

METHODS AND COMPOSITIONS FOR BIOPRODUCTION OF PECTOLINARIGENIN

I. Field of Invention

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The invention is directed to materials and methods for bioproduction of pectolinarigenin. The invention provides methods and materials for cell-based and cell-free production of pectolinarigenin.

II. Reference to Sequence Listing

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The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file named DEBU-023-01WO.xml, created on December 26, 2024, which is 107 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety

III. Background

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Nature is recognized as a source of molecules with relevant potential pharmaceutical applications. Among the various phytochemicals, flavonoids have attracted and are still attracting most of the attention due to their notable biological benefits. Flavonoids have been associated with the role of preventing and managing current diseases such as cancers, diabetes, and cardiovascular disorders. More than 13,000 flavonoids were isolated and identified from plants, some of which
20 like quercetin, kaempferol and scutellarin showed potent pharmacological effects and, therefore, are promising for new drugs.

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The glycosylated flavone pectolinarin was first isolated from *Linaria vulgaris*, a known medicinal Chinese herb used for the internal treatment of digestion problems and urinary disorders, in the external treatment of hemorrhoids, venous skin ulcer, as well as for the washing of festering
wounds and skin rashes. Cheriet et al., I. Isolation and Biological Properties of the Natural
Flavonoids Pectolinarin and Pectolinarigenin—A Review. *Antibiotics* **2020**, *9*, 417. Pectolinarin
and its aglycone, pectolinarigenin, were identified as the major constituents in many medicinal
herbs from different genera around the world.

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Over the last 113 years from its first report, pectolinarin was isolated in most cases from the aerial parts of 87 plants belonging to 29 different genera distributing widely around the world.

Most of these plants are used in folk medicine in different parts of the world. Pectolinarigenin is the aglycone part of pectolinarin, which is obtained by hydrolysis reaction. It is also a natural product, isolated and identified from 136 plants of 71 different genera. Pectolinarigenin proved to be more distributed in the vegetable kingdom than pectolinarin, and this evidence can be explained
5 assuming that not all plants have the *O*-glycosylation enzymes. In most works, pectolinarigenin was isolated from the non-polar fractions of the plant extracts, mainly working on the aerial parts.

IV. Summary of the Invention

The conventional methods for production of pectolinarigenin involve isolation from plants. This increases the cost of production because of the purification required from these mixtures.
10 Moreover, these factors can vary in the source plant from year to year and are influenced by weather and environmental factors. Thus, another challenge for the commercial production of polyphenols from plants is that harvest is often limited to once a year. This means a large volume of extract has to be prepared and stored for an extended time to supply the needs of the food industry throughout the year.

15 Thus, new methods for production of pectolinarigenin are required that are reliable and economically viable. The invention provides methods and compositions for bioproduction of pectolinarigenin. The methods of the invention may lead to production of pectolinarigenin in a host organism comprising certain genetic modifications. The methods of the invention also provide cell-free bioproduction of pectolinarigenin from one or more substrates through one or more
20 substrates. The methods of the invention are beneficial because they provide an economically viable method for production of pectolinarigenin. Advantageously, the methods of the invention are also reliable and are not dependent on the available plants for preparations of pectolinarigenin.

Advantageously, the methods of the invention provide a cell-based or cell-free platform for production of pectolinarigenin. The platform provided in the invention is beneficial because it
25 provides multiple pathways for preparation of pectolinarigenin from naringenin. The availability of different pathways for the production of pectolinarigenin is beneficial because it provides an avenue to prepare and isolate one or more intermediates in the biosynthesis pathway. Thus, the platform provided in the invention unlocks the option of preparation of one or more intermediates which might not otherwise be readily accessible for manufacturing. Beneficially, the pathway for
30 production of pectolinarigenin could also be selected based on the availability of various enzymes

for that particular reaction pathway. In other words, if an enzyme is unavailable (or not readily available), a different pathway, which does not require that particular enzyme, may be selected for production of pectolinarigenin. Thus, the modularity of the biosynthetic pathway is especially beneficial.

5 In certain aspects, the invention provides a method for bioproduction of pectolinarigenin, the method comprising providing one or more enzymes, wherein the one or more enzymes result in transformation of naringenin to pectolinarigenin through one or more intermediates. The method for production of pectolinarigenin includes cell-based and cell-free methods.

10 In certain aspects of the invention, the method for production of pectolinarigenin comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of naringenin to pectolinarigenin through one or more intermediates. In certain embodiments, the genetic modifications in the host cell may also include one or more genetic modifications to enhance the production of naringenin. In certain embodiments, the engineered host cell comprises genetic
15 modifications to enhance the production of naringenin or any precursor leading to production of naringenin in the engineered host cell. The additional genetic modifications leading to an increase in production of naringenin may be beneficial for production of pectolinarigenin because the naringenin may be used a substrate for production of pectolinarigenin. The enhanced amount of naringenin would lead to higher yield of pectolinarigenin.

20 In certain aspects of the invention, the method for production of pectolinarigenin is a cell-free production of pectolinarigenin, wherein the one or more enzymes in a cell-free medium result in transformation of naringenin to pectolinarigenin through one or more intermediates. The ingredients of the components in the cell-free reaction may be generated in a host cell. For example, the substrate, such as naringenin, may be generated in a cell and the subsequent
25 conversion to pectolinarigenin may be conducted in a cell-free medium. In certain embodiments, the one or more intermediates generated in conversion of naringenin to pectolinarigenin may be generated in an engineered host cell, and those one or more intermediates may be subsequently converted to pectolinarigenin by one or more enzymes in a cell-free medium.

In certain aspects, the method comprises a combination of: (i) an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of naringenin to pectolinarigenin or one or more intermediates, and (ii) cell-free production comprising one or more enzymes resulting in transformation of naringenin or a substrate to pectolinarigenin or one or more intermediates for synthesis of pectolinarigenin. In certain embodiments, the engineered host cell comprises genetic modifications to enhance the production of naringenin or any precursor leading to production of naringenin in the engineered host cell.

In certain embodiments of the invention, the one or more intermediates are selected from a group consisting of: apigenin, scutellarein, hispidulin, isosakuranetin, carthamidin, 6-methoxycarthamidin, scutellarein, 4-methoxycarthamidin, acacetin, 4',6-methoxynaringenin, 4'-methoxyscutellarein, and any combination thereof. **FIG. 1** provides exemplary schematic pathways for conversion of naringenin to pectolinarigenin. **FIG. 1** also provides the enzymes mediating the conversion of naringenin to one or more intermediates, or the conversion of one or more intermediates to other intermediates, or the conversion of one or more intermediates to pectolinarigenin. As evident from **FIG. 1**, the methods of the invention provide a modular pathway for conversion of naringenin to pectolinarigenin. The pathway selected for production of pectolinarigenin will determine the identity of the one or more intermediates selected in the process. Each individual segment of the pathway may be controlled by the enzymes selected for that conversion. In certain embodiments, the methods of the invention provide the use of one or more enzymes to mediate the conversion of the starting material to one or more intermediates, or pectolinarigenin. In certain embodiments, the one or more enzymes selected from the group consisting of: flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), O-methyltransferase (OMT), and any combination thereof.

For example, in certain embodiments, the intermediates are apigenin, scutellarein, and hispidulin. In certain embodiments, the method provides a pathway, wherein, naringenin is converted to apigenin; apigenin is converted to scutellarein; scutellarein is converted to hispidulin; and hispidulin is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. For example, as demonstrated in **FIG. 2**, the pathway provided comprises flavone synthase (FNSI) mediated conversion of naringenin to apigenin;

flavone hydroxylase cytochrome P450 (P450) mediated conversion of apigenin to scutellarein; O-methyltransferase (OMT) mediated conversion of scutellarein to hispidulin; and O-methyltransferase (OMT) mediated conversion of hispidulin to pectolinarigenin. Any of these aforementioned steps may be conducted either in an engineered host cell or a cell-free medium.

5 In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are apigenin, scutellarein, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to apigenin; apigenin is converted to scutellarein; scutellarein is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or
10 more enzymes.

 In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are apigenin, scutellarein, and hispidulin. The invention provides that naringenin is converted to apigenin; apigenin is converted to scutellarein; scutellarein is converted to hispidulin; hispidulin is converted to pectolinarigenin. In certain embodiments, one
15 or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

 In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are apigenin, acacetin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to apigenin; apigenin is converted to acacetin; acacetin is
20 converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

 In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are isosakuranetin, 4'-methoxycarthamidin, and 4',6-methoxynaringenin. The invention provides that naringenin is converted to isosakuranetin; isosakuranetin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4',6-methoxynaringenin; 4',6-methoxynaringenin is converted to pectolinarigenin. In certain
25 embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are isosakuranetin, 4'-methoxycarthamidin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to isosakuranetin; isosakuranetin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain
5 embodiments, one or more these steps may be conducted by one or more enzymes. In certain
embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are isosakuranetin, acacetin, and 4'-methoxyscutellarein. The
10 invention provides that naringenin is converted to isosakuranetin; isosakuranetin is converted to
acacetin; acacetin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to
pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or
more enzymes. In certain embodiments, one or more these steps may be conducted by one or more
enzymes.

15 In certain embodiments, the one or more intermediates involved in conversion of
naringenin to pectolinarigenin are carthamidin, 6-methoxycarthamidin, and hispidulin. The
invention provides that naringenin is converted to carthamidin; carthamidin is converted to 6-
methoxycarthamidin; 6-methoxycarthamidin is converted to hispidulin; hispidulin is converted to
pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or
20 more enzymes. In certain embodiments, one or more these steps may be conducted by one or more
enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 6-methoxycarthamidin, and 4',6-methoxynaringenin. The invention provides that naringenin is converted to carthamidin;
25 carthamidin is converted to 6-methoxycarthamidin; 6-methoxycarthamidin is converted to 4',6-
methoxynaringenin; 4',6-methoxynaringenin is converted to pectolinarigenin. In certain
embodiments, one or more these steps may be conducted by one or more enzymes. In certain
embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 6-methoxycarthamidin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to 6-methoxycarthamidin; 6-methoxycarthamidin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain
5 embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, scutellarein, and hispidulin. The invention
10 provides that naringenin is converted to carthamidin; carthamidin is converted to scutellarein; scutellarein is converted to hispidulin; hispidulin is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of
15 naringenin to pectolinarigenin are carthamidin, scutellarein, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to scutellarein; scutellarein is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by
20 one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 4'-methoxycarthamidin, and 4',6-methoxynaringenin. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4',6-methoxynaringenin; 4',6-methoxynaringenin is converted to pectolinarigenin. In certain
25 embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 4'-methoxycarthamidin, and 4'-

methoxyscutellarein. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain
5 embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the invention provides method of production of pectolinarigenin comprises engineered host cells, wherein the host cells comprise one or more genetic modifications. In certain embodiments, the engineered host cell could be prepared from yeast, bacteria, or mammalian cells. In certain embodiments, the engineered host cell is *E. coli*. In certain
10 embodiments, the one or more enzymes are introduced in the host organism by integration into genome of the host organism or on a plasmid. In certain embodiments, the one or more genetic modifications in the engineered host cell are any of the modifications described above. For example, the one or more genetic modification may be overexpression or endogenous enzymes or expression of a variant of an enzyme involved in the conversion of naringenin to pectolinarigenin.
15 The genetic modifications include, without limitation, overexpression or expression of a variant of: flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), O-methyltransferase (OMT), and/or any combination thereof.

In certain embodiments, the one or more genetic modifications in the host cell may include any genetic modification to increase the availability of naringenin. For example, the one or more
20 genetic modifications in an engineered host cell may be selected from a group consisting of: acetyl-CoA carboxylase (ACC), tyrosine ammonia-lyase (TAL), 4-coumarate-CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), and/or any combination thereof. In certain embodiments, the genetic modifications may comprise expressing a variant of one or more of these enzymes. In certain embodiments, the one or more genetic modifications to increase the production
25 of naringenin are provided in PCT/US2022/024591, which is incorporated in its entirety by reference.

In certain embodiments, the engineered host cell comprises one or more genetic modifications to increase the production naringenin and/or pectolinarigenin. In certain embodiments, the engineered host cell comprises one or more genetic modifications to increase
30 the production of naringenin. In these embodiments, the starting material for production of

naringenin would is glycerol. Thus, the methods of the invention beneficially provide an engineered host cell for conversion of glycerol to pectolinarigenin. The engineered host cell may optionally comprise genetic modifications optimized for conversion of glycerol to naringenin through one or more intermediates and subsequent conversion of naringenin to pectolinarigenin through one or more intermediates. The exemplary genetic modifications for production of
5 naringenin are provided in WO2022/221392, which is incorporated by reference in its entirety.

In addition, **FIG. 6** provides an overview of the conversion of glycerol to naringenin through one or more intermediates. In certain preferred embodiments, the engineered host cell for enhanced production of naringenin comprises one or more genetic modifications selected from the group consisting of: overexpression of ACC, TAL, 4CL, CHS, and CHI. In certain embodiments,
10 the engineered host cell may comprise additional genetic modifications for downregulation and/or deletion of one or more enzymes that convert naringenin to other substrates that are not involved in the pathway for bioproduction of pectolinarigenin.

Accordingly, in certain aspects, the invention provides an engineered host cell for
15 conversion of glycerol to pectolinarigenin through one more intermediates. In certain embodiments, the engineered host cell may comprise one more genetic modifications for enzymatic transformation of glycerol to pectolinarigenin through one more intermediates. In certain embodiments, the one more genetic modifications for transformation of glycerol to naringenin. In certain embodiments, the genetic modifications may be selected from the group consisting of overexpression or expression of a variant of: ACC, TAL, 4CL, CHS, and CHI. In
20 certain embodiments, the engineered host cell further comprises one or more genetic modifications for transformation of naringenin to pectolinarigenin. In certain embodiments, the engineered host cell comprises one or more genetic modifications selected from the group consisting of overexpression or expression of a variant of: flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), O-methyltransferase (OMT), and/or any combination thereof. In certain
25 embodiments, the glycerol is from a carbon feedstock. In some embodiments, the glycerol is crude glycerol. In certain embodiments, glycerol obtained as a byproduct of biodiesel processing.

In certain aspects, the invention provides a method for production of pectolinarigenin through transformation of glycerol through one or more intermediates. In certain embodiments,
30 the glycerol is enzymatically transformed to pectolinarigenin through one or more intermediates.

In certain embodiments, the one or more intermediates is naringenin. In certain embodiments, the genetic modifications may be selected from the group consisting of overexpression or expression of a variant of: ACC, TAL, 4CL, CHS, and CHI. In certain embodiments, the engineered host cell further comprises one or more genetic modifications for transformation of naringenin to
5 pectolinarigenin. In certain embodiments, the engineered host cell comprises one or more genetic modifications selected from the group consisting of overexpression or expression of a variant of: flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), O-methyltransferase (OMT), and/or any combination thereof. In certain embodiments, the glycerol is from a carbon feedstock. In some embodiments, the glycerol is crude glycerol. In certain embodiments, glycerol
10 obtained as a byproduct of biodiesel processing.

In certain aspects, the invention provides methods of cell-free production of pectolinarigenin. The cell-free medium for the conversion of naringenin to pectolinarigenin is a cell lysate. In certain embodiments, the cell lysate is a cell lysate from cells from a host organism expressing the one or more enzymes. The one or more enzymes may be the enzymes involved in
15 conversion of naringenin to pectolinarigenin. In certain embodiments, the host organisms expressing the one or more enzymes are cultured until a pre-determined biomass is achieved to produce the requisite quantity of the one or more enzymes. In certain embodiments, the method of the invention further comprising lysing cells followed by removal of cell debris to generate a cell lysate for use in the cell-free medium for cell-free production of pectolinarigenin.

20 In certain embodiments, cell-free medium comprises: buffer, magnesium chloride, one or more substrates, one or more cofactors, the one or more enzymes, and/or water.

In certain embodiments, the buffer in the cell-free medium maintains the pH of the reaction mixture in the optimal range. In certain embodiments, the pH of the reaction medium is from about 5.5 to about 10. In certain embodiments, the pH of the reaction medium is from about 6 to about
25 9.5. In certain embodiments, the pH of cell-free medium is from about 6 to about 9. In certain embodiments, the pH of cell-free medium is from about 6 to about 8. In certain embodiments, the concentration of the buffer in the cell-free medium is from about 1 mM to about 1000 mM. In certain embodiments, the concentration of the buffer is from about 2.5 mM to about 750 mM. In certain embodiments, the concentration of the buffer is from about 5 mM to about 100 mM. In

certain embodiments, the buffer concentration is from about 20 mM to about 100 mM. In certain embodiments, the buffer is a phosphate buffer.

In certain embodiments, the method of the invention comprises conversion of one or more substrates to pectolinarigenin. In certain embodiments, the one or more substrates are present in a concentration of from about 1 mM to about 500 mM. In certain embodiments, the one or more substrates are present in a concentration of from about 1 mM to about 200 mM. In certain embodiments, the one or more substrates are present in a concentration of from about 10 mM to about 100 mM. In certain embodiments, the substrate used for production of pectolinarigenin is naringenin.

In certain embodiments, the cell-free medium comprises magnesium chloride. The magnesium chloride may be present in the range of from about 1 mM to about 50 mM. In certain embodiments, the cell-free medium comprises magnesium chloride in the range of from about 1 mM to about 25 mM. In certain embodiments, the cell-free medium comprises magnesium chloride in the range of from about 1 mM to about 20 mM. In certain embodiments, the cell-free medium comprises magnesium chloride in the range of from about 5 mM to about 25 mM.

In certain embodiments, the cell-free medium comprises the lysate from host microbe with the one or more enzymes for the cell-free reaction medium. In certain embodiments, the cell-free medium comprises lysate with the one or more enzymes in a concentration of from about 1% (v/v) to about 50% (v/v). In certain embodiments, the cell-free medium comprises lysate with the one or more enzymes in a concentration of from about 5% (v/v) to about 40% (v/v). In certain embodiments, the cell-free medium comprises lysate with the one or more enzymes in a concentration of from about 10% (v/v) to about 30% (v/v). In certain embodiments, the cell-medium further comprises the one or more enzymes are present in the range of from about 0.1 μ M to about 5 mM. In certain embodiments, the one or more enzymes are present in the range of from about 0.1 μ M to about 1 mM. In certain embodiments, the one or more enzymes are present in the range of from about 1 μ M to about 100 μ M.

In certain embodiments, the methods of the invention provide a use of one or more cofactors to facilitate the reactions leading to conversion of naringenin to pectolinarigenin. In certain embodiments, wherein the one or more cofactors are present in a concentration of about 1

mM to about 500 mM. In certain embodiments, the one or more cofactors are present in a concentration of about 1 mM to about 200 mM. In certain embodiments, the one or more cofactors are present in a concentration of about 1 mM to about 10 mM.

In certain embodiments, the one or more cofactors for methods of the invention are selected from the group consisting of: 2-oxoglutarate, ascorbic acid, iron(II), FAD, FMN, NADPH, SAM, ATP or any combination thereof. In certain embodiments, the one or more cofactors are 2-oxoglutarate. In certain embodiments, the one or more cofactors are ascorbic acid. In certain embodiments, the one or more cofactors are iron(II). In certain embodiments, the one or more cofactors are heme. In certain embodiments, the one or more cofactors are FAD. In certain embodiments, the one or more cofactors are FMN. In certain embodiments, the one or more cofactors are NADPH. In certain embodiments, the one or more cofactors are s-adenosyl methionine (SAM). In certain embodiments, the one or more cofactors are a divalent metal such as magnesium or manganese. In certain embodiments, the one or more cofactors are ATP.

In certain embodiments, the cell-free reaction for production of polyphenols is conducted for the requisite duration till the desired quantity of the polyphenols are produced in the said reaction. In certain embodiments, the reaction for cell-free production of polyphenols is carried out for a duration of from about 0.1 hours to about 20 hours. In certain embodiments, the reaction for cell-free production of polyphenols is carried out for a duration of from about 0.5 hours to about 20 hours. In certain embodiments, reaction for cell-free production polyphenols is carried out for a duration of from about 1 hours to about 15 hours.

In certain embodiments, the reaction of the cell-free medium is adjusted for an optimal yield of polyphenols to be produced. In certain embodiments, the temperature of the cell-free medium is from about 20 °C to about 40 °C.

In certain embodiments, the reaction is conducted in a bubble column reactor, wherein the one or more enzymes are in a solution. In certain embodiments, the reaction is conducted in a packed bed reactor, wherein the one or more enzymes are immobilized.

