

COMPOSITIONS AND METHODS FOR PRODUCING CHEMICALS AND DERIVATIVES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application serial number 61/704,408, filed August 21, 2012, which is hereby incorporated by reference in its entirety, including all tables, figures, and claims.

INCORPORATION OF SEQUENCE LISTING

[0002] The material in the accompanying Sequence Listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, name SGI1660_1WO_PCT_Sequence Listing_ST25, was created on August __, 2013 and is __ KB. The file can be assessed using Microsoft Word on a computer that uses Windows OS.

BACKGROUND OF THE INVENTION

[0003] In recent years, an increasing effort has been devoted to identify new and effective ways to use renewable feedstocks for the production of organic chemicals. Among a plethora of downstream chemical processing technologies, the conversion of biomass-derived sugars to value-added chemicals is considered very important. In particular, six-carboned carbohydrates, i.e. hexoses such as fructose and glucose, are widely recognized the most abundant monosaccharides existing in nature, therefore can be suitably and economically used as the chemical feedstocks.

[0004] The production of furans and furan derivatives from sugars has attracted increasing attention in chemistry and in catalysis studies, and is believed to have the potential to provide one of the major routes to achieving sustainable energy supply and chemicals production. Indeed, dehydration and/or oxidation of the sugars available within biorefineries with integrated biomass conversion processes can lead to a large family of products including a wide range of furans and furan derivatives.

[0005] Among the furans having the most commercial values, furan-2,5-dicarboxylic acid (also known as 2,5-furandicarboxylic acid, hereinafter abbreviated as FDCA) is a valuable intermediate with various uses in several industries including pharmaceuticals, pesticides, antibacterial agents, fragrances, agricultural chemicals, as well as in a wide range of manufacturing applications of polymer materials, *e.g.* bioplastic resins. As such, FDCA is considered a green alternative of terephthalic acid (TPA), a petroleum-based monomer that is one of the largest-volume petrochemicals produced yearly worldwide. In fact, the US Department of Energy has identified FDCA as one of the top 12 priority compounds made from sugars into a value-added chemical for establishing the “green” chemistry of the future, and as such, it has been named one of the “sleeping giants” of the renewable intermediate chemicals (Werpy and Petersen, *Top Value Added Chemicals from Biomass*. US Department of Energy, Biomass, Vol 1, 2004).

[0006] Although various methods have been proposed for commercial scale production of FDCA (for review, see, *e.g.*, Tong et al., *Appl. Catalysis A: General*, 385, 1-13, 2010), the main industrial synthesis of FDCA currently relies on a chemical dehydration of hexoses, such as glucose or fructose, to the intermediate 5-hydroxymethylfurfural (5-HMF), followed by a chemical oxidation to FDCA. However, it has been reported that current FDCA production processes via dehydration are generally nonselective, unless immediately upon their formation, the unstable intermediate products can be transformed to more stable materials. Thus, the primary technical barrier in the production and use of FDCA is the development of an effective and selective dehydration process from biomass-derived sugars.

[0007] It is therefore desirable to develop methods for production of this highly important compound, as well as many other chemicals and metabolites, by alternative means that not only would substitute renewable for petroleum-based feedstocks, but also use less energy and capital-intensive technologies. In particular, the selective control of sugar dehydration could be a very powerful technology, leading to a wide range of additional, inexpensive building blocks.

SUMMARY OF THE INVENTION

[0008] The present invention provides methods for producing a product of one or more enzymatic pathways. The pathways used in the methods of the invention involve one or more conversion steps such as, for example, an enzymatic conversion of guluronic acid into D-glucarate (Step 7); an enzymatic conversion of 5-ketogluconate (5-KGA) into L-Iduronic acid (Step 15); an enzymatic conversion of L-Iduronic acid into Idaric acid (Step 7b); and an enzymatic conversion of 5-ketocluconate into 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16). In some embodiments the methods of the invention produce 2,5-furandicarboxylic acid (FDCA) as a product. The methods include both enzymatic and chemical conversions as steps. Various pathways are also provided for converting glucose into 5-dehydro-4-deoxy-glucarate (DDG), and for converting glucose into FDCA. The methods can also involve the use of engineered enzymes that perform reactions with high specificity and efficiency.

[0009] In a first aspect the invention provides a method for producing a product of an enzymatic or chemical pathway from a starting substrate. The pathway can contain any one or more of the following conversion steps: an enzymatic conversion of guluronic acid into D-glucarate (Step 7); an enzymatic conversion of 5-ketogluconate (5-KGA) into L-Iduronic acid (Step 15); an enzymatic conversion of L-Iduronic acid into Idaric acid (Step 7b); and an enzymatic conversion of 5-ketocluconate into 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16); an enzymatic conversion of 1,5-gluconolactone to gulurono-lactone (Step 19).

[0010] In one embodiment the product of the enzymatic pathway is 5-dehydro-4-deoxy-glucarate (DDG). In various embodiments the substrate of the method can be glucose, and the product can 5-dehydro-4-deoxy-glucarate (DDG). The method can involve the steps of the enzymatic

conversion of D-glucose to 1,5-gluconolactone (Step 1); the enzymatic conversion of 1,5-gluconolactone to gulurono-lactone (Step 19); the enzymatic conversion of gulurono-lactone to guluronic acid (Step 1B); the enzymatic conversion of guluronic acid to D-glucarate (Step 7); and the enzymatic conversion of D-glucarate to 5-dehydro-4-deoxy-glucarate (DDG) (Step 8).

[0011] In another method of the invention the substrate is glucose and the product is DDG, and the method involves the steps of: the conversion of D-glucose to 1,5-gluconolactone (Step 1); the conversion of 1,5-gluconolactone to gluconic acid (Step 1a); the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14); the conversion of 5-ketogluconate (5-KGA) to L-Iduronic acid (Step 15); the conversion of L-Iduronic acid to Idaric acid (Step 7b); and the conversion of Idaric acid to DDG (Step 8a).

