under the control of a constitutive or inducible promoter. If the endogenous gene is known to be under the control of an inducible promoter, expression can be induced by exposing the cell to a known inducer of the gene. In addition, a nucleic acid encoding the endogenous gene itself can be introduced into a cell to obtain an increased level of expression of the gene due to increased copy number in the genome. Furthermore, certain known inhibitors of the expression of an endogenous gene that are expressed by the cell can be knocked down or even knocked out in the cell using techniques well known in the art, e.g., RNAi, thereby increasing the expression of the endogenous gene.

[0088] In some embodiments, cells and cell lines stably express one or more bitter taste receptors. In some embodiments, the expressed bitter taste receptors increase intracellular free calcium upon activation by an agonist. In some embodiments, a potentiator, agonist or activator can be a small molecule, a chemical moiety, a polypeptide, an antibody, or a food extract. In other embodiments, the expressed bitter taste receptors decrease intracellular free calcium upon inhibition by an antagonist. In some embodiments, an inhibitor, antagonist or blocker can be a small molecule, a chemical moiety, a polypeptide, an antibody, or a food extract. A potentiator, agonist, activator, inhibitor, antagonist or blocker may act upon all or upon a specific subset of bitter taste receptors.

[0089] According to the invention, the bitter taste receptor expressed by a cell or cell line can be from any mammal, including rat, mouse, rabbit, goat, dog, cow, pig or primate. In a preferred embodiment, the bitter taste receptor is human bitter taste receptor.

[0090] In some embodiments, a cell or cell line of the invention may comprise: a nucleotide sequence (SEQ ID NO:2) that encodes a human TAS2R1; a nucleotide sequence (SEQ ID NO:3)

that encodes a human TAS2R3; a nucleotide sequence (SEQ ID NO:4) that encodes a human TAS2R4; a nucleotide sequence (SEQ ID NO:5) that encodes a human TAS2R5; a nucleotide sequence (SEQ ID NO:6) that encodes a human TAS2R7; a nucleotide sequence (SEQ ID NO:7) that encodes a human TAS2R8; a nucleotide sequence (SEQ ID NO:8) that encodes a human TAS2R9; a nucleotide sequence (SEQ ID NO:9) that encodes a human TAS2R10; a nucleotide sequence (SEQ ID NO:10) that encodes a human TAS2R13; a nucleotide sequence (SEQ ID NO:11) that encodes a human TAS2R14; a nucleotide sequence (SEQ ID NO:12) that encodes a human TAS2R16; a nucleotide sequence (SEQ ID NO:13) that encodes a human TAS2R38; a nucleotide sequence (SEQ ID NO:14) that encodes a human TAS2R39; a nucleotide sequence (SEQ ID NO:15) that encodes a human TAS2R40; a nucleotide sequence (SEQ ID NO:16) that encodes a human TAS2R41; a nucleotide sequence (SEQ ID NO:17) that encodes a human TAS2R43; a nucleotide sequence (SEQ ID NO:18) that encodes a human TAS2R44; a nucleotide sequence (SEQ ID NO:19) that encodes a human TAS2R45; a nucleotide sequence (SEQ ID NO:20) that encodes a human TAS2R46; a nucleotide sequence (SEQ ID NO:21) that encodes a human TAS2R47; a nucleotide sequence (SEQ ID NO:22) that encodes a human TAS2R48; a nucleotide sequence (SEQ ID NO:23) that encodes a human TAS2R49; a nucleotide sequence (SEQ ID NO:24) that encodes a human TAS2R50; a nucleotide sequence (SEQ ID NO:25) that encodes a human TAS2R55; a nucleotide sequence (SEQ ID NO:26) that encodes a human TAS2R60; or any combination thereof. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 2-26. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 95% sequence identity to any one of SEQ ID NOs: 2-26. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 2-26, wherein the nucleotide sequence encodes a polypeptide having TAS2R activity. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 95% sequence identity to any one of SEQ ID NOs: 2-26, wherein the nucleotide sequence encodes a polypeptide having TAS2R activity. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that hybridizes under stringent conditions to any one of SEQ ID NOs: 2-26. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that comprises the mature form of any one of SEQ ID NOs: 2-26. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that is a variant of any one of SEQ ID NOs: 2-26. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that is a fragment of any one of SEQ ID NOs: 2-26. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence proteolytic cleavage product of any one of SEQ

ID NOs: 2-26. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that is an ortholog of any one of SEQ ID NOs: 2-26. Such orthologs are well-known in the art. In some embodiments, the nucleotide sequence has five or fewer, four or fewer, three or fewer, two or fewer, or one or fewer conservative substitutions compared to any one of SEQ ID NO: 2-26.

[0091] In some embodiments, a cell or cell line of the invention may comprise a polynucleotide sequence encoding human TAS2R1 (SEQ ID NO: 28); human TAS2R3 (SEQ ID NO:29); human TAS2R4 (SEQ ID NO: 30); human TAS2R5 (SEQ ID NO:31); human TAS2R7 (SEQ ID NO:32); human TAS2R8 (SEQ ID NO:33); human TAS2R9 (SEQ ID NO:34); human TAS2R10 (SEQ ID NO:35); human TAS2R13 (SEQ ID NO:36); human TAS2R14 (SEQ ID NO:37); human TAS2R16 (SEQ ID NO:38); human TAS2R38 (SEQ ID NO:39); human TAS2R39 (SEQ ID NO:40); human TAS2R40 (SEQ ID NO:41); human TAS2R41 (SEQ ID NO:42); human TAS2R43 (SEQ ID NO:43); human TAS2R44 (SEQ ID NO:44); human TAS2R45 (SEQ ID NO:45); human TAS2R46 (SEQ ID NO:46); human TAS2R47 (SEQ ID NO:47); human TAS2R48 (SEQ ID NO:48); human TAS2R49 (SEQ ID NO:49); human TAS2R50 (SEQ ID NO:50); human TAS2R55 (SEQ ID NO:51); human TAS2R60 (SEQ ID NO:52); or any combination thereof. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the nucleotide sequence encoding any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 95% sequence identity to the nucleotide sequence encoding any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the nucleotide sequence encoding any one of SEQ ID NOs: 28-52, wherein the nucleotide sequence encodes a polypeptide having TAS2R activity. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence having 95% sequence identity to the nucleotide sequence encoding any one of SEQ ID NOs: 28-52, wherein the nucleotide sequence encodes a polypeptide having TAS2R activity. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence encoding any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that encodes the mature form of any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that is a variant of the nucleotide sequence encoding any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that is a fragment of the nucleotide sequence encoding any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence proteolytic cleavage product of the

nucleotide sequence encoding any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence that is an ortholog of the nucleotide sequence encoding any one of SEQ ID NOs: 28-52. Such orthologs are well-known in the art. In some embodiments, the nucleotide sequence encodes a TAS2R receptor that has five or fewer, four or fewer, three or fewer, two or fewer, or one or fewer conservative amino acid substitutions compared to any one of SEQ ID NO: 28-52.

[0092] In some embodiments, a cell or cell line of the invention may comprise a human TAS2R1 (SEQ ID NO: 28); human TAS2R3 (SEQ ID NO:29); human TAS2R4 (SEQ ID NO: 30); human TAS2R5 (SEQ ID NO:31); human TAS2R7 (SEQ ID NO:32); human TAS2R8 (SEQ ID NO:33); human TAS2R9 (SEQ ID NO:34); human TAS2R10 (SEQ ID NO:35); human TAS2R13 (SEQ ID NO:36); human TAS2R14 (SEQ ID NO:37); human TAS2R16 (SEQ ID NO:38); human TAS2R38 (SEQ ID NO:39); human TAS2R39 (SEQ ID NO:40); human TAS2R40 (SEQ ID NO:41); human TAS2R41 (SEQ ID NO:42); human TAS2R43 (SEQ ID NO:43); human TAS2R44 (SEQ ID NO:44); human TAS2R45 (SEQ ID NO:45); human TAS2R46 (SEQ ID NO:46); human TAS2R47 (SEQ ID NO:47); human TAS2R48 (SEQ ID NO:48); human TAS2R49 (SEQ ID NO:49); human TAS2R50 (SEQ ID NO:50); human TAS2R55 (SEQ ID NO:51); human TAS2R60 (SEQ ID NO:52); or any combination thereof. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of an amino acid sequence having 80%, 85%, 90%, 95%, 98% or 99% sequence identity to any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of an amino acid sequence having 95% sequence identity to any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of an amino acid sequence having 80%, 85%, 90%, 95%, 98% or 99% sequence identity to any one of SEQ ID NOs: 28-52, wherein the TAS2R receptor has TAS2R receptor activity. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of an amino acid sequence having 95% sequence identity to any one of SEQ ID NOs: 28-52, wherein the TAS2R receptor has TAS2R receptor activity. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of the mature form of any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of a variant of any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of a fragment of any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of a proteolytic cleavage product of any one of SEQ ID NOs: 28-52. In some embodiments, the cell or cell line comprises a TAS2R receptor selected from the group consisting of an ortholog of any one of SEQ ID NOs: 28-52. Such orthologs are well-known in the art. In some embodiments, the TAS2R receptor has five or fewer, four or fewer, three or fewer, two or fewer, or one or fewer conservative amino acid substitutions compared to any one of SEQ ID NO: 28-52.

[0093] Nucleic acids encoding bitter taste receptors can be DNA, synthetic DNA, genomic DNA cDNA, RNA, double-stranded DNA, or single-stranded DNA. In some embodiments, the nucleic acids comprise one or more mutations, as compared to the nucleic acid sequences encoding wild type bitter taste receptors, that may or may not result in an amino acid substitution. In some other embodiments, the nucleic acids comprise one or more naturally-occurring allelic variants, as compared to the most frequently occurring nucleic acid sequences encoding a certain bitter taste receptor in a given population. Naturally-occurring allelic variants include different amino acid sequences of a same bitter taste receptor that are naturally-occurring, e.g., those observed in a given population due to allelic variation or polymorphism. In some embodiments, the nucleic acid encoding the bitter taste receptor is a fragment. In some embodiments the fragment encodes a polypeptide that has TAS2R activity. In some embodiments, the fragment comprises at least 30, at least 40, at least 50, at least 60, at least 80, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 850, or at least 900 nucleotides of SEQ ID NO: 2-26.

[0094] Polymorphism is a common phenomenon in the human genome. Polymorphisms occurring within or near the bitter taste receptor genes may affect their expression or change their function by, e.g., up-regulating or down-regulating their expression levels or by changing their amino acid sequences. Appendix Table 1 shows reference numbers for unique polymorphisms, including single nucleotide polymorphisms ("SNPs") related to human TAS2R genes, position of the SNPs in each reference sequence, and description of the SNPs. The reference numbers are searchable in the Single Nucleotide Polymorphism database ("dbSNP") of the National Center for Biotechnology Information ("NCBI"; Bethesda, MD).

[0095] Allelic variations of human bitter taste receptor genes resulting in coding sequence diversity have been studied and documented. See, e.g., Ueda et al., "Identification of coding single-nucleotide polymorphisms in human taste receptor genes involving bitter tasting", Biochem Biophys Res Commun 285:147-151, 2001; Wooding et al., "Natural selection and molecular evolution in PTC, a bitter-taste receptor gene," Am. J. Hum, Genet. 74:637-646, 2004; and Kim et al., "Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci", Human Mutation 26:199-204, 2005. Appendix Table 2 is a list of natural variations in the coding sequences of different human bitter taste receptors. The human bitter taste receptors, SEQ ID NOS of their coding sequences, and the protein sequences are listed in the first three columns. The nucleotide changes and their positions within each coding sequence as identified by their SEQ ID NOS are indicated in the columns under "Nucleotide change" and "Position of nucleotide change," respectively. The amino acid changes within each bitter taste receptor as identified by their SEQ ID NOS are indicated in the column under "Description" using single-letter abbreviations. Their positions with reference to each corresponding SEQ ID NO are indicated in the column under "Position of amino acid change." In addition, the "Description" column also contains identifiers of those variations that are searchable in dbSNP of NCBI. "Feature identifiers" are unique and stable feature identifiers assigned to some of the variations by the UniProt Protein

Knowledgebase hosted by the European Bioinformatics Institute (Cambridge, United Kingdom). They are searchable within UniProt. "NA" denotes no feature identifiers assigned by UniProt yet.

[0096] Variation in human taste is a well-known phenomenon. Without wishing to be bound by theory, the variation of bitter taste may be related to polymorphisms of the bitter taste receptors. For example, polymorphisms in the hTAS2R38, a receptor for phenylthiocarbamide (PTC), has been linked to the ability to detect propylthiouracil (PROP) (Kim et al., "Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide", Science 299:1221-1225, 2003; Wooding et al., 2004). There are three common polymorphisms in the TAS2R38 gene-A49P, V262A, and I296V-which combine to form two common haplotypes and several other very rare haplotypes. The two common haplotypes are AVI (often called "nontaster") and PAV (often called "taster"). Varying combinations of these haplotypes will yield homozygotes-PAV/PAV and AVI/AVI-and heterozygote PAV/AVI. These genotypes can account for up to 85% of the variation in PTC tasting ability: people possessing two copies of the PAV polymorphism report PTC to be bitterer than TAS2R38 heterozygotes, and people possessing two copies of the AVI/AVI polymorphism often report PTC as being essentially tasteless. These polymorphisms are hypothesized to affect taste by altering G-protein-binding domains. In some embodiments, a cell or cell line of the invention may comprise a polynucleotide sequence encoding human PAV TAS2R38 (SEQ ID NO: 54). In some embodiments, a cell or cell line of the invention may comprise a human PAV TAS2R38 (SEQ ID NO: 54).

[0097] Additionally, a subset of the population, who are very sensitive to the bitterness of the natural plant compounds aloin and aristolochic acid, have inherited certain polymorphisms in the hTAS2R43 gene. People who do not possess this allele do not taste these compounds at low concentrations. The hTAS2R43 sensitivity allele also makes people more sensitive to the bitterness of saccharin. Further, certain hTAS2R44 alleles, which are closely related to hTAS2R43 sensitivity alleles, also make people more sensitive to the bitterness of saccharin. Some subsets of the population do not possess certain hTAS2R genes, contributing to taste variation between individuals. Polymorphisms in bitter genes have also been linked to increased risk of disease, e.g., diabetes (TAS2R9) and alcoholism (TAS2R16). Assays utilizing cells and cell lines stably expressing a heterologous naturally-occurring bitter taste receptor, or an allelic variant or polymorph thereof, or a mutant form thereof having one or more mutations (e.g., random mutations or site-specific mutations) that are not naturally-occurring, are all within the scope of the present invention.

[0098] The cells and cell lines comprising a bitter taste receptor, a mutant form thereof, or a naturally-occurring allelic variant thereof, can be used to identify modulators of bitter taste receptor function, including modulators that are specific for a particular bitter taste receptor mutant form or naturally-occurring allelic variant. The cells and cell lines can thus be used to obtain information about the properties, activities and roles of individual native or mutant forms or naturally-occurring allelic variants of bitter taste receptors and to identify bitter taste receptor modulators with activity for a particular native or mutant form or naturally-occurring allelic variant or for a subset of native

or mutant forms or naturally-occurring allelic variants. These modulators are useful as therapeutics that target differentially modified bitter taste receptor forms in disease states or tissues. Because the polymorphism of bitter taste receptors *in vivo*, for example, may contribute to an undesired activity or disease state, cells and cell lines of this invention also can be used to screen for modulators for therapeutic use where alteration of the response of a mutant form or naturally-occurring allelic variant may be desired. The cells and cell lines are also useful to identify modulators that have activity with only subset of native or mutant forms or naturally-occurring allelic variants of a bitter taste receptor.

[0099] Host cells used to produce a cell or cell line may express in their native state one or more endogenous bitter taste receptor or lack expression of any bitter taste receptor. In the case where the cell or cell line expresses one or more of its own bitter taste receptors, also referred to as "endogenous" bitter taste receptors, the heterologous bitter taste receptor can be the same as one of the cell or cell line's endogenous bitter taste receptor(s). For example, a nucleic acid encoding an bitter taste receptor endogenous to a cell or cell line may be introduced into the cell or the cell line to increase the copy number of the gene encoding the bitter taste receptor in the cell or the cell line so that the bitter taste receptor is expressed at a higher level in the cell or cell line than without the introduced nucleic acid. The host cell may be a primary, germ, or stem cell, including an embryonic stem cell. The host cell may also be an immortalized cell. Primary or immortalized host cells may be derived from mesoderm, ectoderm or endoderm layers of eukaryotic organisms. The host cell may be endothelial, epidermal, mesenchymal, neural, renal, hepatic, hematopoietic, or immune cells. For example, the host cells may be intestinal crypt or villi cells, clara cells, colon cells, intestinal cells, goblet cells, enterochromafin cells, enteroendocrine cells. The host cells may be eukaryotic, prokaryotic, mammalian, human, primate, bovine, porcine, feline, rodent, marsupial, murine or other cells. The host cells may also be non-mammalian, such as yeast, insect, fungus, plant, lower eukaryotes and prokaryotes. Such host cells may provide backgrounds that are more divergent for testing bitter taste receptor modulators with a greater likelihood for the absence of expression products provided by the cell that may interact with the target. In preferred embodiments, the host cell is a mammalian cell. Examples of host cells that may be used to produce a cell or cell line of the invention include but are not limited to: human embryonic kidney 293T cells, established neuronal cell lines, pheochromocytomas, neuroblastomas fibroblasts, rhabdomyosarcomas, dorsal root ganglion cells, NS0 cells, CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), L-cells, HEK-293 (ATCC CRL1573) and PC12 (ATCC CRL-1721), HEK293T (ATCC CRL-11268), RBL (ATCC CRL-1378), SH-SY5Y (ATCC CRL-2266), MDCK (ATCC CCL-34), SJ-RH30 (ATCC CRL-2061), HepG2 (ATCC HB-8065), ND7/23 (ECACC 92090903), CHO (ECACC 85050302), Vero (ATCC CCL 81), Caco-2 (ATCC HTB 37), K562 (ATCC CCL 243), Jurkat (ATCC TIB-152), Per.C6 (Crucell, Leiden, The Netherlands), Huvec (ATCC Human Primary PCS 100-010, Mouse CRL 2514, CRL 2515, CRL 2516), HuH-

7D12 (ECACC 01042712), 293 (ATCC CRL 10852), A549 (ATCC CCL 185), IMR-90 (ATCC CCL 186), MCF-7 (ATC HTB-22), U-2 OS (ATCC HTB-96), T84 (ATCC CCL 248), or any established cell line (polarized or nonpolarized) or any cell line available from repositories such as American Type Culture Collection (ATCC, 10801 University Blvd. Manassas, Va. 20110-2209 USA) or European Collection of Cell Cultures (ECACC, Salisbury Wiltshire SP4 0JG England).

[0100] As will be appreciated by those of skill in the art, any vector that is suitable for use with the host cell may be used to introduce a nucleic acid encoding a TAS2R receptor into the host cell. The vectors comprising the various TAS2R receptors may be the same type or may be of different types. Examples of vectors that may be used to introduce the TAS2R receptor encoding nucleic acids into host cells include but are not limited to plasmids, viruses, including retroviruses and lentiviruses, cosmids, artificial chromosomes and may include for example, Pcmv-Script, pcDNA3.1 Hygro, pcDNA3.1neo, pcDNA3.1puro, pSV2neo, pIRES puro, pSV2 zeo, pFN11A (BIND) Flexi®,pGL4.31,pFC14A (HaloTag® 7) CMV Flexi®,pFC14K (HaloTag® 7) CMV Flexi®,pFN24A (HaloTag® 7) CMVd3 Flexi®,pFN24K (HaloTag® 7) CMVd3 Flexi®,HaloTag™ pHT2,pACT ,pAdVAntage™,pALTER®-MAX, pBIND, pCAT®3-Basic, pCAT®3-Control, pCAT®3-Enhancer,pCAT®3-Promoter,pCI,pCMVTNT™, pG5luc, pSI, pTARGET™, pTNT™, pF12A RM Flexi®, pF12K RM Flexi®, pReg neo, pYES2/GS, pAd/CMV/V5-DEST Gateway® Vector, pAd/PL-DEST™ Gateway®, Vector, Gateway®, pDEST™, 27 Vector, Gateway®, pEF-DEST51 Vector, Gateway®, pcDNA™-DEST47 vector, pCMV/Bsd Vector, pEF6/His A, B, & c, pcDNA™6.2-DEST, pLenti6/TR, pLP-AcGFP1-C, pLPS-AcGFP1-N, pLP-IRESneo, pLP-TRE2, pLP-RevTRE, pLP-LNCX, pLP-CMV-HA, pLP-CMV-Myc, pLP-RetroQ, pLP-CMVneo. In some embodiments, the vectors comprise expression control sequences such as constitutive or conditional promoters. One of ordinary skill in the art will be able to select the appropriate sequences. For example, suitable promoters include but are not limited to CMV, TK, SV40 and EF-1α. In some embodiments, the promoters are inducible, temperature regulated, tissue specific, repressible, heat-shock, developmental, cell lineage specific, eukaryotic, prokaryotic or temporal promoters or a combination or recombination of unmodified or mutagenized, randomized, shuffled sequences of any one or more of the above. In other embodiments, TAS2R receptors are expressed by gene activation, wherein an exogenous promoter is inserted in a host cell's genome by homologous recombination to drive expression of a TAS2R receptor gene that is not normally expressed in that host cell. In some embodiments the gene encoding a TAS2R receptor is episomal. Nucleic acids encoding TAS2R receptor are preferably constitutively expressed.

[0101] Nucleic acids comprising a sequence encoding a TAS2 receptor, or the sequence of a component of the TAS2R signaling pathway, and optionally a nucleic acid encoding a selectable marker may be introduced into selected host cells by well known methods. The methods include but not limited to transfection, viral delivery, protein or peptide mediated insertion, coprecipitation methods, lipid based delivery reagents (lipofection), cytofection, lipopolyamine delivery, dendrimer delivery reagents, electroporation or mechanical delivery. Examples of transfection reagents are GENEPORTER, GENEPORTER2, LIPOFECTAMINE, LIPOFECTAMINE 2000, FUGENE 6,

FUGENE HD, TFX-10, TFX-20, TFX-50, OLIGOFECTAMINE, TRANSFAST, TRANSFECTAM, GENESHUTTLE, TROJENE, GENESILENCER, X-TREMEGENE, PERFECTIN, CYTOFECTIN, SIPORT, UNIFECTOR, SIFECTOR, TRANSIT-LT1, TRANSIT-LT2, TRANSIT-EXPRESS, IFECT, RNAI SHUTTLE, METAFECTENE, LYOVEC, LIPOTAXI, GENEERASER, GENEJUICE, CYTOPURE, JETSI, JETPEI, MEGAFECTIN, POLYFECT, TRANSMESSANGER, RNAiFECT, SUPERFECT, EFFECTENE, TF-PEI-KIT, CLONFECTIN, AND METAFECTINE.

[0102] In another aspect, cells and cell lines expresses a G protein. There are two families of G proteins, heterotrimeric G proteins and monomeric G proteins. Heterotrimeric G proteins are activated by G protein coupled receptors ("GPCRs"), and include three subunits: G_α, G_β and G_γ. As used herein, the term G protein includes any one of these subunits, for example a G_α, or any combination thereof, as well as a heterotrimeric G protein with all three subunits. In the inactive state, G_α, G_β and G_γ form a trimer. The β and γ subunits are closely bound to one another and are referred to as the *beta-gamma complex*. G_α separates from $G_{\beta\gamma}$ after ligand binding to the GPCR. The $G_{\beta\gamma}$ complex is released from the G_α subunit after its GDP-GTP exchange. The $G_{\beta\gamma}$ complex can activate other second messengers or gate ion channels. The four families of G alpha include: G_s (stimulatory) which increase cAMP synthesis by activating adenylate cyclase; G_i (inhibitory) that inhibits adenylate cyclase; the $G_{12/13}$ family regulates various cell movement processes (i.e. cytoskeleton, cell junctions); and G_q, which stimulates calcium signaling and phospholipase C. The monomeric G proteins are homologous to the α subunit of the heterotrimeric G proteins. Any G protein may be expressed in the cells or cell lines of the invention, including, but not limited to, transducin (e.g., GNAT1, GNAT2, and guanine nucleotide-binding protein G(t)), gustducin (e.g., GNAT3 guanine nucleotide binding protein and α transducin 3), human GNA15 (guanine nucleotide binding protein (G protein) α15 (Gq class; synonym GNA16) and mouse Gα15, and their chimera proteins, e.g. Gα15-GNA15 (also known as Gα15-Gα16). In a preferred embodiment, the G protein is mouse Gα15 (SEQ ID NO:53). In another preferred embodiment, the G protein is human GNA15 (SEQ ID NO:1) or is a human G protein encoded by a nucleic acid comprising SEQ ID NO:27. The G protein may also be any mammalian G protein, such as, but not limited to, any mammalian G protein listed in Appendix Table 3. The G protein stably expressed by the cell can be endogenous to the cell. Alternatively, the stable expression of the G protein may be a result of stable transfection of a nucleic acid encoding the G protein into the cell. Cells stably expressing a heterologous G protein are known in the art, e.g., HEK293/Gα15 cells (Chandrashekar et al., "T2Rs function as bitter taste receptors", Cell 100:703-711, 2000; Bufe et al., "The human TAS2R16 receptor mediates bitter taste in response to β-glucopyranosides", Na Genet 32:397-401). In other embodiments, a nucleic acid encoding a G protein and a nucleic acid encoding a bitter taste receptor can be transfected consecutively into a host cell, with either the nucleic acid encoding the G protein transfected first or the nucleic acid encoding the bitter taste receptor transfected first. In other embodiments, a nucleic acid encoding a G protein and a nucleic acid encoding a bitter taste receptor can be co-transfected into a host cell on the same or different vectors. Accordingly, selection of

cells stably expressing both the G protein and the bitter taste receptor, can likewise be carried out consecutively or simultaneously. The cells or cell lines that may be used to stably express a G protein are the same as those that may be used to stably express a bitter taste receptor, as explained above.

[0103] In some embodiments of the invention, cells or cell lines of the invention co-express other proteins with the bitter taste receptor(s). In a preferred embodiment, the other protein is at least one other taste receptor, such as a sweet (TAS1R2/TAS1R3) receptor or an umami (TAS1R1/TAS1R3) receptor. In some embodiments, the cell line panels of the invention include cell lines that express bitter receptors and cell lines that express other taste receptors, such as a sweet (TAS1R2/TAS1R3) receptor or an umami (TAS1R1/TAS1R3) receptor. Proteins that are co-expressed with bitter taste receptors may be expressed by any mechanism, such as, but not limited to, endogenously in the host cell or heterologously from a vector. Also, in other embodiments of the invention, more than one type of bitter taste receptor may be stably expressed in a cell or cell line.

[0104] Also according to the invention, cells and cell lines that express a form of a naturally occurring bitter taste receptor or a naturally-occurring allelic variant thereof, as well as cells and cell lines that express a mutant form of bitter taste receptor, can be characterized for intracellular free calcium levels. In some embodiments, the cells and cell lines of the invention express bitter taste receptor with "physiologically relevant" activity. As used herein, physiological relevance refers to a property of a cell or cell line expressing a bitter taste receptor whereby the bitter taste receptor causes an increase in intracellular free calcium as a naturally occurring bitter taste receptor of the same type would when activated, and responds to modulators in the same ways that naturally occurring bitter taste receptors of the same type would respond when modulated by the same compounds. Bitter taste receptor-expressing cells and cell lines of this invention, including some mutant forms of bitter taste receptor and some naturally-occurring allelic variants of bitter taste receptors, preferably demonstrate comparable function to cells that normally express native bitter taste receptor in a suitable assay, such as an assay measuring intracellular free calcium. Such assays are known to those skilled in the art (Nahorski, "Pharmacology of intracellular signaling pathways," Brit. J. Pharm. 147:S38-S45, 2000)). Such comparisons are used to determine a cell or cell line's physiological relevance. "Sip and spit" taste tests using a panel of trained taste testers also may be used to further validate bitter taste receptor physiological relevance in cells and cell lines of the invention. The results of sip and spit taste tests using modulators identified via screening of native or mutant forms of a bitter taste receptor or a naturally-occurring allelic variant thereof can be used to validate the physiological relevance of these different forms.