In certain aspects, the invention provides A method for bioproduction of pectolinarigenin, the method comprising: providing one or more enzymes, wherein the one or more enzymes result in transformation of one or more substrates to pectolinarigenin optionally through one or more

intermediates. In certain embodiments, one or more substrates are selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof. In certain embodiments, the substrate is apigenin. In certain embodiments, the substrate is scutellarein. In certain embodiments, the substrate is hispidulin.

5 In certain aspects, the method comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates. In certain embodiments, the invention provides a method for cell-free production of pectolinarigenin, wherein the one or more enzymes in a cell-free medium result in
10 transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates.

 In certain aspects, the invention provides a composition for bioproduction of pectolinarigenin, wherein the composition comprises one or more enzymes, wherein the one or more enzymes result in transformation of one or more substrates to pectolinarigenin optionally
15 through one or more intermediates. In certain embodiments, one or more substrates are selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof. In certain embodiments, one or more substrates are selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof. In certain embodiments, the substrate is apigenin. In certain embodiments, the substrate is scutellarein. In certain
20 embodiments, the substrate is hispidulin. In certain embodiments, the composition comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates. In certain embodiments, the composition comprises one or more enzymes in a cell-free medium that results in transformation
25 of the one or more substrates to pectolinarigenin optionally through one or more intermediates.

 In certain aspects, the invention provides a method for production of pectolinarigenin, the method comprises: providing an engineered host cell that comprises one or more genetic modifications, wherein the one or more genetic modifications result in enzymatic transformation of glycerol to pectolinarigenin through one or more intermediates. In certain embodiments, the
30 engineered host cell comprises one or more genetic modifications to enhance the production of naringenin from glycerol the engineered host cell. In certain embodiments, the engineered host

cell comprises one or more genetic modifications to enhance the transformation of naringenin to pectolinarigenin in the engineered host cell through one or more intermediates. In certain embodiments, the one or more genetic modification is expression or overexpression of one or more enzymes. In certain embodiments, one or more enzymes are selected from the group consisting of:
5 FNSI, P450, OMT, and any combination thereof. In certain embodiments, one or more enzymes are selected from the group consisting of expression or overexpression of: FNSI, P450, OMT, ACC, TAL, 4CL, CHS, CHI, and any combination thereof. In certain embodiments, the engineered host cell is *E. coli*.

10 In certain aspects, the invention provides a composition for production of pectolinarigenin, wherein the composition comprises an engineered host cell that comprises one or more genetic modifications, wherein the one or more genetic modifications result in enzymatic transformation of glycerol to pectolinarigenin through one or more intermediates. In certain embodiments, the engineered host cell comprises one or more genetic modifications to enhance the production of
15 naringenin from glycerol the engineered host cell. In certain embodiments, the engineered host cell comprises one or more genetic modifications to enhance the transformation of naringenin to pectolinarigenin in the engineered host cell through one or more intermediates. In certain embodiments, the one or more genetic modification is expression or overexpression of one or more enzymes. In certain embodiments, one or more enzymes are selected from the group consisting of:
20 FNSI, P450, OMT, and any combination thereof. In certain embodiments, one or more enzymes are selected from the group consisting of expression or overexpression of: FNSI, P450, OMT, ACC, TAL, 4CL, CHS, CHI, and any combination thereof. In certain embodiments, the engineered host cell is *E. coli*.

In certain embodiments, the flavone synthase (FNSI) is an enzyme with an amino acid
25 sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

SEQ ID	Accession #	Organism
1	Q7XZQ7.1	<i>Petroselinum crispum</i>
2	NP_001268034.1	<i>Vitis vinifera</i>
3	P41090	<i>Vitaceae</i>

4	Q06942	<i>Rosaceae</i>
5	Q07353	<i>Solanaceae</i>
6	Q9S818	<i>Arabidopsis thaliana</i>
7	Q05963	<i>Asteroideae</i>
8	Q05964	<i>Dianthus caryophyllus</i>
9	Q7XZQ8.1	<i>Petroselinum crispum</i>
10	ABG78791.1	<i>Aethusa cynapium</i>
11	AAX21537.1	<i>Apium graveolens</i>
12	XP_017240664.1	<i>Daucus carota subsp. sativus</i>
13	ABG78790.1	<i>Cuminum cyminum</i>
14	XP_017638014.1	<i>Gossypium arboreum</i>
15	XP_034889682.1	<i>Populus alba</i>

In certain embodiments, flavone hydroxylase cytochrome P450 is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

SEQ ID	Accession #	Organism
16	ASW21051.1	<i>Scutellaria baicalensis</i>
17	KAH6755793.1	<i>Perilla frutescens var. hirtella</i>
18	JX162212	<i>Ocimum basilicum</i>
19	M1KVN4	<i>Mentha piperita</i>
20	XP_051140309.1	<i>Andrographis paniculata</i>
21	XP_047940933.1	<i>Salvia hispanica</i>
22	CAA2982936.1	<i>Olea europaea subsp. europaea</i>
23	AZP01942.1	<i>Panax ginseng</i>
24	O49858	<i>Glycine subgen. Soja</i>
25	OP096437	<i>Lysionotus pauciflorus</i>
26	ASW21050.1	<i>Scutellaria baicalensis</i>

27	KAH6791399.1	<i>Perilla frutescens</i> var. <i>frutescens</i>
28	KAI3451537.1	<i>Paulownia fortunei</i>
29	ASW21052.1	<i>Scutellaria baicalensis</i>
30	KAI3451541.1	<i>Paulownia fortunei</i>

In certain embodiments, O-methyltransferase is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

SEQ ID	Accession #	Organism
31	XP_013456930	<i>Medicago truncatula</i>
32	ARS23163	<i>Plagiochasma appendiculatum</i>
33	APX42106	<i>Plagiochasma appendiculatum</i>
34	C6TAY1.1	<i>Glycine max</i>
35	WLK78566	<i>Salvia plebeia</i>
36	WLK78567	<i>Salvia plebeia</i>
37 - 69		<i>Linea vulgaris</i>

5

The methods of production of pectolinarigenin provided in the invention are beneficial because they provide for an economic and efficient pathway for synthesis of pectolinarigenin. Advantageously, the methods of the invention do not extensive processes for extraction and/or purification of pectolinarigenin from the plants or roots. The methods requiring extraction of the product from plants has complications, such as the processes leading to low yields, a higher number of by-products, and expensive purification and separation protocols.

10

The methods of the current invention beneficially provide cell-based and cell-free approaches for bioproduction of pectolinarigenin. In particular, the product titers for pectolinarigenin produced by the methods of the invention are higher than the product titer produced by any other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least two-fold higher than other

15

known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least five-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least ten-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least hundred-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least five-hundred-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least thousand-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least five-thousand-fold higher than other known methods for production of pectolinarigenin.

In other beneficial aspects of the method of the invention, the methods of the invention provide economically efficient methods of production of pectolinarigenin. Because the methods of the invention also provide for cell-free production of pectolinarigenin, it leads to decreased costs of purification of the reaction products. Moreover, in certain aspects of the invention, the one or more enzymes are included in the same reaction mixture, *i.e.*, the enzymes do not need to be isolated after being expressed in the host organisms. The one or more enzymes could be expressed in the host organisms at the same time and be utilized for the cell-free production without the need of any additional purification and/or separation steps.

In certain other beneficial aspects of the invention, the use of the one or more enzymes for the cell-free production of polyphenols leads to high yield of the product to be produced. The one more enzyme may be further modified to optimize the yield of the product being produced in the cell-free reaction.

In certain aspects, the invention provides compositions for the production of pectolinarigenin from one or more substrates. In certain embodiments, the compositions provided in the invention are compositions suitable for preparing pectolinarigenin from one or more substrates. In certain embodiments, the compositions of the invention comprise engineered host organisms, wherein the engineered host organisms comprise genetic modifications for production of pectolinarigenin from naringenin through one or more intermediates. In certain embodiments,

the compositions of the invention comprise a cell-free medium, which may comprise one or more enzymes, for production of pectolinarigenin from naringenin through one or more intermediates. In certain embodiments, the compositions of the invention comprise a combination of engineered host cells and a cell-free medium for preparation of pectolinarigenin.

5 **V. Brief Description of Drawings**

FIG. 1 provides a description of the modular pathways for conversion of naringenin to pectolinarigenin through one or more intermediates.

FIG. 2 provides a schematic pathway for conversion of naringenin to pectolinarigenin through apigenin, scutellarein, and hispidulin as the intermediates.

10 **FIGS. 3A and 3B** provide HPLC chromatograms for the cell-free production of hispidulin and pectolinarigenin.

FIG. 4 provides HPLC chromatograms for cell production of pectolinarigenin and apigenin from glycerol.

FIG. 5 provides HPLC chromatograms for cell production of pectolinarigenin from
15 hispidulin.

FIG. 6 provides an overview for the enzymatic conversion of glycerol to naringenin.

VI. Detailed Description

 The present application provides compositions and methods for production of
20 pectolinarigenin in a cell-based or cell-free medium, wherein the one or more enzymes result in transformation of one or more substrates to pectolinarigenin. The one or more enzymes may be engineered. The engineered enzyme may be non-naturally occurring.

 The term “non-naturally occurring”, when used in reference to an enzyme is intended to mean that nucleic acids or polypeptides include at least one genetic alteration not normally found
25 in a naturally occurring polypeptide or nucleic acid sequence. Naturally occurring nucleic acids, and polypeptides can be referred to as “wild-type” or “original”. A host cell, organism, or microorganism that includes at least one genetic modification generated by human intervention can also be referred to as “non-naturally occurring”, “engineered”, “genetically engineered,” or “recombinant”.

As used herein, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including,” “includes,” “having,” “has,” “with,” or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

As used herein, “reaction solution” may refer to all components necessary for enzyme-based chemical transformation. This is typically, but not limited to, buffering agent, salts, cofactor, and substrate (starting material).

As used herein, “reaction mixture” may refer to all components from the “reaction solution” plus the enzyme(s) and/or products from the reaction. In some embodiments, the “reaction mixture” may refer to just the reaction solution without any enzymes or reaction products. In some embodiments, “reaction solution” and “reaction mixture” may be used interchangeably.

As used herein, “buffering agents” may refer to chemicals added to water-based solutions that resist changes in pH by the action of acid-base conjugate components.

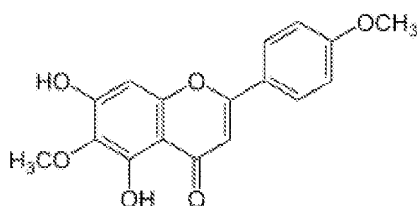
As used herein, “cofactors” may refer to a non-protein chemical compound that may bind to a protein and assist with a biological chemical reaction. Non-limiting examples of cofactors may include but are not limited to NADPH and NADH.

In the cell-free systems described herein, the critical components of the cell, namely cofactors and enzymes, are used in a chemical reaction without cellular components that can directly or indirectly inhibit the desired biochemical reaction. The same enzymes found in plants and other organisms may be created in vivo (typically through protein overexpression in hosts such as bacteria), isolated via chromatography and/or any other methods, and then added into a bioreactor with a substrate (starting material). The enzymes may also be used directly from plants without any isolation. The enzymes transform the substrate in the same way that occurs in the original organism without the organism’s complexity. Additionally, the biochemical reaction may be enhanced by the addition of co-solvents, detergents, or both, which would not be tolerated by, or simply would not work in a whole cell-based manufacturing method. In this way, natural products can be created without the plant, cell, or chemical synthesis.

Pectolinarigenin:

Pectolinarigenin is a flavonoid compound that belongs to the flavone subclass of flavonoids. Flavonoids are a diverse group of phytonutrients found in various fruits, vegetables, and herbs. They are known for their potential health benefits and have been widely studied for their antioxidant, anti-inflammatory, anticancer, and neuroprotective properties.

5 The chemical structure of pectolinarigenin is provided below:



The research regarding the potential applications for pectolinarigenin is still in early stages, and the early data is from in vitro and animal studies. For example, pectolinarigenin is known to have antioxidant, anticancer, anti-inflammatory, and neuroprotective properties.

10 In spite of the promising biological activity, there have been no known economically efficient method for the production of pectolinarigenin. In conventional methods, pectolinarigenin is specifically derived from the plant *Pectis papposa*, which is commonly known as the Guayule plant. This plant is native to the southwestern United States and northern Mexico and has been traditionally used in folk medicine for its medicinal properties. These methods are not cost
15 efficient, and require various steps, including harvesting the plants, preparation of the harvested materials (such as leaves, flowers, or roots), extraction (subjecting the extracted materials to various processes and/or chemicals to bring out pectolinarigenin), and purification (isolating pectolinarigenin from other chemicals). This is an expensive and time-consuming process. The processes involving chemical synthesis of pectolinarigenin are not commercially viable. Thus, a
20 need exists for an economic and efficient process for production of pectolinarigenin.

Production of pectolinarigenin:

Thus, new methods for production of pectolinarigenin are required that are reliable and economically viable. The methods of the invention provides methods and compositions for bioproduction of pectolinarigenin. The methods of the invention may lead to production of
25 pectolinarigenin in a host organism comprising certain genetic modifications. The methods of the

invention also provide cell-free bioproduction of pectolinarigenin from one or more substrates through one or more substrates. The methods of the invention are beneficial because they provide an economically viable method for production of pectolinarigenin. Advantageously, the methods of the invention are also reliable and are not dependent on the available plants for preparations of pectolinarigenin.

Advantageously, the methods of the invention provide a cell-based or cell-free platform for production of pectolinarigenin. The platform provided in the invention is beneficial because it provides multiple pathways for preparation of pectolinarigenin from naringenin. The availability of different pathways for the production of pectolinarigenin is beneficial because it provides an avenue to prepare and isolate one or more intermediates in the biosynthesis pathway. Thus, the platform provided in the invention unlocks the option of preparation of one or more intermediates which might not otherwise be readily accessible for manufacturing. Beneficially, the pathway for production of pectolinarigenin could also be selected based on the availability of various enzymes for that particular reaction pathway. In other words, if an enzyme is unavailable (or not readily available), a different pathway, which does not require that particular enzyme, may be selected for production of pectolinarigenin. Thus, the modularity of the biosynthetic pathway is especially beneficial.

In certain aspects, the invention provides method for bioproduction of pectolinarigenin, the method comprising providing one or more enzymes, wherein the one or more enzymes result in transformation of naringenin to pectolinarigenin through one or more intermediates. The method for production of pectolinarigenin includes cell-based and cell-free methods.

In certain aspects of the invention, the method for production of pectolinarigenin comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of naringenin to pectolinarigenin through one or more intermediates. In certain embodiments, the genetic modifications in the host cell may also include one or more genetic modifications to enhance the production of naringenin. In certain embodiments, engineered host cell comprises genetic modifications to enhance the production of naringenin or any precursor leading to production of naringenin in the engineered host cell. The additional genetic modifications leading to increase in production of naringenin may be beneficial for production of pectolinarigenin because the

naringenin may be used a substrate for production of pectolinarigenin. The enhanced amount of naringenin would lead to higher yield of pectolinarigenin.

In certain aspects of the invention, the method for production of pectolinarigenin is a cell-free production of pectolinarigenin, wherein the one or more enzymes in a cell-free medium result in transformation of naringenin to pectolinarigenin through one or more intermediates. The ingredients of the components in the cell-free reaction may be generated in a host cell. For example, the substrate, such as naringenin, may be generated in a cell and the subsequent conversion to pectolinarigenin may be conducted in a cell-free medium. In certain embodiments, the one or more intermediates generated in conversion of naringenin to pectolinarigenin may be generated in an engineered host cell, and those one or more intermediates may be subsequently converted to pectolinarigenin by one or more enzymes in a cell-free medium.

In certain aspects, the method comprises a combination of: (i) an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of naringenin to pectolinarigenin or one or more intermediates, and (ii) cell-free production comprising one or more enzymes resulting in transformation of naringenin or a substrate to pectolinarigenin or one or more intermediates for synthesis of pectolinarigenin. In certain embodiments, engineered host cell comprises genetic modifications to enhance the production of naringenin or any precursor leading to production of naringenin in the engineered host cell.

In certain embodiments of the invention, the one or more intermediates are selected from a group consisting of: apigenin, scutellarein, hispidulin, isosakuranetin, carthamidin, 6-methoxycarthamidin, scutellarein, 4-methoxycarthamidin, acacetin, 4',6-methoxynaringenin, 4'-methoxyscutellarein, and any combination thereof. **FIG. 1** provides exemplary schematic pathways for conversion of naringenin to pectolinarigenin. As evident from **FIG. 1**, the methods of the invention provide a modular pathway for conversion of naringenin to pectolinarigenin. The pathway selected for production of pectolinarigenin will determine the identity of the one or more intermediates selected in the process. Each individual segment of the pathway may be controlled by the enzymes selected for that conversion. In certain embodiments, the methods of the invention provide the use of one or more enzymes to mediate the conversion of the starting material to one or more intermediates, or pectolinarigenin. In certain embodiments, the one or more enzymes

selected from the group consisting of: flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), O-methyltransferase (OMT), and any combination thereof.

For example, in certain embodiments, the intermediates are apigenin, scutellarein, and hispidulin. In certain embodiments, the method provides a pathway, wherein, naringenin is converted to apigenin; apigenin is converted to scutellarein; scutellarein is converted to hispidulin; and hispidulin is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. For example, as demonstrated in **FIG. 2**, the pathway provided comprises flavone synthase (FNSI) mediated conversion of naringenin to apigenin; flavone hydroxylase cytochrome P450 (P450) mediated conversion of apigenin to scutellarein; O-methyltransferase (OMT) mediated conversion of scutellarein to hispidulin; and O-methyltransferase (OMT) mediated conversion of hispidulin to pectolinarigenin. Any of these aforementioned steps may be conducted either in an engineered host cell or a cell-free medium.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are apigenin, scutellarein, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to apigenin; apigenin is converted to scutellarein; scutellarein is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are apigenin, scutellarein, and hispidulin. The invention provides that naringenin is converted to apigenin; apigenin is converted to scutellarein; scutellarein is converted to hispidulin; hispidulin is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are apigenin, acacetin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to apigenin; apigenin is converted to acacetin; acacetin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In

certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are isosakuranetin, 4'-methoxycarthamidin, and 4',6-methoxynaringenin. The invention provides that naringenin is converted to isosakuranetin; isosakuranetin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4',6-methoxynaringenin; 4',6-methoxynaringenin is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

10 In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are isosakuranetin, 4'-methoxycarthamidin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to isosakuranetin; isosakuranetin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain
15 embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are isosakuranetin, acacetin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to isosakuranetin; isosakuranetin is converted to
20 acacetin; acacetin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of
25 naringenin to pectolinarigenin are carthamidin, 6-methoxycarthamidin, and hispidulin. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to 6-methoxycarthamidin; 6-methoxycarthamidin is converted to hispidulin; hispidulin is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or

more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 6-methoxycarthamidin, and 4',6-methoxynaringenin. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to 6-methoxycarthamidin; 6-methoxycarthamidin is converted to 4',6-methoxynaringenin; 4',6-methoxynaringenin is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

10 In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 6-methoxycarthamidin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to 6-methoxycarthamidin; 6-methoxycarthamidin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain
15 embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, scutellarein, and hispidulin. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to scutellarein; scutellarein is converted to hispidulin; hispidulin is converted to pectolinarigenin. In certain
20 embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, scutellarein, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to scutellarein; scutellarein is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 4'-methoxycarthamidin, and 4',6-methoxynaringenin. The invention provides that naringenin is converted to carthamidin; carthamidin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4',6-methoxynaringenin; 4',6-methoxynaringenin is converted to pectolinarigenin. In certain
5 embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the one or more intermediates involved in conversion of naringenin to pectolinarigenin are carthamidin, 4'-methoxycarthamidin, and 4'-methoxyscutellarein. The invention provides that naringenin is converted to carthamidin;
10 carthamidin is converted to 4'-methoxycarthamidin; 4'-methoxycarthamidin is converted to 4'-methoxyscutellarein; 4'-methoxyscutellarein is converted to pectolinarigenin. In certain embodiments, one or more these steps may be conducted by one or more enzymes. In certain embodiments, one or more these steps may be conducted by one or more enzymes.

In certain embodiments, the invention provides method of production of pectolinarigenin comprises engineered host cells, wherein the host cells comprise one or more genetic modifications. In certain embodiments, the engineered host cell could be prepared from yeast, bacteria, or mammalian cells. In certain embodiments, the engineered host cell is *E. coli*. In certain
15 embodiments, the one or more enzymes are introduced in the host organism by integration into genome of the host organism or on a plasmid. In certain embodiments, the one or more genetic modifications in the engineered host cell are any of the modifications described above. For example, the one or more genetic modification may be overexpression or endogenous enzymes or expression of a variant of an enzyme involved in the conversion of naringenin to pectolinarigenin. The genetic modifications include, without limitation, overexpression or expression of a variant of
20 : flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), O-methyltransferase (OMT), and/or any combination thereof.