[0012] In another method of the invention the substrate is glucose and the product is DDG and the method involves the steps of the conversion of D-glucose to 1,5-gluconolactone (Step 1); the conversion of 1,5-gluconolactone to gluconic acid (Step 1a); the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14); the conversion of 5-ketogluconate (5-KGA) to 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16); the conversion of 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 4); and the conversion of 4-deoxy-5-threo-hexosulose uronate (DTHU) to DDG (Step 5).

[0013] In another method of the invention the substrate is glucose and the product is DDG, and the method involves the steps of: the conversion of D-glucose to 1,5-gluconolactone (Step 1); the conversion of 1,5-gluconolactone to gluconic acid (Step 1a); the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14); the conversion of 5-ketogluconate (5-KGA) to L-Iduronic acid (Step 15); the conversion of L-Iduronic acid to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 7B); and the conversion of 4-deoxy-5-threo-hexosulose uronate (DTHU) to DDG (Step 5).

[0014] Any of the methods disclosed herein can further involve the step of converting the DDG to 2,5-furan-dicarboxylic acid (FDCA). Converting the DDG to FDCA in any of the methods can involve contacting DDG with an inorganic acid to convert the DDG to FDCA.

[0015] In another aspect the invention provides a method for synthesizing derivatized (esterified) FDCA. The method involves contacting DDG with an alcohol, an inorganic acid at a temperature in excess of 60 C to form derivatized FDCA. In different embodiments the alcohol is methanol, butanol or ethanol.

[0016] In another aspect the invention provides a method for synthesizing a derivative of FDCA. The method involves contacting DDG with an alcohol, an inorganic acid, and a co-solvent to produce a derivative of DDG; optionally purifying the derivative of DDG; and contacting the derivative of DDG with an inorganic acid to produce a derivative of FDCA. The inorganic acid can be sulfuric acid and the alcohol can be ethanol or butanol. In various embodiments the co-solvent can

be any of THF, acetone, acetonitrile, an ether, butyl acetate, an dioxane, chloroform, methylene chloride, 1,2-dichloroethane, a hexane, toluene, and a xylene.

[0017] In one embodiment in the derivative of DDG is di-ethyl DDG and the derivative of FDCA is di-ethyl FDCA, and in another embodiment the derivative of DDG is di-butyl DDG and the derivative of FDCA is di-butyl FDCA.

[0018] In another aspect the invention provides a method for synthesizing FDCA. The method involves contacting DDG with an inorganic acid in a gas phase.

[0019] In another aspect the invention provides a method for synthesizing FDCA. The method involves contacting DDG with an inorganic acid at a temperature in excess of 120 C.

[0020] In another aspect the invention provides a method for synthesizing FDCA. The method involves contacting DDG with an inorganic acid under anhydrous reaction conditions.

DESCRIPTION OF THE DRAWINGS

[0021] Figure 1 is a electrophoretic gel of crude lysates and purified enzymes of proteins 474, 475, and 476.

[0022] Figures 2a-h is a schematic illustration of the pathways of Routes 1, 2, 2A, 2C, 2D, 2E, 2F, respectively.

[0023] Figures 3a-c present a schematic illustration of the pathways of Routes 3, 4, and 5, respectively.

[0024] Figure 4 is an HPCL-MS analysis of the dehydration of gluconate with gluconate dehydratase to produce DHG by pSGI-359.

[0025] Figure 5 is a graphical illustration of semicarbizide assay plots for measuring the activity of gluconate dehydratases.

[0026] Figure 6a-b provides Lineweaver-Burk plots for the oxidation of glucuronate and iduronate with three enzymes of the invention.

[0027] Figure 7a shows the results of an HPLC analysis of time points for the isomerization of 5KGA and Iduronate using enzymes DTHU isomerases in the EC 5.3.1.17 family. Controls: dead enzyme is a control with heat inactivated enzyme. Med Bl refers to reactions without isomerase add/n. Time points, x axis 1=0.5 h; 2=1; 3=2 h; 4=16h. Figure 7b shows an HPLC analysis of time points for the isomerization of 5KGA and iduronate using enzymes in the EC 5.3.1.17 family. Controls: dead enzyme is a control with heat inactivated enzyme; Med Bl: refers to reactions without isomerase add/n. Time points, X axis: 1=0 h; 2= 1 h ; 3= 2 h ; 4=17 h.

[0028] Figure 8 shows product formation for the isomerization of 5KGA and iduronate with enzymes in the EC 5.3.1.n1 family. The data were obtained from enzymatic assays.

[0029] Figure 9: HPLC analysis of the formation of 2,5-DDH and the reduction of 5KGA concentration over time. Total ion counts for 2,5-DDH are shown.

[0030] Figure 10 is a HPLC-MS chromatogram showing the production of guluronic acid lactone from 1,5-gluconolactone. An overlay of a trace of authentic guluronic acid is shown.

[0031] Figure 11 is a schematic illustration of the Scheme 6 reaction pathway.

[0032] Figures 12a and 12b are LC-MS chromatograms showing 5-KGA and DDG reaction products, respectively.

[0033] Figure 13 is a an LC-MS chromatogram showing FDCA and FDCA dibutyl ester derivative reaction products.

[0034] Figure 14a is a GC-MS analysis of a crude reaction sample of the diethyl-FDCA synthesis from the reaction of DDG with ethanol. Single peak corresponded to diethyl-FDCA. Figure 14b is an MS fragmentation of the major product from the reaction of DDG with ethanol.

[0035] Figure 15a is a GC-MS analysis of a crude reaction sample of the diethyl-FDCA synthesis from the reaction of DDG with ethanol. Single peak corresponded to diethyl-FDCA. Figure 15b is a MS fragmentation of the major product from the reaction of DDG with ethanol.