[0105] In some embodiments, the cells and cell lines respond to modulators and increase intracellular free calcium with physiological range EC_{50} or IC_{50} values for bitter taste receptors. As used herein, EC_{50} refers to the concentration of a compound or substance required to induce a half-maximal activating response in the cell or cell line. As used herein, IC_{50} refers to the concentration of a compound or substance required to induce a half-maximal inhibitory response in the cell or cell line. EC_{50} and IC_{50} values may be determined using techniques that are well-known in the art, for

example, a dose-response curve that correlates the concentration of a compound or substance to the response of the bitter taste receptor-expressing cell line.

[0106] To make bitter taste receptor expressing cells and cell lines, one can use, for example, the technology described in U.S. Patent 6,692,965 and International Patent Publication WO/2005/079462. Both of these documents are incorporated herein by reference in their entirety for all purposes. This technology provides real-time assessment of millions of cells such that any desired number of clones (from hundreds to thousands of clones) may be selected. Using cell sorting techniques, such as flow cytometric cell sorting (*e.g.*, with a FACS machine) or magnetic cell sorting (*e.g.*, with a MACS machine), one cell per well may be automatically deposited with high statistical confidence in a culture vessel (such as a 96 well culture plate). The speed and automation of the technology allows multigene cell lines to be readily isolated. To make bitter taste receptor expressing cells and cell lines, one can also use, for example, the technology described in International Patent Publications WO 2009/102569 and WO 2010/088633. Both of these documents are incorporated herein by reference in their entirety for all purposes. This technology provides automated methods of generating cells and cell lines matched for physiological properties. Such methods may be used to generate panels of cell lines suitable for high-throughput screening of potential bitter taste receptor modulators.

[0107] Using the technology, the RNA sequence for each bitter taste receptor may be detected using a signaling probe, also referred to as a molecular beacon or fluorogenic probe. In some embodiments, the molecular beacon recognizes a target tag sequence as described above. In another embodiment, the molecular beacon recognizes a sequence within the bitter taste receptor coding sequence itself. Signaling probes may be directed against the RNA tag or bitter taste receptor coding sequence by designing the probes to include a portion that is complementary to the RNA sequence of the tag or the bitter taste receptor coding sequence, respectively. These same techniques may be used to detect the RNA sequence for a G protein, if used.

Methods of Identifying Compounds That Modulate Bitter Taste

[0108] One aspect, the invention provides methods of identifying compounds that modulate bitter taste. In some embodiments, the method is an *in vitro* cell-based assay to, *e.g.*, screen for bitter taste receptor modulators; assess bitterness of substances; produce protein for crystallography and binding studies; and investigate compound selectivity and dosing, receptor/compound binding kinetic and stability, and effects of receptor expression on cellular physiology (*e.g.*, electrophysiology, protein trafficking, protein folding, and protein regulation).

[0109] In some embodiments, the *in vitro* cell-based assays utilize the bitter taste receptor expressing cells and cell lines discussed above. Cells and cell lines expressing various combinations of bitter taste receptors can be used separately or together to identify bitter taste receptor modulators, including those specific for a particular bitter taste receptor or a mutant form or a naturally-occurring allelic variant of bitter taste receptor and to obtain information about the activities of individual forms.

[0110] Modulators include any substance or compound that alters an activity of a bitter taste receptor or a mutant form or a naturally-occurring allelic variant thereof. The modulator can be a bitter taste receptor agonist (potentiator or activator) or antagonist (inhibitor or blocker), including partial agonists or antagonists, selective agonists or antagonists and inverse agonists, and can be an allosteric modulator. A substance or compound is a modulator even if its modulating activity changes under different conditions or concentrations or with respect to different forms (*e.g.*, mutant forms and naturally-occurring allelic variants) of bitter taste receptor. In other aspects, a modulator may change the ability of another modulator to affect the function of a bitter taste receptor. For example, a modulator of a form of bitter taste receptor that is not inhibited by an antagonist may render that form of bitter taste receptor susceptible to inhibition by the antagonist.

[0111] Cells and cell lines may be used to identify the roles of different forms of bitter taste receptors in different bitter taste receptors pathologies by correlating the identity of *in vivo* forms of bitter taste receptor with the identify of known forms of bitter taste receptors based on their response to various modulators. This allows selection of disease- or tissue-specific bitter taste receptor modulators for highly targeted treatment of such bitter taste receptor-related pathologies or other physiological conditions. For example, because many naturally occurring bitter compounds are toxic, bitter taste receptors may serve as warning sensors against the ingestion of toxic food compounds. Bitter taste receptors expressed in the gastrointestinal mucosa might participate in the functional detection of nutrients and harmful substances in the lumen and prepare the gut to absorb them or initiate a protective response. They might also participate in the control of food intake through the activation of gut-brain neural pathways. Accordingly, bitter taste receptor modulators identified using the cell lines and methods of the present invention may be used to regulate nutrient uptake in a number of contexts, e.g., to control the appetite and/or reduce nutrient uptake in the gut of the obese, or to control the hunger feeling and/or to increase the uptake of nutrients and/or energy from food in the malnourished. Bitter taste receptor modulators may also be useful in identifying bitter compounds, further characterizing the specific chemical or structural motifs or key residues of bitter taste receptors that influence their binding properties, identifying bitter taste receptors that are broadly, moderately or selectively tuned for ligand binding, defining groups and subgroups of bitter taste receptors based on their binding profiles, deorphaning orphan bitter taste receptors, using such data for molecular modeling or drug design for bitter taste receptors, and determining in which tissues various bitter taste receptors are active.

[0112] To identify a bitter taste receptor modulator, bitter taste receptor expressing cell or cell line may be exposed to a test compound under conditions in which the bitter taste receptor would be expected to be functional and then detect a statistically significant change (*e.g.*, $p<0.05$) in bitter taste receptor activity compared to a suitable control, *e.g.*, cells that are not exposed to the test compound. Positive and/or negative controls using known agonists or antagonists and/or cells expressing different bitter taste receptor or mutant forms or naturally-occurring allelic variants thereof may also be used. In some embodiments, the bitter taste receptor activity to be detected

and/or measured is change in intracellular free calcium levels. One of ordinary skill in the art would understand that various assay parameters may be optimized, *e.g.*, signal to noise ratio.

[0113] In a further aspect, the invention provides a method of identifying ligands for orphan bitter taste receptors, *i.e.* the invention provides a method of deorphaning bitter taste receptors. A cell or cell line expressing a bitter taste receptor with no known modulator may be screened using a compound or extract library to generate an expression profile for the receptor. Optionally, receptors with similar profiles (if any) are grouped together and screened with known bitter compounds to a ligand(s) that binds a receptor(s). Once a ligand is identified, the results may be further verified with taste tests. Optionally, the cells and cell lines stably express native (*i.e.* untagged) bitter taste receptors so the ligands identified using this method are accurate and relevant.

[0114] In some embodiments, one or more cells or cell lines, including collections of cell lines, are exposed to a test compound. In some embodiments, one or more cells or cell lines, including collections of cell lines, are exposed to a plurality of test compounds, for example, a library of test compounds. A library of test compounds can be screened using the cell lines of the invention to identify one or more modulators. The test compounds can be chemical moieties including small molecules, polypeptides, peptides, peptide mimetics, antibodies or antigen-binding portions thereof. In the case of antibodies, they may be non-human antibodies, chimeric antibodies, humanized antibodies, or fully human antibodies. The antibodies may be intact antibodies comprising a full complement of heavy and light chains or antigen-binding portions of any antibody, including antibody fragments (such as Fab, Fab', $F(ab')_2$, Fd, Fv, dAb and the like), single chain antibodies (scFv), single domain antibodies, all or an antigen-binding portion of a heavy chain or light chain variable region.

[0115] In some embodiments, one or more cells or cell lines, including collections of cell lines, are exposed to a test compound or a plurality of test compounds in the presence of a bitter tastant. In some embodiments, the bitter tastant is a "specific bitter tastant" that activates a subset of the bitter taste receptors. In some embodiments, the bitter tastant is a "universal bitter compound" that activates each bitter taste receptor. Non-limiting examples of universal bitter compounds include denatonium benzoate or denatonium saccharide. A test compound that decreases the induction of bitter taste receptor activity by a bitter tastant is an inhibitor of that bitter tastant. A test compound that increases the induction of bitter taste receptor activity by a bitter tastant is an enhancer of that bitter tastant.

[0116] Some bitter taste modulators and test compounds may exhibit off-target effects. Preferably, the bitter taste modulator or test compound is a selective bitter taste modulator and does not exhibit off-target effects.

[0117] The *in vitro* assays of the invention may be performed using collections of cells or cell lines. In a preferred embodiment, the collection of cells or cell lines includes cells or cell lines expressing each of the 25 bitter taste receptors and/or variants thereof. Such a panel may be used to determine on-target versus off-target activity for a compound, or the role of the receptors in pure bitter versus related (*i.e.*, astringent or metallic) tastes.

[0118] In some embodiments, large compound collections are tested for bitter taste receptor modulating activity in a cell-based, functional, high-throughput screen (HTS), *e.g.*, using a 96 well, 384 well, 1536 well or higher plate format. In some embodiments, a test compound or multiple test compounds including a library of test compounds may be screened using more than one cell or cell line, including collections of cell lines, of the invention. If multiple cells or cell lines, each expressing a different naturally occurring or mutant bitter taste receptor molecule, are used, one can identify modulators that are effective on multiple bitter taste receptors or mutant forms or naturally-occurring allelic variants thereof or alternatively, modulators that are specific for a particular bitter taste receptor or a mutant form or naturally-occurring allelic variant thereof and that do not modulate other bitter taste receptors or other forms of the bitter taste receptor. In the case of a cell or cell line that expresses a human bitter taste receptor, the cells can be exposed to a test compound to identify a compound that modulates bitter taste receptor activity (either increasing or decreasing) for use in the treatment of disease or condition characterized by undesired bitter taste receptor activity, or the decrease or absence of desired bitter taste receptor activity.

[0119] In some embodiments, prior to exposure to a test compound, the cells or cell lines of the invention may be modified by pretreatment with, for example, enzymes, including mammalian or other animal enzymes, plant enzymes, bacterial enzymes, enzymes from lysed cells, protein modifying enzymes, lipid modifying enzymes, and enzymes in the oral cavity, gastrointestinal tract, stomach or saliva. Such enzymes can include, for example, kinases, proteases, phosphatases, glycosidases, oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and the like. Alternatively, the cells and cell lines may be exposed to the test compound first followed by treatment to identify compounds that alter the modification of the bitter taste receptor by the treatment.

[0120] Assays for identifying and measuring GPCR activation are well-known in the art. *See, e.g.*, "G-protein coupled receptors (Signal Transduction Series)," CRC Press 1999; 1st Edition; Eds Haga and Berstein. Any suitable assay for detecting GPCR activation may be used in the methods of the invention to evaluate effect on the activation of bitter taste receptors by potential bitter taste receptor modulators. Examples of such assays include ion sensitive or membrane voltage fluorescent indicators. Under resting state, these dyes are membrane permeable such that exposure to cells allows them to enter cells based on the concentration gradient. Once inside, cellular enzymes convert the dyes into a membrane-impermeable form, trapping the dyes. The membrane impermeable form of the dye is, typically, also highly sensitive to, for example, free intracellular calcium such that calcium binding allows the dye to become fluorescent, when stimulated with lights of specific intensity or wavelengths. Thus, intracellular calcium release in response to GPCR activation may be measured using membrane-permeable dyes that bind to calcium. Such dyes include Indo-1, Fura-2, Fluo-3, Fluo-4, Rhod-2, Rhod-5N, Calcein, Calcein blue, cytoCalcein Violet, Quin-2, Quest Fluo-8H™, Quest Fluo-8L™, Quest Fluo 8™, Quest Rhod-4™, coelenterazine and Calcium-3. In particular embodiments, GPCR activation is measured using Fluo-4 or Calcium-3 fluorescence. In some embodiments, the assay buffer (i.e. load solution) does

not include probenecid. Intracellular calcium levels can be measured by measuring the fluorescence from such dyes in response to calcium binding using, for example, fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Most fluorescent indicators derive from BAPTA chelators that incorporate a photo-induced-electron transfer system that responds to calcium. FLIPR® and FlexStation™ instruments of Molecular Devices Corp., FDSS of Hamamatsu Corp. and NOVOstar™ of BMG Technologies, for example, continuously monitor changes in intracellular calcium levels thereby providing a kinetic read-out of a receptor's activity in the form of a time-dependent fluorescent signal. Accordingly, these instruments enable high throughput measurement of calcium for GPCR research.

[0121] GPCR activity may also be evaluated by measuring adenylate cyclase activity, IP3/Ca^{2+} signaling, phospholipase C/intracellular Ca^{2+} signaling, GTPase activity, GTP binding, microphysiometer/biosensor assays (*see, e.g.*, Hafner, 2000, Biosens. Bioelectron. 15: 149-158), arachinoid acid levels (*see, e.g.*, Gijon et al., 2000, J. Biol.Chem., 275: 20146-20156), cAMP/cGMP levels (by radioimmunoassay or with binding proteins, *see, e.g.*, Horton and Baxendale, 1995, Methods Mol. Biol. 41: 91-105), diacylglycerol (DAG) levels, inositol triphosphate (IP3) levels, protein kinase C activity, and/or MAP kinase activity.

Methods of Identifying Compounds That Modulate Bitter Taste Due to KCl

[0122] According to another aspect, the invention provides a method for identifying a compound that modulates the bitter taste due to KCl. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a bitter taste receptor by KCl. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a bitter taste receptor by KCl followed by downstream signaling. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a signaling pathway after stimulation by KCl. In some embodiments, the method identifies a compound that modulates, inhibits or enhances perception of bitter taste due to KCl. As shown in Example 2 below, KCl activates bitter taste receptors TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. Thus, a compound that modulates KCl's activation of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors should be a modulator of bitter taste due to KCl. In some embodiments, the compound inhibits KCl's activation of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors. In such embodiments, the compound is an inhibitor of bitter taste due to KCl. In some embodiments, the compound enhances KCl's activation of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors. In such embodiments, the compound is an enhancer of bitter taste due to KCl. In some embodiments, the compound activates of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors. In such embodiments, the compound mimics bitter taste due to KCl. In some embodiments, any of the methods of identifying compounds that modulate bitter taste disclosed above is performed using a tastant that activates one or more of the TAS2R4, TAS2R9, TAS2R13,

TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors. In some embodiments, the tastant is selected from KCl, potassium lactate, Acesulfame K, and a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide. In some embodiments, the concentration of the tastant is 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM or 50mM. In some embodiments, the concentration of the tastant is about 5mM, about 10mM, about 15mM, about 20mM, about 25mM, about 30mM, about 35mM, about 40mM, about 45mM or about 50mM. In some embodiments, the concentration of the tastant is at least 5mM, at least 10mM, at least 15mM, at least 20mM, at least 25mM, at least 30mM, at least 35mM, at least 40mM, at least 45mM or at least 50mM. In some embodiments, the concentration of the tastant is at least about 5mM, at least about 10mM, at least about 15mM, at least about 20mM, at least about 25mM, at least about 30mM, at least about 35mM, at least about 40mM, at least about 45mM or at least about 50mM.

[0123] In some embodiments, the test compound modulates, inhibits or enhances KCl-induced activation of two or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances KCl-induced activation of three or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances KCl-induced activation of four or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances KCl-induced activation of five or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances KCl-induced activation of each of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60.

[0124] In any of the methods of identifying compounds that modulate bitter taste due to KCl described in this section, the bitter taste receptors used in the methods may be complexed to a G-protein, as described above. Any G-protein describe above may be used. In some embodiments, the G-protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0125] In any of the methods of identifying compounds that modulate bitter taste due to KCl described in this section, any assay described above may be used to measure bitter taste receptor activity. In some embodiments, the bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is measured using a calcium-sensitive fluorescent dye. In some embodiments, the calcium-sensitive fluorescent dye is selected from Indo-1, Fura-2, Fluo-3, Fluo-4, Rhod-2, Rhod-5N, Calcein, Calcein blue, cytoCalcein Violet, Quin-2, Quest Fluo-8H™, Quest Fluo-8L™, Quest Fluo 8™, Quest Rhod-4™, coelenterazine and Calcium-3. In a particular embodiment, the calcium-sensitive fluorescent dye is Fluo-4 or Calcium-3.

[0126] In some embodiments, the method comprises providing one or more bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the one or more bitter taste receptor with a tastant that activates the one or more bitter taste receptor;

measuring the activity of the one or more bitter taste receptor; washing the one or more bitter taste receptor; contacting the one or more bitter taste receptor with the tastant and a test compound; and measuring activity of the one or more bitter taste receptor. If the activity of the one or more bitter taste receptor due to the tastant differs from the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound modulates bitter taste due to KCl. If the activity of the one or more bitter taste receptor due to the tastant is greater than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound inhibits bitter taste due to KCl. If the activity of the one or more bitter taste receptor due to the tastant is less than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound enhances bitter taste due to KCl. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The receptor may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., the one or more bitter taste receptor may be contacted with the test compound prior to, at the same time as or subsequent to contacting the one or more bitter taste receptor with the tastant.

[0127] In some embodiments, the method comprises providing a first one or more bitter taste receptor and a second one or more bitter taste receptor, each selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the first one or more bitter taste receptor with a tastant that activates the one or more bitter taste receptor; measuring the activity of the first one or more bitter taste receptor; contacting the second one or more bitter taste receptor with the tastant and a test compound; and measuring the second one or more bitter taste receptor activity. If the activity of the first one or more bitter taste receptor differs from the activity of the second one or more bitter taste receptor, then the test compound modulates bitter taste due to KCl. If the activity of the first one or more bitter taste receptor is greater than the activity of the second one or more bitter taste receptor, then the test compound inhibits bitter taste due to KCl. If the activity of the first one or more bitter taste receptor is less than the activity of the second one or more bitter taste receptor, then the test compound enhances bitter taste due to KCl. In some embodiments, the first one more bitter taste receptor is washed after measurement of activity to provide the second one or more bitter taste receptor. The tastant and test compound may be added sequentially or simultaneously, i.e., the second one or more bitter taste receptor may be contacted with the test compound prior to, at the same time as or subsequent to contacting the second one or more bitter taste receptor with the tastant.

[0128] In some embodiments, the method comprises providing a cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the cell with a tastant that activates one or more bitter taste receptor; measuring the activity of the one or more bitter taste receptor; washing the cell; contacting the cell with the tastant and a test compound; and measuring activity of the one or more bitter taste receptor. If the activity of the one or more bitter taste receptor due to the tastant differs from the activity of the one or more bitter taste receptor due to the

tastant and the test compound, then the test compound modulates bitter taste due to KCl. If the activity of the one or more bitter taste receptor due to the tastant is greater than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound inhibits bitter taste due to KCl. If the activity of the one or more bitter taste receptor due to the tastant is less than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound enhances bitter taste due to KCl. In some embodiments, the cell is present in an *in vitro* cell line. In some embodiments, the cell is present in a panel of *in vitro* cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., the cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the cell with the tastant.

[0129] In some embodiments, the method comprises providing a first cell expressing one or more bitter taste receptor and a second cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; contacting the first cell with a tastant that activates one or more bitter taste receptor; measuring the bitter taste receptor activity of the first cell; contacting the second cell with the tastant and a test compound; and measuring the bitter taste receptor activity of the second cell. If the bitter taste receptor activity of the first cell differs from the bitter taste receptor activity of the second cell, then the test compound modulates bitter taste due to KCl. If the bitter taste receptor activity of the first cell is greater than the bitter taste receptor activity of the second cell, then the test compound inhibits bitter taste due to KCl. If the bitter taste receptor activity of the first cell is less than the bitter taste receptor activity of the second cell, then the test compound enhances bitter taste due to KCl. In some embodiments, the first and second cells are present in *in vitro* cell lines. In some embodiments, the first and second cells are present in one or more panels of *in vitro* cell lines. In some embodiments, the first cell is washed after measuring the bitter taste receptor activity to provide the second cell. The tastant and test compound may be added sequentially or simultaneously, i.e., the second cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the second cell with the tastant.

[0130] In some embodiments, the method further comprises providing a third cell expressing one or more bitter taste receptor and a fourth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; wherein the one or more bitter taste receptor in the third and fourth cell are the same; and wherein the one or more bitter taster receptor in the third and fourth cell are different from the bitter taste receptor in the first and second cells. In some embodiments, the method further comprises providing a fifth cell expressing one or more bitter taste receptor and a sixth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; wherein the one or more bitter taste receptor in the fifth and sixth cell are the same; and wherein the one or

more bitter taster receptor in the fifth and sixth cell are different from the bitter taste receptor in the first, second, third and fourth cells. In some embodiments, the method further comprises providing a seventh cell expressing one or more bitter taste receptor and an eighth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; wherein the one or more bitter taste receptor in the seventh and eighth cell are the same; and wherein the one or more bitter taster receptor in the seventh and eighth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, and sixth cells. In some embodiments, the method further comprises providing a ninth cell expressing one or more bitter taste receptor and a tenth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; wherein the one or more bitter taste receptor in the ninth and tenth cell are the same; and wherein the one or more bitter taster receptor in the ninth and tenth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh and eighth cells. In some embodiments, the method further comprises providing an eleventh cell expressing one or more bitter taste receptor and a twelfth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; wherein the one or more bitter taste receptor in the eleventh and twelfth cell are the same; and wherein the one or more bitter taster receptor in the eleventh and twelfth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth cells. In such embodiments, the method comprises contacting the third, fifth, seventh, ninth, and/or eleventh cell with a tastant that activates one or more bitter taste receptor; measuring the bitter taste receptor activity of the third, fifth, seventh, ninth, and/or eleventh cell; contacting the fourth, sixth, eighth, tenth and/or twelfth cell with the tastant and a test compound; and measuring the bitter taste receptor activity of the fourth, sixth, eighth, tenth and/or twelfth cell. If the bitter taste receptor activity of the third, fifth, seventh, ninth, and/or eleventh cell differs from the bitter taste receptor activity of the fourth, sixth, eighth, tenth and/or twelfth cell, respectively, then the test compound modulates bitter taste due to KCl. If the bitter taste receptor activity of the fourth, sixth, eighth, tenth and/or twelfth cell is less than the bitter taste receptor activity of the third, fifth, seventh, ninth, and/or eleventh cell, respectively, then the test compound inhibits bitter taste due to KCl. If the bitter taste receptor activity of the fourth, sixth, eighth, tenth and/or twelfth cell is greater than the bitter taste receptor activity of the third, fifth, seventh, ninth, and/or eleventh cell, respectively, then the test compound enhances bitter taste due to KCl. In some embodiments, the third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh and/or twelfth cells are present in in vitro cell lines. In some embodiments, the third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh and/or twelfth cells are present in one or more panels of in vitro cell lines. In some embodiments, the third, fifth, seventh, ninth, and/or eleventh cell is washed after measuring the bitter taste receptor activity to provide the fourth, sixth, eighth, tenth and/or twelfth cell, respectively. The tastant and test compound may be added sequentially or simultaneously, i.e., the fourth, sixth, eighth, tenth and/or

twelfth cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the fourth, sixth, eighth, tenth and/or twelfth cell with the tastant.

[0131] In some embodiments, the method comprises providing a panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line; contacting each cell line with a tastant that activates four or more of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; measuring the bitter taste receptor activity of each cell line; washing each cell line; contacting each cell line with the tastant and a test compound; and measuring bitter taste receptor activity of each cell line. If the bitter taste receptor activity of four or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 differs when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, the bitter taste receptor activity differs in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the bitter taste receptor activity differs in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, the bitter taste receptor activity is greater in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the bitter taste receptor activity is greater in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, the bitter taste receptor activity is less in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the bitter taste receptor activity is less in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to KCl if the compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity. In some embodiments, the panel is a matched panel of *in vitro* cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line may be contacted

with the test compound prior to, at the same time as or subsequent to contacting the each cell line with the tastant.

[0132] In some embodiments, the method comprises providing a panel of cell lines, wherein the panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, wherein each receptor is expressed in at least one cell line; contacting each cell line with a tastant that activates at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; measuring the bitter taste receptor activity of each cell line; washing each cell line; contacting each cell line with the tastant and a test compound; and measuring bitter taste receptor activity of each cell line. In some embodiments, each cell line in the panel expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line. If the bitter taste receptor activity at least two of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 differs when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in at least three of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in at least four of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in at least five of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines then the test compound selectively modulates bitter taste due to KCl. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is greater in at least three of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is greater in at least four of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is greater in at least five of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste

receptor activity is greater in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines, then the test compound selectively inhibits bitter taste due to KCl. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is less in at least three of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is less in at least four of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is less in at least five of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity is less in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to KCl if the compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity. In some embodiments, the panel is a matched panel of *in vitro* cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line with the tastant.

[0133] In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a tastant that activates four or more of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the tastant and a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. If the bitter taste receptor activity of four or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, the bitter taste receptor activity differs in five or more of the cell lines

selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the bitter taste receptor activity differs in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, the bitter taste receptor activity is greater in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the bitter taste receptor activity is greater in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, the bitter taste receptor activity is less in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the bitter taste receptor activity is less in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to KCl if the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., the each cell line in the second panel may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line in the second panel with the tastant.

[0134] In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a tastant that activates at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the tastant and a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. In some embodiments, each cell line in the first and second panels expresses a bitter

taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. If the bitter taste receptor activity of at least two of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in at least three of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in at least four of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in at least five of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound selectively modulates bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity differs in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines, then the test compound selectively modulates bitter taste due to KCl. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to KCl. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14,

TAS2R44 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, if the bitter taste receptor activity in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 cell lines is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to KCl. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to KCl if the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line in the second panel may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line in the second panel with the tastant.

[0135] In some embodiments, the tastant utilized in any of the above methods of identifying modulators of bitter taste due to KCl is selected from KCl, potassium lactate, Acesulfame K, and a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide. In some embodiments, the concentration of the tastant is 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM or 50mM. In some embodiments, the concentration of the tastant is about 5mM, about 10mM, about 15mM, about 20mM, about 25mM, about 30mM, about 35mM, about 40mM, about 45mM or about 50mM. In some embodiments, the concentration of the tastant is at least 5mM, at least 10mM, at least 15mM, at least 20mM, at least 25mM, at least 30mM, at least 35mM, at least 40mM, at least 45mM or at least 50mM. In some embodiments, the concentration of the tastant is at least about 5mM, at least about 10mM, at least about 15mM, at least about 20mM, at least about 25mM, at least about 30mM, at least about 35mM, at least about 40mM, at least about 45mM or at least about 50mM.

[0136] In another aspect, the invention provides a method of identifying a compound that mimics the bitter taste due to KCl. In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49,

TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a negative control; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. If the test compound induces bitter taste receptor activity of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, then the test compound mimics bitter taste due to KCl. In some embodiments, the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. In some embodiments, the negative control is the assay buffer before addition of the test compound.

[0137] In another aspect, the invention provides a method for determining if KCl is present in a composition. In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a negative control; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the composition; and measuring the bitter taste receptor activity of each cell line in the second panel. KCl is present in the composition if the composition induces TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel and does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the composition is an extract from a food product. In some embodiments, the composition comprises a pharmaceutically active ingredient. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell

lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. In some embodiments, the negative control is the assay buffer before addition of the composition.

[0138] In some embodiments, the method comprises contacting the tastant and tastant plus test compound with two or more different bitter taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with two or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with three or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with three or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with four or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with four or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with five or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with five or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with six or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with six or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60.

[0139] In some embodiments wherein the method of identifying a compound that modulates, inhibits, enhances or mimics bitter flavor due to KCl comprises contacting TAS2R44 or a cell expressing TAS2R44 with a tastant or a test compound, the method also comprises contacting at least one additional bitter taste receptor or a cell expressing at least one additional bitter taste receptor with the tastant or test compound, wherein the at least one additional bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, and TAS2R60.

[0140] In some embodiments, the method further comprises admixing the test compound that modulates, inhibits, enhances or mimic bitter taste due to KCl with foodstuffs, any foodstuff precursor material or any additive employed in the production of foodstuffs. In some embodiments,

the foodstuff is for human consumption. In some embodiments, the foodstuff is for animal consumption, such as pet or livestock consumption. In some embodiments, the method further comprises admixing the test compound that modulates, inhibits, enhances or mimic bitter taste due to KCl with an active agent in a pharmaceutically acceptable form.