In certain embodiments, the one or more genetic modifications in the host cell may include any genetic modification to increase the availability of naringenin. For example, the one or more genetic modifications in an engineered host cell may be selected from a group consisting of: acetyl-CoA carboxylase (ACC), tyrosine ammonia-lyase (TAL), 4-coumarate-CoA ligase (4CL),
30

chalcone synthase (CHS), chalcone isomerase (CHI), and/or any combination thereof. In certain embodiments, the genetic modifications may comprise expressing a variant of one or more of these enzymes. In certain embodiments, the one or more genetic modifications to increase the production of naringenin are provided in PCT/US2022/024591, which is incorporated in its entirety by reference.

In certain aspects, the invention provides methods of cell-free production of pectolinarigenin. The cell-free medium for the conversion of naringenin to pectolinarigenin is a cell lysate. In certain embodiments, the cell lysate is a cell lysate from cells from a host organism expressing the one or more enzymes. The one or more enzymes may be the enzymes involved in conversion of naringenin to pectolinarigenin. In certain embodiments, the host organisms expressing the one or more enzymes are cultured until a pre-determined biomass is achieved to produce the requisite quantity of the one or more enzymes. In certain embodiments, the method of the invention further comprising lysing cells followed by removal of cell debris to generate a cell lysate for use in the cell-free medium for cell-free production of pectolinarigenin.

In certain embodiments, cell-free medium comprises: buffer, magnesium chloride, one or more substrates, one or more cofactors, the one or more enzymes, and/or water.

In certain embodiments, the buffer in the cell-free medium maintains the pH of the reaction mixture in the optimal range. In certain embodiments, the pH of the reaction medium is from about 5.5 to about 10. In certain embodiments, the pH of the reaction medium is from about 6 to about 9.5. In certain embodiments, the pH of cell-free medium is from about 6 to about 9. In certain embodiments, the pH of cell-free medium is from about 6 to about 8. In certain embodiments, the concentration of the buffer in the cell-free medium is from about 1 mM to about 1000 mM. In certain embodiments, the concentration of the buffer is from about 2.5 mM to about 750 mM. In certain embodiments, the concentration of the buffer is from about 5 mM to about 100 mM. In certain embodiments, the buffer concentration is from about 20 mM to about 100 mM. In certain embodiments, the buffer is a phosphate buffer.

In certain embodiments, the method of the invention comprises conversion of one or more substrates to pectolinarigenin. In certain embodiments, the one or more substrates are present in a concentration of from about 1 mM to about 500 mM. In certain embodiments, the one or more

substrates are present in a concentration of from about 1 mM to about 200 mM. In certain embodiments, the one or more substrates are present in a concentration of from about 10 mM to about 100 mM. In certain embodiments, the substrate used for production of pectolinarigenin is naringenin.

5 In certain embodiments, the cell-free medium comprises magnesium chloride. The magnesium chloride may be present in the range of from about 1 mM to about 50 mM. In certain embodiments, the cell-free medium comprises magnesium chloride in the range of from about 1 mM to about 25 mM. In certain embodiments, the cell-free medium comprises magnesium chloride in the range of from about 1 mM to about 20 mM. In certain embodiments, the cell-free medium
10 comprises magnesium chloride in the range of from about 5 mM to about 25 mM.

 In certain embodiments, the cell-free medium comprises the lysate from host microbe with the one or more enzymes for the cell-free reaction medium. In certain embodiments, the cell-free medium comprises lysate with the one or more enzymes in a concentration of from about 1% (v/v) to about 50% (v/v). In certain embodiments, the cell-free medium comprises lysate with the one
15 or more enzymes in a concentration of from about 5% (v/v) to about 40% (v/v). In certain embodiments, the cell-free medium comprises lysate with the one or more enzymes in a concentration of from about 10% (v/v) to about 30% (v/v). In certain embodiments, the cell-medium further comprises the one or more enzymes are present in the range of from about 0.1 μ M to about 5 mM. In certain embodiments, the one or more enzymes are present in the range of from
20 about 0.1 μ M to about 1 mM. In certain embodiments, the one or more enzymes are present in the range of from about 1 μ M to about 100 μ M.

 In certain embodiments, the methods of the invention provide a use of one or more cofactors to facilitate the reactions leading to conversion of naringenin to pectolinarigenin. In certain embodiments, wherein the one or more cofactors are present in a concentration of about 1
25 mM to about 500 mM. In certain embodiments, the one or more cofactors are present in a concentration of about 1 mM to about 200 mM. In certain embodiments, the one or more cofactors are present in a concentration of about 1 mM to about 10 mM.

 In certain embodiments, the cell-free reaction for production of polyphenols is conducted for the requisite duration till the desired quantity of the polyphenols are produced in the said

reaction. In certain embodiments, the reaction for cell-free production of polyphenols is carried out for a duration of from about 0.1 hours to about 20 hours. In certain embodiments, the reaction for cell-free production of polyphenols is carried out for a duration of from about 0.5 hours to about 20 hours. In certain embodiments, reaction for cell-free production polyphenols is carried out for a duration of from about 1 hours to about 15 hours.

In certain embodiments, the reaction of the cell-free medium is adjusted for an optimal yield of polyphenols to be produced. In certain embodiments, the temperature of the cell-free medium is from about 20 °C to about 40 °C.

As described above, the invention provides that all or a certain number of steps for the conversion of a substrate to naringenin may be carried out in an engineered host organism. The host organism may be a bacteria, yeast, or a mammalian cell. In certain embodiments, the engineered host organism are incubated in a culture medium. The methods can further include recovering pectolinarigenin, or one of the intermediates leading to formation of pectolinarigenin, from the culture medium, whole culture, or cells.

The culture comprises cells engineered for the production of pectolinarigenin in a culture medium. In various embodiments the engineered cells can be prokaryotic or eukaryotic cells. The culture medium includes at least one carbon source that is also an energy source. Exemplary carbon sources include glucose, glycerol, sucrose, fructose, and xylose. Such carbon sources may be purified or crude, including a biomass comprising glycerol, for example, crude glycerol produced as a byproduct of biodiesel production from corn waste. In addition, the culture medium can include one or more other carbon sources or compounds to increase precursor generation or cofactor supply such as, without limitation, tyrosine, phenylalanine, coumaric acid, acetate, malonate, succinate, glycine, bicarbonate, biotin, naringenin, 5-aminolevulinic acid, thiamine, pantothenate, alpha-ketoglutarate, and ascorbate. In some embodiments, tyrosine and coumaric acid are provided in the culture medium. In some embodiments, tyrosine, alpha-ketoglutarate, 5-aminolevulinic acid, and ascorbate are provided in the culture medium.

Culture conditions can include aerobic, microaerobic or any combination alternating aerobic/microaerobic growth conditions. Further, culture conditions can include shake flasks, fermentation, and other large scale culture procedures. An exemplary growth condition for

achieving a flavonoid product include aerobic or microaerobic fermentation conditions. The culture conditions can be scaled up and grown continuously for manufacturing flavonoid product. Exemplary growth procedures include, for example, fed-batch fermentation and batch separation. In an exemplary batch fermentation protocol, the cells are grown in a bioreactor that is well
5 controlled for growth temperature, oxygen, pH, carbon sources, and other compounds. The desired temperature can be from, for example, 20-37 °C, depending on the growth characteristics of the production cells and desired conditions for the fermented products. The pH of the bioreactor can be controlled to range from 5-8 or left uncontrolled in some cases. The batch fermentation period can last in the range of several hours to several days, for examples, 8 to 96 hours. Upon completion
10 of the cultivation period, the fermenter contents can be passed through a cell separation unit to remove cells and cell debris. The cells can be lysed or disrupted enzymatically or chemically prior to or after separation of cells from the fermentation broth, as desired, in order to release additional product. In certain embodiments, the engineered host organisms, and their genetic modifications are provided in PCT/US2022/024591, which is incorporated in its entirety by reference.

15 In certain embodiments, the reaction is conducted in a bubble column reactor, wherein the one or more enzymes are in a solution. In certain embodiments, the reaction is conducted in a packed bed reactor, wherein the one or more enzymes are immobilized.

In certain aspects, the methods provided in the invention may be carried out in any reactor suitable for carrying out the cell-free production of pectolinarigenin. In certain embodiments, the
20 reaction for cell-free production of pectolinarigenin is conducted in a bubble column reactor/bioreactor. In certain embodiments, in the bubble column reactor/bioreactor, the one or more enzymes involved in cell-free production of pectolinarigenin are in a solution. In certain embodiments, the reaction for cell-free production of pectolinarigenin is conducted in a bubble column reactor/bioreactor comprises the lysate from the host organism. In certain embodiments, it
25 is advantageous to use the bubble column reactor/bioreactor for cell-free production of pectolinarigenin when the reaction mixture involves the lysate (or lysate with cellular debris removed) from the host cell organisms in which the one or more enzymes responsible for cell-free production of pectolinarigenin were utilized. In certain embodiments, the reaction for cell-free production of pectolinarigenin is conducted in a packed bed reactor/bioreactor. In certain
30 embodiments, the one or more enzymes are immobilized in the packed bed reactor/bioreactor. The

packed bed reactors/bioreactors are preferred for the purified enzymes playing a role in cell-free production of pectolinarigenin. In certain embodiments, the one or more enzymes may be immobilized in a single reactor/bioreactor. In certain other embodiments, the one or more enzymes may be immobilized in different reactors/bioreactors, wherein these reactors/bioreactors are linked sequentially. In certain embodiments, the bioreactor system provided in PCT/US2021/064049, incorporated by reference in its entirety.

In certain aspects, the invention provides A method for bioproduction of pectolinarigenin, the method comprising: providing one or more enzymes, wherein the one or more enzymes result in transformation of one or more substrates to pectolinarigenin optionally through one or more intermediates. In certain embodiments, one or more substrates are selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof. In certain embodiments, the substrate is apigenin. In certain embodiments, the substrate is scutellarein. In certain embodiments, the substrate is hispidulin.

In certain aspects, the method comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates. In certain embodiments, the invention provides a method for cell-free production of pectolinarigenin, wherein the one or more enzymes in a cell-free medium result in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates.

In certain aspects, the invention provides a composition for bioproduction of pectolinarigenin, wherein the composition comprises one or more enzymes, wherein the one or more enzymes result in transformation of one or more substrates to pectolinarigenin optionally through one or more intermediates. In certain embodiments, one or more substrates are selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof. In certain embodiments, one or more substrates are selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof. In certain embodiments, the substrate is apigenin. In certain embodiments, the substrate is scutellarein. In certain embodiments, the substrate is hispidulin. In certain embodiments, the composition comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications

for expression of one or more enzymes resulting in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates. In certain embodiments, the composition comprises one or more enzymes in a cell-free medium that results in transformation of the one or more substrates to pectolinarigenin optionally through one or more intermediates.

5 In certain aspects, the invention provides a method for production of pectolinarigenin, the method comprises: providing an engineered host cell that comprises one or more genetic modifications, wherein the one or more genetic modifications result in enzymatic transformation of glycerol to pectolinarigenin through one or more intermediates. In certain embodiments, the engineered host cell comprises one or more genetic modifications to enhance the production of
10 naringenin from glycerol the engineered host cell. In certain embodiments, the engineered host cell comprises one or more genetic modifications to enhance the transformation of naringenin to pectolinarigenin in the engineered host cell through one or more intermediates. In certain embodiments, the one or more genetic modification is expression or overexpression of one or more enzymes. In certain embodiments, one or more enzymes are selected from the group consisting of:
15 FNSI, P450, OMT, and any combination thereof. In certain embodiments, one or more enzymes are selected from the group consisting of expression or overexpression of: FNSI, P450, OMT, ACC, TAL, 4CL, CHS, CHI, and any combination thereof. In certain embodiments, the engineered host cell is *E. coli*.

In certain aspects, the invention provides a composition for production of pectolinarigenin,
20 wherein the composition comprises an engineered host cell that comprises one or more genetic modifications, wherein the one or more genetic modifications result in enzymatic transformation of glycerol to pectolinarigenin through one or more intermediates. In certain embodiments, the engineered host cell comprises one or more genetic modifications to enhance the production of naringenin from glycerol the engineered host cell. In certain embodiments, the engineered host
25 cell comprises one or more genetic modifications to enhance the transformation of naringenin to pectolinarigenin in the engineered host cell through one or more intermediates. In certain embodiments, the one or more genetic modification is expression or overexpression of one or more enzymes. In certain embodiments, one or more enzymes are selected from the group consisting of: FNSI, P450, OMT, and any combination thereof. In certain embodiments, one or more enzymes
30 are selected from the group consisting of expression or overexpression of: FNSI, P450, OMT,

ACC, TAL, 4CL, CHS, CHI, and any combination thereof. In certain embodiments, the engineered host cell is *E. coli*.

The methods provided in the invention are advantageous over other conventional methods of production of polyphenols. In certain embodiments, the methods of the invention provide cell-free production of polyphenols. Because the methods of the invention are conducted in cell-free medium, they provide significant economic efficiency by reducing the cost of production of polyphenols in other conventional methods. In certain embodiments, because the reaction for production of polyphenols is conducted from the lysates from the host organisms expressing the one or more enzymes involved in the reaction, the methods of the invention are cost-efficient. In certain embodiments, the methods of the invention do not involve the purification of the one or more enzymes. Because purification of individual enzymes is not required in the methods of the invention, provides further economic efficiency by reducing the cost that would have been otherwise required in purifying individual enzymes.

In some embodiments, the isolated pectolinarigenin has a purity of about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 99%, or about 100%.

In other embodiments, the isolated pectolinarigenin has a purity of from about 10% to 95%, or from about 10% to 90%, or from about 10% to 80% or from about 10% to 70%, or from about 10% to 60%, or from about 10% to 50%, or from about 10% to 40%, or from about 20% to 95%, or from about 20% to 90%, or from about 20% to 80% or from about 20% to 70%, or from about 20% to 60%, or from about 20% to 50%, or from about 20% to 40%, or from about 50% to 95%, or from about 50% to 90%, or from about 50% to 80% or from about 50% to 70%, or from about 50% to 60%.

In certain embodiments, the flavone synthase is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

SEQ ID	Accession #	Organism
1	Q7XZQ7.1	<i>Petroselinum crispum</i>
2	NP_001268034.1	<i>Vitis vinifera</i>

3	P41090	<i>Vitaceae</i>
4	Q06942	<i>Rosaceae</i>
5	Q07353	<i>Solanaceae</i>
6	Q9S818	<i>Arabidopsis thaliana</i>
7	Q05963	<i>Asteroideae</i>
8	Q05964	<i>Dianthus caryophyllus</i>
9	Q7XZQ8.1	<i>Petroselinum crispum</i>
10	ABG78791.1	<i>Aethusa cynapium</i>
11	AAX21537.1	<i>Apium graveolens</i>
12	XP_017240664.1	<i>Daucus carota subsp. sativus</i>
13	ABG78790.1	<i>Cuminum cyminum</i>
14	XP_017638014.1	<i>Gossypium arboreum</i>
15	XP_034889682.1	<i>Populus alba</i>

In certain embodiments, flavone hydroxylase cytochrome P450 is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

SEQ ID	Accession #	Organism
16	ASW21051.1	<i>Scutellaria baicalensis</i>
17	KAH6755793.1	<i>Perilla frutescens</i> var. <i>hirtella</i>
18	JX162212	<i>Ocimum basilicum</i>
19	M1KVN4	<i>Mentha piperita</i>
20	XP_051140309.1	<i>Andrographis paniculata</i>
21	XP_047940933.1	<i>Salvia hispanica</i>
22	CAA2982936.1	<i>Olea europaea</i> subsp. <i>europaea</i>
23	AZP01942.1	<i>Panax ginseng</i>
24	O49858	<i>Glycine</i> subgen. <i>Soja</i>
25	OP096437	<i>Lysionotus pauciflorus</i>
26	ASW21050.1	<i>Scutellaria baicalensis</i>

27	KAH6791399.1	<i>Perilla frutescens</i> var. <i>frutescens</i>
28	KAI3451537.1	<i>Paulownia fortunei</i>
29	ASW21052.1	<i>Scutellaria baicalensis</i>
30	KAI3451541.1	<i>Paulownia fortunei</i>

In certain embodiments, O-methyltransferase is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

SEQ ID	Accession #	Organism
31	XP_013456930	<i>Medicago truncatula</i>
32	ARS23163	<i>Plagiochasma appendiculatum</i>
33	APX42106	<i>Plagiochasma appendiculatum</i>
34	C6TAY1.1	<i>Glycine max</i>
35	WLK78566	<i>Salvia plebeia</i>
36	WLK78567	<i>Salvia plebeia</i>
37-69		<i>Linea vulgaris</i>

5

In certain embodiments, acetyl-CoA carboxylase (ACC) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

Organism	GenBank Accession Number
<i>Lipomyces starkeyi</i>	AJT60321.1
<i>Rhodotorula toruloides</i>	GEM08739.1
<i>Ustilago maydis</i>	XP_011390921.1
<i>Mucor circinelloides</i>	EPB82652.1
<i>Kalaharituber pfeilii</i>	KAF8466702.1
<i>Aspergillus fumigatus</i>	KEY77072.1
<i>Rhodotorula diobovata</i>	TNY18634.1

<i>Leucosporidium creatinivorum</i>	ORY74050.1
<i>Microbotryum intermedium</i>	SCV70467.1
<i>Mixia osmundae</i>	GAA98306.1
<i>Puccinia graminis</i>	KAA1079218.1
<i>Suillus occidentalis</i>	KAG1764021.1
<i>Gymnopilus junonius</i>	KAF8909366.1

In certain preferred embodiments, acetyl-CoA carboxylase (ACC) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to the enzyme provided in SEQ ID NO: 70.

- 5 In certain embodiments, tyrosine ammonia lyase (TAL) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

Organism	GenBank Accession Number
<i>Rhodotorula glutini</i>	AGZ04575.1
<i>Flavobacterium johnsoniae</i>	WP_012023194.1
<i>Herpetosiphon aurantiacus</i>	ABX02653.1
<i>Rhodobacter capsulatus</i>	ADE83766.1
<i>Saccharothrix espanaensis</i>	AKE50820.1
<i>Trichosporon cutaneum</i>	AKE50834.1

- 10 In certain preferred embodiments, tyrosine ammonia lyase (TAL) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to the enzyme provided in SEQ ID NO: 71.

In certain embodiments, 4-coumarate-CoA ligase (4CL) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

Organism	GenBank Accession Number
<i>Petroselinum crispum</i>	CAA31697.1
<i>Camellia sinensis</i>	ASU87409.1
<i>Capsicum annuum</i>	KAF3620173.1
<i>Castanea mollissima</i>	KAF3954751.1
<i>Daucus carota</i>	AIT52344.1
<i>Gymura bicolor</i>	BAJ17664.1
<i>Ipomoea purpurea</i>	AHJ60263.1
<i>Lonicera japonica</i>	AGE10594.1
<i>Lycium chinense</i>	QDL52638.1
<i>Nelumbo nucifera</i>	XP_010265453.1
<i>Nyssa sinensis</i>	KAA8540582.1
<i>Solanum lycopersicum</i>	NP_001333770.1
<i>Striga asiatica</i>	GER48539.1

In certain preferred embodiments, 4-coumarate-CoA ligase (4CL) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to the enzyme provided in SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, and/or SEQ ID NO: 76.

In certain embodiments, chalcone synthase (CHS) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

Organism	GenBank Accession Number
<i>Petunia hybrida</i>	AAF60297.1
<i>Acer palmatum</i>	AWN08245.1
<i>Callistephus chinensis</i>	CAA91930.1
<i>Camellia japonica</i>	BAI66465.1
<i>Capsicum annuum</i>	XP_016566084.1

<i>Coffea arabica</i>	XP_027118978.1
<i>Curcuma alismatifolia</i>	ADP08987.1
<i>Dendrobium catenatum</i>	ALE71934.1
<i>Garcinia mangostana</i>	ACM62742.1
<i>Lochroma calycinum</i>	AIY22758.1
<i>Iris germanica</i>	BAE53636.1
<i>Lilium speciosum</i>	BAE79201.1
<i>Lonicera caerulea</i>	ALU09326.1
<i>Lycium ruthenicum</i>	ATB56297.1
<i>Magnolia liliiflora</i>	AHJ60259.1
<i>Matthiola incana</i>	BBM96372.1
<i>Morus alba</i> var. <i>multicaulis</i>	AHL83549.1
<i>Nelumbo nucifera</i>	NP_001305084.1
<i>Nyssa sinensis</i>	KAA8548459.1
<i>Paeonia lactiflora</i>	AEK70334.1
<i>Panax notoginseng</i>	QKV26463.1
<i>Ranunculus asiaticus</i>	AYV99476.1
<i>Rosa chinensis</i>	AEC13058.1
<i>Theobroma cacao</i>	XP_007032052.2

In certain preferred embodiments, chalcone synthase (CHS) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to the enzyme provided in SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, and/or SEQ ID NO: 81.