[0036] Figure 16 is a schematic illustration of the synthesis of FDCA and its derivatives from DTHU.

[0037] Figure 17 is a schematic illustration of Scheme 1. Cell free enzymatic synthesis of DDG from glucose. Enzymes are **ST-1**: glucose oxidase; **ST-1A**: hydrolysis-chemical; **ST-14**: gluconate dehydrogenase (pSGI-504); **ST-15**: 5-dehydro-4-deoxy-D-glucuronate isomerase (DTHU IS, pSGI-434); **ST-7B**: Uronate dehydrogenase (UroDH, pSGI-476)); **ST-8A** Glucarate dehydratase (GlucDH, pSGI-353); **ST-A**: NAD(P)H oxidase (NADH_OX, pSGI-431); **ST-B**: Catalase. Figure 17b shows the concentration of reaction intermediates over the first 3h as analyzed by HPLC. Formation of DDG is shown in both reactions.

DETAILED DESCRIPTION OF THE INVENTION

[0038] The present invention provides methods for producing a product of an enzymatic pathway. The methods can comprise the enzymatic conversion of a substrate into a product. By utilizing the enzymatic and chemical pathways of the invention it is possible to synthesize a wide variety of products in a highly efficient and economical manner. One product that can be produced by the methods and pathways of the invention is 2,5-furanyl dicarboxylic acid (FDCA), which can be produced at commercial scales according to the invention. The methods can comprise one or more enzymatic and/or chemical substrate-to-product conversion steps disclosed herein.

[0039] The pathways of the invention are comprised of one or more steps. It is understood that a step of a pathway of the invention can involve the forward reaction or the reverse reaction, i.e., the substrate A being converted into intermediate B and product C, while in the reverse reaction substrate C is converted into intermediate B and product A. In the methods both the forward and the reverse reactions are described as the step unless otherwise noted.

[0040] The methods involve producing a product of a pathway, which can be an enzymatic pathway. In some embodiments the pathways can include one or more chemical steps. The methods involve one or more enzymatic and/or chemical conversion steps, which convert a substrate to a product. Steps that can be included in the methods include, for example, any one or more of: an enzymatic conversion of guluronic acid into D-glucarate (Step 7); an enzymatic conversion of L-iduronic acid to Idaric acid (7B); an enzymatic conversion of L-Iduronic acid to 4-deoxy-5-threo-hexosulose uronate (DTHU)(7B); an enzymatic conversion of 5-ketogluconate (5-KGA) into L-Iduronic acid (Step 15); an enzymatic conversion of L-Iduronic acid into Idaric acid Step 7B); and an enzymatic conversion of 5-ketocluconate into 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16); an enzymatic conversion of 1,5-gluconolactone to gulurono-lactone (Step 19). Any one or more of the forementioned steps can be included in a method or pathway of the invention. An enzymatic step or pathway is a step or pathway that requires an enzyme as a catalyst in the reaction to make the step proceed. Chemical steps can be performed without an enzyme as a catalyst in the reaction. Any one or more of the steps recited in the methods can be an enzymatic step. In some embodiments every step of the pathway is an enzymatic step, while in other embodiments one or more steps in the pathway is a chemical step.

[0041] In some embodiments any of the methods can include a step involving the addition of the substrate of the reaction to a reaction mix containing the enzyme that performs the conversion. Thus the method of converting guluronic acid into D-glucarate (step 7) can involve the addition of guluronic acid as starting substrate to the reaction mix; the enzymatic conversion of L-iduronic acid to Idaric acid (7B) can involve the addition of L-Iduronic acid as starting substrate to the reaction mix; the enzymatic conversion of L-Iduronic acid to 4-deoxy-5-threo-hexosulose uronate (DTHU) (7B) can involve the addition of DTHU as starting substrate to the reaction mix. Another step that can be included in any of the methods is a step of purifying from the reaction mixture a reaction product. Thus, a step of purifying D-glucarate or L-Iduronic acid, or Idaric acid, or 4,6-dihydroxy 2,5-diketo hexanoate can be included in any of the methods described herein. Any of the methods disclose can include a step of isolating or purifying DDG or FDCA from the reaction mixture.

[0042] The reaction mix used in the methods can be a cell lysate of cells that contain one or more enzymes that perform the enzymatic conversion, but can also be a reaction mixture containing components added by the user to form a reaction mixture, or can contain components purified from a cell lysate, or may be contained in a whole cell biocatalyst.

[0043] In various embodiments the methods of the invention are methods of converting glucose to DDG, or glucose to FDCA, or glucose to DTHU or DEHU, or for converting DDG to FDCA. The methods can involve converting the starting substrate in the method into the product. The starting substrate is the chemical entity considered to begin the method and the product is the chemical entity considered to be the final end product of the method. Intermediates are those

chemical entities that are created in the method (whether transiently or permanently) and that are present between the starting substrate and the product. In various embodiments the methods and pathways of the invention have about four or about five intermediates or 4-5 intermediates, or about 3 intermediates, or 3-5 intermediates, or less than 6 or less than 7 or less than 8 or less than 9 or less than 10 or less than 15 or less than 20 intermediates, meaning these values not counting the starting substrate or the final end product.

[0044] The invention provides methods of producing FDCA and/or DDG, from glucose that have high yields. The theoretical yield is the amount of product that would be formed if the reaction went to completion under ideal conditions. In different embodiments the methods of the invention produce DDG from glucose, fructose, or galactose with a theoretical yield of at least 50% molar, or at least 60% molar or at least 70% molar, or at least 80% molar, at least 90% molar or at least 95% molar or at least 97% molar or at least 98% molar or at least 99% molar, or a theoretical yield of 100% molar. The methods of the invention also can provide product with a carbon conservation of at least 80% or at least 90% or at least 99% or 100%, meaning that the particular carbon atoms present in the initial substrate are present in the end product of the method at the recited percentage. In some embodiments the methods produce DDG and/or FDCA from glucose via dehydration reactions.