Methods of Identifying Compounds That Modulate Bitter Taste Due to Potassium Lactate

[0141] According to another aspect, the invention provides a method for identifying a compound that modulates the bitter taste due to potassium lactate. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a bitter taste receptor by potassium lactate. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a bitter taste receptor by potassium lactate followed by downstream signaling. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a signaling pathway after stimulation by potassium lactate. In some embodiments, the method identifies a compound that modulates, inhibits or enhances perception of bitter taste due to potassium lactate. As shown in Example 3 below, potassium lactate activates bitter taste receptors TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. Thus, a compound that modulates potassium lactate's activation of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors should be a modulator of bitter taste due to potassium lactate. In some embodiments, the compound inhibits potassium lactate's activation of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors. In such embodiments, the compound is an inhibitor of bitter taste due to potassium lactate. In some embodiments, the compound enhances potassium lactate's activation of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors. In such embodiments, the compound is an enhancer of bitter taste due to potassium lactate. In some embodiments, the compound activates of one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors. In such embodiments, the compound mimics bitter taste due to potassium lactate. In some embodiments, any of the methods of identifying compounds that modulate bitter taste disclosed above is performed using a tastant that activates one or more of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptors. In some embodiments, the tastant is selected from KCl, potassium lactate, Acesulfame K, and a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide. In some embodiments, the concentration of the tastant is 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM or 50mM. In some embodiments, the concentration of the tastant is about 5mM, about 10mM, about 15mM, about 20mM, about 25mM, about 30mM, about 35mM, about 40mM, about 45mM or about 50mM. In some embodiments, the concentration of the tastant is at least 5mM, at least 10mM, at least 15mM, at least 20mM, at least 25mM, at least 30mM, at least 35mM, at least 40mM, at least 45mM or at least 50mM. In some embodiments, the

concentration of the tastant is at least about 5mM, at least about 10mM, at least about 15mM, at least about 20mM, at least about 25mM, at least about 30mM, at least about 35mM, at least about 40mM, at least about 45mM or at least about 50mM.

[0142] In some embodiments, the test compound modulates, inhibits or enhances potassium lactate-induced activation of two or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances potassium lactate-induced activation of three or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances potassium lactate-induced activation of four or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances potassium lactate-induced activation of five or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances potassium lactate-induced activation of six or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the test compound modulates, inhibits or enhances potassium lactate-induced activation of each of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60.

[0143] In any of the methods of identifying compounds that modulate bitter taste due to potassium lactate described in this section, the bitter taste receptors used in the methods may be complexed to a G-protein, as described above. Any G-protein describe above may be used. In some embodiments, the G-protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0144] In any of the methods of identifying compounds that modulate bitter taste due to potassium lactate described in this section, any assay described above may be used to measure bitter taste receptor activity. In some embodiments, the bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is measured using a calcium-sensitive fluorescent dye. In some embodiments, the calcium-sensitive fluorescent dye is selected from Indo-1, Fura-2, Fluo-3, Fluo-4, Rhod-2, Rhod-5N, Calcein, Calcein blue, cytoCalcein Violet, Quin-2, Quest Fluo-8H™, Quest Fluo-8L™, Quest Fluo 8™, Quest Rhod-4™, coelenterazine and Calcium-3. In a particular embodiment, the calcium-sensitive fluorescent dye is Fluo-4 or Calcium-3.

[0145] In some embodiments, the method comprises providing one or more bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the one or more bitter taste receptor with a tastant that activates the one or more bitter taste receptor; measuring the activity of the one or more bitter taste receptor; washing the one or more bitter taste receptor; contacting the one or more bitter taste receptor with the tastant and a test compound; and measuring activity of the one or more bitter taste receptor. If the activity of the one or more bitter taste receptor due to the tastant differs from the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound modulates bitter taste due

to potassium lactate. If the activity of the one or more bitter taste receptor due to the tastant is greater than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound inhibits bitter taste due to potassium lactate. If the activity of the one or more bitter taste receptor due to the tastant is less than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound enhances bitter taste due to potassium lactate. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The receptor may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., the one or more bitter taste receptor may be contacted with the test compound prior to, at the same time as or subsequent to contacting the one or more bitter taste receptor with the tastant.

[0146] In some embodiments, the method comprises providing a first one or more bitter taste receptor and a second one or more bitter taste receptor, each selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the first one or more bitter taste receptor with a tastant that activates the one or more bitter taste receptor; measuring the activity of the first one or more bitter taste receptor; contacting the second one or more bitter taste receptor with the tastant and a test compound; and measuring the second one or more bitter taste receptor activity. If the activity of the first one or more bitter taste receptor differs from the activity of the second one or more bitter taste receptor, then the test compound modulates bitter taste due to potassium lactate. If the activity of the first one or more bitter taste receptor is greater than the activity of the second one or more bitter taste receptor, then the test compound inhibits bitter taste due to potassium lactate. If the activity of the first one or more bitter taste receptor is less than the activity of the second one or more bitter taste receptor, then the test compound enhances bitter taste due to potassium lactate. In some embodiments, the first one more bitter taste receptor is washed after measurement of activity to provide the second one or more bitter taste receptor. The tastant and test compound may be added sequentially or simultaneously, i.e., the second one or more bitter taste receptor may be contacted with the test compound prior to, at the same time as or subsequent to contacting the second one or more bitter taste receptor with the tastant.

[0147] In some embodiments, the method comprises providing a cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the cell with a tastant that activates one or more bitter taste receptor; measuring the activity of the one or more bitter taste receptor; washing the cell; contacting the cell with the tastant and a test compound; and measuring activity of the one or more bitter taste receptor. If the activity of the one or more bitter taste receptor due to the tastant differs from the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound modulates bitter taste due to potassium lactate. If the activity of the one or more bitter taste receptor due to the tastant is greater than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound inhibits bitter taste due to potassium lactate. If the activity of the one or more bitter

taste receptor due to the tastant is less than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound enhances bitter taste due to potassium lactate. In some embodiments, the cell is present in an *in vitro* cell line. In some embodiments, the cell is present in a panel of *in vitro* cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., the cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the cell with the tastant.

[0148] In some embodiments, the method comprises providing a first cell expressing one or more bitter taste receptor and a second cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; contacting the first cell with a tastant that activates one or more bitter taste receptor; measuring the bitter taste receptor activity of the first cell; contacting the second cell with the tastant and a test compound; and measuring the bitter taste receptor activity of the second cell. If the bitter taste receptor activity of the first cell differs from the bitter taste receptor activity of the second cell, then the test compound modulates bitter taste due to potassium lactate. If the bitter taste receptor activity of the first cell is greater than the bitter taste receptor activity of the second cell, then the test compound inhibits bitter taste due to potassium lactate. If the bitter taste receptor activity of the first cell is less than the bitter taste receptor activity of the second cell, then the test compound enhances bitter taste due to potassium lactate. In some embodiments, the first and second cells are present in *in vitro* cell lines. In some embodiments, the first and second cells are present in one or more panels of *in vitro* cell lines. In some embodiments, the first cell is washed after measuring the bitter taste receptor activity to provide the second cell. The tastant and test compound may be added sequentially or simultaneously, i.e., the second cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the second cell with the tastant.

[0149] In some embodiments, the method further comprises providing a third cell expressing one or more bitter taste receptor and a fourth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; wherein the one or more bitter taste receptor in the third and fourth cell are the same; and wherein the one or more bitter taster receptor in the third and fourth cell are different from the bitter taste receptor in the first and second cells. In some embodiments, the method further comprises providing a fifth cell expressing one or more bitter taste receptor and a sixth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; wherein the one or more bitter taste receptor in the fifth and sixth cell are the same; and wherein the one or more bitter taster receptor in the fifth and sixth cell are different from the bitter taste receptor in the first, second, third and fourth cells. In some embodiments, the method further comprises providing a seventh cell expressing one or more bitter taste receptor and an eighth cell

expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; wherein the one or more bitter taste receptor in the seventh and eighth cell are the same; and wherein the one or more bitter taster receptor in the seventh and eighth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, and sixth cells. In some embodiments, the method further comprises providing a ninth cell expressing one or more bitter taste receptor and a tenth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; wherein the one or more bitter taste receptor in the ninth and tenth cell are the same; and wherein the one or more bitter taster receptor in the ninth and tenth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh and eighth cells. In some embodiments, the method further comprises providing an eleventh cell expressing one or more bitter taste receptor and a twelfth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; wherein the one or more bitter taste receptor in the eleventh and twelfth cell are the same; and wherein the one or more bitter taster receptor in the eleventh and twelfth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth cells. In some embodiments, the method further comprises providing an thirteenth cell expressing one or more bitter taste receptor and a fourteenth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; wherein the one or more bitter taste receptor in the thirteenth and fourteenth cell are the same; and wherein the one or more bitter taster receptor in the thirteenth and fourteenth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh and twelfth cells. In such embodiments, the method comprises contacting the third, fifth, seventh, ninth, eleventh and/or thirteenth cell with a tastant that activates one or more bitter taste receptor; measuring the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell; contacting the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell with the tastant and a test compound; and measuring the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell. If the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell differs from the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell, respectively, then the test compound modulates bitter taste due to potassium lactate. If the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell is less than the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell, respectively, then the test compound inhibits bitter taste due to potassium lactate. If the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell is greater than the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell, respectively, then the test compound enhances bitter taste due to potassium lactate. In some embodiments, the third, fourth, fifth, sixth, seventh, eighth, ninth, tenth,

eleventh, twelfth, thirteenth, and/or fourteenth cells are present in in vitro cell lines. In some embodiments, the third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, and/or fourteenth are present in one or more panels of in vitro cell lines. In some embodiments, the third, fifth, seventh, ninth, eleventh and/or thirteenth cell is washed after measuring the bitter taste receptor activity to provide the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell, respectively. The tastant and test compound may be added sequentially or simultaneously, i.e., the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the fourth, sixth, eighth, tenth, twelfth, and/or fourteenth cell with the tastant.

[0150] In some embodiments, the method comprises providing a panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line; contacting each cell line with a tastant that activates four or more of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; measuring the bitter taste receptor activity of each cell line; washing each cell line; contacting each cell line with the tastant and a test compound; and measuring bitter taste receptor activity of each cell line. If the bitter taste receptor activity of four or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 differs when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, the bitter taste receptor activity differs in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity differs in six or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity differs in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, the bitter taste receptor activity is greater in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity is greater in six or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity is greater in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less when contacted with the tastant compared to

when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, the bitter taste receptor activity is less in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity is less in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to potassium lactate if the compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity. In some embodiments, the panel is a matched panel of *in vitro* cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line with the tastant.

[0151] In some embodiments, the method comprises providing a panel of cell lines, wherein the panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 wherein each receptor is expressed in at least one cell line; contacting each cell line with a tastant that activates at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; measuring the bitter taste receptor activity of each cell line; washing each cell line; contacting each cell line with the tastant and a test compound; and measuring bitter taste receptor activity of each cell line. In some embodiments, each cell line in the panel expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line. If the bitter taste receptor activity at least two of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 differs when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in at least three of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in at least four of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in at least five of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 then the test compound selectively modulates bitter

taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in at least six of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines then the test compound selectively modulates bitter taste due to potassium lactate. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to potassium lactate. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter

taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to potassium lactate if the compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity. In some embodiments, the panel is a matched panel of in vitro cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line with the tastant.

[0152] In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a tastant that activates four or more of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the tastant and a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. If the bitter taste receptor activity of four or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, the bitter taste receptor activity differs in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity differs in six or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60.

In some embodiments, the bitter taste receptor activity differs in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, the bitter taste receptor activity is greater in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity is greater in six or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity is greater in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, the bitter taste receptor activity is less in five or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity is less in six or more of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the bitter taste receptor activity is less in the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to potassium lactate if the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line of the second panel may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line of the second panel with the tastant.

[0153] In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a tastant that activates at least two of the group selected from

TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the tastant and a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. If the bitter taste receptor activity of at least two of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least three of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least four of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in at least five of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in at least six of the cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound selectively modulates bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity differs in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines, then the test compound selectively modulates bitter taste due to potassium lactate. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to potassium lactate.

In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to potassium lactate. If the bitter taste receptor activity of at least two cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, if the bitter taste receptor activity in each of the TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 cell lines is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to potassium lactate. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to potassium lactate if the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of in vitro cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line of the second panel may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line of the second panel with the tastant.

[0154] In some embodiments, the tastant utilized in any of the above methods of identifying modulators of bitter taste due to potassium lactate is selected from KCl, potassium lactate, Acesulfame K, and a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide. In some embodiments, the concentration of the tastant is 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM or 50mM. In some embodiments, the concentration of the tastant is about 5mM, about 10mM, about 15mM, about 20mM, about 25mM, about 30mM, about 35mM, about 40mM, about 45mM or about 50mM. In some embodiments, the concentration of the tastant is at least 5mM, at least 10mM, at least 15mM, at least 20mM, at least 25mM, at least 30mM, at least 35mM, at least 40mM, at least 45mM or at least 50mM. In some embodiments, the concentration of the tastant is at least about 5mM, at least about 10mM, at least about 15mM, at least about 20mM, at least about 25mM, at least about 30mM, at least about 35mM, at least about 40mM, at least about 45mM or at least about 50mM.

[0155] In another aspect, the invention provides a method of identifying a compound that mimics the bitter taste due to potassium lactate. In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a negative control; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. If the test compound induces bitter taste receptor activity of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60, then the test compound mimics bitter taste due to potassium lactate. In some embodiments, the test compound does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. In some embodiments, the negative control is the assay buffer before addition of the test compound.

[0156] In another aspect, the invention provides a method for determining if potassium lactate is present in a composition. In some embodiments, the method comprises providing a first panel of

cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a negative control; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the composition; and measuring the bitter taste receptor activity of each cell line in the second panel. Potassium lactate is present in the composition if the composition induces TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel and does not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the composition is an extract from a food product. In some embodiments, the composition comprises a pharmaceutically active ingredient. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. In some embodiments, the negative control is the assay buffer before addition of the composition.

[0157] In some embodiments, the method comprises contacting the tastant and tastant plus test compound with two or more different bitter taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with two or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with three or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with three or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with four or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with four or more cells each expressing a different bitter taste receptor selected from

TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with five or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with five or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with six or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with six or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with seven or more different taste receptors selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with seven or more cells each expressing a different bitter taste receptor selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60.

[0158] In some embodiments wherein the method of identifying a compound that modulates, inhibits, enhances or mimics bitter flavor due to potassium lactate comprises contacting TAS2R44 or a cell expressing TAS2R44 with a tastant or a test compound, the method also comprises contacting at least one additional bitter taste receptor or a cell expressing at least one additional bitter taste receptor with the tastant or test compound, wherein the at least one additional bitter taste receptor is selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R46 and TAS2R60.

[0159] In some embodiments, the method further comprises admixing the test compound that modulates, inhibits, enhances or mimic bitter taste due to potassium lactate with foodstuffs, any foodstuff precursor material or any additive employed in the production of foodstuffs. In some embodiments, the foodstuff is for human consumption. In some embodiments, the foodstuff is for animal consumption, such as pet or livestock consumption. In some embodiments, the method further comprises admixing the test compound that modulates, inhibits, enhances or mimic bitter taste due to potassium lactate with an active agent in a pharmaceutically acceptable form.

Methods of Identifying Compounds That Modulate Bitter Taste Due to Acesulfame K

[0160] According to another aspect, the invention provides a method for identifying a compound that modulates the bitter taste due to Acesulfame K. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a bitter taste receptor by Acesulfame K. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a bitter taste receptor by Acesulfame K followed by downstream signaling. In some embodiments, the method identifies a compound that modulates, inhibits or enhances activation of a signaling pathway after stimulation by Acesulfame K. In some embodiments, the method identifies

a compound that modulates, inhibits or enhances perception of bitter taste due to Acesulfame K. As shown in Example 4 below, Acesulfame K activates bitter taste receptors TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. Thus, a compound that modulates Acesulfame K's activation of one or more of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptors should be a modulator of bitter taste due to Acesulfame K. In some embodiments, the compound inhibits Acesulfame K's activation of one or more of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptors. In such embodiments, the compound is an inhibitor of bitter taste due to Acesulfame K. In some embodiments, the compound enhances Acesulfame K's activation of one or more of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptors. In such embodiments, the compound is an enhancer of bitter taste due to Acesulfame K. In some embodiments, the compound activates of one or more of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptors. In such embodiments, the compound mimics bitter taste due to Acesulfame K. In some embodiments, any of the methods of identifying compounds that modulate bitter taste disclosed above is performed using a tastant that activates one or more of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptors. In some embodiments, the tastant is selected from KCl, potassium lactate, Acesulfame K, and a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide. In some embodiments, the concentration of the tastant is 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM or 50mM. In some embodiments, the concentration of the tastant is about 5mM, about 10mM, about 15mM, about 20mM, about 25mM, about 30mM, about 35mM, about 40mM, about 45mM or about 50mM. In some embodiments, the concentration of the tastant is at least 5mM, at least 10mM, at least 15mM, at least 20mM, at least 25mM, at least 30mM, at least 35mM, at least 40mM, at least 45mM or at least 50mM. In some embodiments, the concentration of the tastant is at least about 5mM, at least about 10mM, at least about 15mM, at least about 20mM, at least about 25mM, at least about 30mM, at least about 35mM, at least about 40mM, at least about 45mM or at least about 50mM.

[0161] In some embodiments, the test compound modulates, inhibits or enhances Acesulfame K-induced activation of two or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the test compound modulates, inhibits or enhances Acesulfame K-induced activation of three or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the test compound modulates, inhibits or enhances Acesulfame K-induced activation of four or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the test compound modulates, inhibits or enhances Acesulfame K-induced activation of five or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the test compound modulates, inhibits or enhances Acesulfame K-induced activation of six or more of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44.

In some embodiments, the test compound modulates, inhibits or enhances Acesulfame K-induced activation of each of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44.

[0162] In any of the methods of identifying compounds that modulate bitter taste due to Acesulfame K described in this section, the bitter taste receptors used in the methods may be complexed to a G-protein, as described above. Any G-protein describe above may be used. In some embodiments, the G-protein is a G_q protein, an alpha transducin or an alpha gustducin. In some embodiments, the G_q protein is a $G_{\alpha 15}$ protein.

[0163] In any of the methods of identifying compounds that modulate bitter taste due to Acesulfame K described in this section, any assay described above may be used to measure bitter taste receptor activity. In some embodiments, the bitter taste receptor activity is determined by measuring intracellular calcium concentration. In some embodiments, intracellular calcium concentration is measured using a calcium-sensitive fluorescent dye. In some embodiments, the calcium-sensitive fluorescent dye is selected from Indo-1, Fura-2, Fluo-3, Fluo-4, Rhod-2, Rhod-5N, Calcein, Calcein blue, cytoCalcein Violet, Quin-2, Quest Fluo-8H™, Quest Fluo-8L™, Quest Fluo 8™, Quest Rhod-4™, coelenterazine and Calcium-3. In a particular embodiment, the calcium-sensitive fluorescent dye is Fluo-4 or Calcium-3.

[0164] In some embodiments, the method comprises providing one or more bitter taste receptor selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the one or more bitter taste receptor with a tastant that activates the one or more bitter taste receptor; measuring the activity of the one or more bitter taste receptor; washing the one or more bitter taste receptor; contacting the one or more bitter taste receptor with the tastant and a test compound; and measuring activity of the one or more bitter taste receptor. If the activity of the one or more bitter taste receptor due to the tastant differs from the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound modulates bitter taste due to Acesulfame K. If the activity of the one or more bitter taste receptor due to the tastant is greater than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound inhibits bitter taste due to Acesulfame K. If the activity of the one or more bitter taste receptor due to the tastant is less than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound enhances bitter taste due to Acesulfame K. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The receptor may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., the one or more bitter taste receptor may be contacted with the test compound prior to, at the same time as or subsequent to contacting the one or more bitter taste receptor with the tastant.

[0165] In some embodiments, the method comprises providing a first one or more bitter taste receptor and a second one or more bitter taste receptor, each selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the first one or more bitter taste receptor with a tastant that activates the one or more bitter taste receptor; measuring the

activity of the first one or more bitter taste receptor; contacting the second one or more bitter taste receptor with the tastant and a test compound; and measuring the second one or more bitter taste receptor activity. If the activity of the first one or more bitter taste receptor differs from the activity of the second one or more bitter taste receptor, then the test compound modulates bitter taste due to Acesulfame K. If the activity of the first one or more bitter taste receptor is greater than the activity of the second one or more bitter taste receptor, then the test compound inhibits bitter taste due to Acesulfame K. If the activity of the first one or more bitter taste receptor is less than the activity of the second one or more bitter taste receptor, then the test compound enhances bitter taste due to Acesulfame K. In some embodiments, the first one more bitter taste receptor is washed after measurement of activity to provide the second one or more bitter taste receptor. The tastant and test compound may be added sequentially or simultaneously, i.e., the second one or more bitter taste receptor may be contacted with the test compound prior to, at the same time as or subsequent to contacting the second one or more bitter taste receptor with the tastant.

[0166] In some embodiments, the method comprises providing a cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the cell with a tastant that activates one or more bitter taste receptor; measuring the activity of the one or more bitter taste receptor; washing the cell; contacting the cell with the tastant and a test compound; and measuring activity of the one or more bitter taste receptor. If the activity of the one or more bitter taste receptor due to the tastant differs from the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound modulates bitter taste due to Acesulfame K. If the activity of the one or more bitter taste receptor due to the tastant is greater than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound inhibits bitter taste due to Acesulfame K. If the activity of the one or more bitter taste receptor due to the tastant is less than the activity of the one or more bitter taste receptor due to the tastant and the test compound, then the test compound enhances bitter taste due to Acesulfame K. In some embodiments, the cell is present in an *in vitro* cell line. In some embodiments, the cell is present in a panel of *in vitro* cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., the cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the cell with the tastant.

[0167] In some embodiments, the method comprises providing a first cell expressing one or more bitter taste receptor and a second cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; contacting the first cell with a tastant that activates one or more bitter taste receptor; measuring the bitter taste receptor activity of the first cell; contacting the second cell with the tastant and a test compound; and measuring the bitter taste receptor activity of the second cell. If the bitter taste receptor activity of the first cell differs from the bitter taste receptor activity

of the second cell, then the test compound modulates bitter taste due to Acesulfame K. If the bitter taste receptor activity of the first cell is greater than the bitter taste receptor activity of the second cell, then the test compound inhibits bitter taste due to Acesulfame K. If the bitter taste receptor activity of the first cell is less than the bitter taste receptor activity of the second cell, then the test compound enhances bitter taste due to Acesulfame K. In some embodiments, the first and second cells are present in *in vitro* cell lines. In some embodiments, the first and second cells are present in one or more panels of *in vitro* cell lines. In some embodiments, the first cell is washed after measuring the bitter taste receptor activity to provide the second cell. The tastant and test compound may be added sequentially or simultaneously, i.e., the second cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the second cell with the tastant.

[0168] In some embodiments, the method further comprises providing a third cell expressing one or more bitter taste receptor and a fourth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; wherein the one or more bitter taste receptor in the third and fourth cell are the same; and wherein the one or more bitter taster receptor in the third and fourth cell are different from the bitter taste receptor in the first and second cells. In some embodiments, the method further comprises providing a fifth cell expressing one or more bitter taste receptor and a sixth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; wherein the one or more bitter taste receptor in the fifth and sixth cell are the same; and wherein the one or more bitter taster receptor in the fifth and sixth cell are different from the bitter taste receptor in the first, second, third and fourth cells. In some embodiments, the method further comprises providing a seventh cell expressing one or more bitter taste receptor and an eighth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; wherein the one or more bitter taste receptor in the seventh and eighth cell are the same; and wherein the one or more bitter taster receptor in the seventh and eighth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, and sixth cells. In some embodiments, the method further comprises providing a ninth cell expressing one or more bitter taste receptor and a tenth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; wherein the one or more bitter taste receptor in the ninth and tenth cell are the same; and wherein the one or more bitter taster receptor in the ninth and tenth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh and eighth cells. In some embodiments, the method further comprises providing an eleventh cell expressing one or more bitter taste receptor and a twelfth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; wherein the one or more bitter taste receptor in the eleventh and twelfth cell are the

same; and wherein the one or more bitter taster receptor in the eleventh and twelfth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth cells. In some embodiments, the method further comprises providing an thirteenth cell expressing one or more bitter taste receptor and a fourteenth cell expressing one or more bitter taste receptor, wherein the one or more bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; wherein the one or more bitter taste receptor in the thirteenth and fourteenth cell are the same; and wherein the one or more bitter taster receptor in the thirteenth and fourteenth cell are different from the bitter taste receptor in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh and twelfth cells. In such embodiments, the method comprises contacting the third, fifth, seventh, ninth, eleventh and/or thirteenth cell with a tastant that activates one or more bitter taste receptor; measuring the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell; contacting the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell with the tastant and a test compound; and measuring the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell. If the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell differs from the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell, respectively, then the test compound modulates bitter taste due to Acesulfame K. If the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell is less than the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell, respectively, then the test compound inhibits bitter taste due to Acesulfame K. If the bitter taste receptor activity of the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell is greater than the bitter taste receptor activity of the third, fifth, seventh, ninth, eleventh and/or thirteenth cell, respectively, then the test compound enhances bitter taste due to Acesulfame K. In some embodiments, the third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, and/or fourteenth cells are present in in vitro cell lines. In some embodiments, the third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, and/or fourteenth are present in one or more panels of in vitro cell lines. In some embodiments, the third, fifth, seventh, ninth, eleventh and/or thirteenth cell is washed after measuring the bitter taste receptor activity to provide the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell, respectively. The tastant and test compound may be added sequentially or simultaneously, i.e., the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell may be contacted with the test compound prior to, at the same time as or subsequent to contacting the fourth, sixth, eighth, tenth, twelfth and/or fourteenth cell with the tastant.

[0169] In some embodiments, the method comprises providing a panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line; contacting each cell line with a tastant that activates four or more

of the group selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; measuring the bitter taste receptor activity of each cell line; washing each cell line; contacting each cell line with the tastant and a test compound; and measuring bitter taste receptor activity of each cell line. If the bitter taste receptor activity of four or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 differs when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, the bitter taste receptor activity differs in five or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity differs in six or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity differs in the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, the bitter taste receptor activity is greater in five or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity is greater in six or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity is greater in the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, the bitter taste receptor activity is less in five or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity is less in the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to Acesulfame K if the compound does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor activity. In some embodiments, the panel is a matched panel of *in vitro* cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line with the tastant.

[0170] In some embodiments, the method comprises providing a panel of cell lines, wherein the panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, wherein each receptor is expressed in at least one cell line; contacting each cell line with a tastant that activates at least two of the group selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; measuring the bitter taste receptor activity of each cell line; washing each cell line; contacting each cell line with the tastant and a test compound; and measuring bitter taste receptor activity of each cell line. In some embodiments, each cell line in the panel expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line. If the bitter taste receptor activity at least two of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 differs when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least three of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least four of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least five of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least six of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in each of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines then the test compound selectively modulates bitter taste due to Acesulfame K. If the bitter taste receptor activity of at least two cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater when contacted with the tastant

compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity in each of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines is greater when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively inhibits bitter taste due to Acesulfame K. If the bitter taste receptor activity of at least two cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity in each of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines is less when contacted with the tastant compared to when contacted with the tastant and the test compound, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to Acesulfame K if the compound does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor activity. In some

embodiments, the panel is a matched panel of in vitro cell lines. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line with the tastant.

[0171] In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a tastant that activates four or more of the group selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the tastant and a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. If the bitter taste receptor activity of four or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, the bitter taste receptor activity differs in five or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity differs in six or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity differs in the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, the bitter taste receptor activity is greater in five or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity is greater in six or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity is greater in the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines. If the bitter taste receptor activity of four or more cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, the bitter taste receptor activity is less in five or more of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity is less in six or more of the cell

lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the bitter taste receptor activity is less in the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to Acesulfame K if the test compound does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line in the second panel may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line in the second panel with the tastant.