In certain embodiments, chalcone isomerase (CHI) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to any one of enzymes selected from the group consisting of:

Organism	GenBank Accession Number
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<i>Medicago sativa</i>	AGZ04578.1
<i>Dendrobium hybrid cultivar</i>	AGY46120.1
<i>Abrus precatorius</i>	XP_027366189.1
<i>Antirrhinum majus</i>	BAO32070.1
<i>Arachis duranensis</i>	XP_015942246.1
<i>Astragalus membranaceus</i>	ATY39974.1
<i>Camellia sinensis</i>	XP_028119616.1
<i>Castanea mollissima</i>	KAF3958409.1
<i>Cephalotus follicularis</i>	GAV77263.1
<i>Clarkia gracilis subsp. sonomensis</i>	QPF47150.1
<i>Dianthus caryophyllus</i>	CAA91931.1
<i>Glycyrrhiza uralensis</i>	AXO59749.1
<i>Handroanthus impetiginosus</i>	PIN05040.1
<i>Lotus japonicus</i>	CAD69022.1
<i>Morus alba</i>	AFM29131.1
<i>Phaseolus vulgaris</i>	XP_007142690.1
<i>Punica granatum</i>	ANB66204.1
<i>Rhodamnia argentea</i>	XP_030524476.1
<i>Spatholobus suberectus</i>	TKY50621.1
<i>Trifolium subterraneum</i>	GAU12132.1

In certain preferred embodiments, chalcone isomerase (CHI) is an enzyme with an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or completely identical to the enzyme provided in SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, and/or SEQ ID NO: 86.

The methods of production of pectolinarigenin provided in the invention are beneficial because they provide for an economic and efficient pathway for synthesis of pectolinarigenin. Advantageously, the methods of the invention do not extensive processes for extraction and/or

purification of pectolinarigenin from the plants or roots. The methods requiring extraction of the product from plants has complications, such as the processes leading to low yields, a higher number of by-products, and expensive purification and separation protocols.

The methods of the current invention beneficially provide cell-based and cell-free approaches for bioproduction of pectolinarigenin. In particular, the product titers for pectolinarigenin produced by the methods of the invention are higher than the product titer produced by any other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least two-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least five-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least ten-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least hundred-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least five-hundred-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least thousand-fold higher than other known methods for production of pectolinarigenin. In certain embodiments, the product titers produced by methods of the invention are at least five-thousand-fold higher than other known methods for production of pectolinarigenin.

In other beneficial aspects of the method of the invention, the methods of the invention provide economically efficient methods of production of pectolinarigenin. Because the methods of the invention also provide for cell-free production of pectolinarigenin, it leads to decreased costs of purification of the reaction products. Moreover, in certain aspects of the invention, the one or more enzymes are included in the same reaction mixture, *i.e.*, the enzymes do not need to be isolated after being expressed in the host organisms. The one or more enzymes could be expressed in the host organisms at the same time and be utilized for the cell-free production without the need of any additional purification and/or separation steps.

In certain other beneficial aspects of the invention, the use of the one or more enzymes for the cell-free production of polyphenols leads to high yield of the product to be produced. The one

more enzyme may be further modified to optimize the yield of the product being produced in the cell-free reaction.

In certain aspects, the invention provides compositions for the production of pectolinarigenin from one or more substrates. In certain embodiments, the compositions provided in the invention are compositions suitable for preparing pectolinarigenin from one or more substrates. In certain embodiments, the compositions of the invention comprise engineered host organisms, wherein the engineered host organisms comprise genetic modifications for production of pectolinarigenin from naringenin through one or more intermediates. In certain embodiments, the compositions of the invention comprise a cell-free medium, which may comprise one or more enzymes, for production of pectolinarigenin from naringenin through one or more intermediates. In certain embodiments, the compositions of the invention comprise a combination of engineered host cells and a cell-free medium for preparation of pectolinarigenin.

VII. Examples

Example 1: Cell-Free bioproduction of pectolinarigenin:

The general reaction conditions for cell-free production of pectolinarigenin are provided below. The substrate can be naringenin, or any of the other compounds shown in **FIG. 1**. The enzymes can be any combination of one or more flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), and O-methyltransferase (OMT). Generally, enzymes were expressed from a pET28 vector in BL21-DE3 cells. Starter cultures were incubated overnight at 37 °C, and then diluted 1:100 in TB. After incubation at 37 °C until OD₆₀₀ = 0.8 to 1.2, cultures were induced with IPTG (0.1 to 1 mM) and incubated at 20 to 30 °C overnight. Cultures were then centrifuged and the supernatant discarded. Cells were resuspended in lysis buffer (50 mM sodium phosphate pH 7.5, 300 mM sodium chloride, and 20 mM imidazole). Resuspended cells were sonicated and centrifuged to generate clarified lysate.

Component	Working Range
Buffer	pH 6-10, 50-300 mM
MgCl ₂	1-50 mM
Substrate(s)	1-300 mM

Cofactor(s)	1-500 mM
Enzyme(s)	1-1000 μ M, or 1 to 75% lysate by volume
Time	0.1h-20h
Temperature	20-40 °C

Cell-free conversion of scutellarein to hispidulin: Reactions were performed by combining 200 mM Tris-HCl pH 7.5, 2 mM DTT (dithiothreitol), 4 mM magnesium chloride, 0.5 mM SAM (S-adenosyl methionine), 50% v/v lysate of cells expressing an OMT enzyme (SEQ ID 31 to 69), and
5 0.2 mM scutellarein. Reactions were incubated for 1 hour at 30 °C and then quenched using an equal volume of methanol and filtered. Samples were analyzed by HPLC, demonstrating production of up to 79 μ M hispidulin.

Cell-free conversion of hispidulin to pectolinarigenin: Reactions were performed by combining 25 mM HEPES pH 7.5, 5 mM magnesium chloride, 0.2 mM SAM (S-adenosyl methionine), 0.5
10 mM NAD⁺, 25% v/v lysate of cells expressing an OMT enzyme (SEQ ID 31 to 69), 20% v/v lysate of cells expressing an SAHH enzyme (S-adenosyl homocysteine hydrolase, EC 3.13.2.1), and 0.3 mM hispidulin. Reactions were incubated overnight at 25 °C and then quenched using an equal volume of methanol and filtered. Samples were analyzed by HPLC, demonstrating production of up to 130 μ M pectolinarigenin.

FIG. 3 provides HPLC chromatograms for the formation of hispidulin and pectolinarigenin. **FIG. 3A** provides the HPLC plot demonstrating the production of hispidulin from scutellarein. **FIG. 3B** provides the HPLC plot demonstrating the production of pectolinarigenin from hispidulin.

Example 2: Cellular bioproduction of pectolinarigenin: Cell-based production of
20 **pectolinarigenin from glycerol:** An *E. coli* cell derived from MG1655 was engineered to produce naringenin. The cells may further comprise the genetic modifications comprising overexpression of ACC, TAL, 4CL, CHS, and CHI. To this strain, flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), and two O-methyltransferase (OMT) were added on the chromosome, to produce pectolinarigenin when glycerol was supplied in the culture medium. Cells of an OD 2.0

were cultured in a 48-well plate at 30 degrees for 22 hours with a shaking speed of 600 RPM in minimal medium supplied with 2% glycerol, trace elements, 65 mg/L 5-aminoleuvinic acid, 0.1 mM ferrous sulfate, 0.1 mM 2-oxoglutarate, and 2.5 mM ascorbic acid. Cell cultures were extracted with 50% (v/v) methanol and centrifuged for 15 minutes. The supernatant was analyzed for pectolinarigenin and pathway intermediates by HPLC. **FIG. 4** shows the HPLC chromatograms at 288nm of (a) standards, (b) starting strain producing NAR with titers up to 440 μ M, (c) NAR-producing strain with an FNSI to make apigenin at a titer of up to 210 μ M with 152 μ M NAR leftover, and (d) NAR-producing strain with an FNSI, a P450 and two OMTs to make the final product pectolinarigenin at a titer of up to 30 μ M, with acacetin produced as a byproduct at 38 μ M.

Cell production of apigenin from glycerol: An *E. coli* cell derived from MG1655 was engineered to produce naringenin. The cells optionally further comprise the genetic modifications comprising overexpression of ACC, TAL, 4CL, CHS, and CHI. To this cell, FNSI was added on the chromosome to produce apigenin when glycerol was supplied in the culture medium. Cells of an OD 2.0 were cultured in a 48-well plate at 30 degrees for 22 hours with a shaking speed of 600 RPM in minimal medium supplied with 2% glycerol, trace elements, 65 mg/L 5-aminoleuvinic acid, 0.1 mM ferrous sulfate, 0.1 mM 2-oxoglutarate, and 2.5 mM ascorbic acid. Cell cultures were extracted with 50% (v/v) methanol and centrifuged for 15 minutes. The supernatant was analyzed for apigenin and pathway intermediates by HPLC. **FIG. 4** shows the HPLC chromatograms at 288nm of (a) standards, (b) starting strain producing NAR with titers up to 440 μ M, (c) NAR-producing strain with an FNSI to make apigenin at a titer of up to 210 μ M with 152 μ M NAR leftover, and (d) NAR-producing strain with an FNSI, a P450 and two OMTs to make the final product pectolinarigenin at a titer of up to 30 μ M, with acacetin produced as a byproduct at 38 μ M.

Cell production of pectolinarigenin from hispidulin: An *E. coli* BL21 cell was engineered to express an OMT. To this, hispidulin was fed to the culture. Cells of an OD 2.0 were cultured in a 48-well plate at 30 degrees for 22 hours with a shaking speed of 600 RPM in minimal medium supplied with 2% glycerol, trace elements and 500 μ M of hispidulin. Cell cultures were extracted with 50% (v/v) methanol and centrifuged for 15 minutes. The supernatant was analyzed for pectolinarigenin and pathway intermediates by HPLC. **FIG. 5** shows the HPLC chromatograms

at 330 nm of (a) PNAR standard, (b) hispidulin standard, (c) BL21 cells, and (d) BL21 cells with OMT to produce PNAR at a titer up to 250 μ M.

The table below provides exemplary sequences for one or more enzymes that may be used in accordance with the methods of the invention.

SEQ ID	Enzyme	Accession #	Sequence
1	FNSI	Q7XZQ7.1	MAPSTLTALAEKTLNSKFVRDEDERPKIAYNKF SDEIPVISLAGIDDDSVDKRSQICRKIVEACEDWG IFQVVDHGDIDLISEMTRLARQFFALPAEEKLRF DMTGGKKGGFIVSSHLQGEAVQDWREIVTYFSY PIQARDYSRWDPKPEGWRSITEMYSDELMALAC KLLEVLSEAMGLEKEGLTKACVDMQKVIVNY YPKCPQPNLTLGLKRHTDPGTITLLLQDQVGGGLQ ATRDGGKWTWITVQPVEGAFVVLGDHGHYLSN GRFKNADHQA VVNSNSSRMSIATFQNPAPNATV YPLKIREGEKAVMEEPITFAEMYKRKMSRDIEM ATLKKLAKEKVLQDQVEKAKLQMTPKSADEIF A
2	FNSI	NP_001268034.1	MAPTTLTALAGEKTLQSSFVRDEDERPKVAYND FSNEIPVISLEGIDEVGGRRDEICRKIVEACEDWGI FQVVNHGVDSNLISEMTRLAREFFALPPEEKLRF DMSGGKKGGFIVSSHLQGEAVQDWREIVTYFSY PLRTRDYSRWDPKPEGWRSVTQEYSEKLMGLAC KLLEVLSEAMDLDKDALTNACVDMQKVVVNF YPQCPQPDLTGLKRHTDPGTITLLLQDQVGGGLQ ATRDGGKWTWITVQPVEGAFVVLGDHGHYLSN GRFKNADHQA VVNSNHSRLSIATFQNPAPPEATV YPLKIREGEKAVLEGPITFAEMYRRKMSKDLELA RLKKLAKEQQLQDVEKAKLESKPIDQILA
3	FNSI	P41090	MAPTTLTALAGEKTLQSSFVRDEDERPKVAYND FSNEIPVISLTKESMKLA AVVDEICRKIVEACEDW GIFQVVNHGVDSNLISEMTRLAREFFALPPEENV RFDMSGGKKGGFIVSSHLQGEAVQDWREIVTYF SYPLRTRDYSRWDPKPEGWRSVTQEYSEKLMGL ACKLLEVLSEAMDLDKDALTNACVDMQKVVV NFYPQCPQPDLTGLKRHTDPGTITLLLQDQVGG LQATRDGGKWTWITVQPVEGAFVVLGDHGHYL SNGRFKNADHQA VVNSNHSRLSIATFQNPAPPEAT VYPLKIREGEKAVLEGPITFAEMYRRKMSKDLEL ARLKKLAKEQQLQDVEKAKLESKPIDQILA
4	FNSI	Q06942	MAPATTLTSIAHEKTLQQKFVRDEDERPKVAYN DFSNEIPIISLAGIDEVEGRRGEICKKIVAACEDW GIFQIVDHGVDAELISEMTGLAREFFALPSEEKLR

			FDMSGGKKGGFIVSSHLQGEAVQDWREIVTYFS YPIRHRDYSRWDPDKPEAWREVTKKYSDELMGL ACKLLGVLSEAMGLDTEALTKACVDMQKVVV NFYKCPQPDLTGLKRHTDPGTITLLLQDQVGG LQATRDDGKTWITVQPVGEAFVVNLGDHGHLLS NGRFGNADHQAVVNSNSSRLSIATFQNPQAEIV YPLSVREGEKPILEAPITYTEMYKKKMSKDLELA RLKKLAKEQQSQDLEKAKVDTKPVDDIFA
5	FNSI	Q07353	IPRVTPSTLTALAEKTLQTSFIRDEDERPKVAYN QFSNEIPIISLEGIDDETGKRAEICDKIVKACEDWG VFQVVDHGVDAEVISQMTTFAKEFFALPPEEKL FDMSGGKKGGFIVSSHLQGEVVQDWREIVTYFS YPTRRDYSRWDPDKPEGWIAVTQKYSEKLMELA CKLLDVLSEAMGLEKEALTKACVDMQKVVVN FYPKCPEPDLTGLKRHTDPGTITLLLQDQVGG QATKDNGKTWITVQPVGEAFVVNLGDHGHFLS NGRFGNADHQAVVNSNSSRLSIATFQNPQAEIV YPLKIREGEKSIMDEPITFAEMYRRKMSKDLELA RLKKQAKEQQQLQAEVAAEKAKLESKPIEILA
6	FNSI	Q9S818	MAPGTLTELAGESKLNSKFVRDEDERPKVAYNV FSDEIPVISLAGIDVDGKRGEICRQIVEACENWG IFQVVDHGVDTNLVADMTRLARDFFALPPEDKL RFDMSGGKKGGFIVSSHLQGEAVQDWREIVTYF SYPVRNRDYSRWDPDKPEGWVKVTEEYSERLMSL ACKLLEVLSEAMGLEKESLTNACVDMQKIVVN YYPKCPQPDLTGLKRHTDPGTITLLLQDQVGG QATRDNGKTWITVQPVGEAFVVNLGDHGHFLSN GRFGNADHQAVVNSNSSRLSIATFQNPAPDATV YPLKVREGEKAILEEPITFAEMYKRKMGRDLELA RLKKLAKEERDHEVDKPVDDQIFA
7	FNSI	Q05963	MAAPISLKWEEHSLHENKFVRDEDERPKVPYNT FSNEIPVISLAGIDGCRRAEICDEIVKACEDWGIFQ VVDHGVDTKLLSDMTGLARDFFHLPTQEKLRF MTGGKKGGFIVSSHLQGEAVQDWREIVTYFSYPI KARDYSRWDPDKPNEWRAVTEEYSKVLMLGLACK LLEVLSEAMGLEKEALTKACVDMQKVVVNYY PKCPQPDLTGLKRHTDPGTITLLLQDQVGG QATRDNGKTWITVQPVGEAFVVNLGDHGHFLSN GRFGNADHQAVVNSNSTSRLSIATFQNPQAEIVYPL KINEGEKSIMEEPMTFMEMYKKKMSTDLELARL KKLAKDKQQDLEVVKPIQNIFA
8	FNSI	Q05964	MVAEKPCTLTSLEGDDKLNSNFVRDEDERPKVA YNEFSNDIPVISLAGIDGKRGEICRKIVEACEDW GIFQVVDHGVGDDLIADMTRLAREFFALPAEEKL RFDMSGGKKGGFIVSSHLQGEVVQDWREIVTYF SYPTNSRDYTRWDPDKPEGWIKVTEEYSNKLMTL

			ACTLLGVLSEAMGLELEALTKACVDMQKIVVN YYPKCPQPDLTGLKRHTDPGTITLLLQDQVGGL QATRDGGKWTWITVQVPGAFVVNLGDHGHFLSN GRFKNADHQA VVNSECSRLSIATFQNPSPDATVY PLAIREGENSIMEEPITFADLYRRKMAKDLEIARH KRLAKEEMPFKELDEAKFESKSIDQILA
9	FNSI	Q7XZQ8.1	MAPTTITALAKEKTLNLDVFRDEDERPKVAYNQ FSNEIPIISLAGLDDSDGRRPEICRKIVKACEDW GIFQVVDHGDGLISEMTRLSREFFALPAEEKLE YD TTGGKRGGFTISTVLQGGDAMDWREFV TYFS YPINARDYSRWPKKPEGWRSTTEVYSEKLMVLG AKLLEVLSEAMGLEKGD LTKACVDMEQKVLIN YYPTCPQPDLT LGVRRHTDPGTITILLQDMVGGL QATRDGGKWTWITVQVPGAFVVNLGDHGHYLS NGRFRNADHQA VVNSTSSRLSIATFQNP AQNAIV YPLKIREGEKAILDEAITYAEMYKKCM TKHIEVA TRKKLAKEKRLQDEKAKLEMKSKSADENLA
10	FNSI	ABG78791.1	MAPTTITALS QEKS LNLDFVRDEDERPKVAYNQF SNEIPIISLAGMDDDSNGRRPEICRKIVEAFEDWGI FQVVDHGDIDKGLISQMSRLSREFFALPAEEKLRY DTTGGKRGGFTISTHLQGGDV KDWREFV TYFSY PIEDRDYSRWPEKPEGWRSTTEVYSEKLMVLGA KLLEVLSEAMGLEKEALTKACVNMEQKVLINYY PTCPEPDLT LGVRRHTDPGTITILLQDMVGGLQA TRDGGKWTWITVQVPGAFVVNLGDHGHYLSNG RFKNADHQA VVNSTSSRLSIATFQNP AQNAIVYP LKIREGEKAILDEAITYAEMYKKNM TKHIEVAAL KKLAKEKRLQDEKAKLEM
11	FNSI	AAX21537.1	MAPSTITALS QEKT LNLDFVRDEDERPKVAYNQF SNEVP IISLAGLDDDSNGRRAEICRKIVEAFE EWG IFQVVDHGDIDGLISEMSRLSREFFALPAEEKLVY DTTGEKKGGFTISTHLQGGDV RDWREFV TYFSY PISARDYSRWPKKPEGWRSTTEVYSEKLMVLGA KLLEVLSEAMGLEKEALTKACVEME QKVLINYY PTCPEPDLT LGVRRHTDPGTITILLQDMVGGLQA TRDGGKWTWITVQVPGAFVVNLGDHGHYLSNG RFRNADHQA VVNSTSTRLSIATFQNP AQNAIVYP LKIREGEKAILDEAITYAEMYKKNM TKHIAVATQ KKLAKEKRLQDEKAKMKI
12	FNSI	XP_017240664.1	MAPTTITALAKEKTLNSDFVRDEDERPKVAYNQ FSTEIPIISLAGIDDDSNRRPEVCRKIVEAFEDWG IFQVVDHGDIDGLIAEMSRLSREFFALPAEEKLRY DTTGGKRGGFTISTHLQGGDV KDWREFV VYFSY PVDARDYSRWPKPEGWRSVTEVYSEKLMALG AKLLEVLSEAMGLEKEALTEACVNMEQKVLIN YPTCPQPDLT LGVRRHTDPGTITILLQDMVGGLQ