Synthesis Routes

[0045] The invention also provides specific pathways for synthesizing and producing a desired product. Any of the following described routes or pathways can begin with glucose and flow towards a desired product. In some embodiments D-glucose is the starting substrate and the direction of the pathway towards any intermediate or final product of the pathway is considered to be in the downstream direction, while the opposite direction towards glucose is considered the upstream direction. It will be realized that routes or pathways can flow in either the downstream or upstream direction. It is also understood that glucose, fructose, galactose, or any intermediate in any of the pathways can be the starting substrate in a method of the invention, and DDG, FDCA, or any intermediate in any of the routes or pathways of the invention can be the final end product of a method of the invention. The disclosed methods therefore include any one or more steps disclosed in any of the routes or pathways of the invention for converting any starting substrate or intermediate into any end product or intermediate in the disclosed routes or pathways using one or more of the steps in the disclosed routes or pathways. Thus, for example the methods can be methods for converting glucose to DDG, or glucose to guluronic acid, or glucose to galactarate, or glucose to DTHU, or glucose to DEHU, or for converting glucose to guluronic acid, or for converting glucose to iduronic acid, or for converting glucose to idaric acid, or for converting glucose to glucaric acid, or for converting galactarate to DDG, or for converting guluronic acid to D-glucarate, or for converting 5-KGA to L-Iduronic acid, or for converting L-Iduronic acid to Idaric acid, or for converting 5-KGA to 2,5-DDH or DTHU, or for converting DHG to DEHU. In these embodiments the methods utilize

the steps disclosed in the methods and pathways of the invention from glucose as starting substrate to the relevant end product.

[0046] Route 1 is illustrated in Figure 2a. Route 1 converts D-glucose (or any intermediate in the pathway) into 5-dehydro-4-deoxy-glucarate (DDG) via an enzymatic pathway via a series of indicated steps. Route 1 converts D-glucose into DDG via a pathway having 1,5-gluconolactone, gluconic acid, 3-dehydro-gluconic acid (DHG), 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH), and 4-deoxy-L-threo-hexosulose uronate (DTHU) as intermediates and DDG as the final end product. For any of the pathways additional intermediates not shown can also be present. The steps are the enzymatic conversion of D-glucose to 1,5-gluconolactone (Step 1); the enzymatic conversion of 1,5-gluconolactone to gluconic acid (Step 1A); the enzymatic conversion of gluconic acid to 3-dehydro-gluconic acid (DHG) (Step 2); the enzymatic conversion of 3-dehydro-gluconic acid (DHG) to 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 3); the enzymatic conversion of 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) to 4-deoxy-L-threo-hexosulose uronate (DTHU) (Step 4); and the enzymatic conversion of 4-deoxy-L-threo-hexosulose uronate (DTHU) to 5-dehydro-4-deoxy glucarate (DDG) (Step 5). Route 1 also comprises sub-routes where the glucose or any intermediate in the pathway is converted into any other downstream intermediate as final product, and each substrate to product sub-route is considered disclosed as if each is set forth herein in full.

[0047] Route 2 is illustrated in Figure 2b and converts D-glucose into DDG. The steps in the Route 2 pathway are the enzymatic conversion of D-glucose into 1,5-gluconolactone (Step 1); the enzymatic conversion of 1,5-gluconolactone to gluconic acid (Step 1A); the enzymatic conversion of gluconic acid to guluronic acid (Step 6); the enzymatic conversion of guluronic acid to D-glucarate (Step 7); the enzymatic conversion of D-glucarate to DDG (Step 8). Route 2 also comprises sub-routes where glucose or any intermediate in the pathway is converted into any other downstream intermediate as final product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0048] Route 2A is illustrated in Figure 2c. The steps in Route 2A are the enzymatic conversion of D-glucose to 1,5-gluconolactone (Step 1); the enzymatic conversion of 1,5-gluconolactone to guluronic acid lactone (Step 19); the enzymatic conversion of guluronic acid lactone to guluronic acid (Step 1B); the enzymatic conversion of guluronic acid to D-glucarate (Step 7); the enzymatic conversion of D-glucarate to 5-dehydro-4-deoxy-glucarate (DDG) (Step 8). Route 2A also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0049] Route 2B is illustrated in Figure 2d. The steps in Route 2B are the enzymatic conversion of D-glucose into gluconic acid (Steps 1 and 1A); the enzymatic conversion of gluconic acid into 5-ketogluconate (5-KGA) (Step 14); the enzymatic conversion of 5-KGA into L-Iduronic