[0172] In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a tastant that activates at least two of the group selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the tastant and a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. In some embodiments, each cell line in the first and second panels expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60, wherein each receptor is expressed in at least one cell line of each panel. If the bitter taste receptor activity of at least two of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 differs in the first panel compared to the second panel, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least three of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least four of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then

the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least five of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in at least six of the cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound selectively modulates bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity differs in each of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines, then the test compound selectively modulates bitter taste due to Acesulfame K. If the bitter taste receptor activity of at least two cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity in each of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines is greater in the first panel compared to the second panel, then the test compound selectively inhibits bitter taste due to Acesulfame K. If the bitter taste receptor activity of at least two cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least three cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least four cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least five cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less

in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity of at least six cell lines selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, if the bitter taste receptor activity in each of the TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 cell lines is less in the first panel compared to the second panel, then the test compound selectively enhances bitter taste due to Acesulfame K. In some embodiments, the test compound selectively modulates, inhibits or activates bitter taste due to Acesulfame K if the test compound does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of in vitro cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. The tastant and test compound may be added sequentially or simultaneously, i.e., each cell line in the second panel may be contacted with the test compound prior to, at the same time as or subsequent to contacting each cell line in the second panel with the tastant.

[0173] In some embodiments, the tastant utilized in any of the above methods of identifying modulators of bitter taste due to Acesulfame K is selected from KCl, potassium lactate, Acesulfame K, and a universal bitter compound. In some embodiments, the universal bitter compound is denatonium benzoate or denatonium saccharide. In some embodiments, the concentration of the tastant is 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM or 50mM. In some embodiments, the concentration of the tastant is about 5mM, about 10mM, about 15mM, about 20mM, about 25mM, about 30mM, about 35mM, about 40mM, about 45mM or about 50mM. In some embodiments, the concentration of the tastant is at least 5mM, at least 10mM, at least 15mM, at least 20mM, at least 25mM, at least 30mM, at least 35mM, at least 40mM, at least 45mM or at least 50mM. In some embodiments, the concentration of the tastant is at least about 5mM, at least about 10mM, at least about 15mM, at least about 20mM, at least about 25mM, at least about 30mM, at least about 35mM, at least about 40mM, at least about 45mM or at least about 50mM.

[0174] In another aspect, the invention provides a method of identifying a compound that mimics the bitter taste due to Acesulfame K. In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49,

TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a negative control; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with a test compound; and measuring the bitter taste receptor activity of each cell line in the second panel. If the test compound induces bitter taste receptor activity of TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44, then the test compound mimics bitter taste due to Acesulfame K. In some embodiments, the test compound does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. In some embodiments, the negative control is the assay buffer before addition of the test compound.

[0175] In another aspect, the invention provides a method for determining if Acesulfame K is present in a composition. In some embodiments, the method comprises providing a first panel of cell lines and a second panel of cell lines, wherein each cell line expresses a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor, wherein each receptor is expressed in at least one cell line, and wherein the first and second panels comprise the same cell lines; contacting each cell line in the first panel with a negative control; measuring the bitter taste receptor activity of each cell line in the first panel; contacting each cell line in the second panel with the composition; and measuring the bitter taste receptor activity of each cell line in the second panel. Acesulfame K is present in the composition if the composition induces TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter taste receptor activity in the second panel compared to the first panel and does not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, and TAS2R60 bitter taste receptor activity in the second panel compared to the first panel. In some embodiments, the composition is an extract from a food product. In some embodiments, the composition comprises a pharmaceutically active ingredient. In some embodiments, the first and second panels are matched panels of *in vitro* cell lines. In some embodiments, the first panel of cell lines is washed after it is measured for bitter taste receptor activity to provide the second panel of cell lines. In other words, the first and second panels of cell

lines are the same, with a washing step between first measuring step and the second contacting step. The skilled worker would recognize that, in such embodiments, the testing order does not matter. The cell lines may be contacted with the test compound either before or after washing. In some embodiments, the negative control is the assay buffer before addition of the composition.

[0176] In some embodiments, the method comprises contacting the tastant and tastant plus test compound with two or more different bitter taste receptors selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with two or more cells each expressing a different bitter taste receptor selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with three or more different taste receptors selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with three or more cells each expressing a different bitter taste receptor selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with four or more different taste receptors selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with four or more cells each expressing a different bitter taste receptor selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with five or more different taste receptors selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with five or more cells each expressing a different bitter taste receptor selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with six or more different taste receptors selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with six or more cells each expressing a different bitter taste receptor selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with seven or more different taste receptors selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the method comprises contacting the tastant and tastant plus test compound with seven or more cells each expressing a different bitter taste receptor selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44.

[0177] In some embodiments wherein the method of identifying a compound that modulates, inhibits, enhances or mimics bitter flavor due to Acesulfame K comprises contacting TAS2R44 or a cell expressing TAS2R44 with a tastant or a test compound, the method also comprises contacting

at least one additional bitter taste receptor or a cell expressing at least one additional bitter taste receptor with the tastant or test compound, wherein the at least one additional bitter taste receptor is selected from TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, and TAS2R16.

[0178] In some embodiments, the method further comprises admixing the test compound that modulates, inhibits, enhances or mimic bitter taste due to Acesulfame K with foodstuffs, any foodstuff precursor material or any additive employed in the production of foodstuffs. In some embodiments, the foodstuff is for human consumption. In some embodiments, the foodstuff is for animal consumption, such as pet or livestock consumption. In some embodiments, the method further comprises admixing the test compound that modulates, inhibits, enhances or mimic bitter taste due to Acesulfame K with an active agent in a pharmaceutically acceptable form.

Panels of Cell Lines For Identifying Compounds That Modulate Bitter Taste

[0179] According to another aspect, the invention provides panels of cell lines for identifying a compound that modulates the bitter taste.

[0180] In some embodiments, the panel of cell lines is for identifying a compound that modulates the bitter taste due to KCl. In some embodiments, the panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, each of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 is expressed in at least one cell line in the panel. In some embodiments, the panel consists essentially of cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60. In some embodiments, the panel of cell lines further comprises a negative control cell line. In some embodiments, the negative control is a negative control for a method of identifying a compound that modulates the bitter taste due to KCl.

[0181] In some embodiments, the panel of cell lines is for identifying a compound that modulates the bitter taste due to potassium lactate. In some embodiments, the panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, each of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 is expressed in at least one cell line in the panel. In some embodiments, the panel consists essentially of cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60. In some embodiments, the panel of cell lines further comprises a negative control cell line. In some embodiments, the negative control is a negative control for a method of identifying a compound that modulates the bitter taste due to potassium lactate.

[0182] In some embodiments, the panel of cell lines is for identifying a compound that modulates the bitter taste due to Acesulfame K In some embodiments, the panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, each of TAS2R1,

TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 is expressed in at least one cell line in the panel. In some embodiments, the panel consists essentially of cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44. In some embodiments, the panel of cell lines further comprises a negative control cell line. In some embodiments, the negative control is a negative control for a method of identifying a compound that modulates the bitter taste due to Acesulfame K.

Examples

[0183] In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

[0184] A panel of cell lines expressing each of the twenty-five bitter taste receptors was generated as described in International Patent Application Publication WO 2010/088633 (*see, e.g.*, Example 26). Accordingly, each cell line of the panel expressed a human bitter taste receptor and mouse $G\alpha_{15}$ signaling protein.

[0185] Each of the Examples below utilized the following functional assay:

Day1: Stable cell lines were seeded onto black wall/clear bottom 96-well, plates (*e.g.*, Corning, 3904). Approximately 40K cells were added per well and incubated at 37°C, 5% CO_2 overnight in cell growth media (DMEM (Sigma, D5796) supplemented with 10% Fetal Bovine Serum (Sigma, 5178), 2mM Glutamine (Sigma, G7513)).

Day2: Growth media was discarded and cells were incubated at 37°C, 5% CO_2 for 60 minutes in 100ul of load solution containing 1X Ca-3 dye (Molecular Devices, R8090), 1% DMSO in buffer containing 130mM NaCl, 2mM $CaCl_2$, 5mM KCl, 1.2mM $MgCl_2$, 10mM HEPES and 10mM Glucose (pH 7.0). Probenecid was not included in the load solution.

Functional assay: After dye loading, cell plates were placed in a fluorescent plate reader (*e.g.*, FDSS6000 (Hamamatsu, Japan)) and receptor stimulation was measured by adding 50ul of 3X concentrated ligand/agonist stock. Fluorescence was monitored continuously for 10s before agonist addition and for 100-250s after stimulation with agonist.

[0186] Receptor Activation is defined as follows:

% Activation = [((Maximum signal fluorescence – Minimum signal fluorescence) – (Maximum control fluorescence – Minimum control fluorescence)) / (Maximum buffer fluorescence – Minimum buffer fluorescence)]*100

Functional Response = [(Maximum signal fluorescence – Minimum signal fluorescence) – (Maximum control fluorescence – Minimum control fluorescence)]

Signal fluorescence: refers to the change in fluorescence seen with addition of ligand

Control fluorescence: refers to the change in fluorescence seen with addition of buffer (negative control)

Calcium signals from ligand and control were normalized to the basal fluorescence of the cells prior to the stimulation.

[0187] A concentration analysis was performed and EC_{50} values were calculated by nonlinear regression using the formula: Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*Hill Slope)), X= log of dose or concentration, Y = Response (increasing as X increases), Top = maximum signal, Bottom = minimum signal. EC_{50} (half maximal effective concentration) refers to the molar concentration of the agonist which produces 50% of the maximum possible effective response from that agonist.

Example 1 Verification of Functional Signaling in the Bitter Taste Receptor Panel.

[0188] To confirm that each of the 25 bitter receptor cell lines functionally and stably express the G protein ($G\alpha_{15}$), the entire panel was tested, as described above, with increasing concentrations of isoproterenol, an agonist of β2-adrenergic receptor, which is endogenously expressed in the HEK293T host cell line. Isoproterenol is known to signal via $G\alpha_{15}$ signaling cascade to increase intracellular calcium levels.

[0189] As shown in Figure 1, all twenty-five bitter taste receptor cell lines present in the panel uniformly respond to isoproterenol in a functional assay. For each of the twenty-five cell lines, the EC_{50} value was approximately 4.9 ± 0.41 nM, which is in close agreement with published literature values. The EC_{50} values show remarkable reproducibility between the twenty-five clonal cell lines and confirm that the panel of bitter cell lines is useful for functional assays.

Example 2 Identification of the Bitter Taste Receptors That Respond to KCl

[0190] To identify the set of receptors that are sensitive to KCl and likely mediate bitter taste due to KCl, the entire panel of twenty-five bitter receptor expressing cell lines were independently and simultaneously exposed to 20mM KCl. Bitter taste receptor activation was measured by changes in intracellular calcium using the fluorescent reporter system described above. As shown in Figure 2, only six bitter taste receptor expressing cell lines out of the twenty-five strongly and specifically responded to KCl stimulation. KCl elicited a weak or background response in the other nineteen cell lines. KCl induced TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter receptor activity (Figures 2 and 6). KCl did not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, or TAS2R55 bitter taste receptor activity (Figures 2 and 6). Heretofore, no ligand had been identified for TAS2R60, which has now been deorphaned.

[0191] To further characterize KCl-induced signaling by TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, a functional profile was created by measuring KCl's activation of each of the receptors by stimulating them with increasing concentrations of KCl and calculating

EC_{50} values as described above. As shown in Figure 3, the EC_{50} value for each cell line was approximately 5-15mM. Due to the difference in the nature of the receptors and ligands, the dose-response curves in the TAS2R activity assays are not directly comparable to the dose-response curves in Figure 1.

Example 3 Identification of the Bitter Taste Receptors That Respond to Potassium Lactate

[0192] To identify the set of receptors that are sensitive to potassium lactate and likely mediate bitter taste due to potassium lactate, the entire panel of twenty-five bitter receptor expressing cell lines were independently and simultaneously exposed to 20mM potassium lactate. Bitter taste receptor activation was measured by changes in intracellular calcium using the fluorescent reporter system described above. As shown in Figure 4, only seven bitter taste receptor expressing cell lines out of the twenty-five strongly and specifically responded to potassium lactate stimulation. Potassium lactate elicited a weak or background response in the other eighteen cell lines. Potassium lactate induced TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44, TAS2R46 and TAS2R60 bitter receptor activity (Figures 4 and 6). Potassium lactate did not induce TAS2R1, TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R16, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R55 bitter taste receptor activity (Figures 4 and 6). Heretofore, no ligand had been identified for TAS2R60, which has now been deorphaned.

Example 4 Identification of the Bitter Taste Receptors That Respond to Acesulfame K

[0193] To identify the set of receptors that are sensitive to Acesulfame K and likely mediate bitter taste due to Acesulfame K, the entire panel of twenty-five bitter receptor expressing cell lines were independently and simultaneously exposed to 20mM Acesulfame K. Bitter taste receptor activation was measured by changes in intracellular calcium using the fluorescent reporter system described above. As shown in Figure 5, only seven bitter taste receptor expressing cell lines out of the twenty-five strongly and specifically responded to Acesulfame K stimulation. Acesulfame K elicited a weak or background response in the other eighteen cell lines. Acesulfame K induced TAS2R1, TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R16, and TAS2R44 bitter receptor activity (Figures 5 and 6). Acesulfame K did not induce TAS2R3, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R43, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, TAS2R55, or TAS2R60 bitter taste receptor activity (Figures 5 and 6).

SEQUENCE LISTING

[0194] Human GNA15

[0195] MARSLTWRCCPWCLTEDEKAAARVDQEINRILLEQKKQDRGELKLLLLGPGESGKSTFIKQM
RIIHGAGYSEEERKGFRPLVYQNIFVSMRAMIEAMERLQIPFSRPESKHHASLVMSQDPYKVTTFEKRY
AAAMQWLWRDAGIRACYERRREFHLLDSAVYYLSHLERITEEGYVPTAQDVLRSRMPTTGINEYCFSV
QKTNLRIVDVGGQKSERKKWIHCFENVIALIYLASLSEYDQCLEENNQENRMKESLALFGTILELPWFK
STSVILFLNKTDILEEKIPTSHLATYFPSFQGPKQDAEAAKRFILDMYTRMYTGCVDGPEGSKKGARSRR
LFSHYTCATDTQNIRKVFKDVRDSVLARYLDEINLL (SEQ ID NO:1)

[0196] TAS2R1 CDS

[0197] ATGCTAGAGTCTCACCTCATTATCTATTTTCTTCTTGCAGTGATACAATTTCTTCTTGGGAT
TTTCACAAATGGCATCATTGTGGTGGTGAATGGCATTGACTTGATCAAGCACAGAAAAATGGCTCC
GCTGGATCTCCTTCTTTCTTGTCTGGCAGTTTCTAGAATTTTTCTGCAGTTGTTCATCTTCTACGTTA
ATGTGATTGTTATCTTCTTCATAGAATTCATCATGTGTTCTGCGAATTGTGCAATTCTCTTATTTATA
AATGAATTGGAACTTTGGCTTGCCACATGGCTCGGCGTTTTCTATTGTGCCAAGGTTGCCAGCGTC
CGTCACCCACTCTTCATCTGGTTGAAGATGAGGATATCCAAGCTGGTCCCATGGATGATCCTGGGG
TCTCTGCTATATGTATCTATGATTTGTGTTTTCCATAGCAAATATGCAGGGTTTATGGTCCCATACT
TCCTAAGGAAATTTTTCTCCCAAAATGCCACAATTCAAAAAGAAGATACACTGGCTATACAGATTT
TCTCTTTTGTTGCTGAGTTCTCAGTGCCATTGCTTATCTTCCTTTTTGCTGTTTTGCTCTTGATTTTCT
CTCTGGGGAGGCACACCCGGCAAATGAGAAACACAGTGGCCGGCAGCAGGGTTCCTGGCAGGGGT
GCACCCATCAGCGCGTTGCTGTCTATCCTGTCCTTCCTGATCCTCTACTTCTCCCACTGCATGATAA
AAGTTTTTCTCTCTTCTCTAAAGTTTCACATCAGAAGGTTCATCTTTCTGTTCTTCATCCTTGTGATT
GGTGTATACCCTTCTGGACACTCTCTCATCTTAATTTTAGGAAATCCTAAATTGAAACAAAATGCA
AAAAAGTTCCTCCTCCACAGTAAGTGCTGTCAGTGA (SEQ ID NO:2)

[0198] TAS2R3 CDS

[0199] ATGATGGGACTCACCGAGGGGGTGTTCCTGATTCTGTCTGGCACTCAGTTCACACTGGGA
ATTCTGGTCAATTGTTTCATTGAGTTGGTCAATGGTAGCAGCTGGTTCAAGACCAAGAGAATGTCT
TTGTCTGACTTCATCATCACCACCCTGGCACTCTTGAGGATCATTCTGCTGTGTATTATCTTGACTG
ATAGTTTTTTAATAGAATTCTCTCCCAACACACATGATTCAGGGATAATAATGCAAATTATTGATG
TTTCCTGGACATTTACAAACCATCTGAGCATTTGGCTTGCCACCTGTCTTGGTGTCCTCTACTGCCT
GAAAATCGCCAGTTTCTCTCACCCCACATTCCTCTGGCTCAAGTGGAGAGTTTCTAGGGTGATGGT
ATGGATGCTGTTGGGTGCACTGCTCTTATCCTGTGGTAGTACCGCATCTCTGATCAATGAGTTTAAG
CTCTATTCTGTCTTTAGGGGAATTGAGGCCACCAGGAATGTGACTGAACACTTCAGAAAGAAGAG
GAGTGAGTATTATCTGATCCATGTTCTTGGGACTCTGTGGTACCTGCCTCCCTTAATTGTGTCCCTG
GCCTCCTACTCTTTGCTCATCTTCTCCCTGGGGAGGCACACACGGCAGATGCTGCAAAATGGGACA
AGCTCCAGAGATCCAACCACTGAGGCCCACAAGAGGGCCATCAGAATCATCCTTTCCTTCTTCTTT
CTCTTCTTACTTTACTTTCTTGCTTTCTTAATTGCATCATTTGGTAATTTCCTACCAAAAACCAAGAT
GGCTAAGATGATTGGTGAAGTAATGACAATGTTTTATCCTGCTGGCCACTCATTTATTCTCATTCTG

GGGAACAGTAAGCTGAAGCAGACATTTGTAGTGATGCTCCGGTGTGAGTCTGGTCATCTGAAGCCT
GGATCCAAGGGACCCATTTTCTCTTAG (SEQ ID NO:3)

[0200] TAS2R4 CDS

[0201] ATGCTTCGGTTATTCTATTTCTCTGCTATTATTGCCTCAGTTATTTTAAATTTTGTAGGAAT
CATTATGAATCTGTTTATTACAGTGGTCAATTGCAAAACTTGGGTCAAAAGCCATAGAATCTCCTC
TTCTGATAGGATTCTGTTCAGCCTGGGCATCACCAGGTTTCTTATGCTGGGACTATTTCTGGTGAAC
ACCATCTACTTCGTCTCTTCAAATACGGAAAGGTCAGTCTACCTGTCTGCTTTTTTTGTGTTGTGTTT
CATGTTTTTGGACTCGAGCAGTGTCTGGTTTGTGACCTTGCTCAATATCTTGTACTGTGTGAAGATT
ACTAACTTCCAACACTCAGTGTTTCTCCTGCTGAAGCGGAATATCTCCCCAAAGATCCCCAGGCTG
CTGCTGGCCTGTGTGCTGATTTCTGCTTTCACCACTTGCCTGTACATCACGCTTAGCCAGGCATCAC
CTTTTCCTGAACTTGTGACTACGAGAAATAACACATCATTTAATATCAGTGAGGGCATCTTGTCTTT
AGTGGTTTCTTTGGTCTTGAGCTCATCTCTCCAGTTCATCATTAATGTGACTTCTGCTTCCTTGCTAA
TACACTCCTTGAGGAGACATATACAGAAGATGCAGAAAAATGCCACTGGTTTCTGGAATCCCCAG
ACGGAAGCTCATGTAGGTGCTATGAAGCTGATGGTCTATTTCCTCATCCTCTACATTCCATATTCAG
TTGCTACCCTGGTCCAGTATCTCCCCTTTTATGCAGGGATGGATATGGGGACCAAATCCATTTGTCT
GATTTTTGCCACCCTTTACTCTCCAGGACATTCTGTTCTCATTATTATCACACATCCTAAACTGAAA
ACAACAGCAAAGAAGATTCTTTGTTTCAAAAAATAG (SEQ ID NO:4)

[0202] TAS2R5 CDS

[0203] ATGCTGAGCGCTGGCCTAGGACTGCTGATGCTGGTGGCAGTGGTTGAATTTCTCATCGGTT
TAATTGGAAATGGAAGCCTGGTGGTCTGGAGTTTTAGAGAATGGATCAGAAAATTCAACTGGTCCT
CATATAACCTCATTATCCTGGGCCTGGCTGGCTGCCGATTTCTCCTGCAGTGGCTGATCATTTTGGA
CTTAAGCTTGTTTCCACTTTTCCAGAGCAGCCGTTGGCTTCGCTATCTTAGTATCTTCTGGGTCCTG
GTAAGCCAGGCCAGCTTATGGTTTGCCACCTTCCTCAGTGTCTTCTATTGCAAGAAGATCACGACC
TTCGATCGCCCGGCCTACTTGTGGCTGAAGCAGAGGGCCTATAACCTGAGTCTCTGGTGCCTTCTG
GGCTACTTTATAATCAATTTGTTACTTACAGTCCAAATTGGCTTAACATTCTATCATCCTCCCCAAG
GAAACAGCAGCATTCGGTATCCCTTTGAAAGCTGGCAGTACCTGTATGCATTTCAGCTCAATTCAG
GAAGTTATTTGCCTTTAGTGGTGTTTCTTGTTTCCTCTGGGATGCTGATTGTCTCTTTGTATACACAC
CACAAGAAGATGAAGGTCCATTCAGCTGGTAGGAGGGATGTCCGGGCCAAGGCTCACATCACTGC
GCTGAAGTCCTTGGGCTGCTTCCTCTTACTTCACCTGGTTTATATCATGGCCAGCCCCTTCTCCATC
ACCTCCAAGACTTATCCTCCTGATCTCACCAGTGTCTTCATCTGGGAGACACTCATGGCAGCCTATC
CTTCTCTTCATTCTCTCATATTGATCATGGGGATTCCTAGGGTGAAGCAGACTTGTCAGAAGATCCT
GTGGAAGACAGTGTGTGCTCGGAGATGCTGGGGCCCATGA (SEQ ID NO:5)

[0204] TAS2R7 CDS

[0205] ATGGCAGATAAAGTGCAGACTACTTTATTGTTCTTAGCAGTTGGAGAGTTTTCAGTGGGGA
TCTTAGGGAATGCATTCATTGGATTGGTAAACTGCATGGATTGGGTCAAGAAGAGGAAAATTGCCT
CCATTGATTTAATCCTCACAAGTCTGGCCATATCCAGAATTTGTCTATTGTGCGTAATACTATTAGA
TTGTTTTATATTGGTGCTATATCCAGATGTCTATGCCACTGGTAAAGAAATGAGAATCATTGACTTC
TTCTGGACACTAACCAATCATTTAAGTATCTGGTTTGCAACCTGCCTCAGCATTTACTATTTCTTCA

AGATAGGTAATTTCTTTCACCCACTTTTCCTCTGGATGAAGTGGAGAATTGACAGGGTGATTTCCT
GGATTCTACTGGGGTGCGTGGTTCTCTCTGTGTTTATTAGCCTTCCAGCCACTGAGAATTTGAACGC
TGATTTCAGGTTTTGTGTGAAGGCAAAGAGGAAAACAAACTTAACTTGGAGTTGCAGAGTAAATA
AAACTCAACATGCTTCTACCAAGTTATTTCTCAACCTGGCAACGCTGCTCCCCTTTTGTGTGTGCCT
AATGTCCTTTTTCCTCTTGATCCTCTCCCTGCGGAGACATATCAGGCGAATGCAGCTCAGTGCCACA
GGGTGCAGAGACCCCAGCACAGAAGCCCATGTGAGAGCCCTGAAAGCTGTCATTTCCTTCCTTCTC
CTCTTTATTGCCTACTATTTGTCCTTTCTCATTGCCACCTCCAGCTACTTTATGCCAGAGACGGAATT
AGCTGTGATTTTTGGTGAGTCCATAGCTCTAATCTACCCCTCAAGTCATTCATTTATCCTAATACTG
GGGAACAATAAATTAAGACATGCATCTCTAAAGGTGATTTGGAAAGTAATGTCTATTCTAAAAGG
AAGAAAATTCCAACAACATAAACAAATCTGA (SEQ ID NO:6)

[0206] TAS2R8 CDS

[0207] ATGTTCAGTCCTGCAGATAACATCTTTATAATCCTAATAACTGGAGAATTCATACTAGGAA
TATTGGGGAATGGATACATTGCACTAGTCAACTGGATTGACTGGATTAAGAAGAAAAAGATTTCC
ACAGTTGACTACATCCTTACCAATTTAGTTATCGCCAGAATTTGTTTGATCAGTGTAATGGTTGTAA
ATGGCATTGTAATAGTACTGAACCCAGATGTTTATACAAAAAATAAACAACAGATAGTCATTTTTA
CCTTCTGGACATTTGCCAACTACTTAAATATGTGGATTACCACCTGCCTTAATGTCTTCTATTTTCT
GAAGATAGCCAGTTCCTCTCATCCACTTTTTCTCTGGCTGAAGTGGAAAATTGATATGGTGGTGCA
CTGGATCCTGCTGGGATGCTTTGCCATTTCCTTGTTGGTCAGCCTTATAGCAGCAATAGTACTGAGT
TGTGATTATAGGTTTCATGCAATTGCCAAACATAAAAGAAACATTACTGAAATGTTCCATGTGAGT
AAAATACCATACTTTGAACCCTTGACTCTCTTTAACCTGTTTGCAATTGTCCCATTTATTGTGTCAC
TGATATCATTTTTCCTTTTAGTAAGATCTTTATGGAGACATACCAAGCAAATAAAACTCTATGCTAC
CGGCAGTAGAGACCCCAGCACAGAAGTTCATGTGAGAGCCATTAAAACTATGACTTCATTTATCTT
CTTTTTTTTCCTATACTATATTTCTTCTATTTTGATGACCTTTAGCTATCTTATGACAAAATACAAGT
TAGCTGTGGAGTTTGGAGAGATTGCAGCAATTCTCTACCCCTTGGGTCACTCACTTATTTTAATTGT
TTTAAATAATAAACTGAGGCAGACATTTGTCAGAATGCTGACATGTAGAAAAATTGCCTGCATGAT
ATGA (SEQ ID NO:7)

[0208] TAS2R9 CDS

[0209] ATGCCAAGTGCAATAGAGGCAATATATATTATTTTAATTGCTGGTGAATTGACCATAGGG
ATTTGGGGAAATGGATTCATTGTACTAGTTAACTGCATTGACTGGCTCAAAAGAAGAGATATTTCC
TTGATTGACATCATCCTGATCAGCTTGGCCATCTCCAGAATCTGTCTGCTGTGTGTAATATCATTAG
ATGGCTTCTTTATGCTGCTCTTTCCAGGTACATATGGCAATAGCGTGCTAGTAAGCATTGTGAATGT
TGTCTGGACATTTGCCAATAATTCAAGTCTCTGGTTTACTTCTTGCCTCAGTATCTTCTATTTACTCA
AGATAGCCAATATATCGCACCCATTTTTCTTCTGGCTGAAGCTAAAGATCAACAAGGTCATGCTTG
CGATTCTTCTGGGGTCCTTTCTTATCTCTTTAATTATTAGTGTTCCAAAGAATGATGATATGTGGTA
TCACCTTTTCAAAGTCAGTCATGAAGAAAACATTACTTGGAAATTCAAAGTGAGTAAAATTCCAGG
TACTTTCAAACAGTTAACCCTGAACCTGGGGGTGATGGTTCCCTTTATCCTTTGCCTGATCTCATTT
TTCTTGTTACTTTTCTCCCTAGTTAGACACACCAAGCAGATTCGACTGCATGCTACAGGGTTCAGAG
ACCCCAGTACAGAGGCCCACATGAGGGCCATAAAGGCAGTGATCATCTTTCTGCTCCTCCTCATCG
TGTACTACCCAGTCTTTCTTGTTATGACCTCTAGCGCTCTGATTCCTCAGGGAAAATTAGTGTTGAT