			ATRDGGKTWITVQPVEGAFVVNLGDHGHYLSN GRFKNADHQA VVNSTSSRLSIATFQNPQAIVY PLKIREGEKPILEEAMTYAEMYKKNMTKHIEVAT QKKLAKEKRLQNEKAKLETKF
13	FNSI	ABG78790.1	MAPTTITALAQEKTLSDFVRDEDERPKVAYNQ FSTEIPIISLAGIDDDSKGRRPEVCRKIVEAFEDWG IFQVVDHGVDSALISEMSRLSREFFALPAEEKLRY DTTGGKRGFTISTHQQGDDVRDWREFVTYFSY PVDARDYSRWPEKPEGWRSVTEVYSEKLMVLG AKLLEVLSEAMGLDKGALTKACVNMEQKVLIN YYPTCPEPDLTLGVRRHTDPGTITILLQDMVGGL QATRDGGKTWITVQPVEGVFVVNLGDHGHYLS NGRFRKNADHQA VVNSTSSRLSIATFQNPQAIV YPLKIREGEKPILEEAITYAEMYKKNMTKHIEVA TQKKLAKEKRLQEEKAKLETKTKSADGILA
14	FNSI	XP_017638014.1	MAPSTLTALAEKTLQASFVRDEDERPKVAYNQ FSNDIPVISLAGIDDDVDGKRGEICKKIVEACEDWG VFQVVDHGVDTKL VSEMTRLAREFFALPAEKL RFDMSGGKKGGFIVSSHLQGEAVQDWREIVTYF SYPLRSRDYSRWPDKPQGWVEVTKEYSEKLMG LACKLLEVLSEAMGLEKEALTKACVDMQKV VNFYPKCPQPDLTGLKRHTDPGTITILLQDQVG GLQATRDNGKTWITVQPVEGAFVVNLGDHGHY LSNGRFRKNADHQA VVNSDCSRLSIATFQNPAPD ATVYPLKIREGEKPILEEPITFAEMYRRKM SKDLE LARLKKLAKEQQQLKEKEAENEKPKLEAKPLEE ILA
15	FNSI	XP_034889682.1	MAPSTLTALAEKTLRASVVRDEDERPKVAYNE FSNEIPVISIAGIDDGGEKRAEICNKIVEACEDWG VFQIVDHGVDAKL VSEMTTLAKEFFALPPEEKLR FDMMSGGKKGGFIVSSHLQGEAVQDWREIVTFFS YPIRTRDYSRWPDKPEAWKAVTEEYSKKLMELA CKLLGVLSEAMGLETEALTKACVDMQKV VVN YYPKCPQPDLTGLKRHTDPGTITILLQDTVGG QATRDNGKTWITVQPVEGAFVVNLGDHGHYLS NGRFRKNADHQA VVNSSRLSIATFQNPAPDAT VYPLKIREGEKSVLDEPITFAEMYRRKM SKDIEIA NKKKMAKEQQQLKDLEKAKLETKPIEEILA
16	P450	ASW21051.1	MELSSVIYGAIALLSLFYCYLHFSKPKKSSLNAPP EAGGARFITGHLHLM DGRSASDKLPHINLGLLAD QHGPIFTIRLG VHRVAVVSSWELAKEIFTTHDTA VMARPRIADDYLSYDGASLGFSYPYGPYWREIR KLVTTELYKLWEEKKDGSGRVLVDMKQWLG NL SLNLVSRMVVGKRFYGGDDSETTKRWGVMRE FFQLIGQFIPGDGLPFLRWDLGGFEKRTRDTAY ELDKIIMWLAEYRKREYSGDDKEQCFMALMLS

			LVQANPTLQLHYDADTIKATCQVLISAASDTT VILIWVISLLLNNADVLLKKVQEELDEQVGRERRV EESDISNLPYLQAVVKETMRLYPPAPFAGVRAFS EDCTVGGYHIQKGTFLIVNLWKLHRDPRVWSDD ALEFKPQRFDDKKVEVKQDFELMPFGGRRMC PGSNLGMHMHVFLANILQAFDITTGSTVDMTE SVGLTNMKATPLDAILTPRLSPTLY
17	P450	KAH6755793.1	MELIFAIYGAITIFFIFFLYQLLNPTGKHKAPPKA GGARPFTGHLHVIGGGSSTLPHINLATLADKHG PIFRIRLGVNRAVVVSSWELAKELFTTHDVAISSR PMVRSTKYLSDLA VFGFTPYGSYWQQLRKVV ASELLAARKIELQRNV RVSETMQSINEL YRVYEE KNDGSGKVLLDMKRWLGDLSLNVILRLVVGKR FHC RVDAEQTRRCREVMRDFHLAGLFLPADAF PFLGWLD FGGYEKKMKQTGEELDKILGGWLAE HREMEYSGEDCTTQNFMGVMQSVVDGANLQG KYD VDTIHKATCQVLISGGTDTTAVMLIWAISLIL NNRHVLKKTQEELDEQVGRERRVNESDINNLY LQAVVQETLRLYPAGPLGGIREFSKDCFVGGYH VPKGTWLLVNLWKLHRDPQVWPDDPLEFKPER FLSRQKDVDVKQDFELIPFGAGRRICPGANFGL HMLHLVLASLLQAFDLSTINNEKVDMTESAGLT NLKATPLDVLVAPRLSPSLYLSS
18	P450	JX162212	MEFISFVYTIAFSSLLYFYLIWSESAPKTTTHK APPEASGAWPVIGHLRIMSGHPSAGIPHVNLGML ADKHGPIFSIRLGVHRVVVVSSPEVIKELFTTNDV AVSSRPSVKAGKHLAYDNAMLGFASYGAYWRQ LRKIVSLELLSNRRLELQSHVSMSETGQFVKELY KLWEKKKSDGSGTEVGEVGVVDMKRWL GELN MNVVMRMVAGKRFGSGDNAEETKRCRRVMGD FFYLAGFFVPADALPYLGWLDLGGHEKRMKKA AKELDEVVGEWLA EHREREFSGEGKAQDFMDV MISVVKGADLQCEFDVDTIHKATCGTLIAGGTD TAVVFVWALSLLL N HSHVLKKAQQELDKHV GK DRRVKESDLNNLIYLQAI VKETLRLYPPGPLAGT RRFTEDCVVGGYYIPKDTWLIVNLWKLQRDPRV WSDPLEFRPERFLAGDKTFDVKQDFELIPFGAG RRICPGLSFG LQMLHLVLASLLQAFDMSTVSDEA VDMSESAGLTNMKATPLDVVVT PRLPPRLYNEI VEIY
19	P450	M1KVN4	MDFNAAVCAALAFISLLSYLIWLRSASTHQAPP EAGGAWPFLGHLNISGHTGLPHVSLGNLADKH GPIFGIRIGVHRAVVVSSEVIKELFTTNDAAVSS RPSVKAGKHLAYDYAMLGFSSYGT YWRQMRKL VSLELFSARRVELQRNV RVSETAHFINELYSAWE ERRDGSSRVSVEMKELFGELSMNVILKMVAGKR

			FSGGDDAEEARRCRRVMREFFHLAGLFVLSDAF PYLGWLDLGGHERRMKRTAEEMDELVGEWLAE HRRKEYSGEGKAQDFMDVMLSEVKGANFECEY DVDTIKATCGTLIAGGTDTTAVVFIWALALLN NPHVLQKAQHELDTHVGKQRRVNESDLNNLVY LQAITKETLRLYPPGPLGGTRRLTQDCHVGGYHI PKETWLIVNLWKLHRDPRVWSDPSEFRPERFLN GEKSMDVKGQDFELIPFSAGRRCIPGTNFGQLML HLVLASLLQAFDLSRVSNEEIDMSESAGLTNIKA TPLDVLIAAPRLPPSLYI
20	P450	XP_051140309.1	MEFSSAIGSAAAAAILILYYYLLRRRSLNPKYK LPPEAGGARLILGHLHLMGDAARPPHFYLADMA DQHGPIFTIRLGVRRAVVVSSWSIAKELFTSCDA AVSSRPKMRAIKHLSYGFAMFGFSPYGDYWREI RKIANTELLSSHRVEVM SHVYEP AIAQSVKEIYS RWEDERNIYKDGLSGRVLVDMKKWFAELNLNV ILAMVAGKRFSAAAGEDDGEEMRRRCRAVFRDFF HLLGLFVPADALPWLRFDLGGLEKRMKRTAK ELDGIMA EWVVEHRRKGGDTAARDFMDVMVS AVRGSEQLTRQADADTVIKSTCQMIIAGGADTSS VVLTWALSLLLNNRRVLAKAQEELDKHVGRTR RVKESDIPNLVYLQAVVKEILRLYPAGPLGGPRE FTQDCSIKGYHIPKGTWLMVNTWKLQRDPDVW SDDALEFKPERFLTTRHGNIDVKGNFELIPFGA GRRICPGTNLALHMMHLVLANLLQAFEVGTVCD EAVDMSESLGLSNLKATPLDVLVAPRLSPEHY
21	P450	XP_047940933.1	MDLSFFIGAGALITVLPFLYFFYTNSTNHNASGI HAPPKAGGAWPFTGHLHVMSSGNLPHIALGAM ADKYGPVFQIGLVQRQALVSDGKLAKELFTTC DLAVSSRPKMASSRNLYEMAMFGFAPYGGQYW REVRKVVSTELLSARRLELQKHVLVSETDLVNE LFGVWSQKADVSGRVLVEMKQWFGDLNLNAV LRMVVGKRCFGSGNGDIDEARRCQRVMRDFFD LAGVFVAGDAMPYLRWLDVGGYEKKMRETSKE LDSL VGQWLEEHRESGISDKGEDFMDVMLCVVR GTKLQNHDSDIHKSTCSNLISGASDTT SIMLVW ALALLNNNYALRKVQQLDDQVGRDKRVNDS DIDKLLYLQAVVKETLRLYPAAPLSGPREFTQDC VLGTYNVPKGTILFVNIWKLHRDPKVWSEDPLEF RPERFLTTHRQVDVKSPNFDYIPFGGGRRICPGIN YGM RMLHLVLASLLQAFDISTPNGEVVDMSSEG GLTNGKATPLDVLVSPRLCPSLY
22	P450	CAA2982936.1	MDFTFSKTGPSITAIFALFFIFYLRFRSKGSKNNAP PEAGGSWPFIGHLHLIGGGSRLPHITLGEMADKY GPIFTIRLGVHRTL VVSSWELAKELFTTWDVAVS SRPKFLAAKHMGYNFAMFAFSPYGAYWREIRKL

			IAVELFSIRSLELLKHVRVSETELSMKELYEYWN AKKDGSGQLLVEMKQWFGYLNLNVIIRMIAGKR YFGTTADGDKIEARQCQKVLRRDFFHLAGLFVVA DTIPFLGWLDVGGYEKRMKETGKEMDRIVEKW LEEKQKANSKSKHDFMDVMLSAVEGTALQDY DPGTIIKSTCLSLIAGGGDTVTVMVWTVSLLN NRHVLKKAQQELDIHIGKERRVKESDIDKLIYQ AIVKETLRLYPAGFLGSPREFTEDCKLADYHIPK GTRLIINLWKTHRDPQVWFDPLEFRPERFLTTHK DFDVKGQNFELIPFGAGRRCPGISFSLHMLHLVL ANLLHAFELSTPSHESVNMTVNAGLTNMKATPL DVLLSPRLSRTLY
23	P450	AZP01942.1	METFLAQLYSTTTIAALFVLLVLYFSPWTRINK KNVAPEAGGGWPIIHLHLLGGSKLPHLVFGSM ADKYGPFTVRLGVQVRSVVSSWEMVKDIFTTN DVIVSGRPKFLAAKHSYNYAMFGFSPYGSFWL ELRKITSLQLLSNRRLELLKHVRVSEMEISMRQL YKLWSEKKNNGSGRVLMDMKKWFGELNLNVTF RMVAGKRYFGGGAASNDEEARRCRRVVREFFR LLGVVVVADSLPFLRWLDLGGYERAMKETARE MDSIVSVWLEEHRISDSSGDDANMEQDFMDV MLSAVKNVDLCGFDAHTVIKATCMVISSGTDIT TVELTWALCLLNRRHVLKKAQEELDNVVGKQ RRVKESDLNLIYLAIVKETLRLYPAGQLGGQR EFSDDCTVGGYHVPKRTRLVVNLWKLHRDPRIW SDPTEFRPERFLERHKEIDVKGQHFELIPFGARR VCPGITFGLQMFHLVLASLLHGFDISTPSDAPVD MAEGAGLTNAKITPLEILAPRLSPSLYE
24	P450	O49858	MDLLNCLNLNTIAIASILSLIFLCFLYRKNSRG KDAPVVSAGWPILGHLSSLNGSQTPHKVLGALA DKYGPLFTIKLGMKPALVLSNWEMSKELFTTND LAVSSRPKLVAVEVMSYNQAFVGLAPYGPYWR ELRKIVTFEFLSNRRIEQRNHIRVSEVRTSIKELFD IWSNGNKNESRYTLVDIKQWLAYLTFNMVVRM VVGKRYFGVMHVEGKDKAQRFMKNIREFMNL MGFTFTVADGVPCLRWLDLGGHEKAMKANAKE VDKLLSEWLEEHRQKLLGENVESDRDFMDVMI SALNGAQIGAFDADTICKATSLELILGGTDSTAVT LTWALSLLLRNPLALGKAKEEIDMQIGKDEYIRE SDISKLVYLQAIVKETLRLYPAPFSSPREFTENCI LGGYHIKKGTRLIHNLWKIHRDPSVWSDPLEFKP ERFLTTHKDVDLRGHNFELLPGSGRRVCAGMS LGLNMVHFTLANLLHSFDILNPSAEPVDMTEFFG FTNTKATPLEILVKPRQSPNYETL
25	P450	OP096437	MVFIFLISGLLLAAFLYITSWRRNPKNRPKNLL SLPQPPQAWPILGHLHLLGGDAHTCLTFSKLAEK

			YGPVFSRLGFQHAVVISDRRAVMEFTVRDKS FANRPRNSSGEHLVYDNAAIGFANGVYWRDMR KLVLTEVLSTRSIEKMRNLRVSEIDMSIKELYFQA VRNQNKPVNLSQWTEQMTLNIIVKTLAGKRYGS DPDGSVPEEVSVFRRLISDITNLTGQFVLADVFPF FLKWLDPKGYIKRMKKVSEELDGILEVWIEEHLR RRSSGGEVGSYEQDFVDVLLSNIDDRLVKIHGKA KMIIKATLVNVILGGFDTTSVFLSWALSLLLQHK EAMNLAQKEIDSKIGKQRWVQESDLKNLVYLQA IVKETLRLYPSLPLSVPHEAVEDSELGYPVPKG ALLFVNLWKLHRDPQFWPEPEEFRPERFLSGQA AQMDVLGQQFEYTPFGSGRRVCPGITFAHVGN LTLARLLQGFDLVTPGDQKVDMSVGFVGTIPRA NPLEVLITPRLHTKLYES
26	P450	ASW21050.1	MELSSVIYGAIALLSLFYCYLHFSKPKKSSLNAPP EAGGARFITGHLHLMMDGRSASDKLPHINLGLLAD QHGIPTIRLGVHRAVVVSSWELAKEIFTTHDTA VMARPLIADDYLSYDGASLGFSPYGPYWREIR KLVTELLSARRIELQRATRVREITQFTGELYKL WEEKKDGSGRVLVDMKQWLGNLSLNLVSRMV VGKRFYGGDDSETTKRWRGVMREFFQLIGQFIP GDGLPFLRWLDLGGFEKRTRDTAYELDKIIAMW LAEYRKREYSGDDKEQCFMALMLSLVQANPTL QLHYDADTIKATCQVLISAASDTTTLIWIWVISLL LNNADVLLKKVQEELDEQVGRERRVEESDISNLP YLQAVVKETMRLYPPAPFAGVRAFSEDCTVGGY HIQKGTFLIVNLWKLHRDPRVWSDDALEFKPQR FFDKKVEVKGQDFELMPFGGRRMCPGSNLGM HMHVFLANILQAFDITTGSTVDMTESVGLTNM KATPLDAILTPRLSPTLY
27	P450	KAH6791399.1	MELIFAIYGAVTIFFIFFLYQLLLNP TGKHKAPPK AGGARPFTGHLHVIGGGSSANLPHINLATLADKH GPIFTIWLGVNRAVVVSSWELAKELFTTHDMAIS SRPMVRSAYLSYDLAVFGFTPYGSYWQQLRKV VASELLAARKIELQRNVRVSETMQSINELYRVYE EKNDGSGKVLLDMKGWLGDLNLVILRLVVGK RFHGGVDAEETRRYREVMRDFHFLARLFVPADA FPFLGWLDGFGYEKKMKQTGEELDKIFGGWLAE HREMEYSNEDCTSHDFMGVMQSVVDGTLNQG YDAHTTIKATCQVLISGGTDTTAVMLIWAISLILN NRHVLKKAQEELDEQVGRERRVNESDINNLYYL QAVVQETLRLYPAGPLGGIREFSKDCFVGGYHV PKGTWLLVNLWKLHRDPRVWPDDPLEFKPERFL NRQKDVDVKGQDFELIPFGAGRRICPGANFGLH MLHLVLASLLQAFDLSTINNEKVDMTESAGLTN LKATPLDVLGAPRLSRSLYLSP

28	P450	KAI3451537.1	MFMDNHPANQYSAMEFISVLCGVIGFSVLFYNY LVWFRSAKPKTGIDKAPPEAGGAWPFTGHLHLM GGGSSAELPHINLAALADKYGPVFTIRLGVHRAL VSSWELAKELFTTYDLAVSSRPRLRAAKHLGY NFAMFAFSPYGAYWRGLRKLVSVELLSSSRVEL LRHVCVSETAQAINELYTLWEEKKDGSVSVTV MKQWFGNLLNVVLGMVVGKRIYGGGRANAEE TRRCREVVRDFVHLLGLFVAADALPYLGWLDLG GYEKRMKETAKELDGIVGGWLAEHREKAYSGE DKPQDFMDVMLSVVQGEGLQGQYDADTIHKSTC QSLIAGGSDTTTVMLVWALSLLLNNRRVLKKAQ EELDKHIGRERQVNKSDINKLDYLQAIKETLRL YPAAPLSAAREFSEDSHVGGYHISKGTWLMNL WKLHRDPRVWGEDSLEFKPERFLSTHRKVDVRG QDFELIPFGGRRICPGTNFGLNVLHLVLANLLQ AFELSTVSEEVVDMTESAGLTNIKATPLDVLVAP RLSPSLY
29	P450	ASW21052.1	MLYDNSQYLAMEFSSAIYGAIAFFLFLYCLLYT TSSKPNTRAYKAPPEAGGARLFSGHLHLMAGGT TGELPHINLANLADKHGPVFTIRLGVKRALVSS WESAKELFTTCDVAVSSRPRLMKAACKLLGYDFA MFGFAPYGAYWRELRLISVELLSTRRMELQKE VRDSETRESIKELYKLWEERSEGSVLDVMKQ WFGDLNLLNVVLRMVAGKRWIGPETGRWREVL RDFYLAGMFVPADAFPLGWLDLGGHEKMRMQ TAKELDGIVGGWVAEHREKEYSGEDKPKDFVD VMLSVVQGSRLRADIYDADTIKATCEALIVGGSD TTTVMILWTLFLLNNRHVLRKAQEELDKHVGR ERRVNQSDINNLYYLQAIKETLRLYPAGPIGGIR EFTQDCQVGGYHVPKGTWLVNLWKLHRDPKV WSEDCLEFRPERFLNKNIDVRGQDFELIPFGGGR RICPGANFGLHMLHLVLANLLQAFELTTVSDQVI DMTESAGMTNMKATPLNVLVAPRLSPTLY
30	P450	KAI3451541.1	MDSHNANYQYLTMEFSSAICGAIVFFFLFYSSL WFRSTKPKNGTKAPPEAGGAWPFTGHLHLMGG GSSAKLPHFNLAALADKYGPVFTIRLGVHRALV VSSWELAKELFTTFDMVSSRPRLRAADHMSYN FAMFGFSPYGTYWREMRKLVSVELLSSRRVELL RHMVSETAQFINELYKLWEEKKDGSVSVLVD MKQWFGDLNLLNVVLRMVAGKRFHGGGRADAE TRRCREVMRDFHLAGLFVAADALPYLGWLDL GGHEKKMKENIKELDGIVGGWLAEHREKKNNG KDKPQDFMDVMLSVLQGADIEGPYDADTIKAT CESLIAGGTDTTVMMLVWALSLLLNNRHVLKKA QEELDEHIGRDRRVIESDINNLYYLQAIKETLRL YPAGPLGGTREFSEDSNVGGYHVPKGTWLVNL