acid (Step 15); the enzymatic conversion of L-Iduronic acid into Idaric acid (Step 7B); the enzymatic conversion of Idaric acid into DDG (Step 8A). Route 2B also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0050] Route 2C is illustrated in Figure 2e. The steps in Route 2C are the enzymatic conversion of D-glucose to gluconic acid (Steps 1 and 1A); the enzymatic conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14); the enzymatic conversion of 5-KGA to 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16); the enzymatic conversion of 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 4); the enzymatic conversion of DTHU to DDG (Step 5). Route 2C also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0051] Route 2D is illustrated in Figure 2f. The steps in Route 2D are the enzymatic conversion of D-glucose to gluconic acid (Steps 1 and 1A); the enzymatic conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14); the enzymatic conversion of 5-KGA to Iduronic acid (Step 15); the enzymatic conversion of L-Iduronic acid to DTHU (Step 17); the enzymatic conversion of DTHU to DDG (Step 5). Route 2D also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0052] Route 2E is illustrated in Figure 2g. The steps in Route 2D are the enzymatic conversion of D-glucose to 1,5-gluconolactone (Step 1); the enzymatic conversion of 1,5-gluconolactone to guluronic acid lactone (Step 19); the enzymatic conversion of guluronic acid lactone to guluronic acid (Step 1B); the enzymatic conversion of guluronic acid to 4-deoxy-erythro-hexosulose uronate (DEHU) (Step 17A); the enzymatic conversion of DEHU to 3-deoxy-D-erythro-2-hexulosaric acid (DDH) (Step 7A). Route 2E also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0053] Route 2F is illustrated in Figure 2h. The steps in Route 2F are the enzymatic conversion of D-glucose to gluconic acid (Steps 1 and 1A); the enzymatic conversion of gluconic acid to guluronic acid (Step 6); the enzymatic conversion of guluronic acid to 4-deoxy-erythro-hexosulose uronate (DEHU) (Step 17); the enzymatic conversion of DEHU to 3-deoxy-D-erythro-2-hexulosaric acid (DDH) (Step 7A). Route 2F also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0054] Route 3 is illustrated in Figure 3a. The steps in Route 3 are the enzymatic conversion of D-glucose to gluconic acid (Steps 1 and 1A); the enzymatic conversion of gluconic acid to 3-dehydro-gluconic acid (DHG) (Step 2); the enzymatic conversion of DHG to 4-deoxy-erythro-hexosulose uronate (DEHU) (Step 6A); the enzymatic conversion of DEHU to DDG (Step 7A). Route 3 also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0055] Route 4 is illustrated in Figure 3b. The steps in Route 4 are the enzymatic conversion of D-glucose to a-D-gluco-hexodialdo-1,5-pyranose (Step 9); the enzymatic conversion of a-D-gluco-hexodialdo-1,5-pyranose to a-D-glucopyranuronic acid (Step 10); the enzymatic conversion of a-D-glucopyranuronic acid to D-glucaric acid 1,5-lactone (Step 11); the enzymatic conversion of D-glucaric acid 1,5-lactone to D-glucarate (Step 1C); the enzymatic conversion of D-glucarate to DDG (Step 8). Route 4 also comprises sub-routes where glucose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final end product, and each sub-route is considered disclosed as if each is set forth herein in full.

[0056] Route 5 is illustrated in Figure 3c. The steps in Route 5 are the enzymatic conversion of D-galactose to D-galacto-hexodialdose (Step 9A); the enzymatic conversion of D-galacto-hexodialdose to galacturonate (Step 10A); the enzymatic conversion of galacturonate to galactarate (Step 11A); the enzymatic conversion of galactarate to DDG (Step 13). Route 5 also comprises sub-routes where galactose or any intermediate in the pathway as starting substrate is converted into any other downstream intermediate as final product, and each sub-route is considered disclosed as if each is set forth herein in full.

The Enzymatic Steps

[0057] There are disclosed a wide variety of enzymes (and nucleic acids that encode the enzymes) that can perform the steps of the methods outlined herein. In addition to the families and classes of enzymes disclosed herein for performing the steps of the invention, additional enzymes (or nucleic acids encoding the enzymes) having a sequence identity to any enzyme or member of a class of enzymes disclosed herein will also be useful in the invention that has a sequence identity of at least 40% or at least 50% or at least 60% or at least 70% or at least 80% or at least 90% or at least 95% or at least 97% or at least 98% or at least 99% to any enzyme or member of an enzyme class disclosed herein. Percent sequence identity or homology with respect to amino acid or nucleotide sequences is defined herein as the percentage of amino acid or nucleotide residues in the candidate sequence that are identical with the known polypeptides, after aligning the sequences for maximum percent identity and introducing gaps, if necessary, to achieve the maximum percent identity or homology. Homology or identity at the nucleotide or amino acid sequence level may be determined using methods known in the art, including but not limited to BLAST (Basic Local Alignment Search Tool) analysis using the

algorithms employed by the programs blastp, blastn, blastx, tblastn and tblastx (Altschul (1997), *Nucleic Acids Res.* 25, 3389-3402, and Karlin (1990), *Proc. Natl. Acad. Sci. USA* 87, 2264-2268), which are tailored for sequence similarity searching. Alternatively a functional fragment of any of the enzymes (or nucleic acids encoding such enzymes) disclosed herein may also be used. The term “functional fragment” refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, where the remaining amino acid sequence has at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the corresponding positions in the reference sequence, and that retains about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the activity of the full-length polypeptide. Functional fragments may comprise, *e.g.*, 90% or less, 80% or less, 70% or less, 60% or less, 50% or less, 40% or less, 30% or less, or 20% or less of the full-length polypeptide, and can include, for example, up to about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the full-length polypeptide. The EC numbers provided use the enzyme nomenclature of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology.

[0058] Step 1 – Conversion (oxidation or dehydrogenation) of glucose to 1,5-gluconolactone. This step can be performed with various enzymes, such as those of the family oxygen dependent glucose oxidases (EC 1.1.3.4) or NAD(P)-dependent glucose dehydrogenases (EC 1.1.1.118, EC 1.1.1.119). *Gluconobacter oxydans* has been shown to efficiently oxidize glucose to gluconic acid and 5-ketogluconate (5-KGA) when grown in a fermentor. Enzymes of the family of soluble and membrane-bound PQQ-dependent enzymes (EC 1.1.99.35 and EC 1.1.5.2) found in *Gluconobacter* and other oxidative bacteria can be used. Quinoprotein glucose is another enzyme that is useful in performing this step. The specific enzyme selected will be dependent on the desired reaction conditions and necessary co-factors that will be present in the reaction, which are illustrated in Table 1.