GATTGGTGACATAGTAACTGTCATTTTCCCATCAAGCCATTCATTCATTCTAATTATGGGAAATAGC
AAGTTGAGGGAAGCTTTTCTGAAGATGTTAAGATTTGTGAAGTGTTTCCTTAGAAGAAGAAAGCCT
TTTGTTCCATAG (SEQ ID NO:8)

[0210] TAS2R10 CDS

[0211] ATGCTACGTGTAGTGGAAGGCATCTTCATTTTTGTTGTAGTTAGTGAGTCAGTGTTTGGGG
TTTTGGGGAATGGATTTATTGGACTTGTAAACTGCATTGACTGTGCCAAGAATAAGTTATCTACGA
TTGGCTTTATTCTCACCGGCTTAGCTATTTCAAGAATTTTTCTGATATGGATAATAATTACAGATGG
ATTTATACAGATATTCTCTCCAAATATATATGCCTCCGGTAACCTAATTGAATATATTAGTTACTTT
TGGGTAATTGGTAATCAATCAAGTATGTGGTTTGCCACCAGCCTCAGCATCTTCTATTTCCTGAAG
ATAGCAAATTTTTCCAACTACATATTTCTCTGGTTGAAGAGCAGAACAAATATGGTTCTTCCCTTCA
TGATAGTATTCTTACTTATTTCATCGTTACTTAATTTTGCATACATTGCGAAGATTCTTAATGATTAT
AAAATGAAGAATGACACAGTCTGGGATCTCAACATGTATAAAAGTGAATACTTTATTAAACAGAT
TTTGCTAAATCTGGGAGTCATTTTCTTCTTTACACTATCCCTAATTACATGTATTTTTTTAATCATTT
CCCTTTGGAGACACAACAGGCAGATGCAATCAAATGTGACAGGATTGAGAGACTCCAACACAGAA
GCTCATGTGAAGGCAATGAAAGTTTTGATATCTTTCATCATCCTCTTTATCTTGTATTTTATAGGCA
TGGCCATAGAAATATCATGTTTTACTGTGCGAGAAAACAAACTGCTGCTTATGTTTGGAATGACAA
CCACAGCCATCTATCCCTGGGGTCACTCATTTATCTTAATTCTAGGAAACAGCAAGCTAAAGCAAG
CCTCTTTGAGGGTACTGCAGCAATTGAAGTGCTGTGAGAAAAGGAAAAATCTCAGAGTCACATAG
(SEQ ID NO:9)

[0212] TAS2R13 CDS

[0213] ATGGAAAGTGCCCTGCCGAGTATCTTCACTCTTGTAATAATTGCAGAATTCATAATTGGGA
ATTTGAGCAATGGATTTATAGTACTGATCAACTGCATTGACTGGGTCAGTAAAAGAGAGCTGTCCT
CAGTCGATAAACTCCTCATTATCTTGGCAATCTCCAGAATTGGGCTGATCTGGGAAATATTAGTAA
GTTGGTTTTTAGCTCTGCATTATCTAGCCATATTTGTGTCTGGAACAGGATTAAGAATTATGATTTT
TAGCTGGATAGTTTCTAATCACTTCAATCTCTGGCTTGCTACAATCTTCAGCATCTTTTATTTGCTCA
AAATAGCGAGTTTCTCTAGCCCTGCTTTTCTCTATTTGAAGTGGAGAGTAAACAAAGTGATTCTGA
TGATACTGCTAGGAACCTTGGTCTTCTTATTTTTAAATCTGATACAAATAAACATGCATATAAAAG
ACTGGCTGGACCGATATGAAAGAAACACAACTTGGAATTTCAGTATGAGTGACTTTGAAACATTTT
CAGTGTCGGTCAAATTCACTATGACTATGTTCAGTCTAACACCATTTACTGTGGCCTTCATCTCTTT
TCTCCTGTTAATTTTCTCCCTGCAGAAACATCTCCAGAAAATGCAACTCAATTACAAAGGACACAG
AGACCCCAGGACCAAGGTCCATACAAATGCCTTGAAAATTGTGATCTCATTCCTTTTATTCTATGCT
AGTTTCTTTCTATGTGTTCTCATATCATGGATTTCTGAGCTGTATCAGAACACAGTGATCTACATGC
TTTGTGAGACGATTGGAGTCTTCTCTCCTTCAAGCCACTCCTTTCTTCTGATTCTAGGAAACGCTAA
GTTAAGACAGGCCTTTCTTTTGGTGGCAGCTAAGGTATGGGCTAAACGATGA (SEQ ID NO:10)

[0214] TAS2R14 CDS

[0215] ATGGGTGGTGTCATAAAGAGCATATTTACATTCGTTTTAATTGTGGAATTTATAATTGGAA
ATTTAGGAAATAGTTTCATAGCACTGGTGAACTGTATTGACTGGGTCAAGGGAAGAAAGATCTCTT
CGGTTGATCGGATCCTCACTGCTTTGGCAATCTCTCGAATTAGCCTGGTTTGGTTAATATTCGGAAG

CTGGTGTGTGTCTGTGTTTTTCCCAGCTTTATTTGCCACTGAAAAAATGTTCAGAATGCTTACTAAT
ATCTGGACAGTGATCAATCATTTTAGTGTCTGGTTAGCTACAGGCCTCGGTACTTTTTATTTTCTCA
AGATAGCCAATTTTTCTAACTCTATTTTTCTCTACCTAAAGTGGAGGGTTAAAAAGGTGGTTTTGGT
GCTGCTTCTTGTGACTTCGGTCTTCTTGTTTTTAAATATTGCACTGATAAACATCCATATAAATGCC
AGTATCAATGGATACAGAAGAAACAAGACTTGCAGTTCTGATTCAAGTAACTTTACACGATTTTCC
AGTCTTATTGTATTAACCAGCACTGTGTTCATTTTCATACCCTTTACTTTGTCCCTGGCAATGTTTCT
TCTCCTCATCTTCTCCATGTGGAAACATCGCAAGAAGATGCAGCACACTGTCAAAATATCCGGAGA
CGCCAGCACCAAAGCCCACAGAGGAGTTAAAAGTGTGATCACTTTCTTCCTACTCTATGCCATTTT
CTCTCTGTCTTTTTTCATATCAGTTTGGACCTCTGAAAGGTTGGAGGAAAATCTAATTATTCTTTCC
CAGGTGATGGGAATGGCTTATCCTTCATGTCACTCATGTGTTCTGATTCTTGGAAACAAGAAGCTG
AGACAGGCCTCTCTGTCAGTGCTACTGTGGCTGAGGTACATGTTCAAAGATGGGGAGCCCTCAGGT
CACAAAGAATTTAGAGAATCATCTTGA (SEQ ID NO:11)

[0216] TAS2R16 CDS

[0217] ATGATACCCATCCAACTCACTGTCTTCTTCATGATCATCTATGTGCTTGAGTCCTTGACAAT
TATTGTGCAGAGCAGCCTAATTGTTGCAGTGCTGGGCAGAGAATGGCTGCAAGTCAGAAGGCTGA
TGCCTGTGGACATGATTCTCATCAGCCTGGGCATCTCTCGCTTCTGTCTACAGTGGGCATCAATGCT
GAACAATTTTTGCTCCTATTTTAATTTGAATTATGTACTTTGCAACTTAACAATCACCTGGGAATTT
TTTAATATCCTTACATTCTGGTTAAACAGCTTGCTTACCGTGTTCTACTGCATCAAGGTCTCTTCTTT
CACCCATCACATCTTTCTCTGGCTGAGGTGGAGAATTTTGAGGTTGTTTCCCTGGATATTACTGGGT
TCTCTGATGATTACTTGTGTAACAATCATCCCTTCAGCTATTGGGAATTACATTCAAATTCAGTTAC
TCACCATGGAGCATCTACCAAGAAACAGCACTGTAACTGACAAACTTGAAAATTTTCATCAGTATC
AGTTCCAGGCTCATACAGTTGCATTGGTTATTCCTTTCATCCTGTTCCTGGCCTCCACCATCTTTCTC
ATGGCATCACTGACCAAGCAGATACAACATCATAGCACTGGTCACTGCAATCCAAGCATGAAAGC
GCGCTTCACTGCCCTGAGGTCCCTTGCCGTCTTATTTATTGTGTTTACCTCTTACTTTCTAACCATAC
TCATCACCATTATAGGTACTCTATTTGATAAGAGATGTTGGTTATGGGTCTGGGAAGCTTTTGTCTA
TGCTTTCATCTTAATGCATTCCACTTCACTGATGCTGAGCAGCCCTACGTTGAAAAGGATTCTAAA
GGGAAAGTGCTAG (SEQ ID NO:12)

[0218] TAS2R38 CDS

[0219] ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTATGAAGTCAGGAGTACATTTCTGTTCA
TTTCAGTCCTGGAGTTTGCAGTGGGGTTTCTGACCAATGCCTTCGTTTTCTTGGTGAATTTTTGGGA
TGTAGTGAAGAGGCAGGCACTGAGCAACAGTGATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCT
TTTCCTGCATGGACTGCTGTTCCTGAGTGCTATCCAGCTTACCCACTTCCAGAAGTTGAGTGAACCA
CTGAACCACAGCTACCAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACCTCTGGCTT
GCTGCCTGCCTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCCTGATCTGCTT
GGCAAGCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGCATCTGC
ACTGTCCTCTGTGTTTGGTGCTTTTTTAGCAGACCTCACTTCACAGTCACAACTGTGCTATTCATGA
ATAACAATACAAGGCTCAACTGGCAGATTAAAGATCTCAATTTATTTTATTCCTTTCTCTTCTGCTA
TCTGTGGTCTGTGCCTCCTTTCCTATTGTTTCTGGTTTCTTCTGGGATGCTGACTGTCTCCCTGGGAA
GGCACATGAGGACAATGAAGGTCTATACCAGAAACTCTCGTGACCCCAGCCTGGAGGCCCACATT

AAAGCCCTCAAGTCTCTTGTCTCCTTTTTCTGCTTCTTTGTGATATCATCCTGTGCTGCCTTCATCTC
TGTGCCCCTACTGATTCTGTGGCGCGACAAAATAGGGGTGATGGTTTGTGTTGGGATAATGGCAGC
TTGTCCCTCTGGGCATGCAGCCATCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGAC
CATTCTGCTCTGGGCTCAGAGCAGCCTGAAGGTAAGAGCCGACCACAAGGCAGATTCCCGGACAC
TGTGCTGA (SEQ ID NO:13)

[0220] TAS2R39 CDS

[0221] ATGCTAGGGAGATGTTTTCCTCCAGACACCAAAGAGAAGCAACAGCTCAGAATGACTAAA
CTCTGCGATCCTGCAGAAAGTGAATTGTCGCCATTTCTCATCACCTTAATTTTAGCAGTTTTACTTG
CTGAATACCTCATTGGTATCATTGCAAATGGTTTCATCATGGCTATACATGCAGCTGAATGGGTTC
AAAATAAGGCAGTTTCCACAAGTGGCAGGATCCTGGTTTTCCTGAGTGTATCCAGAATAGCTCTCC
AAAGCCTCATGATGTTAGAAATTACCATCAGCTCAACCTCCCTAAGTTTTTATTCTGAAGACGCTG
TATATTATGCATTCAAAATAAGTTTTATATTCTTAAATTTTTGTAGCCTGTGGTTTGCTGCCTGGCTC
AGTTTCTTCTACTTTGTGAAGATTGCCAATTTCTCCTACCCCCTTTTCCTCAAACTGAGGTGGAGAA
TTACTGGATTGATACCCTGGCTTCTGTGGCTGTCCGTGTTTATTTCCTTCAGTCACAGCATGTTCTG
CATCAACATCTGCACTGTGTATTGTAACAATTCTTTCCCTATCCACTCCTCCAACTCCACTAAGAAA
ACATACTTGTCTGAGATCAATGTGGTCGGTCTGGCTTTTTTCTTTAACCTGGGGATTGTGACTCCTC
TGATCATGTTCATCCTGACAGCCACCCTGCTGATCCTCTCTCTCAAGAGACACACCCTACACATGG
GAAGCAATGCCACAGGGTCCAACGACCCCAGCATGGAGGCTCACATGGGGGCCATCAAAGCTATC
AGCTACTTTCTCATTCTCTACATTTTCAATGCAGTTGCTCTGTTTATCTACCTGTCCAACATGTTTGA
CATCAACAGTCTGTGGAATAATTTGTGCCAGATCATCATGGCTGCCTACCCTGCCAGCCACTCAAT
TCTACTGATTCAAGATAACCCTGGGCTGAGAAGAGCCTGGAGCGGCTTCAGCTTCGACTTCATCTT
TACCCAAAAGAGTGGACTCTGA (SEQ ID NO:14)

[0222] TAS2R40 CDS

[0223] ATGGCAACGGTGAACACAGATGCCACAGATAAAGACATATCCAAGTTCAAGGTCACCTTC
ACTTTGGTGGTCTCCGGAATAGAGTGCATCACTGGCATCCTTGGGAGTGGCTTCATCACGGCCATC
TATGGGGCTGAGTGGGCCAGGGGCAAAACACTCCCCACTGGTGACCGCATTATGTTGATGCTGAG
CTTTTCCAGGCTCTTGCTACAGATTTGGATGATGCTGGAGAACATTTTCAGTCTGCTATTCCGAATT
GTTTATAACCAAAACTCAGTGTATATCCTCTTCAAAGTCATCACTGTCTTTCTGAACCATTCCAATC
TCTGGTTTGCTGCCTGGCTCAAAGTCTTCTATTGTCTTAGAATTGCAAACTTCAATCATCCTTTGTTC
TTCCTGATGAAGAGGAAAATCATAGTGCTGATGCCTTGGCTTCTCAGGCTGTCAGTGTTGGTTTCCT
TAAGCTTCAGCTTTCCTCTCTCGAGAGATGTCTTCAATGTGTATGTGAATAGCTCCATTCCTATCCC
CTCCTCCAACTCCACGGAGAAGAAGTACTTCTCTGAGACCAATATGGTCAACCTGGTATTTTTCTA
TAACATGGGGATCTTCGTTCCTCTGATCATGTTCATCCTGGCAGCCACCCTGCTGATCCTCTCTCTC
AAGAGACACACCCTACACATGGGAAGCAATGCCACAGGGTCCAGGGACCCCAGCATGAAGGCTC
ACATAGGGGCCATCAAAGCCACCAGCTACTTTCTCATCCTCTACATTTTCAATGCAATTGCTCTATT
TCTTTCCACGTCCAACATCTTTGACACTTACAGTTCCTGGAATATTTTGTGCAAGATCATCATGGCT
GCCTACCCTGCCGGCCACTCAGTACAACTGATCTTGGGCAACCCTGGGCTGAGAAGAGCCTGGAA
GCGGTTTCAGCACCAAGTTCCTCTTTACCTAAAAGGGCAGACTCTGTGA (SEQ ID NO:15)

[0224] tAS2R41 CDS

[0225] ATGCAAGCAGCACTGACGGCCTTCTTCGTGTTGCTCTTTAGCCTGCTGAGTCTTCTGGGGA
TTGCAGCGAATGGCTTCATTGTGCTGGTGCTGGGCAGGGAGTGGCTGCGATATGGCAGGTTGCTGC
CCTTGGATATGATCCTCATTAGCTTGGGTGCCTCCCGCTTCTGCCTGCAGTTGGTTGGGACGGTGCA
CAACTTCTACTACTCTGCCCAGAAGGTCGAGTACTCTGGGGGTCTCGGCCGACAGTTCTTCCATCT
ACACTGGCACTTCCTGAACTCAGCCACCTTCTGGTTTTGCAGCTGGCTCAGTGTCCTGTTCTGTGTG
AAGATTGCTAACATCACACACTCCACCTTCCTGTGGCTGAAGTGGAGGTTCCCAGGGTGGGTGCCC
TGGCTCCTGTTGGGCTCTGTCCTGATCTCCTTCATCATAACCCTGCTGTTTTTTTGGGTGAACTACCC
TGTATATCAAGAATTTTTAATTAGAAAATTTTCTGGGAACATGACCTACAAGTGGAATACAAGGAT
AGAAACATACTATTTCCCATCCCTGAAACTGGTCATCTGGTCAATTCCTTTTTCTGTTTTTCTGGTCT
CAATTATGCTGCTGATTAATTCTCTGAGGAGGCATACTCAGAGAATGCAGCACAACGGGCACAGC
CTGCAGGACCCCAGCACCCAGGCTCACACCAGAGCTCTGAAGTCCCTCATCTCCTTCCTCATTCTTT
ATGCTCTGTCCTTTCTGTCCCTGATCATTGATGCCGCAAAATTTATCTCCATGCAGAACGACTTTTA
CTGGCCATGGCAAATTGCAGTCTACCTGTGCATATCTGTCCATCCCTTCATCCTCATCTTCAGCAAC
CTCAAGCTTCGAAGCGTGTTCTCACAGCTCCTGTTGTTGGCAAGGGGCTTCTGGGTGGCCTGA
(SEQ ID NO:16)

[0226] TAS2R43 CDS

[0227] ATGATAACTTTTCTGCCCATCATTTTTTCCAGTCTGGTAGTGGTTACATTTGTTATTGGAAA
TTTTGCTAATGGCTTCATAGCACTGGTAAATTCCATTGAGTGGTTCAAGAGACAAAAGATCTCCTT
TGCTGACCAAATTCTCACTGCTCTGGCGGTCTCCAGAGTTGGTTTGCTCTGGGTATTATTATTAAAC
TGGTATTCAACTGTGTTGAATCCAGCTTTTAATAGTGTAGAAGTAAGAACTACTGCTTATAATATCT
GGGCAGTGATCAACCATTTCAGCAACTGGCTTGCTACTACCCTCAGCATATTTTATTTGCTCAAGAT
TGCCAATTTCTCCAACTTTATTTTTCTTCACTTAAAGAGGAGAGTTAAGAGTGTCATTCTGGTGATG
TTGTTGGGGCCTTTGCTATTTTTGGCTTGTCATCTTTTTGTGATAAACATGAATGAGATTGTGCGGA
CAAAAGAATTTGAAGGAAACATGACTTGGAAGATCAAATTGAAGAGTGCAATGTACTTTTCAAAT
ATGACTGTAACCATGGTAGCAAACTTAGTACCCTTCACTCTGACCCTACTATCTTTTATGCTGTTAA
TCTGTTCTTTGTGTAAACATCTCAAGAAGATGCAGCTCCATGGTAAAGGATCTCAAGATCCCAGCA
CCAAGGTCCACATAAAAGCTTTGCAAACTGTGATCTCCTTCCTCTTGTTATGTGCCATTTACTTTCT
GTCCATAATGATATCAGTTTGGAGTTTTGGAAGTCTGGAAAACAAACCTGTCTTCATGTTCTGCAA
AGCTATTAGATTCAGCTATCCTTCAATCCACCCATTCATCCTGATTTGGGGAAACAAGAAGCTAAA
GCAGACTTTTCTTTCAGTTTTTTGGCAAATGAGGTACTGGGTGAAAGGAGAGAAGACTTCATCTCC
ATGA (SEQ ID NO:17)

[0228] tAS2R44 CDS

[0229] ATGACAACTTTTATACCCATCATTTTTTCCAGTGTGGTAGTGGTTCTATTTGTTATTGGAAA
TTTTGCTAATGGCTTCATAGCATTGGTAAATTCCATTGAGCGGGTCAAGAGACAAAAGATCTCTTT
TGCTGACCAGATTCTCACTGCTCTGGCGGTCTCCAGAGTTGGTTTGCTCTGGGTATTATTATTAAAT
TGGTATTCAACTGTGTTTAATCCAGCTTTTTATAGTGTAGAAGTAAGAACTACTGCTTATAATGTCT
GGGCAGTAACCGGCCATTTCAGCAACTGGCTTGCTACTAGCCTCAGCATATTTTATTTGCTCAAGA
TTGCCAATTTCTCCAACCTTATTTTTCTTCACTTAAAGAGGAGAGTTAAGAGTGTCATTCTGGTGAT

GCTGTTGGGGCCTTTACTATTTTTGGCTTGTCAACTTTTTGTGATAAACATGAAAGAGATTGTACGG
ACAAAAGAATATGAAGGAAACTTGACTTGGAAGATCAAATTGAGGAGTGCAGTGTACCTTTCAGA
TGCGACTGTAACCACGCTAGGAAACTTAGTGCCCTTCACTCTGACCCTGCTATGTTTTTTGCTGTTA
ATCTGTTCTCTGTGTAAACATCTCAAGAAGATGCAGCTCCATGGTAAAGGATCTCAAGATCCCAGC
ACCAAGGTCCACATAAAAGCTTTGCAAACTGTGATCTTTTTCCTCTTGTTATGTGCCGTTTACTTTC
TGTCCATAATGATATCAGTTTGGAGTTTTGGGAGTCTGGAAAACAAACCTGTCTTCATGTTCTGCA
AAGCTATTAGATTCAGCTATCCTTCAATCCACCCATTCATCCTGATTTGGGGAAACAAGAAGCTAA
AGCAGACTTTTCTTTCAGTTTTGCGGCAAGTGAGGTACTGGGTGAAAGGAGAGAAGCCTTCATCTC
CATGA (SEQ ID NO:18)

[0230] tAS2R45 CDS

[0231] ATGATAACTTTTCTGCCCATCATATTTTCCATTCTAGTAGTGGTTACATTTGTTATTGGAAA
TTTTGCTAATGGCTTCATAGCGTTGGTAAATTCCACCGAGTGGGTGAAGAGACAAAAGATCTCCTT
TGCTGACCAAATTGTCACTGCTCTGGCGGTCTCCAGAGTTGGTTTGCTCTGGGTGTTATTATTAAAT
TGGTATTCAACTGTGTTGAATCCAGCTTTTTGTAGTGTAGAATTAAGAACTACTGCTTATAATATCT
GGGCAGTAACCGGCCATTTCAGCAACTGGCCTGCTACTAGCCTCAGCATATTTTATTTGCTCAAGA
TTGCCAATTTCTCCAACCTTATTTTTCTTCGCTTAAAGAGGAGAGTTAAGAGTGTCATTCTGGTGAT
GCTGTTGGGGCCTTTGCTATTTTTGGCTTGTCATCTTTTTGTGGTAAACATGAATCAGATTGTATGG
ACAAAAGAATATGAAGGAAACATGACTTGGAAGATCAAATTGAGGCGTGCAATGTACCTTTCAGA
TACGACTGTAACCATGCTAGCAAACTTAGTACCCTTTACTGTAACCCTGATATCTTTTCTGCTGTTA
GTCTGTTCTCTGTGTAAACATCTCAAGAAGATGCACCTCCATGGCAAAGGATCTCAAGATCCCAGT
ACCAAGGTCCACATAAAAGTTTTGCAAACTGTGATCTCCTTCCTCTTGTTATGTGCCATTTACTTTG
TGTCTGTAATAATATCAGTTTGGAGTTTTAAGAATCTGGAAAACAAACCTGTCTTCATGTTCTGCCA
AGCTATTGGATTCAGCTGTTCTTCAGCCCACCCGTTCATCCTGATTTGGGGAAACAAGAAGCTAAA
GCAGACTTATCTTTCAGTTTTGTGGCAAATGAGGTACTGA (SEQ ID NO:19)

[0232] TAS2R46 CDS

[0233] ATGATAACTTTTCTGCCCATCATTTTTTCCATTCTAATAGTGGTTACATTTGTGATTGGAAA
TTTTGCTAATGGCTTCATAGCATTGGTAAATTCCATTGAGTGGTTTAAGAGACAAAAGATCTCTTTT
GCTGACCAAATTCTCACTGCTCTGGCAGTCTCCAGAGTTGGTTTACTCTGGGTATTAGTATTAAATT
GGTATGCAACTGAGTTGAATCCAGCTTTTAACAGTATAGAAGTAAGAATTACTGCTTACAATGTCT
GGGCAGTAATCAACCATTTCAGCAACTGGCTTGCTACTAGCCTCAGCATATTTTATTTGCTCAAGA
TTGCCAATTTCTCCAACCTTATTTTTCTTCACTTAAAGAGGAGAGTTAAGAGTGTTGTTCTGGTGAT
ACTATTGGGGCCTTTGCTATTTTTGGTTTGTCATCTTTTTGTGATAAACATGAATCAGATTATATGG
ACAAAAGAATATGAAGGAAACATGACTTGGAAGATCAAACTGAGGAGTGCAATGTACCTTTCAAA
TACAACGGTAACCATCCTAGCAAACTTAGTTCCCTTCACTCTGACCCTGATATCTTTTCTGCTGTTA
ATCTGTTCTCTGTGTAAACATCTCAAAAAGATGCAGCTCCATGGCAAAGGATCTCAAGATCCCAGC
ATGAAGGTCCACATAAAAGCTTTGCAAACTGTGACCTCCTTCCTCTTGTTATGTGCCATTTACTTTC
TGTCCATAATCATGTCAGTTTGGAGTTTTGAGAGTCTGGAAAACAAACCTGTCTTCATGTTCTGCG
AAGCTATTGCATTCAGCTATCCTTCAACCCACCCATTCATCCTGATTTGGGGAAACAAGAAGCTAA
AGCAGACTTTTCTTTCAGTTTTGTGGCAAATGAGGTACTGA (SEQ ID NO:20)

[0234] TAS2R47 CDS

[0235] ATGATAACTTTTCTGCCCATCATTTTTTCCATTCTAATAGTGGTTATATTTGTTATTGGAAA
TTTTGCTAATGGCTTCATAGCATTGGTAAATTCCATTGAGTGGGTCAAGAGACAAAAGATCTCCTT
TGTTGACCAAATTCTCACTGCTCTGGCGGTCTCCAGAGTTGGTTTGCTCTGGGTGTTATTACTACAT
TGGTATGCAACTCAGTTGAATCCAGCTTTTTATAGTGTAGAAGTAAGAATTACTGCTTATAATGTCT
GGGCAGTAACCAACCATTTCAGCAGCTGGCTTGCTACTAGCCTCAGCATGTTTTATTTGCTCAGGA
TTGCCAATTTCTCCAACCTTATTTTTCTTCGCATAAAGAGGAGAGTTAAGAGTGTTGTTCTGGTGAT
ACTGTTGGGGCCTTTGCTATTTTTGGTTTGTCATCTTTTTGTGATAAACATGGATGAGACTGTATGG
ACAAAAGAATATGAAGGAAACGTGACTTGGAAGATCAAATTGAGGAGTGCAATGTACCATTCAAA
TATGACTCTAACCATGCTAGCAAACTTTGTACCCCTCACTCTGACCCTGATATCTTTTCTGCTGTTA
ATCTGTTCTCTGTGTAAACATCTCAAGAAGATGCAGCTCCATGGCAAAGGATCTCAAGATCCCAGC
ACCAAGGTCCACATAAAAGCTTTGCAAACTGTGACCTCCTTTCTTCTGTTATGTGCCATTTACTTTC
TGTCCATGATCATATCAGTTTGTAATTTTGGGAGGCTGGAAAAGCAACCTGTCTTCATGTTCTGCCA
AGCTATTATATTCAGCTATCCTTCAACCCACCCATTCATCCTGATTTTGGGAAACAAGAAGCTAAA
GCAGATTTTTCTTTCAGTTTTGCGGCATGTGAGGTACTGGGTGAAAGACAGAAGCCTTCGTCTCCA
TAGATTCACAAGAGGGGCATTGTGTGTCTTCTGA (SEQ ID NO:21)

[0236] TAS2R48 CDS

[0237] ATGATGTGTTTTCTGCTCATCATTTCATCAATTCTGGTAGTGTTTGCATTTGTTCTTGGAAA
TGTTGCCAATGGCTTCATAGCCCTAGTAAATGTCATTGACTGGGTTAACACACGAAAGATCTCCTC
AGCTGAGCAAATTCTCACTGCTCTGGTGGTCTCCAGAATTGGTTTACTCTGGGTCATGTTATTCCTT
TGGTATGCAACTGTGTTTAATTCTGCTTTATATGGTTTAGAAGTAAGAATTGTTGCTTCTAATGCCT
GGGCTGTAACGAACCATTTCAGCATGTGGCTTGCTGCTAGCCTCAGCATATTTTGTTTGCTCAAGAT
TGCCAATTTCTCCAACCTTATTTCTCTCCACCTAAAGAAGAGAATTAAGAGTGTTGTTCTGGTGATA
CTGTTGGGGCCCTTGGTATTTCTGATTTGTAATCTTGCTGTGATAACCATGGATGAGAGAGTGTGG
ACAAAAGAATATGAAGGAAATGTGACTTGGAAGATCAAATTGAGGAATGCAATACACCTTTCAAG
CTTGACTGTAACTACTCTAGCAAACCTCATACCCTTTACTCTGAGCCTAATATGTTTTCTGCTGTTA
ATCTGTTCTCTTTGTAAACATCTCAAGAAGATGCGGCTCCATAGCAAAGGATCTCAAGATCCCAGC
ACCAAGGTCCATATAAAAGCTTTGCAAACTGTGACCTCCTTCCTCATGTTATTTGCCATTTACTTTC
TGTGTATAATCACATCAACTTGGAATCTTAGGACACAGCAGAGCAAACTTGTACTCCTGCTTTGCC
AAACTGTTGCAATCATGTATCCTTCATTCCACTCATTCATCCTGATTATGGGAAGTAGGAAGCTAA
AACAGACCTTTCTTTCAGTTTTGTGGCAGATGACACGCTGA (SEQ ID NO:22)