			WKLQRDPHVWGEDSLEFKPERFLNTHKNMDVK GHDFELIPFGAGRRICPGTSFGLDVLHLVLANFLQ AFEVSTVFDEMVDMTESAGLTNSKATPLDVLVA PRLSPSLY
31	OMT	XP_013456930	MAFSTNGSESELYHAQIHL YKHVYNFVSSMAL KSAMELGIADAIHNHGKPMTLSELASSLKLHPSK VNILHRFLRLLTHNGFFAKTIVKGKEGDEEEEIAY SLTPPSKLLISGKPTCLSSIVKGALHPSSLDMWSS SKKWFNEDKEQTLFECATGESFWDFLNKDSESS TLSMFQDAMASDSRMFKLVLQENKRVFEGLESL VDVGGGTGGVTKLIHEIFPHLKCTVFDQPQVVG NL TGNENLNFVGGDMFKSIPSADAVLLKWVLHD WNDEQSLKILKNSKEAISHKGKDGKVIIDISIDET SDDRGLTELQLDYDLVMLTMFLGKERTKQEW KLIYDAGFSSYKITPISGFKSLIEVYP
32	OMT	ARS23163	MAVSTNGKTSEGVSDVSR TKNITNGNGMDMSV SANVASIGSVSATPLSPEDKRDHVVAINFLLGWV QTWALKAVVTMRIPDIISKAPMDALTAREIISIQ CKNPSNAVPLLERLMRFMVCKGFFSESVNGENEI AYELNGVSKMYVTDHEMSVVEWVNVAFHRSGI PALTRMHEAITEDVCPWDQAHGMPYAHNP KFP ELGDFHFKAMIVHSNFLTKILAE EYEGFKDFKVL VDVGGSLGNNLALILRFHPLRGINFDMPSVIAG APSPFGVEHVGGNFYESVPSGDAIMLMAILHNNS DENCVKILRSVHAALPEDGKLIVAEYIMTNEADH NGERIRLLDMLMLVQFRGGEERTFEHHKTL YEA AGFRHYKLIKTS GPMDIIEVRK
33	OMT	APX42106	MAPEVLSRTWSCITALASSTIWREGHAGDGREK LLLKSVLDNAEKNPQSVMR TIDQFARRSWLMNI GDDKGLLLDRAVSNRKPRVVLELGAYVGYSAV RIASQLDEGSKLISLEMSAENSNITRQVVHHAGL ADKVQVVEGFLKDKVEEVQRLLQELNAKTFDFV FIDHDKNCYLPDFLILKEHGLVGKGTVLFADNM KIPGSPEYRDYVQKNVDDFETIEHKTRFEYFSWL PDSVVISTLK
34	OMT	C6TAY1.1	MASPLNNGRKASEIFQGGALLYKHLLGFIDSKCL KWMVELDIPDIIHSHSHGQPITFSELVSILQVPPTK TRQVQSLMRYLAHNGFFEIVRIHDNIEAYALTAA SELLVKSEL SLAPMVEYFLEPNCQGAWNQLKR WVHEEDLTVFGVSLGTPFWDFINKDPAYNKSFN EAMACDSQMLNLAFRDCNWVFEGLESIVDVGG GTGITAKIICEAFPKLKCMVLERPNVVENLSGSN NLTFVGGDMFKCIPKADAVLLKLV LHNWNDND CMKILENCKE AISGESKTGKVVIDTVINENKDE RQVTELKLLMDVHMACIINGKERKEEDWKKLF MEAGFQSYKISPFTGYLSLIEIYP

35	OMT	WLK78566	MDRKTQYILETSVYPREHEAMKELREITAKHPW NVMTPADEGQFLIMLLKLINAKNTMEIGVYT YSLALATALPEDGKILAMDVNRENYELGLPVIQ KAGVAHKIDFREGPALPVLDEIVKDSKKHGTFDF IFVDADKDNLYLNYHKRLIELVKVGGVIGYDNTL WHGSVVAPPDAPLKKYIRYYRDFVLELNKALAA DQRIEICQLPVG DGVTLCRRIS
36	OMT	WLK78567	MAQTAAATENQAGRHQEVGHKSLLQSDDL YQY ILETSVYPREPEPMKELREITANHPWNIMTTSADE GQFLNMLLKLINAKNTMEIGVYTGYSLALATA LPDDGKILAMDINKENYELGLPVIEKAGVAHKID FKEGPALPVL DQMIADGKYHGSFDFIFVDADKD NYLNYHKRLIDL VKVGGVIGYDNTLWNGSVVA PPDAPLRKYVRYYRDFVLELNKALAADPRIEICQ LPVG DGITLCRRIS
37	OMT		MANDAKVVSKLLVMNCMQAFEGLKLIVDVGGG TGTMAKTMIGACSQLKCVVLDLLHWILHNWSD EECVKILGKCKEAIPSTENG GKVIFIEMVVCCHK EDDTAYETQLFWD TLMMAQTTGKERNEKEWA KLFLDSGFSSYKITSGLGLRSLIEVFP
38	OMT		MGGGFELKNAVLEGGVPFDRVHGTHAFEYPSKD SRFNEVFNKAMIGHTNIIMKQVLEQYKGFENLN KLVDVGGGLGITLSMITSTYPNIKGINFDLPHVIK NAPAYPGVEHVGGDMFESVPQGD AIFMKWILHD WSDDHCVKLLKNCHNALPENGKIIVVEAML PVK PETTA AFISTKAI
39	OMT		MVGGGGGGGGYWLTPASRLLLRDDPLSMVSFA LGVLDP IYVDPWHHVCDWFQND EETAFTAHG VTFFEHFGMDPSMNKLFNEAMASGSHLVNQFLI QEGLKCVVLELSHV VAGFQDSGNITLVVGD MF SIPQADAVFLKNCKEAIPSQGDVVNVII VDMIIND KGADQVAVEAQILFDMEMMILTTVKERTEKEW AKLFIDSGFTSYKVIHVLRLMSLIEVFP
40	OMT		MSRCIMRYMFELKNAVLEGGVPFDRVHGTHAF EYPSKDSRFNEVFNKAMIGHTNIIMKQVLEQYK FENLNKLVDVGGGLGITLSMITSTYPHIKGINFDL PHVIKNAPAYPGVEHVGGDMFESVPQGD AIFMK WILHDWSDDHCVKLLKNCHNALPENGKIIVVEA MLPVK PETTA AFISTSQSDLVMMTQNP GGKERT EKEFLALATGAGFRGITICCSVCSYRVMEFYK
41	OMT		MAADRSIVEFCNKLLAPILYRWFELKNAVLEGG VPFVRAHGVSGFEYISRDQNLNEVF NKGMTGYT TFSMKHIVEQYKGFEVVNKLVDVGGGLGVT LST IVSKHPNIKGINFDLPHVIDNAPPYPGVEHVGGN MFESVPEGDAIFMKWILHDW NDAHCLKLLKNC YAVLPKDGKVIVVEAVLPVKPEKSTS FISASQAD

			LLMMTQSPGGKERTEKEFLALAKGAGFSGIKKC CLVWNYWVMEFYK
42	OMT		MKCKFLVGQFVLNYDDALDFILSTQYHLKDAV LDGGIPFNKAYGMTAFDYHGTDPFRNKVFNQG MSNHSTIIMKKILEIYDGFEGLSVVDVGGGTGA TLNMIVSKYPSIKGINFDLPHVIEDAPTYPGVEHV GGDMFVSVPEGDAIFMKWICHWDSDTHCLKFLK KCYEALPENGKVILAECVLPEVPDTGLPTKNVIH VDVIMLAHNPPGGKERTQKDFEALAKGAGFTKFQ KVCCAYNSWIMELRK
43	OMT		MAMQNGTSSSAHDDVLNAHEHVWNHMFNYISS MSLKCVVDLDIPKIIHKHKGKPMTLSELVDALPID KAKSECIDRLMRLLLHSKFFVKVNISEGEGEEKEG YWLTPSSRILLKDAPFSLEPLVHLALDPTFMKPW HHVTEWLSNDRHPTAYETTYGQPYWEHASQLPS LNHLFNEGMASDARLVNVVLLRDCKHVFEGFDS FVDVGGGTGMTARAIADVFPDMKCIVLDLPHVV AGLEESKNLTYVAGDMFEAVPPADVLLKVIYV LFYFNHIEELVMELGDNSALLSDC
44	OMT		MEDEKQQSCIYATQLVNSSVLPMLHSTQLDV LEILAKAGPDAQLSASEIVSRMDEVKNLGAAM LDRMLSLLVGHSVLTCSVSDAEKHYGLAPTAKY FVKNEDGASFGALMALLQDKVFIDSWFELKNAV LEGGVPFDRVHGTHAFEYPSKDSRFNEVFNKAM IGHTNIIMKQVLEQYKGFENLNKLVDVGGGLGIT LSMITSTYPNIKGINFDLPHVIKNAPAYPGVEHVG GDMFESVPQGDALFMKWILHDWSDDHCVKLLK NCHNALPENGKIIVVEAMLPVKPETTAAFISTKAI
45	OMT		MSSTFDHTNANPEEDANCLFAMKLATGAVLPM VLKTAIELDLLELIKAGPNASVSASELASHLPTK NPNAASMIDRILRLVSAHSILACSLKRLPNDCVER RYS LAPVCKFLTKNEDGVSLSNLVLFNNDKVSM ELWYHLKDAVIAGGNPFNRAYGINNFESHSHDL SHNEIFNRAMSDHSNIFTKKIVEIYNGFEGLSLV DVGGGIGATISTIISNYPSIKGINFDLPQVIQKAPS YPVIIFIITPASPPRRHHHHHRIFGVWLDDVELLR SFTAEPSTPGIHWTVKVMAMRIMAMESIPRRRI
46	OMT		MAIVKCAIELQIPDVLESHGGSMTHSELAAAIDC SSTALRRIMRYLTHRRIFKQTPANQDSSSIRYTQT PLSRLLMRTGSNSMAGFVLLESSQVMLAPWHGL SARTLANEAAPPPFEAANGEDVWKYAAANPGHS KLINEAMACHARVAISAIVDQYPEAFMGVGSV DVGGGDGTSVGSVKACPWITRGINFDLPHVVS VALRCDGVEHVGGDMFQSVPKADVAFMLMWVL HDWSDDCITILRNCRDAIPKDNGKVIIAEAVIEE

			GEEYEDDKYDDVRLALDMVMLAHTKKGKERTL KEWEYVLNSAGFSRYTLKRIDAIVAVIEAYP
47	OMT		MATAVIINTNGSITHDHHHDEEEQKACIYATQL MTSSVLPIVLNTVFQLQVFEILENAGPNGQLSASE IVSQMQPAPKNPDAAAMLDRMLSLLVGHAVLT CSFTSNDEKLYGLTLVTKYFVRKENGASFGHRM DLLQDQVFFQSWYELKNAVLEGGVPFERVGFEN LNTLVDVGGSFGVSLRTILSRFPNIKGLNFDQPHV IKDAPPIPGVEHVGGDMFESVPDEGDAIFMKYIL HDWSDAHCLKVLKNCYKALPKNGKVIVVEAVL QDKPEKSASFRSTSQIDVYIMTQTLGGKERTEKE FLSLATQAGFGKGMNMCCFVCNNWVFEFYK
48	OMT		MWCPVPFNASILPHQAQFWEKLVLIDVQLSASQI VSKLQAENPDAAVMLDRMLRLLVGYSVLTCSVS DHGQRLYGLAPVAKYFVRSEAKASFGPMMALIQ DKVFFESWFELKNAVLEGGVPFERVHGSHTFEY ASGDPRFSEVLNKAMTGYTTFLMKEILARYKGF EKLDKLVGVGSLGVTLSMIISKYPNTKGINFDL PPVIKEAPLYPGLHVEGDMFESVPQGDALFMK WILHDWSDPHCLKLLKNCYESLPETGKVIVVEGI LPVKPDKGGSFISTSQLDVFMMAQTPGGKERTEE EFLTLAIEAGFRGMTKCLVCNFWVIEFYK
49	OMT		MGSISEHPATPTTDPSEEEESCLFAMQLASASILPM VLKSAJELDLELIKKDGLVSPSQLVAQLPASNP APVMLDRILRLASYSVLNCSLKTVDGGIERLY SLAPVCRFLTKNKDGVSMAPLLLMNQDKIFMDI WYHLTDVVDGGVPFNKTHGMTAFDYCGTDPR FNKAFNLGMSNHSTIIMKKILETYYGFECLKSVV DVGGGTGATLNMIVSKYPSIKGINFDLPHVIEEAS SYPGVEHIRGDMFVSVPKGDAIFMRWICHWDSD APCLKLLKKCYEALHKKWKSISRVCFARGGKE RTQKDFETLAKRAGFTKFEKVCCALQYMDHGIA
50	OMT		MATAVIKTNGSISHDHHQDEEEQKACIYATQL MTSSALPMVLNNVFQLQVFEILENAGPNGQLSAS KIVSQMQPAPKNPDAAAMLDRMLSLLAGHAVL TCSFTSDDEKVYSLTLVTKYFVRKENGASFGCR MDLLQDQVFFQSWYELNNAVLEGGVPFVRVYG THPCDYTSRGPGFENLNTLVDVGASFGVSLRTIL SRFPNIKGINFDQPHVIKDAPPFPLEHVEGDMFE SVPEGDVVFMKYILHDWSDAHCLKVLKNCYKA LPKDGKVIVVEAVLQDKPENSASFRSTSQIDVYI MTQTMGGKERTEKEFLSLAKRAGFKSMNMCCF VCNNWVFEFYK
51	OMT		MVLDEEAQAKIDVWSYTTGFIHERVVKCAVD FEIPDIEKHGGPMSLTDLSAAIQCPPDALYRIMRFLT AHKIFKITNGSTNPNPPLYAQTPLSRLFARDKMAP

			YILLHGDPPGPVGGLTVKALRTGTRDDLGHGEVI EQDGAEWTADEFKDHGDIFTEAMACHARVTIAAII ENYPGVKGVGSLVDVGGRNGTAVGALVKAFP TVKGINFDLPQVVKDAPPRDGVVHVGGDMFVS VPKADAAMLMRVLHDWSDEACVDVLKKCKEA VPAKTGKVIVLDAVIHEEDEDEGHDEYEGVRLEL DMTMMAVTVKGKERTYKEWVHLLKAAGFSKY KFTSIKAVEFVIEAFA
52	OMT		MEKEEEEEELQSLMYAAQLVNSSVLPMVLHAVIQ LEVFDIMAEAGPNEQLSASEIVSGMADVKNPNA AVMLDRMLSLLVGHSVLTCSVSDTEKRYGLAPV GKYFVKNEGASLVDMMALLQDKVFIDSWFELK NAVLEGGVPFDRVHGINAFEYPAKDPRFNEVFN KAMISHTNIVMLQVLELYKGFENLNLVDVGGG LGITLRIITSSYPNIKGINFDLPHVIKNAPAYPGVE HVGGMDFESVPQGD AIFMKSILHDWSDDHCEKL LKNCHNALPKNGKIIVVEAMLPKPEPNTAFIITS QSDMVMMTQNPGGKERTEEEFRALATGAGFRGI NIVVLLCNRYRVMFYK
53	OMT		MDVESTTIAKSEVKGIQAKVEMWKYALGFTPM AIVKCAIDLQIADVLENHGGAMTLSELSDALGCV PSLLYRIMRYLIHRGIFKQTPTSQDSSFSYIQTPLS RLLMKNESDSMAAFVLLESTPVM LGPWQHLSAR VSVNGDLPFVAANGKDLWEYAAANPGHSKLIN DAMACRARM DIEAIVNQYSEVFKGIGSLVDVGG GDGTTLSVVVKACPWIHGINFDLPHVASVAPRIE GVEHVGGNMFKSVPKADAAILMAVLHDWNDEE CIDILKNCREAIDKETGKVIAEAVLEEAEDNMFS DVRALDMVMM AHTSTGKERTLKEWEHVVTA AGFTRCVKKHIEAITDVIEVYP
54	OMT		MEAKSIVKPRSEVKGIEAQVEMWKYALGLTPMA IVKCAIELQIADVLENHGGSMALPELSTALGCAP SLLYRVMRYLIHRGIFKQTPTSTDSSFAYVQTPLS RLLMRNEVDSMAALVLLESTPVM LGPWHGLSA RVSTNGDFPF EVANGKDLWEYAAANPGHSKLIN DAMASRARM DIYAIVDQYPEAFEGIGSLVDVGG GDGTTISVVVQACPWICGINFDLPHVTSVASRIEG VEHIGGMDFESVPKADAAILMSVLHDWSDEECI DILKNCREAIDKETGKVIAEVVLEEGEDNMCS D VRLALDMVMLAHTSKGKERTLKEWEYVVTAAG FTRYVVKHIEAITDVIEVYP
55	OMT		MKHDHDEDSEAVVHALNGCLALIFPMVLRASIK LDLFEIIAGGSLTPSAIAAKLPVSVPPERIGVLDSM LRSLTAHSFLTCTNDDEGRIEKRYALSPAGRCFV KDESSGGVSLAPFQAFNCTPHTMDFWFDLEGAV LEKGTSTFERVYGKHAYEYINSHPELGGMFNDAM

			AAHSALLMKKAVETYNGFEGLSLVNVGGGYG VSLDMIVTKYPSIRGINFDLPEVIQNAPSYKGIEHI GGNMFNQMPEGDAILLKAILHNWSDEKCIILLKN CYKALPNKGKVIAIECIIPDHDHQTDDVHVKFTS QMDIIMQTVFGAKARTKDELRALAMKAGFSEFK IVSYVYGMWILEFLKLE
56	OMT		MALPNGIQSHHEFFEAQAHVWNHIFSFINMSMLK CAVQLGIPDTIHKYGKPMTLSQLVNSLPINKAKS HGIYRLMRVLTHSKFFVNIKSSDEDEEERYWLTS ASRLLLRDDPMSMVPFLAFQLHPVSTDWLWHHFD AWFQNE DATPLVTSTGWTLWEGAHQDPNMNNL FNEAMASDSKVMRNVNCTQALEGLKSIVDVAG GTGTMAKAIAGACPGIKCVVLDLPHVIAGLEETE NLSFVGGMNFESIPKADVVMKWILHDWNDEEC VKILGKCKEAIPSKENGGKVILIEMVVS DGD KED HKAYETQLFWDMLMMAQITGKERTEKEWAKLF VDSGFSSYKITPGLGLRSLIEVFP
57	OMT		MEVKPKTETREESLAQIDIWNYVFGFTPMAIVKC AIELQIPDVLESHGGSMTSELAAINCSTALRRI MRYLTHGRIFKQTPANQDSSSIRYTQTPLSRLLM RTGSNSMAGFVLLESSQVMLAPWHGLSARTLAN EAAPPPFEAANGEDLWKYAAANPGHSKLINEAM ACHARVAISAIVGQYPEAFMGVGS LVDVGGGDG TSVGS LVKACPWITRGINFDLPHVVSVALRCDGV EHVGGDMFQSVPKADVAFLMWVLHDWSDDECI TILRNCRDAIPKDNKGVIIAEAVIEEGEEYEDDKY NDVRLALDMVMLAHTKEGKERTLKEWGYVLNS AGFSRYTVKCIDDIVAVIEAYP
58	OMT		MALPNGIESHQELLEAQAHVWNHIFSFINSMTLK CAIQLDIPDTIHKHKGKPMKLPelikALPINKAKSH GIYRLMRILTHSKFFVKIKNSDEDEEDGYWLTPA SRLLLRDDPLSMVPFALCSLHPIYTDPGNHVCEW FQDDNETTFVTAHGVTVWEYAGKEPTTNKLFND ALASDSSLVNQILVDKCKHVFEGLSMVDVGGG IGIVAKAIADACSGLKCVVLDLPHVVADLKDSSES LTFVGGMNFESIPQADVFLKCILHDWNDGECV KILKKSKEAIPSNDEGGKVIIIVDMIVHDERDDHE AVKTQLLCDMEMMILANGKERTEKEWAKLFFD AGFTSYKITPVLGLRSLIEAFP
59	OMT		MEAGKRSYTEEEQQHSIYATQLVTASVMPMVLN TVIQLGVFEILAKGGPKAQLSASQIVSQMASATN LDASAMLDRLSLLVGYSVLTCVSDGQRVQKL YGLAPVAKYFVRNQDGASFGPLIALFQDKVFTES WFELKNAVLEGGVPFERVYGTHAFEYPSRDHRF NEVFNKAMIGYTTFLVKQILEQYKGFEGNLKLV DVGGGLGVALGMIISRYPNIKGINFDLPHVINNAP