[0059] Step 1A – Conversion (*e.g.*, hydrolysis) of 1,5-gluconolactone to gluconate. This step can be performed chemically in aqueous media and the rate of hydrolysis is dependent on pH (Shimahara, K, Takahashi, T., *Biochim. Biophys. Acta* (1970), 201, 410). Hydrolysis is faster in basic pH (*e.g.* pH 7.5) and slower in acid pH. Many microorganisms also contain specific 1,5-glucono lactone hydrolases, and a few of them have been cloned and characterized (EC 3.1.1.17; Shinagawa, E *Biosci. Biotechnol. Biochem.* 2009, 73, 241-244).

[0060] Step 1B – Conversion of Guluronic acid lactone to guluronic acid. The chemical hydrolysis of guluronic acid lactone can be done by a spontaneous reaction in aqueous solutions. An enzyme capable of catalyzing this hydrolysis is identified amongst the large number of lactonases (EC 3.1.1. XX and more specifically 3.1.1.17, 3.1.1.25).

[0061] Step 2 – Conversion of gluconic acid to 3-dehydro gluconic acid (DHG): Several enzymes, such as gluconate dehydratases, can be used in the dehydration of gluconic acid to dehydro gluconic acid (DHG). Examples include those belonging to the gluconate dehydratase family (EC 4.2.1.39). A specific example of such a dehydratase has been shown to dehydrate gluconate (Kim, S. Lee, S.B. *Biotechnol. Bioprocess Eng.* (2008), 13, 436). Particular examples of enzymes from this family and their cloning are shown in Example 1.

[0062] Step 3: Conversion of 3-dehydro-gluconic acid (DHG) to 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH). Enzymes, 2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase (or DHG dehydrogenases) (EC 1.1.1.127) for performing this conversion have been described.

[0063] Step 4: Conversion of 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) to 4-deoxy-L-threo-hexosulose uronate (DTHU). Enzymes of the family EC 5.3.1.12 can be used in this step, and Step 15 shows that five such enzymes were cloned and shown to have activity for the dehydration of 5-KGA. These enzyme will also show activity towards 2,5-DDH and DTHU.

[0064] Step 5: Conversion of DTHU to 5-dehydro-4-deoxy-glucarate (DDG). DDG can be produced from the chemical or enzymatic oxidation of DTHU, for example with a mild chemical catalyst capable of oxidizing aldehydes in the presence of alcohols. Aldehyde oxidases can be used to catalyze this oxidation. Oxidative bacteria such as *Acetobacter* and *Gluconobacter* (Hollmann *et al Green Chem.* 2011, 13, 226) will be useful in screening. Enzymes of the following families can perform this reaction: aldehyde oxidase EC1.2.3.1, aldehyde ferredoxin oxidoreductase (EC1.2.7.5), and in all the families of EC1.2.1.-XX. Enzymes of the family of uronate dehydrogenases (EC 1.1.1.203) (e.g. see Step 7) will also have this activity. Other enzymes with both alcohol and aldehyde oxidation activity can be used, including enzymes in the alditol oxidase family (see Steps 19 and 6). Other broad substrate oxidases include soluble and membrane bound PQQ-dependent alcohol/aldehyde oxidases. More specifically soluble periplasmic PQQ oxidases enzymes and their homologs belonging into Type I (EC 1.1.9.1) and II (EC 1.1.2.8) families as well as membrane bound PQQ oxidases belonging into EC 1.1.5.X families are useful. In other embodiments aldehyde dehydrogenases/oxidases that act on DTHU can be used.

[0065] Steps 6 and 6A: Conversion of gluconic acid to guluronic acid (6) and conversion of 3-dehydro-gluconic acid (DHG) to 4-deoxy-5-erythro-hexosulose uronate (DEHU)(6A). The enzymes described in Step 5 are useful for these conversions. Other useful enzymes include NAD(P)-dependent dehydrogenases in the EC 1.1.1.XX families and more specifically glucuronate dehydrogenase (EC 1.1.1.19), glucuronolactone reductase (EC 1.1.1.20). In addition, a large number O₂-dependent alcohol oxidases with broad substrate range including sugars will be useful (EC 1.1.3.XX), including sorbitol/mannitol oxidases (EC 1.1.3.40), hexose oxidases (EC 1.1.3.5), alcohol oxidases (EC 1.1.3.13) and vanillin oxidase (EC 1.1.3.38). PQQ-dependent enzymes and enzymes present in oxidative bacteria can also be used for these conversions.

[0066] Steps 7 and 7B: Conversion of guluronic acid to D-glucaric acid (7) and conversion of L-Iduronic acid to Idaric acid (7B). These steps can be accomplished with enzymes of the family of uronate dehydrogenases (EC 1.1.1.203) or the oxidases, as described herein.

[0067] Step 7A: Conversion of 4-deoxy-5-erythro-hexosulose uronate (DEHU) to 3-deoxy-D-erythro-2-hexulosaric acid (DDH). The same enzymes described in Step 5 will be useful for performing this conversion.

[0068] Steps 8 and 8A: Conversion of D-glucaric acid to 5-dehydro-4-deoxy-glucarate (DDG) (Step 8) and conversion of Idaric acid to DDG (Step 8A). Enzymes of the family of glucarate dehydratases (EC 4.2.1.40) can be used to perform these steps. Enzymes of this family have been cloned and have been shown to efficiently convert glucarate to DDG. Two D-glucarate dehydratases (EC 4.2.1.40) were cloned as shown in the Table of cloned glucarate dehydratases below. Both enzymes showed very high activity for the dehydration of Glucarate to DDG using the semicarbazide assay, as described in Step 2.