[0238] TAS2R49 CDS

[0239] ATGATGAGTTTTCTACACATTGTTTTTTCCATTCTAGTAGTGGTTGCATTTATTCTTGGAAA
TTTTGCCAATGGCTTTATAGCACTGATAAATTTCATTGCCTGGGTCAAGAGACAAAAGATCTCCTC
AGCTGATCAAATTATTGCTGCTCTGGCAGTCTCCAGAGTTGGTTTGCTCTGGGTAATATTATTACAT
TGGTATTCAACTGTGTTGAATCCAACTTCATCTAATTTAAAAGTAATAATTTTTATTTCTAATGCCT
GGGCAGTAACCAATCATTTCAGCATCTGGCTTGCTACTAGCCTCAGCATATTTTATTTGCTCAAGAT
CGTCAATTTCTCCAGACTTATTTTTCATCACTTAAAAAGGAAGGCTAAGAGTGTAGTTCTGGTGAT

AGTGTTGGGGTCTTTGTTCTTTTTGGTTTGTCACCTTGTGATGAAACACACGTATATAAATGTGTGG
ACAGAAGAATGTGAAGGAAACGTAACTTGGAAGATCAAACTGAGGAATGCAATGCACCTTTCCAA
CTTGACTGTAGCCATGCTAGCAAACTTGATACCATTCACTCTGACCCTGATATCTTTTCTGCTGTTA
ATCTACTCTCTGTGTAAACATCTGAAGAAGATGCAGCTCCATGGCAAAGGATCTCAAGATCCCAGC
ACCAAGATCCACATAAAAGCTCTGCAAACTGTGACCTCCTTCCTCATATTACTTGCCATTTACTTTC
TGTGTCTAATCATATCGTTTTGGAATTTTAAGATGCGACCAAAAGAAATTGTCTTAATGCTTTGCCA
AGCTTTTGGAATCATATATCCATCATTCCACTCATTCATTCTGATTTGGGGGAACAAGACGCTAAA
GCAGACCTTTCTTTCAGTTTTGTGGCAGGTGACTTGCTGGGCAAAAGGACAGAACCAGTCAACTCC
ATAG (SEQ ID NO:23)

[0240] TAS2R50 CDS

[0241] ATGATAACTTTTCTATACATTTTTTTTTCAATTCTAATAATGGTTTTATTTGTTCTCGGAAA
CTTTGCCAATGGCTTCATAGCACTGGTAAATTTCATTGACTGGGTGAAGAGAAAAAAGATCTCCTC
AGCTGACCAAATTCTCACTGCTCTGGCGGTCTCCAGAATTGGTTTGCTCTGGGCATTATTATTAAAT
TGGTATTTAACTGTGTTGAATCCAGCTTTTTATAGTGTAGAATTAAGAATTACTTCTTATAATGCCT
GGGTTGTAACCAACCATTTCAGCATGTGGCTTGCTGCTAACCTCAGCATATTTTATTTGCTCAAGAT
TGCCAATTTCTCCAACCTTCTTTTTCTTCATTTAAAGAGGAGAGTTAGGAGTGTCATTCTGGTGATA
CTGTTGGGGACTTTGATATTTTTGGTTTGTCATCTTCTTGTGGCAAACATGGATGAGAGTATGTGGG
CAGAAGAATATGAAGGAAACATGACTGGGAAGATGAAATTGAGGAATACAGTACATCTTTCATAT
TTGACTGTAACTACCCTATGGAGCTTCATACCCTTTACTCTGTCCCTGATATCTTTTCTGATGCTAAT
CTGTTCTCTGTGTAAACATCTCAAGAAGATGCAGCTCCATGGAGAAGGATCGCAAGATCTCAGCAC
CAAGGTCCACATAAAAGCTTTGCAAACTCTGATCTCCTTCCTCTTGTTATGTGCCATTTTCTTTCTAT
TCCTAATCGTTTCGGTTTGGAGTCCTAGGAGGCTGCGGAATGACCCGGTTGTCATGGTTAGCAAGG
CTGTTGGAAACATATATCTTGCATTCGACTCATTCATCCTAATTTGGAGAACCAAGAAGCTAAAAC
ACACCTTTCTTTTGATTTTGTGTCAGATTAGGTGCTGA (SEQ ID NO:24)

[0242] TAS2R55 CDS

[0243] ATGGCCACCGAATTGGACAAAATCTTTCTGATTCTGGCAATAGCAGAATTCATCATCAGC
ATGCTGGGGAATGTGTTCATTGGACTGGTAAACTGCTCTGAAGGGATCAAGAACCAAAAGGTCTT
CTCAGCTGACTTCATCCTCACCTGCTTGGCTATCTCCACAATTGGACAACTGTTGGTGATACTGTTT
GATTCATTTCTAGTGGGACTTGCTTCACATTTATATACCACATATAGACTAGGAAAAACTGTTATTA
TGCTTTGGCACATGACTAATCACTTGACAACCTGGCTTGCCACCTGCCTAAGCATTTTCTATTTCTT
TAAGATAGCCCACTTCCCCCACTCCCTTTTCCTCTGGCTGAGGTGGAGGATGAACGGAATGATTGT
TATGCTTCTTATATTGTCTTTGTTCTTACTGATTTTTGACAGTTTAGTGCTAGAAATATTTATTGATA
TCTCACTCAATATAATAGATAAAAGTAATCTGACTTTATATTTAGATGAAAGTAAAACTCTCTTTG
ATAAACTCTCTATTTTAAAAACTCTTCTCAGCTTGACCAGTTTTATCCCCTTTTCTCTGTCCCTGACC
TCCTTGCTTTTTTTATTTCTGTCCTTGGTGAGACATACTAGAAATTTGAAGCTCAGTTCCTTGGGCTC
TAGAGACTCCAGCACAGAGGCCCATAGGAGGGCCATGAAAATGGTGATGTCTTTCCTTTTCCTCTT
CATAGTTCATTTTTTTTCCTTACAAGTGGCCAATTGGATATTTTTTATGTTGTGGAACAACAAGTAC
ATAAAGTTTGTCATGTTAGCCTTAAATGCCTTTCCCTCGTGCCACTCATTTATTCTCATTCTGGGAA

ACAGCAAGCTGCGACAGACAGCTGTGAGGCTACTGTGGCATCTTAGGAACTATACAAAAACACCA
AATGCTTTACCTTTGTGA (SEQ ID NO:25)

[0244] TAS2R60 CDS

[0245] ATGAATGGAGACCACATGGTTCTAGGATCTTCGGTGACTGACAAGAAGGCCATCATCTTG
GTTACCATTTTACTCCTTTTACGCCTGGTAGCAATAGCAGGCAATGGCTTCATCACTGCTGCTCTGG
GCGTGGAGTGGGTGCTACGGAGAATGTTGTTGCCTTGTGATAAGTTATTGGTTAGCCTAGGGGCCT
CTCGCTTCTGTCTGCAGTCAGTGGTAATGGGTAAGACCATTTATGTTTTCTTGCATCCGATGGCCTT
CCCATACAACCCTGTACTGCAGTTTCTAGCTTTCCAGTGGGACTTCCTGAATGCTGCCACCTTATGG
TCCTCTACCTGGCTCAGTGTCTTCTATTGTGTGAAAATTGCTACCTTCACCCACCCTGTCTTCTTCTG
GCTAAAGCACAAGTTGTCTGGGTGGCTACCATGGATGCTCTTCAGCTCTGTAGGGCTCTCCAGCTT
CACCACCATTCTATTTTTCATAGGCAACCACAGAATGTATCAGAACTATTTAAGGAACCATCTACA
ACCTTGGAATGTCACTGGCGATAGCATACGGAGCTACTGTGAGAAATTCTATCTCTTCCCTCTAAA
AATGATTACTTGGACAATGCCCACTGCTGTCTTTTTCATTTGCATGATTTTGCTCATCACATCTCTG
GGAAGACACAGGAAGAAGGCTCTCCTTACAACCTCAGGATTCCGAGAGCCCAGTGTGCAGGCACA
CATAAAGGCTCTGCTGGCTCTCCTCTCTTTTGCCATGCTCTTCATCTCATATTTCCTGTCACTGGTGT
TCAGTGCTGCAGGTATTTTTCCACCTCTGGACTTTAAATTCTGGGTGTGGGAGTCAGTGATTTATCT
GTGTGCAGCAGTTCACCCCATCATTCTGCTCTTCAGCAACTGCAGGCTGAGAGCTGTGCTGAAGAG
TCGTCGTTCCTCAAGGTGTGGGACACCTTGA (SEQ ID NO:26)

[0246] HUMAN GNA15 CDS

[0247] ATGGCCCGGTCCCTGACTTGGGGCTGCTGTCCCTGGTGCCTGACAGAGGAGGAGAAGACT
GCCGCCAGAATCGACCAGGAGATCAACAGGATTTTGTTGGAACAGAAAAAACAAGAGCGCGAGG
AATTGAAACTCCTGCTGTTGGGGCCTGGTGAGAGCGGGAAGAGTACGTTCATCAAGCAGATGCGC
ATCATTCACGGTGTGGGCTACTCGGAGGAGGACCGCAGAGCCTTCCGGCTGCTCATCTACCAGAAC
ATCTTCGTCTCCATGCAGGCCATGATAGATGCGATGGACCGGCTGCAGATCCCCTTCAGCAGGCCT
GACAGCAAGCAGCACGCCAGCCTAGTGATGACCCAGGACCCCTATAAAGTGAGCACATTCGAGAA
GCCATATGCAGTGGCCATGCAGTACCTGTGGCGGGACGCGGGCATCCGTGCATGCTACGAGCGAA
GGCGTGAATTCCACCTTCTGGACTCCGCGGTGTATTACCTGTCACACCTGGAGCGCATATCAGAGG
ACAGCTACATCCCCACTGCGCAAGACGTGCTGCGCAGTCGCATGCCCACCACAGGCATCAATGAG
TACTGCTTCTCCGTGAAGAAAACCAAACTGCGCATCGTGGATGTTGGTGGCCAGAGGTCAGAGCG
TAGGAAATGGATTCACTGTTTCGAGAACGTGATTGCCCTCATCTACCTGGCCTCCCTGAGCGAGTA
TGACCAGTGCCTAGAGGAGAACGATCAGGAGAACCGCATGGAGGAGAGTCTCGCTCTGTTCAGCA
CGATCCTAGAGCTGCCCTGGTTCAAGAGCACCTCGGTCATCCTCTTCCTCAACAAGACGGACATCC
TGGAAGATAAGATTCACACCTCCCACCTGGCCACATACTTCCCCAGCTTCCAGGGACCCCGGCGAG
ACGCAGAGGCCGCCAAGAGCTTCATCTTGGACATGTATGCGCGCGTGTACGCGAGCTGCGCAGAG
CCCCAGGACGGTGGCAGGAAAGGCTCCCGCGCGCCGCTTCTTCGCACACTTCACCTGTGCCACG
GACACGCAAAGCGTCCGCAGCGTGTTCAAGGACGTGCGGGACTCGGTGCTGGCCCGGTACCTGGA
CGAGATCAACCTGCTGTGA (SEQ ID NO:27)

[0248] TAS2R1

[0249] MLESHLIIYFLLAVIQFLLGIFTNGIIVVVNGIDLIKHRKMAPLDLLLSCLAVSRIFLQLFIFYVNV IVIFFIEFIMCSANCAILLFINELELWLATWLGVFYCAKVASVRHPLFIWLKMRISKLVPWMILGSLLYVS MICVFHSKYAGFMVPYFLRKFFSQNATIQKEDTLAIQIFSFVAEFSVPLLIFLFAVLLLIFSLGRHTRQMR NTVAGSRVPGRGAPISALLSILSFLILYFSHCMIKVFLSSLKFHIRRFIFLFFILVIGIYPSGHSLILILGNPKL KQNAKKFLLHSKCCQ (SEQ ID NO:28)

[0250] TAS2R3

[0251] MMGLTEGVFLILSGTQFTLGILVNCFIELVNGSSWFKTKRMSLSDFIITTLALLRIILLCIILTDSF LIEFSPNTHDSGIIMQIIDVSWTFTNHLSIWLATCLGVLYCLKIASFSHPTFLWLKWRVSRVMVWMLLG ALLLSCGSTASLINEFKLYSVFRGIEATRNVTEHFRKKRSEYYLIHVLGTLWYLPPLIVSLASYSLLIFSLG RHTRQMLQNGTSSRDPTTEAHKRAIRIILSFFFLFLLYFLAFLIASFGNFLPKTKMAKMIGEVMTMFYPA GHSFILILGNSKLKQTFVVMLRCESGHLKPGSKGPIFS (SEQ ID NO:29)

[0252] TAS2R4

[0253] MLRLFYFSAIIASVILNFVGIIMNLFITVVNCKTWVKSHRISSSDRILFSLGITRFLMLGLFLVNTI YFVSSNTERSVYLSAFFVLCFMFLDSSSVWFVTLLNILYCVKITNFQHSVFLLLKRNISPKIPRLLLACVLI SAFTTCLYITLSQASPFPELVTTRNNTSFNISEGILSLVVSLVLSSSLQFIINVTSASLLIHSLRRHIQKMQK NATGFWNPQTEAHVGAMKLMVYFLILYIPYSVATLVQYLPFYAGMDMGTKSICLIFATLYSPGHSVLIII THPKLKTTAKKILCFKK (SEQ ID NO:30)

[0254] TAS2R5

[0255] MLSAGLGLLMLVAVVEFLIGLIGNGSLVVWSFREWIRKFNWSSYNLIILGLAGCRFLLQWLIIL DLSLFPLFQSSRWLRYLSIFWVLVSQASLWFATFLSVFYCKKITTFDRPAYLWLKQRAYNLSLWCLLGY FIINLLLTVQIGLTFYHPPQGNSSIRYPFESWQYLYAFQLNSGSYLPLVVFLVSSGMLIVSLYTHHKKMK VHSAGRRDVRAKAHITALKSLGCFLLLHLVYIMASPFSITSKTYPPDLTSVFIWETLMAAYPSLHSLILIM GIPRVKQTCQKILWKTVCARRCWGP (SEQ ID NO:31)

[0256] TAS2R7

[0257] MADKVQTTLLFLAVGEFSVGILGNAFIGLVNCMDWVKKRKIASIDLILTSLAISRICLLCVILLD CFILVLYPDVYATGKEMRIIDFFWTLTNHLSIWFATCLSIYYFFKIGNFFHPLFLWMKWRIDRVISWILLG CVVLSVFISLPATENLNADFRFCVKAKRKTNLTWSCRVNKTQHASTKLFLNLATLLPFCVCLMSFFLLI LSLRRHIRRMQLSATGCRDPSTEAHVRALKAVISFLLLFIAYYLSFLIATSSYFMPETELAVIFGESIALIY PSSHSFILILGNNKLRHASLKVIWKVMSILKGRKFQQHKQI (SEQ ID NO:32)

[0258] TAS2R8

[0259] MFSPADNIFIILITGEFILGILGNGYIALVNWIDWIKKKKISTVDYILTNLVIARICLISVMVVNGI VIVLNPDVYTKNKQQIVIFTFWTFANYLNMWITTCLNVFYFLKIASSSHPLFLWLKWKIDMVVHWILLG CFAISLLVSLIAAIVLSCDYRFHAIAKHKRNITEMFHVSKIPYFEPLTLFNLFAIVPFIVSLISFFLLVRSLW RHTKQIKLYATGSRDPSTEVHVRAIKTMTSFIFFFFLYYISSILMTFSYLMTKYKLAVEFGEIAAILYPLG HSLILIVLNNKLRQTFVRMLTCRKIACMI (SEQ ID NO:33)

[0260] TAS2R9

[0261] MPSAIEAIYIILIAGELTIGIWGNGFIVLVNCIDWLKRRDISLIDIILISLAISRICLLCVISLDGFFML LFPGTYGNSVLVSIVNVVWTFANNSSLWFTSCLSIFYLLKIANISHPFFFWLKLKINKVMLAILLGSFLISL IISVPKNDDMWYHLFKVSHEENITWKFKVSKIPGTFKQLTLNLGVMVPFILCLISFFLLLFSLVRHTKQIR LHATGFRDPSTEAHMRAIKAVIIFLLLLIVYYPVFLVMTSSALIPQGKLVLMIGDIVTVIFPSSHSFILIMG NSKLREAFLKMLRFVKCFLRRRKPFVP (SEQ ID NO:34)

[0262] TAS2R10

[0263] MLRVVEGIFIFVVVSESVFGVLGNGFIGLVNCIDCAKNKLSTIGFILTGLAISRIFLIWIIITDGFIQI FSPNIYASGNLIEYISYFWVIGNQSSMWFATSLSIFYFLKIANFSNYIFLWLKSRTNMVLPFMIVFLLISSL LNFAYIAKILNDYKMKNDTVWDLNMYKSEYFIKQILLNLGVIFFFTLSLITCIFLIISLWRHNRQMQSNV TGLRDSNTEAHVKAMKVLISFIILFILYFIGMAIEISCFTVRENKLLLMFGMTTTAIYPWGHSFILILGNSK LKQASLRVLQQLKCCEK (SEQ ID NO:35)

[0264] TAS2R13

[0265] MESALPSIFTLVIIAEFIIGNLSNGFIVLINCIDWVSKRELSSVDKLLIILAISRIGLIWEILVSWFLA LHYLAIFVSGTGLRIMIFSWIVSNHFNLWLATIFSIFYLLKIASFSSPAFLYLKWRVNKVILMILLGTLVFL FLNLIQINMHIKDWLDRYERNTTWNFSMSDFETFSVSVKFTMTMFSLTPFTVAFISFLLLIFSLQKHLQK MQLNYKGHRDPRTKVHTNALKIVISFLLFYASFFLCVLISWISELYQNTVIYMLCETIGVFSPSSHSFLLIL GNAKLRQAFLLVAAKVW (SEQ ID NO:36)

[0266] TAS2R14

[0267] MGGVIKSIFTFVLIVEFIIGNLGNSFIALVNCIDWVKGRKISSVDRILTALAISRISLVWLIFGSWC VSVFFPALFATEKMFRMLTNIWTVINHFSVWLATGLGTFYFLKIANFSNSIFLYLKWRVKKVVLVLLLV TSVFLFLNIALINIHINASINGYRRNKTCSSDSSNFTRFSSLIVLTSTVFIFIPFTLSLAMFLLLIFSMWKHRK KMQHTVKISGDASTKAHRGVKSVITFFLLYAIFSLSFFISVWTSERLEENLIILSQVMGMAYPSCHSCVLI LGNKKLRQASLSVLLWLRYMFKDGEPSGHKEFRESS (SEQ ID NO:37)

[0268] TAS2R16

[0269] MIPIQLTVFFMIIYVLESLTIIVQSSLIVAVLGREWLQVRRLMPVDMILISLGISRFCLQWASMLN NFCSYFNLNYVLCNLTITWEFFNILTFWLNSLLTVFYCIKVSSFTHHIFLWLRWRILRLFPWILLGSLMIT CVTIIPSAIGNYIQIQLLTMEHLPRNSTVTDKLENFHQYQFQAHTVALVIPFILFLASTIFLMASLTKQIQH HSTGHCNPSMKARFTALRSLAVLFIVFTSYFLTILITIIGTLFDKRCWLWVWEAFVYAFILMHSTSLMLS SPTLKRILKGKC (SEQ ID NO:38)

[0270] TAS2R38

[0271] MLTLTRIRTVSYEVRSTFLFISVLEFAVGFLTNAFVFLVNFWDVVKRQALSNSDCVLLCLSISRL FLHGLLFLSAIQLTHFQKLSEPLNHSYQAIIMLWMIANQANLWLAACLSLLYCSKLIRFSHTFLICLASW VSRKISQMLLGIILCSCICTVLCVWCFFSRPHFTVTTVLFMNNNTRLNWQIKDLNLFYSFLFCYLWSVPP FLLFLVSSGMLTVSLGRHMRTMKVYTRNSRDPSLEAHIKALKSLVSFFCFFVISSCAAFISVPLLILWRD KIGVMVCVGIMAACPSGHAAILISGNAKLRRAVMTILLWAQSSLKVRADHKADSRTLC (SEQ ID NO:39)

[0272] TAS2R39

[0273] MLGRCFPPDTKEKQQLRMTKLCDPAESELSPFLITLILAVLLAEYLIGIIANGFIMAIHAAEWVQ NKAVSTSGRILVFLSVSRIALQSLMMLEITISSTSLSFYSEDAVYYAFKISFIFLNFCSLWFAAWLSFFYFV KIANFSYPLFLKLRWRITGLIPWLLWLSVFISFSHSMFCINICTVYCNNSFPIHSSNSTKKTYLSEINVVGL AFFFNLGIVTPLIMFILTATLLILSLKRHTLHMGSNATGSNDPSMEAHMGAIKAISYFLILYIFNAVALFIY LSNMFDINSLWNNLCQIIMAAYPASHSILLIQDNPGLRRAWKRLQLRLHLYPKEWTL (SEQ ID NO:40)

[0274] TAS2R40

[0275] MATVNTDATDKDISKFKVTFTLVVSGIECITGILGSGFITAIYGAEWARGKTLPTGDRIMLMLS FSRLLLQIWMMLENIFSLLFRIVYNQNSVYILFKVITVFLNHSNLWFAAWLKVFYCLRIANFNHPLFFLM KRKIIVLMPWLLRLSVLVSLSFSFPLSRDVFNVYYVNSSIPIPSSNSTEKKYFSETNMVNLVFFYNMGIFVP LIMFILAATLLILSLKRHTLHMGSNATGSRDPSMKAHIGAIKATSYFLILYIFNAIALFLSTSNIFDTYSSW NILCKIIMAAYPAGHSVQLILGNPGLRRAWKRFQHQVPLYLKGQTL (SEQ ID NO:41)

[0276] TAS2R41

[0277] MQAALTAFFVLLFSLLSLLGIAANGFIVLVLGREWLRYGRLLPLDMILISLGASRFCLQLVGTV HNFYYSAQKVEYSGGLGRQFFHLHWHFLNSATFWFCSWLSVLFCVKIANITHSTFLWLKWRFLGWVP WLLLGSVLISFIITLLFFWVNYPVYQEFLIRKFSGNMTYKWNTRIETYYFPSLKLVIWSIPFSVFLVSIMLL INSLRRHTQRMQHNGHSLQDPSTQAHTRALKSLISFLILYALSFLSLIIDAAKFISMQNDFYWPWQIAVY LCISVHPFILIFSNLKLRSVFSQLLLLARGFWVA (SEQ ID NO:42)

[0278] TAS2R43

[0279] MITFLPIIFSSLVVVTFVIGNFANGFIALVNSIESFKRQKISFADQILTALAVSRVGLLWVLLLNW YSTVLNPAFNSVEVRTTAYNIWAVINHFSNWLATTLSIFYLLKIANFSNFIFLHLKRRVKSVILVMLLGP LLFLACHLFVINMNEIVRTKEFEGNMTWKIKLKSAMYFSNMTVTMVANLVPFTLTLLSFMLLICSLCKH LKKMQLRGKGSQDPSTKVHIKALQTVISFLLLCAIYFLSIMISVWSFGSLENKPVFMFCKAIRFSYPSIHP FILIWGNKKLKQTFLSVFWQMRYWVKGEKTSSP (SEQ ID NO:43)

[0280] TAS2R44

[0281] MTTFIPIIFSSVVVVLFVIGNFANGFIALVNSIERVKRQKISFADQILTALAVSRVGLLWVLLLN WYSTVFNPAFYSVEVRTTAYNVWAVTGHFSNWLATSLSIFYLLKIANFSNLIFLHLKRRVKSVILVMLL GPLLFLACQLFVINMKEIVRTKEYEGNMTWKIKLRSAVYLSDATVTTLGNLVPFTLTLLCFLLLICSLCK HLKKMQLHGKGSQDPSTKVHIKALQTVIFFLLLCAVYFLSIMISVWSFGSLENKPVFMFCKAIRFSYPSI HPFILIWGNKKLKQTFLSVLRQVRYW (SEQ ID NO:44)

[0282] TAS2R45

[0283] MITFLPIIFSILVVVTFVIGNFANGFIALVNSTEWVKRQKISFADQIVTALAVSRVGLLWVLLLN WYSTVLNPAFCSVELRTTAYNIWAVTGHFSNWPATSLSIFYLLKIANFSNLIFLRLKRRVKSVILVVLLG PLLFLACHLFVVNMNQIVWTKEYEGNMTWKIKLRRAMYLSDTTVTMLANLVPFTVTLISFLLLVCSLC

KHLKKMQLHGKGSQDPSTKVHIKVLQTVISFFLLRAIYFVSVIISVWSFKNLENKPVFMFCQAIGFSCSS AHPFILIWGNKKLKQTYLSVLWQMRY (SEQ ID NO:45)

[0284] TAS2R46

[0285] MITFLPIIFSILIVVTFVIGNFANGFIALVNSIEWFKRQKISFADQILTALAVSRVGLLWVLVLNW YATELNPAFNSIEVRITAYNVWAVINHFSNWLATSLSIFYLLKIANFSNLIFLHLKRRVKSVVLVILLGPL LFLVCHLFVINMNQIIWTKEYEGNMTWKIKLRSAMYLSNTTVTILANLVPFTLTLISFLLLICSLCKHLK KMQLHGKGSQDPSMKVHIKALQTVTSFLLLCAIYFLSIIMSVWSFESLENKPVFMFCEAIAFSYPSTHPFI LIWGNKKLKQTFLSVLWHVRYWVVKGEKPSSS (SEQ ID NO:46)

[0286] TAS2R47

[0287] MITFLPIIFSILIVVIFVIGNFANGFIALVNSIEWVKRQKISFVDQILTALAVSRVGLLWVLLLHW YATQLNPAFYSVEVRITAYNVWAVTNHFSSWLATSLSMFYLLRIANFSNLIFLRIKRRVKSVVLVILLGP LLFLVCHLFVINMDETVWTKEYEGNVTWKIKLRSAMYHSNMTLTMLANFVPLTLTLISFLLLICSLCKH LKKMQLHGKGSQDPSTKVHIKALQTVTSFLLLCAIYFLSMIISVCNLGRLEKQPVFMFCQAIIFSYPSTHP FILILGNKKLKQIFLSVLRHVRYWVKDRSLRLHRFTRAALCKG (SEQ ID NO:47)

[0288] TAS2R48

[0289] MMCFLLIISSILVVFAFVLGNVANGFIALVNVIDWVNTRKISSAEQILTALVVSRIGLLWVMLFL WYATVFNSALYGLEVRIVASNAWAVTNHFSMWLAASLSIFCLLKIANFSNLISLHLKKRIKSVVLVILL GPLVFLICNLAVITMDERVWTKEYEGNVTWKIKLRNAIHLSSLTVTTLANLIPFTLSLICFLLLICSLCKH LKKMRLHSKGSQDPSTKVHIKALQTVTSFLMLFAIYFLCIITSTWNLRTQQSKLVLLLCQTVAIMYPSFH SFILIMGSRKLKQTFLSVLWQMTR (SEQ ID NO:48)