			SYPGLEHVGGDMFESVPQGDAIFMKWILHDWSD DHCLKLLRNCYKALPENGKVIVVDGILPDEPEQN ASFISTSQIDVFMMTQNPGGKERTKEFFTLATSA GFRGMNMSCLVCNFWVMEFYK
60	OMT		MELSRDELLDAQAHVWNHIFNFINSMSLKCAIQL GIPDIIHNHKGKPTLSELTSALPSLNKAKSKNLYRL MRILVHSKFFVRDEEERYWLTPASRLLLVTTSEP RSVAPFALSMLDPFLMDPWHGLDKWFQNDESTP YANTHKKELWDHALKDPRLNDFNEGMASSAR LVNTILHGSEECKDVFGLESIVDVAGGTGTVAK AIAEAYPGLKCAVLDPHVADLQGNNGNLTYY AGDMFKFIPPANAVFMKWILHDWSDEECVKILE KCKEAIPSKSNGGKVIIVDIVVDEGKQANDKDNQ EAVETKLCFDMMLMMVVVKGKERTKEWAKIFF DAGFTSYNITPDEFFGLRSLIQVYP
61	OMT		MALQNVNTNFTSDDLGAQEHVWNHMFHYINSM SLKCVVDLGIPEIIHKHKGKPMTLSELIDALPIEKT SQCIYRLMRVLLQSKFFVKVNISESEEREYGLT PASRLLLRDAPLSLKPLVQLILDPILAKPWHHLTE WLSNDRHTTAFETAHGQSFWEHAVESSFNHLF NEGMASSDTRLVNEVLLRDYKHFEGFESFVDVG GGTGMTASGIADAFPMKCVVLDLPHVVAGLEE SKNLTYYAGDMFEAVPPADVLLKWILHDWND EESVKILKRCRDATGDSKGGKIIIDMVLGNND DDTAKESQLYFDMLMMVHLTGKERTKEWAM LFSEAGFSNYKITHALGLRSIIEVYP
62	OMT		MEAGKRSYTEEEEEAQQHSIYATQLVSASVMPM VLNTVIQLGVFEILAKGGPKAQLSASQIVSQMAS ATNLDAASAMLDRLSLLVGYSVLTCSVSDRQSV QKLYGLAPVAKYFVRNQDGASFGPLIALFQDKV FTESWFELKNAVLEGGVPFERVYGTNVFEYTSK DPRFNEALNKGMLGYTTFFAKQFLEQYKGFENL NKLVDVGGGLGVSLSTIVSKYPNIKGINFDLPHV VNNAPSYPGLEHVGGDMFESVPEGDVIFMKWIL HDWSDAHC SKLLKNCYKALPENGKVIVVDAVM PANPDNNSFSISTSQIDLFMLTQVPGGKERTKEF LATEAGFKGINRCCLICNFWVMEFYK
63	OMT		MGSITEYPATPTTDPSEEEESCLFAMQLASASVLP MVLKSAIELDLLELIKAGLVSPAELAAQLPTSN PEAPVMLDRILRLASYSVLNCSLKNTSDGGVER LYSLAPVCRFLTNGDGVSMAPLLLMNQDKILM ESWYHLKDAVLDGGIPFNKAYGMTAFDYHGTD PRFNKVFNQGMSNHSTIIMKKILEIYDGFELKSV VDVGGGTGATLNMIVSKYPSIKGINFDLPHVIED APTYPGVEHVGGDMFVSVPEDGAIFMKWICH WSDTHCLKFLKKCYEALPENGKVILAECVLPEVP

			DTGLPTKNVIHVDVIMLAHNPPGGKERTQKDFEA LAKGAGFTKFQKVCCAYNSWIMELRK
64	OMT		MSSTFDHTNASPKEDANCLFAMKLATGAVLPM VLKTAIELDLLELIKKAGPNASLSASELASHLPTK NPNAASMIDRILRLLSAHSILACSLKTLPNDCVER RYS LAPVCKFLTKNEDGVSLSNLVLFNNDK VSM ELWYHLKDAVIGGGNPFNRAYGINNFESHSHDL SHNEIFNRAMSDHSNIFTKKIVEIYNGFEGLSLV DVGGGIGATISTIISKYPSIKGINFDLPQVIQKAPS YPGVEHVGGDMFVSVPKADAVFMRWILHDWSD EDCQKVLKNCYESIPENGKVIIADSIFLEEPNSELE SSLVAVVDLIMMVITQGGKERTKEFEQELAKGA GFTKFIKVSSAFNTWIMELYK
65	OMT		MEVGKVSYSSEEVECCNYAGQLVTL SALPMVLN TVIELQVFEILAKAAATSGDPNNVQLSASQIVSL MPAGSAKNPDAAAMLDRMLSLLVGYSVLTCSL DDDQKLYGLAPVAKYFVLNKDGASFAPLMALF HHKVLIENTWELKNAVLEGGVPFVRAHGVSGFE YISRQNLNEVFNGMTGYTTFSMKHIVEQYKG FEVVKLVLDVGGGLGVTLSTIVSKHPNIKGINF LPHVIDNAPPYPGVEHVGGNMFESVPEGDAIFM KWILHDWNDACHLKLKNCYAVLPKDGKVIVV EAVLPVKPEKSTSFISASQADLLMMTQSPGGKER TEKEFLALAKGAGFSGIKKCLVWNYWVMEFY K
66	OMT		MSSAIYRNTTNSSIDQPDEDANCLFAMKLATGA VLPMVLKSAIELDLFELIKKAGPNAFVSASELAS QLLTKNPNAASMIDRILRLLSAPSILVCRLNTLPN GCEERQYSMA PVCKFLTKNEDGVSLSNLLLFTH DKVSMELWYHLRDAVIEGGDPFNRAHGMNLF YHCIHDIRYNNICNRAMSDHSTIFTKKIITYKGF EGLKSLVDVGGGIGATIATIVSKYPSIEGINDFPE VAQKAPSYPGVEHIGGNMFVGVPKADAVFMKW ILHDWSEDCQKVLKNCYESIPENGKVIIADSLFL EEQNNELESSLVTVSDLSMMATTPGGKERTKE LQELAKGAGFSKFVKVLSAFDTWIMELYK
67	OMT		MSTSMIRTNGSTISHDHHHDEEEQKACIYATQL MTSSVLPMLNNTVFQLQVFEILENAGPNAQLSAS GIVSQMQPAPKNPDAAAMLDRMLSLLVGHAVLT CSFTDDDDDEKLYGLTLVTKYFVRKENGASFGHR MDLLQDQVFFQSWYELKNAVLEGGVPFERVY GTHLYEYTSRDPFRNGVFNKAMTGYTTILMNEIVE HYKGFENLETLDVGGSGFVALRTIVSRFPNIKI NFDQPHVVKDAPPIPLEHVAGDMFESVPQGD AI FMKYILHDWSDACHLKV LKNCYKALPENGKVIV VEAVLQDKPEKSASYMSTSQIDVYIMTQTLGGK

			ERTTEKEFLSLAKKAGFQSMNMCCFVCNNWVFE FYK
68	OMT		MSDHSNIFTKKIVEIYNGFEGLKSLVDVGGGIGA TISTIISKYPSIKGINFDLPQVIQKAPSYPGVEHVG GDMFVSVPKADAVFMRWILHDWSEDCQKVLK NCYESIPENGKVIIADSIFLEEPNSELESSLVAVVD LIMMVITQGEYVVISSTPTHYKKIGSFPTGNPIGN DPDPVRKDPFPTARRSEKSARRYFGRKVGRHFSR PAFRPDSSRNIFDQNPVVLPMVLKSAIELNLELI KKAGPTAFVSASELASQLPTTNPNAASMIDRILH LLSAHSILVCTLKPLPKDRVERRYS LAPVCKFLT KNEDGVSLSTLLL FHNDRTVMEIWHYHLKDAVIE GGNAFNRAYGINVFESHSHDLSYNETFNRAMSD NSNIFTKKIIEAYKGFEGGLKSLVDVGGGTGATIST IISKYPSIKGFNFDLPQVIQEAAPYPGVEHIGGDM FVSVPKADAI FMKGILHDWGDEQCQKLLKNCYE SLPINGKVIIVEFLFSEEPNSGMEYSWVAVMDLV MMAITSSGKERTEKEFQELAKSAGFTKFIKVSSA FSAWIMELYK
69	OMT		MENQINTNIINHAVDQND EAVIHALNACGIFVLP MVLNAMVELGVLEVINKVGRDGRACLSPSQIAS QLQVTC SKDGVCVLDSMLRYLATHSFVTCLTRT LDNGETETCYGLAPAGRCFVEDESGVSLVPFLT TCKSAKRVEFWYNLKDAVLGDETLHKRLYGNS PYEHVTSNERRNKMFNKAMGAHSVVFMMKKILA TYQGFEGLSSLVNVGGADGVSLNMIIAKYPYIRG TNYDLPKVIQAAPSYNGIEHIGGDMFHEVPRGHA ILLKKKSEAVVHALNGCLALIFPMVLRASIKLDL FEIIAGGSLTPSAIAAKLPVSVPPERIGVLD SMLRS LTAHSFLTCTNDDEGRIEKRYALSPAGRCFVKDE SSGGVSLAPFQAFNCTPHTMDFWFDLEGAVLEK GTSFERVYGKHAYEYINSHPELGGMFNDAMAA HSALLMKKAVETYNGFEGLGSLNVNGGGYGV LDMIVTKYPSIRGINFDLPEVIQNAPSYKGIEHIGG NMFNQMPGDAILLKAILHNWSDEKCIILLKNCY KALPNKGKVIAIECIIPDHDIHQTD DVHVKFTSQ MDIIMQTVFGAKARTKDEL RALAMKAGFSEFKI VSYVYGMWILEFLKLE
70	ACC	EPB82652.1	MVEHRSLPGHFLGGSLESAPQGPVKDFVQAHE GHTVISKVLIANNGMAAMKEIRSVRKWAYETFG NERAIEFTVMATPEDLKANA EYIRMADNFVEVP GGSNNNNYANVELIVDVAERTAVHAVWAGWG HASENPRLPEMLAKSKHKCLFIGPPASAMRSLGD KISSTIVAQSAQVPTMGWSGDGITETEFDAAGHV IVPDNAYNEACVKTAEQGLKAAEKIGFPVMIKAS EGGGGKGIRMVKDGSNFAQLFAQVQGEIPGSPIF

			IMKLAGNARHLEVQLLADQYGNAISLFGRDCSV QRRHQKIIIEAPVTIAKPDVFEQMEKAAVRLGKL VGYVSAGTVEYLYSHHDDQFYFLELNPRLQVEH PTTEMVSGVNLPAACLQIAMGIPLHRIRDIRVLY GVQPNASASEIDFGFEHPTSLTSHRRPTPKGHVIAC RITAENPDAGFKPSSGIMQELNFRSSTNVWGYFS VVSAGGLHEYADSQFGHIFAYGENRQQARKNM VIALKELSIRADFRSTVEYIIRLLETPDFEENTINT GWLDMMLISKKLTAERPDTMLAVFCGAVTKAHM ASLDCFQQYKQSLEKGQVPSKGSCLKTVFTVDFIY EEVRYNFTVTQSAPGIYTLYLNGTKTQVGIRDLS DGGLLISIDGKSHTTYSRDEVQATRMMVDGKTC LLEKESDPTQLRSPSPGKLVNLLVENGDLNAGD AYAEIEVMKMYMPLIATEDGHVQFIKQAGATLE AGDIIGILSLDDPSRVKHALPFNGTVPAFGAPHIT GDKPVQRFNATKLTQLHILQGYDNQALVQTVVK DFADILNNPDLPYSELNSVLSALSGRIPQRLEASI HKLADESKAANQEFPAAQFEKLVEDFAREHITLQ SEATAYKNSVAPLSSIFARYRNGLTEHAYSNYVE LMEAYYDVEILFNQQREEEVILSLRDQHKDDLD KVLAVTLSHAKVNIKNNVILMLLDLINPVSTGSA LDKYFTPIPKRLSEIESRATQKVTLKARELLILCQ LPSYEERQAQMYQILKNSVTESVYGGGSEYRTPS YDAFKDLIDTKFNVFDVLPFFFYHADPYIALAAI EVYCRRSYHAYKILDVAYNLEHKPYVVAWKFL LQTAANGIDSNKRIASYSDLTFLNKTETEEPIRTG AMTACNSLADLQAEPRILTAEEEEPLPPMLQRN AAPKEERMENILNIAVRADEDMDDTAFRTKICE MITANADVFRQAHLRRLSVVVCRDNQWPDYYT FRERENYQEDETIRHIEPAMAYQLELARLSNFDIK PCFIENRQMHVYYAVAKENPSDCRFFIRALVRPG RVKSSMRTADYLISESDRLTDLDTLEIVSHEYK NSDCNHLFINFIPTFAIEADDVEHALKDFVDRHG KRLWKL RVTGAEIRFNVQSKKPDAPIIPMRFTVD NVSGFILKVEVYQEVKTEKSGWILKSVNKIPGAM HMQPLSTPYPTKEWLQPRRYKAHLMGTTYVYD FPELFRQSVQNQWTQAIKRNPLLKQPSHLVEAKE LVLDEDDVLQEIDRAPGTNTVGMVAWIMTIRTP EYPSGRRIIAIAINDITFKIGSFGVAEDQVFYKASEL ARALGIPRIYLSANS GARIGLADELISQFRAAWK DASNPTAGFKYLYLTPAEYDVLAQQGDAKSVLV EEIQDEGETRLRITDVIGHTDGLGVENLKGSLIA GATSRAYDDIFTITLVTCRSVGIGAYLVRLGQRTI QNEGQPIILTGAPALNKVLGREVYTSNLQLGGTQ IMYKNGVSHLTAENDLEGIKIVQWLSFVPDVR NAPVSMRLGADPIDRDIEYTPPKGPSDPRFFLAG
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			KSENGKWLSGFFDQDSFVETLSGWARTVVVGR ARLGGIPMGVVSVEPTRVENIVPADPANSSTEQ VFMEAGGVWFPNSAYKTAQAINDFNKGEQLPL MIFANWRGFSGGQRDMYNEVLKYGAQIVDAL NYKQPVFVYIIPNGELRGGAWVVVDPTINKDMM EMYADNNARGGVLEPEGIVEIKYRKPALLATME RLDATYASLKKQLAEEGKTDEEKAALKVQVEA REQELLPVYQQISIQFADLHDRAGRMKAKGVIRK ALDWRRARHYFYWRVRRRLCEEYTFRKIVTATS AAPMPREQMLDLVKQWFTNDNETVNFEDADEL VSEWFEKRASVIDQRISKLKSDATKEQIVSLGNA DQEAVIEGFSQLIENLSEDARAEILRKLNSRF
71	TAL	ABC88669.1	MTQVVERQADRLSSREYLARVVRSGWDAGLT SCTDEEIVRMGASARTIEEYLKSDKPIYGLTQGF PLVLFDADSELEQGGSLISHLGTGQGAPLAPEVS RLILWLRIQNMKGYSVSPVFWQKLADLWNK GFTPAIPRHGTVSASGDLQPLAHAALAFTGVGEA WTRDADGRWSTVPAVDALAALGAEPDWPVRE ALAFVNGTGASLAVAVLNHRSAALRLVRACAVLS ARLATLLGANPEHYDVGHGVARGQVQGLTAAE WIRQGLPRGMVRDGSRLQEPYSLRCAPQVLGA VLDQLDGAGDVLAREVDGCQDNPIITYEGELLHG GNFHAMPVGFASDQIGLAMHMAAYLAERQLGL LVSPVTNGDLPPMLTPRAGRAGLAGVQISATSF VSRIRQLVFPA SLTTLPTNGWNQDHVPMALNGA NSVFEALELGWLTVGSLAVGVAQLAAMTGHA EGVWAE LAGICPPLDADRPLGAEVRAARDLLSA HADQLLVDEADGKDFG
72	4CL	P14912.1	MGDCVAPKEDLIFRSKLPDIYIPKHLPLHTYCFEN ISKVGDKSCLINGATGETFTYSQVELLSRKVASG LNKLGIQQGDTIMLLLNSPEYFFAFLGASYRGAI STMANPFFTSAEVIKQLKASQAKLIITQACYVDK VKDYAAEKNIQIICIDDA PQDCLHFSKLMEADES EMPEVVINSDDVVALPYSSGTTGLPKGVM LTHK GLVTSVAQQVDGDNPNLYMHSEDMICILPLFHI YSLNAVLCCGLRAGVTILIMQKFDIVPFLELIQKY KVTIGPFVPIVLAIAKSPVVDKYDLSSVRTVMSG AAPLGKELEDAVRAKFPNAKLGGYGMTEAGP VLAMCLAFAKEPYEIKSGACGTVVRNAEMKIVD PETNASLPRNQGEICIRGDQIMKGYLNDPESTRT TIDEEGWLHTGDIGFIDDDDEL FIVDRLKEIKYK GFQVAPAELEALLLTHPTISDAAVVPMIDEKAGE VPVAFVVRTNGFTTTEEEIKQFVSKQVVFYKRIF RVFFVDAIPKSPSGKILRKDLRARIASGDLPK
73	4CL	AIT52344.1	MGDCAAPKQEIIFRSKLPDIYIPKHLPLHSYCFENI SKVSDRACLINGATGETFSYAQVELISRRVASGL

			NKLGHIHQGDTMMILLPNTPEYFFAFLGASYRGAV STMANPFFTSPEVIKQLKASQAKLIITQACYVEKV KEYAAENNITVVCIDEAPRDCLHFTTLMEADEAE MPEVAIDSDDVVALPYSSGTTGLPKGVMLTHKG LVTSAQQRVDGENPNLYIHSEDMICILPLFHIYS LNAVLCCLGRAGATILIMQKFDIVPFLELIQKYK VTIGPFVPPIVLAIKSPVVDNYDLSSVRTVMSG APLKELEDVRAKFPNAKLGQGYGMTEAGPV LAMCLAFAKEPFEIKSGACGTVVRNAEMKIVDP ETHASLPRNQSGEICIRGDQIMKGYLNDPESTKTT IDEEGWLHTGDIGFIDEDDELFIVDRLKEIKYKG FQVAPAEIEALLLTHPTISDAAVVPMIDEKAGEVP VAFVVRLNGSTTTEEEIKQFVSKQVVFYKRVFRV FFVDAIPKSPSGKILRKELRARIASGDLPK
74	4CL	GER48539.1	MEPTTKSKDIIFRSKLPDIYIPKHLPLHTYCFENIS RFGSRPCLINGSTGEILTYDQVELASRRVGSGLHR LGIRQGDITIMLLLPNSPEFVLAFLGASHIGAVSTM ANPFFTPAEVVKQAAASRAKLIVTQACHVDKVR DYAAEHGVKVVCDGAPPEECLPFSEVASGDEA ELPAVKISPDDVVALPYSSGTTGLPKGVMLTHKG LVTSAQQVDGENPNLYIHSDDVIMCVLPLFHIY SLNSIMLCGLRVGAAILIMQKFEIVPFLELIQRYR VTIGPFVPPIVLAIKSPVVEKYDLSSVRTVMSG APLGRELEDAVRLKFPNAKLGQGYGMTEAGPVL AMCLAFAKEPFEIKSGACGTVVRNAEMKIVDTE TGASLGRNQPGEICIRGDQIMKGYLNDPESTERTI DKEGWLHTGDIGFIDDDDELFIVDRLKEIKYKGF QVAPAELEALLLNHPNISDAAVVSMKDEQAGEV PVAYVVKSNGSTITEDEIKQFVSKQVIFYKRINRV FFIDAIPKSPSGKILRKDLRLAAGVPN
75	4CL	KAF3620179.1	MPMENEAKQGDIIIFRSKLPDIYIPNHLSLHSYCFE NISEFSSRPCLINGANNQIYTYADVELNSRKVAA GLHKQFGIQKQDTIMILLPNSEFVFAFLGASYLG AISTMANPLFTPAEVVKQVKASNAEIIVTQACHV NKVKDYALENDVKIVCIDSAPEGCVHFSELIQAD EHDIPVQIKPDDVVALPYSSGTTGLPKGVMLTH KGLVTSVAQQVDGENPNLYIHSEDMICILPLF HIYSLNSVLLCGLRVGAAILIMQKFDIVPFLELIQ NYKVTIGPFVPPIVLAIKSPMVDNYDLSSVRTV MSGAPLKELEDTVRAKFPNAKLGQGYGMTE AGPVLAMCLAFAKEPFEIKSGACGTVVRNAEMK IVDPDTGNSLHRNQSGEICIRGDQIMKGYLNDPE ATAGTIDKEGWLHTGDIGYIDNDELFIVDRLKE LIKYKGFQVAPAELEALLLNHPNISDAAVVPMK DEQAGEVPVAFVVRSNGSTITEDEVKEFISKQVIF