Cloned glucarate dehydratases

Organism	pSGI (Vector)	Gene ID	WT/SYN
E. coli	353 (pET28)	P0AES2	WT
Pseudomonas (SGI)	244	#8114	WT

[0069] Step 9 and 9A: Conversion of D-glucose to α -D-gluco-hexodialdo-1,5-pyranose (9) and conversion of D-galactose to D-galacto-hexodialdose (9A). Oxidases such as those of the galactose oxidase family (EC 1.1.3.9) can be used in this step. Mutant galactose oxidases are also engineered to have activity on glucose and have been described (Arnold, F.H. et al *ChemBioChem*, 2002, 3(2), 781).

[0070] Step 10: Conversion of α -D-gluco-hexodialdo-1,5-pyranose to α -D-glucopyranuronic acid (step 10) and D-galacto-hexodialdose to galacturonate (10A). This step can be performed using an enzyme of the family of aldehyde dehydrogenases.

[0071] Step 11 and 11A: Conversion of α -D-glucopyranuronic acid to glucuronic acid 1,5-lactone. Aldehyde dehydrogenases and oxidases as described in Step 5 will be useful in performing this step. Uronate dehydrogenases described in Steps 7 and 7B can also be useful in performing this step. Step-11A is the conversion of galacturonate to galactarate. The uronate dehydrogenase (EC 1.1.1.203), for example those described in Steps 7 and 7B, will be useful in performing this step.

[0072] Step 12: Conversion of fructose to glucose. Glucose and fructose isomerases (EC 5.3.1.5) will be useful in performing this step.

[0073] Step 13: Conversion of galactarate to 5-dehydro-4-deoxy-D-glucarate (DDG). Enzymes of the family of galactarate dehydrogenases (EC 4.2.1.42) can be used to perform this step, and additional enzymes can be engineered for performing this step.

[0074] Step 14: Conversion of gluconate to 5-ketogluconate (5-KGA). A number of enzymes of the family of NAD(P)- dependent dehydrogenases (EC1.1.1.69) have been cloned and shown to have activity for the oxidation of gluconate or the reduction of 5KGA. For example, the NADPH-dependent gluconate 5-dehydrogenase from *Gluconobacter* (Expasy P50199) was synthesized for optimal expression in *E. coli* as shown herein and was cloned in pET24 (pSGI-383). The enzyme was expressed and shown to have the required activities. Additional enzymes useful for performing this step include those of the family of PQQ-dependent enzymes present in *Gluconobacter* (Peters, B. et al. *Appl. Microbiol Biotechnol.*, (2013), 97, 6397), as well as the enzymes described in Step 6. Enzymes from these families can also be used to synthesize 5KGA from gluconate.

[0075] Step 15: Conversion of 5-KGA to L-Iduronic acid. This step can be performed with various enzymes from different isomerase families, as further described in Example 4.

[0076] Step 16: Conversion of 5-KGA to (4S)-4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH). This dehydration can be performed with enzymes in the gluconate dehydratase family (EC 4.2.3.39), such as those described in Example 5 or Step 17.

[0077] Step 17 and 17A: L-Iduronate to 4-deoxy-5-threo-hexosulose uronate (DTHU) and Guluronate to 4-deoxy-5-hexoulose uronate (DHU).

[0078] Enzymes of the family of dehydratases are identified that can be used in the performance of this step. Enzymes from the families of gluconate or glucarate dehydratases will have the desired activity for performing these steps. Furthermore, many dehydratases of the family (EC 4.2.1.X) will be useful in the performance of these steps. In particular, enzymes that dehydrate 1,2-dihydroxy acids to selectively produce 2-keto-acids will be useful, such as enzymes of the families: EC 4.2.1.6 (galactonate dehydratase), EC 4.2.1.8 (mannonate dehydratase), EC 4.2.1.25 (arabonate dehydratase), EC 4.2.1.39 (gluconate dehydratase), EC 4.2.1.40 (glucarate dehydratase), EC 4.2.1.67 (fuconate dehydratase), EC 4.2.1.82 (xylonate dehydratase), EC 4.2.1.90 (rhamnonate dehydratase) and dihydroxy acid dehydratases (4.2.1.9). Since known enzyme selectivity is the production of an alpha-keto acid the identified enzymes will produce DEHU and DTHU, respectively, as the reaction products.

[0079] Step 19: Conversion of 1,5-gluconolactone to guluronic acid lactone. This step can be performed by enzymes of the family of alditol oxidases (EC 1.1.3.41) or the enzymes described in Step 6.

Methods of Converting DDG to FDCA and of making esterified DDG and FDCA

[0080] The present invention also provides novel methods of converting DDG to FDCA and FDCA esters. Esters of FDCA include diethyl esters, dibutyl esters, and other esters. The methods involve converting DDG into a DDG ester by contacting DDG with an alcohol, an inorganic acid, and optionally a co-solvent to produce a derivative of DDG. The alcohol can be methanol, ethanol, propanol, butanol, or any C1-C20 alcohol. The inorganic acid can be sulfuric acid. The co-solvent

can be any of or any mixture of THF, acetone, acetonitrile, an ether, butyl acetate, an dioxane, chloroform, methylene chloride, 1,2-dichloroethane, a hexane, toluene, and a xylene. The esterified DDG can then be converted into esterified FDCA. The DDG can be optionally purified as a step prior to performing the method. Purifying the DDG can comprise removing water from the solvent comprising the DDG, for example removing greater than 87% of the water or greater than 90% of the water or greater than 95% of the water or greater than 97% or greater than 98% or greater than 99% of the water from the solvent comprising the DDG. Yields of greater than 25% or 30% or 35% or 40% or 45% molar can be obtained.

DDG Purification

[0081] DDG purification for dehydration or esterification was performed by acidifying the DDG, e.g., by lowering the pH of the reaction with the addition of conc HCl to pH ~2.5. At this pH proteins and any residual glucarate precipitate are removed by filtration and the mixture is lyophilized to give a white powder consisting of DDG and the reaction salts. This DDG can be dehydrated to give 2,5-FDCA, or be esterified to dibutyl-DDG (or di-ethyl DDG) prior to dehydration. This method of purifying or esterifying DDG can be added as a step in any of the methods and pathways disclosed herein that produce DDG.