[0290] TAS2R49

[0291] MMSFLHIVFSILVVVAFILGNFANGFIALINFIAWVKRQKISSADQIIAALAVSRVGLLWVILLH WYSTVLNPTSSNLKVIIFISNAWAVTNHFSIWLATSLSIFYLLKIVNFSRLIFHHLKRKAKSVVLVIVLGSL FFLVCHLVMKHTYINVWTEECEGNVTWKIKLRNAMHLSNLTVAMLANLIPFTLTLISFLLLIYSLCKHL KKMQLHGKGSQDPSTKIHIKALQTVTSFLILLAIYFLCLIISFWNFKMRPKEIVLMLCQAFGIIYPSFHSFI LIWGNKTLKQTFLSVLWQVTCWAKGQNQSTP (SEQ ID NO:49)

[0292] TAS2R50

[0293] MITFLYIFFSILIMVLFVLGNFANGFIALVNFIDWVKRKKISSADQILTALAVSRIGLLWALLLN WYLTVLNPAFYSVELRITSYNAWVVTNHFSMWLAANLSIFYLLKIANFSNLLFLHLKRRVRSVILVILL GTLIFLVCHLLVANMDESMWAEEYEGNMTGKMKLRNTVHLSYLTVTTLWSFIPFTLSLISFLMLICSLY KHLKKMQLHGEGSQDLSTKVHIKALQTLISFLLLCAIFFLFLIVSVWSPRRLRNDPVVMVSKAVGNIYL AFDSFILIWRTKKLKHTFLLILCQIRC (SEQ ID NO:50)

[0294] TAS2R55

[0295] MATELDKIFLILAIAEFIISMLGNVFIGLVNCSEGIKNQKVFSADFILTCLAISTIGQLLVILFDSFL VGLASHLYTTYRLGKTVIMLWHMTNHLTTWLATCLSIFYFFKIAHFPHSLFLWLRWRMNGMIVMLLIL SLFLLIFDSLVLEIFIDISLNIIDKSNLTLYLDESKTLYDKLSILKTLLSLTSFIPFSLFLTSLLFLFLSLVRHTR

NLKLSSLGSRDSSTEAHRRAMKMVMSFLFLFIVHFFSLQVANGIFFMLWNNKYIKFVMLALNAFPSCHS FILILGNSKLRQTAVRLLWHLRNYTKTPNALPL (SEQ ID NO:51)

[0296] TAS2R60

[0297] MNGDHMVLGSSVTDKKAIILVTILLLLRLVAIAGNGFITAALGVEWVLRRMLLPCDKLLVSLG ASRFCLQSVVMGKTIYVFLHPMAFPYNPVLQFLAFQWDFLNAATLWSSTWLSVFYCVKIATFTHPVFF WLKHKLSGWLPWMLFSSVGLSSFTTILFFIGNHRMYQNYLRNHLQPWNVTGDSIRSYCEKFYLFPLKM ITWTMPTAVFFICMILLITSLGRHRKKALLTTSGFREPSVQAHIKALLALLSFAMLFISYFLSLVFSAAGIF PPLDFKFWVWESVIYLCAAVHPIILFSNCRLRAVLKSRRSSRCGTP (SEQ ID NO:52)

[0298] Mouse Gna15 (Gα15)

[0299] MARSTWGCCWCTKTAARDNRKKRKGGSGKSTKMRHGVGYSDRRARYNVSMAMDAMDRS RDSKHASVMTDYKVSTKYAVAMYWRDAGRACYRRRHDSAVYYSHRSDSYTADVRSRMTTGNYCSV KKTKRVDVGGRSRRKWHCNVAYASSYDCNDNRMSASTWKSTSVNKTDDKHTSHATYSGRRDAAAK SDMYARVYASCADGGRKGSRARRAHTCATDTSVRSVKDVRDSVARYDN (SEQ ID NO:53)

[0300] TAS2R38 (PAV Haplotype)

[0301] MLTLTRIRTVSYEVRSTFLFISVLEFAVGFLTNAFVFLVNFWDVVKRQPLSNSDCVLLCLSISRL FLHGLLFLSAIQLTHFQKLSEPLNHSYQAIIMLWMIANQANLWLAACLSLLYCSKLIRFSHTFLICLASW VSRKISQMLLGIILCSCICTVLCVWCFFSRPHFTVTTVLFMNNNTRLNWQIKDLNLFYSFLFCYLWSVPP FLLFLVSSGMLTVSLGRHMRTMKVYTRNSRDPSLEAHIKALKSLVSFFCFFVISSCAAFISVPLLILWRD KIGVMVCVGIMAACPSGHAAVLISGNAKLRRAVMTILLWAQSSLKVRADHKADSRTLC (SEQ ID NO:54)

Appendix Table 1 Polymorphisms related to human bitter taste receptor genes

Gene	Reference sequence number	Position in reference sequence	SNP
TAS2R1	rs10543720	pos=401	alleles="-/CTATCTAT"
	rs2234228	pos=101	alleles="A/G"
	rs2234229	pos=101	alleles="C/T"
	rs2234230	pos=101	alleles="A/C"
	rs2234231	pos=101	alleles="C/T"
	rs2234232	pos=101	alleles="A/G"
	rs2234233	pos=301	alleles="C/T"
	rs2234234	pos=101	alleles="C/T"
	rs2234235	pos=301	alleles="C/T"
	rs34440745	pos=301	alleles="A/T"
	rs35186690	pos=301	alleles="-/G"
	rs35524938	pos=401	alleles="-/ATCT"
	rs36214451	pos=401	alleles="-/TATCTATC"
	rs41464	pos=201	alleles="A/G"
	rs41465	pos=201	alleles="A/G"
	rs41466	pos=301	alleles="A/G"
	rs41467	pos=301	alleles="G/T"
	rs41468	pos=301	alleles="C/T"
	rs41469	pos=301	alleles="A/G"
	rs41470	pos=201	alleles="A/G"
	rs56300050	pos=253	alleles="-/ATCT"
	rs57183738	pos=101	alleles="G/T"
	rs58046500	pos=101	alleles="C/T"
	rs58171988	pos=201	alleles="A/G"
TAS2R3	rs11514837	pos=458	alleles="A/G"
	rs11763979	pos=501	alleles="G/T"
	rs11771020	pos=501	alleles="C/T"
	rs11771072	pos=201	alleles="A/C"
	rs12667706	pos=201	alleles="A/G"
	rs12703406	pos=277	alleles="A/G"
	rs13311828	pos=367	alleles="A/G"
	rs13311829	pos=367	alleles="C/G"
	rs13311831	pos=342	alleles="A/G"
	rs17162469	pos=101	alleles="A/G"
	rs17162471	pos=101	alleles="A/C"
	rs17162473	pos=101	alleles="A/G"
	rs17162483	pos=101	alleles="A/G"
	rs2270009	pos=301	alleles="C/T"
	rs28480612	pos=201	alleles="A/G"
	rs4726475	pos=609	alleles="C/T"
	rs56917574	pos=101	alleles="G/T"
	rs58640454	pos=101	alleles="A/G"
	rs60922375	pos=101	alleles="A/C"
	rs6962760	pos=301	alleles="C/T"
	rs6965618	pos=259	alleles="C/T"
	rs765007	pos=301	alleles="C/T"
	rs765008	pos=301	alleles="G/T"
	rs7793232	pos=714	alleles="A/G"

Gene	Reference sequence number	Position in reference sequence	SNP
TAS2R4	rs10485837	pos=101	alleles="A/G"
	rs2233990	pos=301	alleles="A/G"
	rs2233991	pos=101	alleles="C/T"
	rs2233992	pos=101	alleles="A/G"
	rs2233993	pos=101	alleles="A/G"
	rs2233994	pos=101	alleles="A/G"
	rs2233995	pos=301	alleles="A/G"
	rs2233996	pos=101	alleles="C/G"
	rs2233997	pos=101	alleles="A/C"
	rs2233998	pos=301	alleles="C/T"
	rs2233999	pos=101	alleles="A/T"
	rs2234000	pos=101	alleles="C/T"
	rs2234001	pos=301	alleles="C/G"
	rs2234002	pos=301	alleles="A/G"
	rs2234003	pos=101	alleles="A/G"
	rs33920115	pos=301	alleles="A/G"
	rs34855644	pos=301	alleles="-/T"
	rs3840580	pos=61	alleles="-/AA"
	rs57597591	pos=201	alleles="-/T"
	rs59513189	pos=201	alleles="G/T"
	rs61582517	pos=201	alleles="-/TGTAGATA"
TAS2R5	rs10952507	pos=201	alleles="A/G"
	rs11761380	pos=301	alleles="A/C"
	rs11769235	pos=201	alleles="A/C"
	rs2227264	pos=301	alleles="G/T"
	rs2234004	pos=101	alleles="C/T"
	rs2234005	pos=101	alleles="A/G"
	rs2234006	pos=682	alleles="C/T"
	rs2234007	pos=494	alleles="A/G"
	rs2234008	pos=101	alleles="A/G"
	rs2234009	pos=101	alleles="C/T"
	rs2234010	pos=101	alleles="A/G"
	rs2234011	pos=101	alleles="C/T"
	rs2234012	pos=301	alleles="A/G"
	rs2234013	pos=101	alleles="A/G"
	rs2234014	pos=101	alleles="C/T"
	rs2234015	pos=301	alleles="A/G"
	rs2234016	pos=101	alleles="G/T"
	rs2234017	pos=201	alleles="C/G"
	rs2234018	pos=101	alleles="A/T"
	rs2234019	pos=101	alleles="A/G"
	rs2234020	pos=101	alleles="C/T"
	rs34529840	pos=301	alleles="A/G"
	rs3801001	pos=61	alleles="A/C"
	rs4726476	pos=201	alleles="C/G"
	rs60900504	pos=101	alleles="C/T"
	rs62477710	pos=251	alleles="C/T"
	rs62477711	pos=251	alleles="G/T"
TAS2R7	rs10161483	pos=201	alleles="A/G"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs10772362	pos=501	alleles="C/T"
	rs11054041	pos=201	alleles="A/C"
	rs11838055	pos=301	alleles="A/G"
	rs2418107	pos=501	alleles="C/G"
	rs2588350	pos=301	alleles="C/T"
	rs34212148	pos=301	alleles="-/G"
	rs36067388	pos=301	alleles="-/G"
	rs3759251	pos=101	alleles="A/T"
	rs3759252	pos=61	alleles="A/C"
	rs619381	pos=519	alleles="C/T"
	rs7303054	pos=201	alleles="C/T"
TAS2R8	rs12314840	pos=224	alleles="C/T"
	rs1548803	pos=780	alleles="C/T"
	rs1838344	pos=277	alleles="C/T"
	rs1838345	pos=322	alleles="A/G"
	rs2537817	pos=301	alleles="C/T"
	rs40313	pos=176	alleles="C/T"
	rs41324347	pos=65	alleles="G/T"
	rs60652912	pos=201	alleles="A/C"
	rs620878	pos=283	alleles="G/T"
	rs7972779	pos=424	alleles="C/T"
TAS2R9	rs11054042	pos=201	alleles="C/G"
	rs11054043	pos=201	alleles="G/T"
	rs11054044	pos=201	alleles="C/G"
	rs11402198	pos=401	alleles="-/G"
	rs17207899	pos=101	alleles="G/T"
	rs17742870	pos=101	alleles="A/T"
	rs1838346	pos=301	alleles="A/G"
	rs2159903	pos=84	alleles="A/G"
	rs36044129	pos=301	alleles="-/T"
	rs3741845	pos=179	alleles="C/T"
	rs3944035	pos=100	alleles="A/G"
	rs40313	pos=176	alleles="C/T"
	rs60652912	pos=201	alleles="A/C"
	rs61320953	pos=201	alleles="-/T"
	rs655046	pos=301	alleles="A/G"
	rs667123	pos=301	alleles="A/G"
	rs667128	pos=201	alleles="C/T"
TAS2R10	rs10845219	pos=301	alleles="C/T"
	rs12307411	pos=301	alleles="C/T"
	rs35370388	pos=301	alleles="-/TGTG"
	rs58719830	pos=225	alleles="-/TGTG"
	rs597468	pos=301	alleles="A/G"
	rs60832178	pos=101	alleles="C/T"
	rs61912242	pos=251	alleles="G/T"
	rs689118	pos=301	alleles="C/T"
TAS2R13	rs1015442	pos=519	alleles="C/T"
	rs1015443	pos=946	alleles="C/T"
	rs10566346	pos=401	alleles="-/TG"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs10591343	pos=501	alleles="-/GT"
	rs10845238	pos=258	alleles="G/T"
	rs10845239	pos=346	alleles="A/T"
	rs10845240	pos=449	alleles="C/G"
	rs11054070	pos=2000	alleles="C/G"
	rs11054071	pos=201	alleles="C/G"
	rs11830286	pos=301	alleles="A/G"
	rs34885344	pos=301	alleles="C/T"
	rs35172210	pos=301	alleles="-/T"
	rs56987993	pos=101	alleles="C/G"
	rs7308212	pos=256	alleles="C/T"
	rs7968736	pos=201	alleles="A/T"
	rs7978678	pos=201	alleles="A/G"
TAS2R14	rs10492104	pos=101	alleles="C/G"
	rs11610105	pos=201	alleles="A/G"
	rs16925868	pos=101	alleles="C/T"
	rs3033010	pos=501	alleles="-/C/CT/G"
	rs34789740	pos=301	alleles="A/G"
	rs35386049	pos=301	alleles="-/C"
	rs35405135	pos=301	alleles="-/T"
	rs35804287	pos=301	alleles="A/G"
	rs35926739	pos=301	alleles="-/T"
	rs3741843	pos=301	alleles="A/G"
	rs3851583	pos=501	alleles="A/G"
	rs3851584	pos=500	alleles="G/T"
	rs3851585	pos=501	alleles="C/G"
	rs3863321	pos=21	alleles="C/T"
	rs3936285	pos=537	alleles="A/T"
	rs4140968	pos=101	alleles="C/T"
	rs56393802	pos=241	alleles="-/TG"
	rs60186756	pos=201	alleles="-/T"
	rs60288130	pos=201	alleles="-/TT"
	rs61659284	pos=226	alleles="-/CTCT"
	rs7138535	pos=301	alleles="A/T"
	rs7487884	pos=239	alleles="C/T"
TAS2R16	rs10487745	pos=101	alleles="A/C"
	rs1204014	pos=201	alleles="A/G"
	rs1357949	pos=497	alleles="A/G"
	rs1525489	pos=301	alleles="A/G"
	rs2233988	pos=301	alleles="C/T"
	rs2233989	pos=201	alleles="C/T"
	rs2692396	pos=301	alleles="C/G"
	rs28371571	pos=94	alleles="A/G"
	rs28371572	pos=114	alleles="C/G"
	rs28371573	pos=126	alleles="C/T"
	rs28371574	pos=133	alleles="A/G"
	rs28371575	pos=140	alleles="C/T"
	rs28371576	pos=136	alleles="C/T"
	rs28371577	pos=140	alleles="A/C"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs28371578	pos=138	alleles="A/G"
	rs28371579	pos=139	alleles="C/T"
	rs28371580	pos=139	alleles="A/G"
	rs28371581	pos=139	alleles="G/T"
	rs34032423	pos=301	alleles="-/CT"
	rs34215184	pos=301	alleles="A/C"
	rs34638781	pos=301	alleles="-/C"
	rs35947098	pos=301	alleles="C/T"
	rs58410964	pos=101	alleles="A/G"
	rs59108896	pos=101	alleles="G/T"
	rs59743922	pos=101	alleles="A/G"
	rs60714340	pos=101	alleles="C/T"
	rs6466849	pos=201	alleles="C/T"
	rs702423	pos=301	alleles="A/G"
	rs846664	pos=301	alleles="G/T"
	rs846665	pos=284	alleles="C/G"
	rs846666	pos=392	alleles="G/T"
	rs860170	pos=301	alleles="A/G"
	rs978739	pos=535	alleles="A/G"
TAS2R38	rs10246939	pos=301	alleles="C/T"
	rs1726866	pos=301	alleles="C/T"
	rs35251805	pos=301	alleles="-/G"
	rs4613903	pos=301	alleles="G/T"
	rs61464348	pos=201	alleles="A/C"
	rs713598	pos=301	alleles="C/G"
TAS2R39	rs10608369	pos=401	alleles="-/GT"
	rs34169190	pos=301	alleles="C/T"
	rs35474877	pos=301	alleles="A/G"
	rs4103817	pos=451	alleles="A/G"
	rs4726600	pos=301	alleles="A/G"
	rs56782833	pos=283	alleles="-/A"
	rs59031091	pos=201	alleles="C/G"
	rs6964922	pos=227	alleles="C/T"
TAS2R40	rs10225801	pos=201	alleles="A/G"
	rs10260248	pos=301	alleles="A/C"
	rs17164164	pos=301	alleles="C/G"
TAS2R41	rs10278721	pos=301	alleles="C/T"
	rs13243940	pos=501	alleles="A/T"
	rs13362832	pos=201	alleles="C/T"
	rs13362858	pos=301	alleles="C/G"
	rs1404634	pos=301	alleles="A/G"
	rs1404635	pos=301	alleles="A/G"
	rs1473653	pos=301	alleles="A/G"
	rs33922222	pos=401	alleles="-/C"
	rs34170633	pos=301	alleles="-/A"
	rs34281448	pos=301	alleles="-/A"
	rs34863914	pos=301	alleles="C/T"
	rs5888105	pos=401	alleles="-/G"
	rs5888106	pos=401	alleles="-/C"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs59826238	pos=101	alleles="C/T"
	rs60096100	pos=201	alleles="A/C"
	rs6947971	pos=5600	alleles="G/T"
	rs6949267	pos=526	alleles="C/G"
TAS2R43	rs10556970	pos=401	alleles="-/AT"
	rs1965231	pos=265	alleles="C/T"
	rs34115566	pos=301	alleles="-/GT"
	rs35720106	pos=301	alleles="C/G"
TAS2R44	rs10591850	pos=401	alleles="-/AAAT"
	rs10743938	pos=201	alleles="A/T"
	rs10772422	pos=501	alleles="C/T"
	rs10772423	pos=301	alleles="C/T"
	rs10845293	pos=301	alleles="A/G"
	rs10845294	pos=301	alleles="C/G"
	rs10845295	pos=201	alleles="A/G"
	rs10845296	pos=371	alleles="A/G"
	rs11522329	pos=301	alleles="A/G"
	rs11537117	pos=201	alleles="A/T"
	rs11537118	pos=218	alleles="A/G"
	rs11560815	pos=231	alleles="C/T"
	rs11612527	pos=301	alleles="A/T"
	rs12315036	pos=201	alleles="G/T"
	rs12318612	pos=301	alleles="C/G"
	rs12370363	pos=201	alleles="A/G"
	rs12819202	pos=301	alleles="C/T"
	rs1965230	pos=663	alleles="A/G"
	rs2418291	pos=501	alleles="C/T"
	rs2418292	pos=500	alleles="A/G"
	rs2418293	pos=500	alleles="C/T"
	rs2418294	pos=500	alleles="C/T"
	rs2418295	pos=500	alleles="C/G"
	rs2418296	pos=500	alleles="A/G"
	rs2418297	pos=500	alleles="C/T"
	rs2418298	pos=500	alleles="A/C"
	rs2418299	pos=500	alleles="A/T"
	rs2418300	pos=500	alleles="A/C"
	rs2418301	pos=500	alleles="C/T"
	rs28409955	pos=201	alleles="C/T"
	rs28679275	pos=201	alleles="C/T"
	rs2900583	pos=501	alleles="C/T"
	rs2900584	pos=501	alleles="C/T"
	rs2900585	pos=501	alleles="C/T"
	rs2952703	pos=201	alleles="G/T"
	rs33998340	pos=401	alleles="-/AGT"
	rs34066385	pos=401	alleles="-/ACAC"
	rs34763234	pos=301	alleles="A/G"
	rs35241999	pos=301	alleles="A/G"
	rs3759246	pos=61	alleles="C/G"
	rs3759247	pos=61	alleles="A/G"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs3983336	pos=500	alleles="A/G"
	rs3983337	pos=500	alleles="A/C"
	rs3983338	pos=500	alleles="A/C"
	rs3983339	pos=500	alleles="C/T"
	rs3983340	pos=500	alleles="C/T"
	rs3983341	pos=500	alleles="A/G"
	rs3983342	pos=500	alleles="G/T"
	rs3983343	pos=500	alleles="C/T"
	rs5024225	pos=401	alleles="A/T"
	rs56079155	pos=201	alleles="-/CA"
	rs56873588	pos=201	alleles="-/AATA"
	rs5796420	pos=401	alleles="-/ACAC"
	rs7952952	pos=301	alleles="A/G"
	rs7953498	pos=301	alleles="C/G"
TAS2R46	rs11560816	pos=201	alleles="A/G"
	rs2244875	pos=500	alleles="C/T"
	rs2598002	pos=301	alleles="A/C"
	rs2599402	pos=201	alleles="A/G"
	rs2708378	pos=201	alleles="C/T"
	rs2708379	pos=201	alleles="A/G"
	rs2708380	pos=301	alleles="A/T"
	rs2708381	pos=301	alleles="A/G"
	rs2708382	pos=495	alleles="A/G"
	rs34033169	pos=301	alleles="-/G"
	rs34164014	pos=301	alleles="-/C"
	rs35602687	pos=301	alleles="-/C"
	rs35801645	pos=301	alleles="-/T"
	rs61912070	pos=251	alleles="G/T"
	rs62760561	pos=401	alleles="-/TCT"
	rs63450660	pos=401	alleles="-/T"
	rs7970996	pos=201	alleles="C/T"
TAS2R47	rs10645657	pos=401	alleles="-/AC"
	rs1669404	pos=201	alleles="A/G"
	rs1669405	pos=201	alleles="G/T"
	rs1960613	pos=502	alleles="G/T"
	rs2218819	pos=37	alleles="C/T"
	rs2597924	pos=201	alleles="A/G"
	rs2597925	pos=201	alleles="A/G"
	rs2597926	pos=201	alleles="G/T"
	rs2597927	pos=201	alleles="G/T"
	rs2599396	pos=301	alleles="A/G"
	rs2599397	pos=301	alleles="C/G"
	rs2599404	pos=301	alleles="A/C"
	rs2600355	pos=301	alleles="G/T"
	rs2600356	pos=301	alleles="A/C"
	rs2600357	pos=301	alleles="C/T"
	rs2600358	pos=301	alleles="A/G"
	rs2708351	pos=201	alleles="G/T"
	rs2708371	pos=201	alleles="C/G"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs2708372	pos=201	alleles="C/T"
	rs2923236	pos=201	alleles="C/T"
	rs2952701	pos=201	alleles="C/T"
	rs2952702	pos=201	alleles="C/T"
	rs34383190	pos=401	alleles="-/TC"
	rs34570579	pos=301	alleles="-/C"
	rs34656404	pos=301	alleles="A/G"
	rs34960146	pos=301	alleles="-/C"
	rs35267335	pos=301	alleles="A/G"
	rs35413568	pos=301	alleles="-/C"
	rs35632581	pos=301	alleles="-/C"
	rs35884825	pos=401	alleles="-/AG"
	rs36109559	pos=301	alleles="-/A"
	rs36123978	pos=301	alleles="-/AG"
	rs3759244	pos=201	alleles="C/T"
	rs3759245	pos=201	alleles="C/T"
	rs3863323	pos=501	alleles="G/T"
	rs4092162	pos=91	alleles="A/G"
	rs4763238	pos=201	alleles="A/C"
	rs5796422	pos=401	alleles="-/AG"
	rs61928449	pos=251	alleles="A/C"
	rs7296647	pos=201	alleles="A/G"
	rs7313796	pos=201	alleles="A/C"
	rs7980677	pos=301	alleles="C/T"
	rs977473	pos=209	alleles="A/T"
	rs977474	pos=512	alleles="A/G"
TAS2R48	rs10743937	pos=301	alleles="C/T"
	rs10772419	pos=301	alleles="A/C"
	rs10772420	pos=301	alleles="A/G"
	rs11054169	pos=335	alleles="A/G"
	rs11054170	pos=337	alleles="G/T"
	rs11054171	pos=356	alleles="A/G"
	rs12313469	pos=301	alleles="A/G"
	rs12424373	pos=301	alleles="G/T"
	rs12578654	pos=301	alleles="C/T"
	rs1868768	pos=301	alleles="A/C"
	rs1868769	pos=312	alleles="A/G"
	rs34254748	pos=301	alleles="-/G"
	rs35032794	pos=301	alleles="-/C"
	rs36057973	pos=301	alleles="-/G"
	rs3863330	pos=499	alleles="A/T"
	rs3863333	pos=301	alleles="G/T"
	rs4763235	pos=201	alleles="C/G"
	rs56985810	pos=201	alleles="C/T"
	rs60770813	pos=101	alleles="C/G"
	rs61624520	pos=201	alleles="-/T"
	rs7131800	pos=267	alleles="A/G"
	rs7961372	pos=201	alleles="A/C"
	rs9330646	pos=301	alleles="A/T"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs9777804	pos=301	alleles="C/G"
	rs9777906	pos=301	alleles="A/T"
TAS2R49	rs10772407	pos=201	alleles="A/C"
	rs10845278	pos=356	alleles="C/T"
	rs10845279	pos=301	alleles="A/C"
	rs10845280	pos=301	alleles="A/G"
	rs10845281	pos=301	alleles="C/T"
	rs11054139	pos=501	alleles="C/T"
	rs11054140	pos=301	alleles="C/T"
	rs11054141	pos=261	alleles="C/T"
	rs11054142	pos=301	alleles="A/G"
	rs11054143	pos=301	alleles="C/T"
	rs12226919	pos=301	alleles="G/T"
	rs12226920	pos=301	alleles="G/T"
	rs12311429	pos=301	alleles="A/G"
	rs12311490	pos=301	alleles="A/G"
	rs12312963	pos=201	alleles="C/T"
	rs1450839	pos=301	alleles="A/G"
	rs1463237	pos=348	alleles="C/T"
	rs34365504	pos=301	alleles="-/T"
	rs34579433	pos=301	alleles="-/A"
	rs34813278	pos=301	alleles="-/A"
	rs34965724	pos=301	alleles="-/A"
	rs35021650	pos=301	alleles="-/C"
	rs35875890	pos=301	alleles="-/ATG"
	rs4388985	pos=401	alleles="A/G"
	rs4418898	pos=401	alleles="C/T"
	rs4506739	pos=401	alleles="A/G"
	rs4763604	pos=201	alleles="G/T"
	rs4763605	pos=201	alleles="A/G"
	rs58133495	pos=501	alleles="-/GAT"
	rs59686635	pos=101	alleles="A/C"
	rs61912291	pos=251	alleles="G/T"
	rs7135018	pos=251	alleles="C/T"
	rs7135941	pos=301	alleles="C/T"
	rs7301234	pos=301	alleles="A/G"
TAS2R50	rs10772396	pos=362	alleles="C/T"
	rs10772397	pos=301	alleles="C/T"
	rs10772398	pos=201	alleles="C/T"
	rs10772399	pos=201	alleles="C/T"
	rs11054131	pos=201	alleles="C/G"
	rs11054132	pos=201	alleles="A/G"
	rs11054133	pos=201	alleles="C/T"
	rs11421487	pos=401	alleles="-/T"
	rs12426805	pos=301	alleles="A/G"
	rs1376251	pos=301	alleles="C/T"
	rs2167263	pos=245	alleles="C/G"
	rs35533340	pos=301	alleles="-/C/G"
	rs35633248	pos=301	alleles="-/T"

Gene	Reference sequence number	Position in reference sequence	SNP
	rs35638884	pos=301	alleles="-/A"
	rs35852119	pos=301	alleles="-/T"
	rs35970171	pos=301	alleles="-/T"
	rs55748583	pos=201	alleles="C/T"
	rs58805611	pos=101	alleles="C/T"
TAS2R60	rs10241042	pos=316	alleles="C/G"
	rs10241523	pos=316	alleles="A/C"
	rs11978402	pos=337	alleles="A/G"
	rs12534427	pos=301	alleles="C/G"
	rs12671578	pos=201	alleles="A/G"
	rs34328217	pos=301	alleles="-/C"
	rs34465195	pos=301	alleles="A/G"
	rs34910453	pos=301	alleles="C/T"
	rs35195910	pos=301	alleles="-/TCT"
	rs36004042	pos=301	alleles="-/G"
	rs4541818	pos=401	alleles="C/G"
	rs4595035	pos=301	alleles="C/T"
	rs58270521	pos=251	alleles="C/T"

Appendix Table 2 Allelic variations in coding sequences of human bitter taste receptors