			YKRIKRVFFVDAVPKSPSGKILRKDLRAKLAAGF PN
76	4CL	ASU87409.1	MDTKTTQQEIIFRSKLPDIYIPKQLPLHSYCFENIS QFSSKPCLINGSTGKVYTYSDVELTSRKVAAGFH NLGIQQRDTIMLLLPCPEFVFAFLGASYLGAIIT MANPFFTPAETIKQAKASNSKLIITQSSYTSKVLD YSSENNVKIICIDSPPDGCLHFSELIQSNETQLPEV EIDSNEVVALPYSSGTTGLPKGVMLTHKGLVTSV AQQVDGENPNLYIHSEDMMMCVLPLFHIYSLNS VLLCGLRVGAAILIMQKFEIGSFLKLIQRYKVTIG PFVPPIVLAIKSEVVDDYDLSTIRTMMSGAAPL GKELEDAVRAKFPKAKLGQGYGMTEAGPVLAM CLAFAKKPFEIKSGACGTVVRNAEMKIVDPDAG FSLPRNQPGEICIRGDQIMKGYLNDPEATERTIDK QGWLHTGDIGYIDDDDELFIVDRLKELIKYKGFQ VAPAELEALLLNHPTISDAAVVPMKDESAGEVP VAFVVRTNGFEVTENEIKKYISEQVVFYKKINRV YFVDAIPKAPSGKILRKDLRARLAAGIPS
77	CHS	AAF60297.1	MVTVEEYRKAQRAEGPATVMAIGTATPTNCVD QSTYPDYYFRITNSEHKTDLKEKFKRMCEKSMIK KRYMHLTEEILKENPSMCEYMAPSLDARQDIVV VEVPKLGKEAAQKAIKEWGQPKSKITHLVFCTTS GVDMPGCDYQLTKLLGLRPSVKRLMMYQQGCF AGGTVLRLAKDLAENNKGARVLVVCSEITAVTF RGPNDTHLDSL VGQALFGDGAGAIIGSDPIPGVE RPLFELVSAAQTLLPD SHGAIDGHLREVGLTFHL LKDVPGGLISKNIEKSLEEAFRPLSISDWNLSLFWIA HPGGPAILDQVEIKLGLKPEKCLKATRNVL SNYGN MSSACVLFILDEM RKASAKEGLGTTGEGLEWGV LFGFGPGLTVETVVLH SVAT
78	CHS	XP_016566084.1	MVTVEEYRKAQRAEGPATVMAIGTATPSNCVD QSTYPDYYFRITNSEHKTTELKEKFKRMCEKSMIK TRYMHLTEEILKENPNMCAYMAPSLDARQDIVV VEVPKLGKEAAQKAIKEWGQPKSKITHLVFCTTS GVDMPGCDYQLAKLLGLRPSVKRLMMYQQGCF AGGTVLRLAKDLAENNKGARVLVVCSEITAVTF RGPSESHLDSL VGQALFGDGAAAIIIMGSDPIPGV ERPLFQLVSAAQTLLPDSEGAIDGHLREVGLTFH LLKDVPGGLISKNIEKSLVEAFQPLGISDWNLSLFWI AHPGGPAILDQVELKLGLKPEKCLKATREVL SNY GNMSSACVLFILDEM RKASTKEGLGTS GEGLEW GVLFFGPGLTVETVVLH SVAI
79	CHS	AEC13058.1	MVTVEEVRKAQRAEGPATVLAIGTATPPNCIDQS TYPDYYFRITKSEHKAELKEKFQRMCDKSMIKK RYMYLTEEILKENPSMCEYMAPSLDARQDMVV VEIPKLGKEAATKAIKEWGQPKSKITHLVFCTTS

			GVDMPGADYQLTKLLGLRPSVKRLMMYQQGCF AGGTVLRLAKDLAENNKGARVLVVCSEITAVTF RGPSDTHLDSL VGQALFGDGAAAIIVGSDPLPEV EKPLFELVSAAQTILPDSG AIDGHLREVGLTFHL LKDV PGLISK NIEKSLNEAFKPLNITDWNLSFWIA HPGGPAILDQVEAKLGLKPEKLEATRHLSEYGN MSSACVLFILDEVRRKSAANGHKTTGEGLEWGV LFGFGPGLTVETVVLHVSAA
80	CHS	AHL83549.1	MSMTPSVHEIRKAQRSEGPATVLSIGTATPTNFV PQADYDPYYFRITNSDHMTDLKDKFKRMCEKS MITKRHMYLTEEILKENPKMCEYMAPSLDARQD IVVVEVPKLGKEAAAKAIKEWGQPKSKITHLIFC TTSGVDMPGADYQLTKLLGLRPSVKRFMMYQQ GCFAGGTVLRLAKDLAENNKGARVLVVCSEITA VTRGPSHTHLDL VGQALFGDGAAAVILGADP DTSVERPIFELVSAAQTILPDSEGAIDGHLREVGL TFHLLKDV PGLISK NIEKSLVEAFTPIGISDWN SIF WIAHPGGPAILDQVEAKLGLKQEKLSATRHVLSE YGNMSSACVLFILDEVRRKKSVEEGKATTGEGLE WGVLF GFGPGLTVETIVLHSLPAV
81	CHS	ALE71934.1	MAPPAMEEIRRAQRAEGPATVLAIGASTPPNALY QADYDPYYFRITKSEHLTELKEFKQMCDKSMI RKRYMYLTEEILKENPNICAFMAPSLDARQDIVV TEVPKLAKESARAIKEWGQPKSRITHLIFCTTSG VDMPGADYQLTRLLGLRPSVNRIMLYQQGCFAG GTVLRLAKDLAENNAGARVLVVCSEITAVTFRG PSESHLDL VGQALFGDGAAAIIVGSDPDLTTERP LFQLVSASQTILPESEGAIDGHLREMGLTFHLLKD VPGLISKNIQKSLVETFKPLGIHDWNSIFWIAHPG GPAILDQVEIKLGLKEEKLASSRNVLA EYGNMSS ACVLFILDEMRRRS AEAGQATTGEGLEWGVLF G FGPGLTVETVVLRSVPIAGAV
82	CHI	P28012.1	MAASITAITVENLEYPVVTSPTVGKSYFLGGAG ERGLTIEGNFIKFTAIGVYLEDIAVASLAAKWKG KSSEELLETDFYRDIISGPFEKLIRGSKIRELSGPE YSRKVMENCVAHLKSVGTYGDAEAEAMQKFAE AFKPVNFPPGASVFYRQSPDGILGLSFSPDTSIPEK EAALIENKAVSSAVLETMIGEHA VSPDLKRCLAA RLPALLNEGAFKIGN
83	CHI	PNX83855.1	MSAITAIHVENIEFPAVITSPVTGKSYFLGGAGER GLTIEGNFIKFTAIGVYLEDVAVASLATKWKGKS SEELLETDFYRDIISGPFEKLIRGSKIRELSGPEYS RKVTENCVAHLKSVGTYGDAEVEAMEKFVEAF KPINFPFGASVFYRQSPDGILGVSIHFFP
84	CHI	XP_027366189.1	MAAASLTAVQVENLEFPAVVTSPTATGKTYFLGG AGVRGLTIEGNFIKFTGIGVYLEDQAVASLATKW

			KGKSSEELVESLDFFRDIISGPFEKLIRGSKIRQLS GPEYSKKVMENCVAHMKSVGTYGDAEAAGIEE FAQAFKPVNFPPGASVFYRQSPDGVGLSFSQDA TIPEEEAAVIKNKPVSAAVLETMIGEHAVSPDLK RSLAARLPAVL SHGVFKIGN
85	CHI	XP_015942246.1	MAAEPSITAIQFENLVFPAVVTPPGSSKSYFLAGA GERGLTIDGKFIKFTGIGVYLEDKAVPSLAGKWK DKSSQQLLQTLHFYRDIISGPFEKLIRGSKILALSG VEYSRKVMENCVAHMKSVGTYGDAEAIAIQF AEAFKNVNFKPGASVFYRQSPLGHLGLSFSQDG NIPEKEAAVIENKPLSSAVLETMIGEHAVSPDLKC SLAARLPAVLQQGHIIVTPPQHN
86	CHI	GAV77263.1	MGPSPSVTELQVENVTFPFSVKPPGSTKTLFLGG AGERGLEIQGKFIKFTAIGVYLEGDAVASLAVKW KGKSKEELTDSVEFFRDIVTGPFKFTQVTTILPL TGQQYSEKVSENCVAFWKSVDGIYTDAEAKAIEK FIEVFKEETFPPGSSILFTQSPNGALTIAFSKDGVIP EVGKAVIENKLLAEGLLESIIIGKHGVSPVAKQCL ATRLSELL

Incorporation by Reference

References and citations to other documents, such as patents, patent applications, patent
5 publications, journals, books, papers, web contents, publicly accessible databases, have been
made throughout this disclosure. All such documents are hereby incorporated herein by reference
in their entirety for all purposes.

Equivalents

Various modifications of the invention and many further embodiments thereof, in addition
10 to those shown and described herein, will become apparent to those skilled in the art from the full
contents of this document, including references to the scientific and patent literature cited herein.
The subject matter herein contains important information, exemplification and guidance that can
be adapted to the practice of this invention in its various embodiments and equivalents thereof.

CLAIMS:

1. A method for bioproduction of pectolinarigenin, the method comprising providing one or more enzymes, wherein the one or more enzymes result in transformation of naringenin to pectolinarigenin through one or more intermediates.
2. The method of claim 1, wherein the method comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of naringenin to pectolinarigenin through one or more intermediates.
3. The method of claim 1, wherein the method is a cell-free production of pectolinarigenin, wherein the one or more enzymes in a cell-free medium result in transformation of naringenin to pectolinarigenin through one or more intermediates.
4. The method of claim 1, wherein the method comprises a combination of: (i) an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of naringenin to pectolinarigenin or one or more intermediates, and (ii) cell-free production comprising one or more enzymes resulting in transformation of naringenin or a substrate to pectolinarigenin or one or more intermediates for synthesis of pectolinarigenin.
5. The method of claim 2, wherein the engineered host cell comprises genetic modifications to enhance the production of naringenin or any precursor leading to production of naringenin in the engineered host cell.
6. The method of any of claims 1-4, wherein the one or more intermediates are selected from a group consisting of: apigenin, scutellarein, hispidulin, isosakuranetin, carthamidin, 6-methoxycarthamidin, scutellarein, 4-methoxycarthamidin, acacetin, 4',6-methoxynaringenin, 4'-methoxyscutellarein, and any combination thereof.

7. The method of any of claims 1-4, wherein the intermediates are apigenin, scutellarein, and hispidulin.
8. The method of any of claims 1-4, wherein the one or more enzymes selected from the group consisting of: flavone synthase (FNSI), flavone hydroxylase cytochrome P450 (P450), O-methyltransferase (OMT), and any combination thereof.
9. The method of any of claims 1-4, wherein the one or more intermediates are apigenin, scutellarein, and 4'-methoxyscutellarein.
10. The method of any of claims 1-4, wherein the one or more intermediates are apigenin, scutellarein, and hispidulin.
11. The method of any of claims 1-4, wherein the one or more intermediates are apigenin, acacetin, and 4'-methoxyscutellarein.
12. The method of any of claims 1-4, wherein the one or more intermediates are isosakuranetin, 4'-methoxycarthamidin, and 4',6-methoxynaringenin.
13. The method of any of claims 1-4, wherein the one or more intermediates are isosakuranetin, 4'-methoxycarthamidin, and 4'-methoxyscutellarein.
14. The method of any of claims 1-4, wherein the one or more intermediates are isosakuranetin, acacetin, and 4'-methoxyscutellarein.
15. The method of any of claims 1-4, wherein the one or more intermediates are carthamidin, 6-methoxycarthamidin, and hispidulin.
16. The method of any of claims 1-4, wherein the one or more intermediates are carthamidin, 6-methoxycarthamidin, and 4',6-methoxynaringenin.
17. The method of any of claims 1-4, wherein the one or more intermediates are carthamidin, 6-methoxycarthamidin, and 4'-methoxyscutellarein.

18. The method of any of claims 1-4, wherein the one or more intermediates are carthamidin, scutellarein, and hispidulin.
19. The method of any of claims 1-4, wherein the one or more intermediates are carthamidin, scutellarein, and 4'-methoxyscutellarein.
20. The method of any of claims 1-4, wherein the one or more intermediates are carthamidin, 4'-methoxycarthamidin, and 4',6-methoxynaringenin.
21. The method of any of claims 1-4, wherein the one or more intermediates are carthamidin, 4'-methoxycarthamidin, and 4'-methoxyscutellarein.
22. The method of claim 2, wherein the engineered host cell is bacteria, yeast, and/or mammalian cells.
23. The method of claim 3, wherein the cell-free medium is a cell lysate.
24. The method of claim 23, wherein the cell lysate is a cell lysate from cells from a host organism expressing the one or more enzymes.
25. The method of claim 24, wherein the host organism is selected from a group consisting of: bacteria, yeast, and/or mammalian cells.
26. The method of claim 24, wherein the one or more enzymes are introduced in the host organism by integration into genome of the host organism or on a plasmid.
27. The method of claim 26, wherein the host organisms expressing the one or more enzymes are cultured until a pre-determined biomass is achieved to produce the requisite quantity of the one or more enzymes.
28. The method of claim 27, further comprising lysing cells followed by removal of cell debris to generate a cell lysate for use in the cell-free medium for cell-free production of pectolinarigenin.

29. The method of claim 3, wherein the cell-free medium comprises: buffer, magnesium chloride, one or more substrates, one or more cofactors, the one or more enzymes, and/or water.
30. The method of claim 29, wherein the buffer is a phosphate buffer.
31. The method of claim 29, wherein pH of cell-free medium is from about 5.5 to about 10.
32. The method of claim 29, wherein pH of cell-free medium is from about 6.5 to about 9.
33. The method of 29, wherein the buffer concentration is from about 10 mM to about 500 mM.
34. The method of 29, wherein the buffer concentration is from about 50 mM to about 300 mM.
35. The method of claim 29, wherein the buffer concentration is from about 20 mM to about 100 mM.
36. The method of claim 29, wherein magnesium chloride is present in the range of from about 1 mM to about 50 mM.
37. The method of claim 29, wherein magnesium chloride is present in the range of from about 5 mM to about 25 mM.
38. The method of claim 29, wherein the substrate is naringenin.
39. The method of claim 29, wherein the one or more substrates are present in a concentration of about 1 mM to about 500 mM.
40. The method of claim 29, wherein the one or more substrates are present in a concentration of about 1 mM to about 200 mM.

41. The method of claim 29, wherein the one or more substrates are present in a concentration of about 10 mM to about 100 mM.
42. The method of claim 29, wherein the one or more cofactors are present in a concentration of about 1 mM to about 500 mM.
43. The method of claim 29, wherein the one or more cofactors are present in a concentration of about 1 mM to about 200 mM.
44. The method of claim 29, wherein the one or more cofactors are present in a concentration of about 1 mM to about 10 mM.
45. The method of claim 29, wherein the one or more enzymes are present in the range of from about 0.1 μ M to about 5 mM.
46. The method of claim 29, wherein the one or more enzymes are present in the range of from about 0.1 μ M to about 1 mM.
47. The method of claim 29, wherein the one or more enzymes are present in the range of from about 1 μ M to about 100 μ M.
48. The method of claim 29, wherein reaction for cell-free production of pectolinarigenin is conducted for a duration of from about 0.1 hours to about 20 hours.
49. The method of claim 29, wherein reaction for cell-free production of pectolinarigenin is conducted for a duration of from about 1 hour to about 10 hours.
50. The method of claim 29, wherein temperature of the cell-free medium is from about 20 °C to about 40 °C.
51. The method of claim 3, wherein reaction is conducted in a bubble column reactor, wherein the one or more enzymes are in a solution.

52. The method of claim 3, wherein reaction is conducted in a packed bed reactor, wherein the one or more enzymes are immobilized.
53. The method of any of claims 1-4, wherein the enzyme is flavone synthase enzyme with an amino acid sequence at least 95% identical to any one of enzymes selected from the group consisting of SEQ ID 1 to 15.
54. The method of any of claims 1-4, wherein the enzyme is flavone hydroxylase cytochrome P450 enzyme with an amino acid sequence at least 95% identical to any one of enzymes selected from the group consisting of SEQ ID 16 to 30.
55. The method of any of claims 1-4, wherein the enzyme is O-methyltransferase enzyme with an amino acid sequence at least 95% identical to any one of enzymes selected from the group consisting of SEQ ID 31 to 69.
56. A method for bioproduction of pectolinarigenin, the method comprising: providing one or more enzymes, wherein the one or more enzymes result in transformation of one or more substrates to pectolinarigenin optionally through one or more intermediates.
57. The method of claim 56, wherein the one or more substrates is selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof.
58. The method of claim 56, wherein the one or more substrate is apigenin.
59. The method of claim 56, wherein the one or more substrate is scutellarein.
60. The method of claim 56, wherein the one or more substrate is hispidulin.
61. The method of any of claims 56 – 60, wherein the method comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates.

62. The method of any of claims 56 – 60, wherein the method is a cell-free production of pectolinarigenin, wherein the one or more enzymes in a cell-free medium result in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates.
63. A composition for bioproduction of pectolinarigenin, wherein the composition comprises one or more enzymes, wherein the one or more enzymes result in transformation of one or more substrates to pectolinarigenin optionally through one or more intermediates.
64. The composition of claim 63, wherein the one or more substrates is selected from the group consisting of naringenin, apigenin, scutellarein, hispidulin, or any combination thereof.
65. The composition of claim 63, wherein the one or more substrate is apigenin.
66. The composition of claim 63, wherein the one or more substrate is scutellarein.
67. The composition of claim 63, wherein the one or more substrate is hispidulin.
68. The composition of any of claims 63 - 67, wherein the composition comprises an engineered host cell, wherein the engineered host cell comprises one or more genetic modifications for expression of one or more enzymes resulting in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates.
69. The composition of any of claims 63 - 67, wherein the composition is for a cell-free production of pectolinarigenin, wherein the one or more enzymes in a cell-free medium result in transformation of the one or more substrate to pectolinarigenin optionally through one or more intermediates.
70. A method for production of pectolinarigenin, the method comprises: providing an engineered host cell that comprises one or more genetic modifications, wherein the one or

more genetic modifications result in enzymatic transformation of glycerol to pectolinarigenin through one or more intermediates.

71. The method of claim 70, wherein the engineered host cell comprises one or more genetic modifications to enhance the production of naringenin from glycerol the engineered host cell.
72. The method of claim 71, wherein the engineered host cell comprises one or more genetic modifications to enhance the transformation of naringenin to pectolinarigenin in the engineered host cell through one or more intermediates.
73. The method of claims 71 or 72, wherein the one or more genetic modification is expression or overexpression of one or more enzymes.
74. The method of claim 73, wherein the one or more enzymes selected from the group consisting of: FNSI, P450, OMT, and any combination thereof.
75. The method of claim 74, wherein the one or more enzymes selected from the group consisting of expression or overexpression of: FNSI, P450, OMT, ACC, TAL, 4CL, CHS, CHI, and any combination thereof.
76. The method of any of claims 70 – 75, wherein the engineered host cell is *E. coli*.
77. A composition for production of pectolinarigenin, wherein the composition comprises an engineered host cell that comprises one or more genetic modifications, wherein the one or more genetic modifications result in enzymatic transformation of glycerol to pectolinarigenin through one or more intermediates.
78. The composition of claim 77, wherein the engineered host cell comprises one or more genetic modifications to enhance the production of naringenin from glycerol the engineered host cell.

79. The composition of claim 72, wherein the engineered host cell comprises one or more genetic modifications to enhance the transformation of naringenin to pectolinarigenin in the engineered host cell through one or more intermediates.
80. The composition of claims 78 or 79, wherein the one or more genetic modification is expression or overexpression of one or more enzymes.
81. The composition of claim 80, wherein the one or more enzymes selected from the group consisting of: FNSI, P450, OMT, and any combination thereof.
82. The composition of claim 80, wherein the one or more enzymes selected from the group consisting of expression or overexpression of: FNSI, P450, OMT, ACC, TAL, 4CL, CHS, CHI, and any combination thereof.
83. The composition of any of claims 70 – 75, wherein the engineered host cell is *E. coli*.