Human bitter taste receptor	Nucleic Acid SEQ ID NO	Protein SEQ ID NO	Position of nucleotide change	Nucleotide change	Position of amino acid change	Description	Feature identifier
TAS2R1	6	32	332	G → A	111	R → H: dbSNP rs41469.	VAR_020198
			422	G → A	141	C → Y: dbSNP rs2234232.	VAR_053340
			616	C → T	206	R → W: dbSNP rs2234233.	VAR_020199
TAS2R3	7	33	349	C → T	117	P → S	NA
TAS2R4	8	34	8	G → A	3	R → Q: dbSNP rs2233995.	VAR_034535
			17	A → C	6	Y → S: dbSNP rs 2233997	NA
			20	C → T	7	F → S: dbSNP rs2233998.	VAR_034536
			186	T → A	62	F → L: dbSNP rs2233999.	VAR_053341
			221	C → T	74	T → M: dbSNP rs2234000.	VAR_020200
			286	G → C	96	V → L: dbSNP rs2234001.	VAR_020201
			512	G → A	171	S → N: dbSNP rs2234002.	VAR_020202
			571	A → G	191	I → V: dbSNP rs2234003.	VAR_053342
TAS2R5	9	35	58	G → A	20	G → S: dbSNP rs2234013.	VAR_053343
			77	G → T	26	S → I: dbSNP rs2227264.	VAR_020203
			235	C → T	79	R → C	NA
			338	C → T	113	P → L: dbSNP rs2234014.	VAR_034537
			500	A → G	167	Y → C: dbSNP rs34529840.	VAR_034538
			638	G → A	213	R → Q: dbSNP rs2234015.	VAR_024184
			881	G → T	294	R → L: dbSNP rs2234016.	VAR_053344
TAS2R7	10	36	254	T → C	85	I → T	NA
			538	G → T	180	A → T	NA
			640	C → T	214	R → stop codon	NA
			787	A → T	263	T → S: dbSNP rs3759251.	VAR_021852
			788	C → T	263	T → M	NA
			912	G → A	304	M → I: dbSNP rs619381.	VAR_024185
TAS2R8	11	37	142	C → T	48	L → F	NA
			370	T → G	124	W → G	NA
			496	A → G	166	R → G	NA
			829	T → C	277	Y → H	NA
			922	A → G	308	M → V: dbSNP rs2537817.	VAR_024186
TAS2R9	12	38	201	C → A	67	F → L	NA

Human bitter taste receptor	Nucleic Acid SEQ ID NO	Protein SEQ ID NO	Position of nucleotide change	Nucleotide change	Position of amino acid change	Description	Feature identifier
			381	C → A	127	N → K	NA
			450	T → A	150	D → E	NA
			508	A → C	170	K → Q: dbSNP rs11054043.	VAR_053345
			560	T → C	187	V → A: dbSNP rs3741845.	VAR_020204
			697	G → A	233	A → T	NA
			712	C → G	238	L → V: dbSNP rs11054042.	VAR_053346
			867	G → T	289	L → F	NA
			880	C → A	294	L → M	NA
TAS2R10	13	39	467	T → C	156	M → T: dbSNP rs597468.	VAR_030009
			521	A → C	174	K → T	NA
			691	T → C	231	S → P	NA
TAS2R13	14	40	446	A → G	149	N → S	VAR_036432
			776	A → G	259	N → S: dbSNP rs1015443.	VAR_021853
TAS2R14	15	41	256	A → G	86	T → A: dbSNP rs16925868.	VAR_053347
			589	A → G	197	M → V	NA
TAS2R16	16	42	301	G → A	101	V → M	NA
			481	C → T	161	P → S	NA
			516	T → G	172	N → K:. dbSNP rs846664.	VAR_034539
			665	G → A	222	R → H: dbSNP rs860170.	VAR_020205
TAS2R38	17	43	145	G → C	49	A → P: dbSNP rs713598.	VAR_017860
			239	A → G	80	H → R	NA
			785	C → T	262	A → V: dbSNP rs1726866.	VAR_017861
			820	C → T	274	R → C	NA
			886	A → G	296	I → V: dbSNP rs10246939.	VAR_017862
TAS2R39	18	44	578	C → T	193	S → F: dbSNP rs35474877.	VAR_053348
			589	A → G	197	K → E: dbSNP rs34169190.	VAR_053349
TAS2R40	19	45	67	G → C	23	V → L: dbSNP rs17164164.	VAR_053350
			560	C → A	187	S → Y: dbSNP rs10260248.	VAR_053351
			817	A → G	273	T → A	NA
			871	G → A	291	G → S	NA
TAS2R41	20	46	380	C → T	127	P → L	NA
			584	T → A	195	V → D	NA
TAS2R43	21	47	599	G → T	200	C → F	NA
			635	G → A	212	R → H	NA
			889	A → G	297	M → V	NA
			916	A → C	306	T → P	NA

Human bitter taste receptor	Nucleic Acid SEQ ID NO	Protein SEQ ID NO	Position of nucleotide change	Nucleotide change	Position of amino acid change	Description	Feature identifier
TAs2R44	22	48	103	C → T	35	R → W: dbSNP rs10845295.	VAR_030684
			484	T → A	162	L → M: dbSNP rs10743938.	VAR_030685
			599	G → A	200	C → Y	NA
			649	C → G	217	Q → E: dbSNP rs10845294.	VAR_030686
			656	C → T	219	P → L	
			680	C → T	227	A → V: dbSNP rs10845293.	VAR_030687
			718	G → A	240	V → I: dbSNP rs10772423.	VAR_030688
			827	C → G	276	P → R	NA
			843	G → T	281	W → C	NA
TAS2R45	23	49	176	T → G	59	L → R	NA
			227	A → G	76	Y → S	NA
			394	G → A	132	V → M	NA
			630	G → C	210	Q → H	NA
			703	T → C	235	F → L	NA
			712	T → C	238	C → R	NA
TAS2R46	24	50	106	T → G	36	F → V	NA
			682	T → A	228	L → M	NA
			749	G → A	250	W → stop codon	NA
			834	C → G	278	I → M	NA
			862	C → T	288	Q → stop codon	NA
TAS2R47	25	51	521	A → G	174	H → R	NA
			577	A → G	193	I → V	NA
			756	T → G	252	F → L	NA
TAS2R48	26	52	94	G → A	32	V → I	NA
			113	C → A	38	T → K	NA
			376	A → C	126	K → Q: dbSNP rs12424373.	VAR_053354
			456	A → T	152	R → S	NA
			673	A → G	225	I → V	NA
			719	T → C	240	I → T	NA
			799	G → C	267	V → L	NA
			815	C → T	272	P → L	NA
			895	C → T	299	R → C: dbSNP rs10772420	VAR_053355
TAS2R49	27	53	235	A → G	79	K → E: dbSNP rs7135018.	VAR_053356
			421	G → A	141	V → I	NA
			429	C → A	143	H → Q: dbSNP rs12226920.	VAR_053357
			442	C → A	148	H → N: dbSNP rs12226919.	VAR_053358
			516	G → A	172	M → I	NA
			706	A → G	236	I → V: dbSNP rs10845281.	VAR_053359

Human bitter taste receptor	Nucleic Acid SEQ ID NO	Protein SEQ ID NO	Position of nucleotide change	Nucleotide change	Position of amino acid change	Description	Feature identifier
			755	T → C	252	F → S: dbSNP rs10845280.	VAR_053360
			764	G → T	255	R → L: dbSNP rs10845279.	VAR_053361
			808	A → G	270	I → V	NA
TAS2R50	28	54	155	C → T	52	A → V	NA
			181	G → T	61	A → S	NA
			608	G → A	203	C → Y: dbSNP rs1376251	VAR_024187
TAS2R55	29	55	524	T → A	175	F → Y	NA
			587	T → C	196	F → S: dbSNP rs5020531.	VAR_053352
			763	G → T	255	G → W	NA
			794	A → G	265	Y → C: dbSNP rs1451772.	VAR_053353
TAS2R60	30	56	595	A → T	199	M → L	

Appendix Table 3 Mammalian G proteins, their families and descriptions

Class	Family/*Subtype*	Protein # (UniProt)	Description
G-alpha	**G_s**		
	Gs	P04896	Galpha-s-Bos taurus
	Gs	P16052	Galpha-s-Cricetulus longicaudatus
	Gs	P63092	Galpha-s-Homo sapiens-2
	Gs	P63091	Galpha-s-Canis familiaris
	Gs	P63093	Galpha-s-Mesocricetus auratus
	Gs	P63094	Galpha-s-Mus musculus-2
	Gs	P63095	Galpha-s-Rattus norvegicus-2
	Gs	P29797	Galpha-s-Sus scrofa
	Gs	O60726	Galpha-s-Homo sapiens-4
	Gs	O75632	Galpha-s-Homo sapiens-5
	Gs	O75633	Galpha-s-Homo sapiens-6
	Gs	Q14433	Galpha-s-Homo sapiens-7
	Gs	Q14455	Galpha-s-Homo sapiens
	Gs	Q8R4A8	Galpha-s-Cricetulus griseus
	Gs	Q9JJ33	Galpha-s-Mus musculus
	Gs	Q9JLG1	Galpha-s-Rattus norvegicus-1
	Gs	Q5JWF2	Galpha-s-Homo sapiens-3
	Golf	P38405	Galpha-olf-Homo sapiens-2
	Golf	Q8CGK7	Galpha-olf-Mus musculus
	Golf	P38406	Galpha-olf-Rattus norvegicus
	Golf	Q86XU3	Galpha-olf-Homo sapiens-1
	$G_{i/o}$		
	Gi	Q29047	Galpha-i-Sus scrofa
	Gi1	P38401	Galpha-i1-Cavia porcellus
	Gi1	P50146	Galpha-i1-Gallus gallus
	Gi1	P63096	Galpha-i1-Homo sapiens-1
	Gi1	P63097	Galpha-i1-Bos taurus
	Gi1	P10824	Galpha-i1-Rattus norvegicus
	Gi1	O43383	Galpha-i1-Homo sapiens-2
	Gi1	Q61018	Galpha-i1-Mus musculus
	Gi2	P38400	Galpha-i2-Canis familiaris
	Gi2	P38402	Galpha-i2-Cavia porcellus
	Gi2	P50147	Galpha-i2-Gallus gallus
	Gi2	P04899	Galpha-i2-Homo sapiens-2
	Gi2	P08752	Galpha-i2-Mus musculus-2
	Gi2	P04897	Galpha-i2-Rattus norvegicus
	Gi2	Q7M3G8	Galpha-i2-Sus scrofa
	Gi2	Q7M3G9	Galpha-i2-Bos taurus-2
	Gi2	Q7M3H0	Galpha-i2-Bos taurus-1
	Gi2	Q8JZT4	Galpha-i2-Mus musculus-1
	Gi2	Q96C71	Galpha-i2-Homo sapiens-1
	Gi3	P38403	Galpha-i3-Cavia porcellus
	Gi3	Q60397	Galpha-i3-Cricetulus griseus
	Gi3	P08754	Galpha-i3-Homo sapiens
	Gi3	P08753	Galpha-i3-Rattus norvegicus
	Gi3	Q9DC51	Galpha-i3-Mus musculus
	Go	P59215	Galpha-o-Rattus norvegicus

	Go	Q8N6I9	Galpha-o-Homo sapiens
	Go1	P08239	Galpha-o1-Bos taurus
	Go1	P59216	Galpha-o1-Cricetulus longicaudatus
	Go1	P09471	Galpha-o1-Homo sapiens
	Go1	P18872	Galpha-o1-Mus musculus
	Gz	P19086	Galpha-z-Homo sapiens-2
	Gz	O70443	Galpha-z-Mus musculus
	Gz	P19627	Galpha-z-Rattus norvegicus
	Gz	Q8IY73	Galpha-z-Homo sapiens-3
	Gz	Q8N652	Galpha-z-Homo sapiens-1
	Gz	Q95LC0	Galpha-z-Sus scrofa
	Gt	Q16162	Galpha-t-Homo sapiens
	Gt	Q9D7B3	Galpha-t-Mus musculus
	Gt1	P04695	Galpha-t1-Bos taurus
	Gt1	Q28300	Galpha-t1-Canis familiaris
	Gt1	P11488	Galpha-t1-Homo sapiens
	Gt1	P20612	Galpha-t1-Mus musculus
	Gt2	P04696	Galpha-t2-Bos taurus
	Gt2	P19087	Galpha-t2-Homo sapiens
	Gt2	P50149	Galpha-t2-Mus musculus-2
	Gt2	Q8BSY7	Galpha-t2-Mus musculus-1
	Ggust	P29348	Galpha-gust-Rattus norvegicus
	$G_{q/11}$		
	Gq	Q6NT27	Galpha-q-Homo sapiens-2
	Gq	Q28294	Galpha-q-Canis familiaris
	Gq	P50148	Galpha-q-Homo sapiens-1
	Gq	P21279	Galpha-q-Mus musculus
	Gq	P82471	Galpha-q-Rattus norvegicus
	G11	Q71RI7	Galpha-11-Gallus gallus
	G11	P38409	Galpha-11-Bos taurus
	G11	P52206	Galpha-11-Canis familiaris
	G11	P29992	Galpha-11-Homo sapiens
	G11	P45645	Galpha-11-Meleagris gallopavo
	G11	P21278	Galpha-11-Mus musculus-2
	G11	Q9JID2	Galpha-11-Rattus norvegicus
	G11	Q8SPP3	Galpha-11-Macaca mulatta
	G11	Q91X95	Galpha-11-Mus musculus-1
	G14	P38408	Galpha-14-Bos taurus
	G14	O95837	Galpha-14-Homo sapiens
	G14	P30677	Galpha-14-Mus musculus-2
	G14	Q8C3M7	Galpha-14-Mus musculus-3
	G14	Q8CBT5	Galpha-14-Mus musculus-4
	G14	Q8R2X9	Galpha-14-Mus musculus-1
	G15	P30678	Galpha-15-Mus musculus
	G15	O88302	Galpha-15-Rattus norvegicus
	G16	P30679	Galpha-16-Homo sapiens
	$G_{12/13}$		
	G12	Q03113	Galpha-12-Homo sapiens
	G12	P27600	Galpha-12-Mus musculus
	G12	Q63210	Galpha-12-Rattus norvegicus

	G13	Q14344	Galpha-13-Homo sapiens
	G13	P27601	Galpha-13-Mus musculus-2
	G13	Q8C5L2	Galpha-13-Mus musculus-3
	G13	Q9D034	Galpha-13-Mus musculus-1
G-beta	**B_{1-5}**		
	B1	Q6TMK6	Gbeta-1-Cricetulus griscus
	B1	P62871	Gbeta-1-Bos taurus
	B1	P62872	Gbeta-1-Canis familiaris
	B1	P62873	Gbeta-1-Homo sapiens
	B1	P62874	Gbeta-1-Mus musculus
	B1	P54311	Gbeta-1-Rattus norvegicus-2
	B1	Q9QX36	Gbeta-1-Rattus norvegicus-1
	B2	P11017	Gbeta-2-Bos taurus
	B2	P62879	Gbeta-2-Homo sapiens
	B2	P62880	Gbeta-2-Mus musculus
	B2	P54313	Gbeta-2-Rattus norvegicus-2
	B2	Q9QX35	Gbeta-2-Rattus norvegicus-1
	B3	P79147	Gbeta-3-Canis familiaris
	B3	P16520	Gbeta-3-Homo sapiens-1
	B3	Q61011	Gbeta-3-Mus musculus
	B3	P52287	Gbeta-3-Rattus norvegicus
	B3	Q96B71	Gbeta-3-Homo sapiens-2
	B4	Q9HAV0	Gbeta-4-Homo sapiens
	B4	P29387	Gbeta-4-Mus musculus
	B4	O35353	Gbeta-4-Rattus norvegicus
	B5	O14775	Gbeta-5-Homo sapiens-2
	B5	P62881	Gbeta-5-Mus musculus-2
	B5	P62882	Gbeta-5-Rattus norvegicus
	B5	Q60525	Gbeta-5-Mesocricetus auratus
	B5	Q96F32	Gbeta-5-Homo sapiens-1
	B5	Q9CSQ0	Gbeta-5-Mus musculus-3
	B5	Q9CU21	Gbeta-5-Mus musculus-1
	$B_{unclassified}$		
	B unclassified	Q61621	unclassified_Gbeta-Mus musculus-1
	B unclassified	Q8BMQ1	unclassified_Gbeta-Mus musculus-2
	B unclassified	Q9UFT3	unclassified_Gbeta-Homo sapiens
G-gamma	γ_{1-12}		
	γ1	Q8R1U6	Ggamma-1-Mus musculus
	γ2	P59768	Ggamma-2-Homo sapiens
	γ2	P63212	Ggamma-2-Bos taurus
	γ2	P63213	Ggamma-2-Mus musculus
	γ2	O35355	Ggamma-2-Rattus norvegicus
	γ3	P63214	Ggamma-3-Bos taurus
	γ3	P63215	Ggamma-3-Homo sapiens
	γ3	P63216	Ggamma-3-Mus musculus
	γ3	O35356	Ggamma-3-Rattus norvegicus
	γ4	P50150	Ggamma-4-Homo sapiens
	γ4	P50153	Ggamma-4-Mus musculus
	γ4	O35357	Ggamma-4-Rattus norvegicus
	γ5	P63217	Ggamma-5-Bos taurus

	γ5	P63218	Ggamma-5-Homo sapiens-2
	γ5	Q80SZ7	Ggamma-5-Mus musculus
	γ5	P63219	Ggamma-5-Rattus norvegicus
	γ5	Q9Y3K8	Ggamma-5-Homo sapiens-1
	γ7	P30671	Ggamma-7-Bos taurus
	γ7	O60262	Ggamma-7-Homo sapiens
	γ7	Q61016	Ggamma-7-Mus musculus
	γ7	P43425	Ggamma-7-Rattus norvegicus
	γ8	Q9UK08	Ggamma-8-Homo sapiens-2
	γ8	P63078	Ggamma-8-Mus musculus-2
	γ8	P63077	Ggamma-8-Rattus norvegicus
	γ8	P50154	Ggamma-8-Bos taurus
	γ8	O14610	Ggamma-8-Homo sapiens-1
	γ8	Q61017	Ggamma-8-Mus musculus-1
	γ10	P50151	Ggamma-10-Homo sapiens-2
	γ10	O35358	Ggamma-10-Rattus norvegicus
	γ10	Q96BN9	Ggamma-10-Homo sapiens-1
	γ10	Q9CXP8	Ggamma-10-Mus musculus
	γ11	P61952	Ggamma-11-Homo sapiens
	γ11	P61953	Ggamma-11-Mus musculus
	γ11	P61954	Ggamma-11-Rattus norvegicus
	γ12	Q28024	Ggamma-12-Bos taurus
	γ12	Q9UBI6	Ggamma-12-Homo sapiens
	γ12	Q9DAS9	Ggamma-12-Mus musculus
	γ12	O35359	Ggamma-12-Rattus norvegicus
	γ13	Q9P2W3	Ggamma-13-Homo sapiens
	γ13	Q9JMF3	Ggamma-13-Mus musculus
	γt1	P02698	Ggamma-t1-Bos taurus
	γt1	P63211	Ggamma-t1-Homo sapiens
	γt1	P63210	Ggamma-t1-Canis familiaris
	γt1	Q61012	Ggamma-t1-Mus musculus
	$\gamma_{unclassified}$		
	γ unclassified	Q7M3H1	unclassified_Ggamma-Bos indicus

CLAIMS

1. A method for identifying a compound that inhibits the bitter taste due to KCl, comprising:

a) providing a first and a second cell,

wherein the first and second cell each expresses one or more bitter taste receptors selected from the group consisting of:

TAS2R4 (SEQ ID NO: 30), TAS2R9 (SEQ ID NO: 34), TAS2R13 (SEQ ID NO: 36), TAS2R14 (SEQ ID NO: 37), TAS2R44 (SEQ ID NO: 44) and TAS2R60 (SEQ ID NO: 56), or a naturally-occurring allelic variant thereof, wherein the first and second cell express the same one or more bitter taste receptors;

b) contacting said first cell with a tastant,

wherein the tastant activates the one or more bitter taste receptor;

c) contacting said second cell with a test compound and the tastant;

d) assaying said first and second cells for bitter taste receptor activation; and

e) comparing the bitter taste receptor activation of said first cell to the bitter taste receptor activation of said second cell,

wherein the test compound is an inhibitor of bitter taste due to KCl, if bitter taste receptor activity of said second cell is less than the bitter taste receptor activity of said first cell.

2. The method of claim 1, wherein the bitter taste receptor is complexed to a G protein.

3. The method according to claim 2, wherein said G protein is a Gq protein, an alpha transducin or an alpha gustducin.

4. The method according to claim 3, wherein the Gq protein is a Gα15 protein (SEQ ID NO: 1).

5. The method according to any one of claims 1-4, wherein bitter taste receptor activity is determined by measuring intracellular calcium concentration.

6. The method according to claim 5, wherein the concentration of intracellular calcium is determined using a calcium-sensitive fluorescent dye.

7. The method according to claim 6, wherein the calcium-sensitive fluorescent dye is selected from the group consisting of Indo-1, Fura-2, Fluo-3, Fluo-4, Rhod-2, Rhod-5N, Calcein, Calcein blue, cytoCalcein Violet, Quin-2, Quest Fluo-8H™, Quest Fluo 8L™, Quest Fluo 8™, Quest Rhod-4™, coelenterazine and Calcium-3.

8. The method according to any one of claims 1-7, wherein said first and second cells are present in in vitro cell lines.

9. The method according to any one of claims 1-7, wherein said first and second cells are present in panels of in vitro cell lines.

10. A method for identifying a compound that selectively acts on receptors to inhibit the bitter taste due to KCl comprising:

a) providing a first and second panel of cell lines,

wherein each of said first and second panel comprises cell lines that express a bitter taste receptor selected from the group consisting of: TAS2R4 (SEQ ID NO: 30), TAS2R9 (SEQ ID NO: 34), TAS2R13 (SEQ ID NO: 36), TAS2R14 (SEQ ID NO: 37), TAS2R44 (SEQ ID NO: 44) and TAS2R60 (SEQ ID NO: 56), or a naturally-occurring allelic variant thereof;

wherein TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, or a naturally-occurring allelic variant thereof are each expressed in at least one cell line, and

wherein the first and second panels comprise the same cell lines;

b) contacting each cell line in the first panel with a tastant,

wherein the tastant activates at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, or a naturally-occurring allelic variant thereof;

c) contacting each cell line in the second panel with a test compound and the tastant;

d) assaying each cell line for bitter taste receptor activation;

wherein, the test compound is a selective inhibitor of bitter taste due to KCl if the bitter taste receptor activity of at least two of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, or a naturally-occurring allelic variant thereof is less in the second panel compared to the first panel.

11. The method of claim 10, wherein the bitter taste receptor activity of at least three of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, or a naturally-occurring allelic variant thereof is less in the second panel compared to the first panel.

12. The method of claim 10, wherein the bitter taste receptor activity of at least four of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, or a naturally-occurring allelic variant thereof is less in the second panel compared to the first panel.

13. The method of claim 10, wherein the bitter taste receptor activity of at least five of the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, or a naturally-occurring allelic variant thereof is less in the second panel compared to the first panel.

14. The method of claim 10, wherein the bitter taste receptor activity of each member in the group selected from TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60, or a naturally-occurring allelic variant thereof is less in the second panel compared to the first panel.

15. The method of any one of claims 10-14, wherein the first and second panel comprise TAS2R1 (SEQ ID NO: 28), TAS2R3 (SEQ ID NO: 29), TAS2R4 (SEQ ID NO: 30), TAS2R5 (SEQ ID NO: 31), TAS2R7 (SEQ ID NO: 32), TAS2R8 (SEQ ID NO: 33), TAS2R9 (SEQ ID NO: 34), TAS2R10 (SEQ ID NO: 35), TAS2R13 (SEQ ID NO: 36), TAS2R14 (SEQ ID NO: 37), TAS2R16 (SEQ ID NO: 38), TAS2R38 (SEQ ID NO: 39), TAS2R39 (SEQ ID NO: 40), TAS2R40 (SEQ ID NO: 41), TAS2R41 (SEQ ID NO: 42), TAS2R43 (SEQ ID NO: 43), TAS2R44 (SEQ ID NO: 44), TAS2R45 (SEQ ID NO: 45), TAS2R46 (SEQ ID NO: 46), TAS2R47 (SEQ ID NO: 47), TAS2R48 (SEQ ID NO: 48), TAS2R49 (SEQ ID NO: 49), TAS2R50 (SEQ ID NO: 50), TAS2R55 (SEQ ID NO: 51), and TAS2R60 (SEQ ID NO: 52) bitter taste receptors, or naturally-occurring allelic variants thereof.

16. The method of any of claims 10-15, wherein the bitter taste receptor is complexed to a G protein.

17. The method according to claim 16, wherein said G protein is a Gq protein, an alpha transducin or an alpha gustducin.

18. The method according to claim 17, wherein the Gq protein is a Gα15 protein (SEQ ID NO: 1).

19. The method according to any one of claims 10-18, wherein bitter taste receptor activity is determined by measuring intracellular calcium concentration.

20. The method according to claim 19, wherein the concentration of intracellular calcium is determined using a calcium-sensitive fluorescent dye.

21. The method according to claim 20, wherein the calcium-sensitive fluorescent dye is selected from the group consisting of Indo-1, Fura-2, Fluo-3, Fluo-4, Rhod-2, Rhod-5N, Calcein, Calcein blue, cytoCalcein Violet, Quin-2, Quest Fluo-8H™, Quest Fluo 8L™, Quest Fluo 8™, Quest Rhod-4™, coelenterazine and Calcium-3.

22. A method for identifying a compound that enhances the bitter taste due to KCl comprising:

a) providing a first and a second cell,

wherein the first and second cell each expresses one or more bitter taste receptors selected from the group consisting of:

TAS2R4 (SEQ ID NO: 30), TAS2R9 (SEQ ID NO: 34), TAS2R13 (SEQ ID NO: 36), TAS2R14 (SEQ ID NO: 37), TAS2R44 (SEQ ID NO: 44) and TAS2R60 (SEQ ID NO: 56), or a naturally-occurring allelic variant thereof, wherein the first and second cell express the same one or more bitter taste receptors;

b) contacting said first cell with a tastant,

wherein the tastant activates one or more of TAS2R4, TAS2R9, TAS2R13, TAS2R14, TAS2R44 and TAS2R60 bitter taste receptors, or a naturally-occurring allelic variant thereof;

c) contacting said second cell with a test compound and the tastant;

d) assaying said first and second cells for bitter taste receptor activation; and

c) comparing the bitter taste receptor activation of said first cell to the bitter taste receptor activation of said second cell,

wherein the test compound is an enhancer of bitter taste due to KCl, if bitter taste receptor activity of said second cell is more than the bitter taste receptor activity of said first cell.

23. The method according to any one of claims 1-22, wherein (i) the allelic variant of TAS2R4 comprises a R3Q, a Y6S, a F7S, a F62L, a T74M, a V96L, a S171N, or a I191V mutation or a combination thereof; (ii) the TAS2R9 variant comprises a F67L, N127K, D150E, K170Q, V187A, A233T, L238V, L289F, or a L294M mutation or a combination thereof; (iii) the TAS2R13 variant comprises a N149S or a N259S mutation or a combination thereof; (iv) the TAS2R14 variant comprises a T86A or a M197V mutation or a combination thereof; (v) the TAS2R44 variant comprises a R35W, a L162M, a C200Y, a Q217E, a P219L, a A227V, a V240I, a P276R, or a W281C mutation or a combination thereof; or (vi) the TAS2R60 variant comprises a M199L mutation.

24. The method according to any one of claims 1-22, wherein (i) the allelic variant of TAS2R4 has an amino acid sequence having at least 95% sequence identity to SEQ ID NO:30, (ii) the allelic variant of TAS2R9 has an amino acid sequence having at least 95% sequence identity to SEQ ID NO:34, (iii) the allelic variant of TAS2R13 has an amino acid sequence having at least 95% sequence identity to SEQ ID NO:36, (iv) the allelic variant of TAS2R14 has an amino acid sequence having at least 95% sequence identity to SEQ ID NO:37, (v) the allelic variant of TAS2R44 has an amino acid sequence having at least 95% sequence identity to SEQ ID NO:44, or (vi) the allelic variant of TAS2R60 has an amino acid sequence having at least 95% sequence identity to SEQ ID NO:52.