Methods for synthesizing FDCA and FDCA Derivatives

[0082] The invention also provides various methods of synthesizing FDCA. One method for synthesizing FDCA involves contacting DDG with an alcohol, an inorganic acid at a high temperature to form FDCA. The alcohol can be any alcohol, and examples include (but are not limited to) methanol, ethanol, propanol, and butanol. Diols can also be used. The high temperature can be a temperature greater than 70 °C or greater than 80 °C or greater than 90 °C or greater than 100 °C or greater than 110 °C or greater than 120 °C or greater than 130 °C or greater than 140 °C or greater than 150 °C to form FDCA. Reaction yields of greater than 20% or greater than 30% or greater than 35% or greater than 40% can be achieved.

[0083] The invention also provides methods for synthesizing derivatives of FDCA. The methods involve contacting a derivative of DDG with an inorganic acid to produce a derivative of FDCA. The inorganic acid can be, for example, sulfuric acid. Optionally, the derivative of DDG can be purified prior to contacting it with the second inorganic acid. Non-limiting examples of the derivative of DDG that can be used include methyl DDG, ethyl DDG, propyl DDG, butyl DDG, isobutyl DDG, di-methyl DDG, di-ethyl DDG, di-propyl DDG, di-butyl DDG. The derivative of FDCA produced can be methyl FDCA, ethyl FDCA, propyl FDCA, butyl FDCA, di-methyl FDCA, di-ethyl FDCA, di-propyl FDCA, di-butyl FDCA, and isobutyl FDCA. The derivative of FDCA produced corresponds to the derivative of DDG used in the method. The derivative of FDCA can then be de-esterified to produce FDCA. The method can also be conducted in the gas phase, e.g., using the parameters described below.

[0084] Another method for synthesizing FDCA or derivatives of FDCA involves contacting DDG or derivatives of DDG (any described herein) with an inorganic acid in a gas phase, which can be done with a short residence time, e.g., of less than 10 seconds or less than 8 seconds, or less than 6 seconds or less than 5 seconds or less than 4 seconds or less than 3 seconds or less than 2 seconds or less than 1 second. The residence time refers to the time that the sample is present in the reaction zone of the high temperature flow through reactor. The method can also be conducted at high temperatures, for example at temperatures greater than 150 °C, greater than 200 °C, greater than 250 °C, greater than 300 °C or greater than 350 °C. Yields of greater than 25% or greater than 30% or greater than 40% or greater than 45% or greater than 50% molar are obtainable. Another method for synthesizing FDCA involves contacting DDG with an inorganic acid at a temperature in excess of 80 °C or 90 °C or 100 °C or 110 °C or 120 °C. Another method for synthesizing FDCA involves contacting DDG with an inorganic acid under anhydrous reaction conditions. In various embodiments the anhydrous conditions can be established by lyophilizing the DDG in any method of synthesizing FDCA disclosed herein so that the DDG contains less than 10% or less than 9% or less than 8% or less than 7% or less than 6% or less than 5% or less than 4% or less than 3% water or less than 2% water, by weight.

[0085] The methods of the invention for synthesizing FDCA described herein provide a significantly higher yield than has been available. In different embodiments molar yields of FDCA (v. DDG) can be obtained of greater than 10% or greater than 15% or greater than 20% or greater than 25% or greater than 30% or greater than 35% or greater than 40% or greater than 45% or greater than 50%.

EXAMPLES

Example 1 – Step 2, Gluconic Acid to 3-dehydro-gluconic acid (DHG)

[0086] Enzymes with natural activity for the dehydration of gluconate have been discovered (EC 4.2.1.39). Three enzymes from this family were cloned as shown in Table 1. Enzyme pSGI-365 was cloned and shown to be a dehydratase with broad substrate range having strong activity for the dehydration of gluconate (Kim, S. Lee, S.B. *Biotechnol. Bioprocess Eng.* **2008**, 13, 436).

Table 1: Enzymes used in this experiment and identity homology. All expressed in *P. fluorescens*

Organism	pSGI (Vector)	Gene ID	W T/SYN	Expression Host
<i>Achromobacter</i>	365 (pRANGER)	E3HJU7	Syn	<i>P.fluorescens</i>
<i>Achromobacter</i>	359 (pRANGER)	#0385	wt	<i>P.fluorescens</i>
<i>Acinetobacter</i>	360 (pRANGER)	#0336	wt	<i>P.fluorescens</i>

	359_Achromob	365_E3HJU7
pSGI-360_Acinetobacter (SGI)	78	79
pSGI-359_Achromobacter (SGI)		95
pSGI-365_Achromobacter		

[0087] Proteins 359, 360, and 365 showed 2-5 $\mu\text{mole}/\text{min}$ per mg of crude enzyme lysate activity for the synthesis of dehydration of gluconate (gel not shown). pSGI-359 was isolated by precipitation with ammonium sulfate and re-dissolving in buffer and assayed by the semicarbazide assay. Activities of 46.2 U/mL or 5.3 U/mg (1 unit= $\mu\text{mole}/\text{min}$) for the dehydration of gluconate were calculated from semicarbazide assay plots. Reaction buffer (93 mL) containing Kpi 10 mM pH 8.0 with 2 mM MgCl_2 and 3.5 gr (0.016 mole) of sodium gluconate was mixed with 7 mL of the previous gluconate dehydratase solution. The reaction was incubated at 45 °C for 16 h before one aliquot was analyzed by HPLC-MS (Figure 4). As shown in Figure 4 one new major product with the molecular weight of DHG was produced. The product was also shown to have activity with DHG dehydratases.

[0088] All proteins were cloned on the pRANGER™ (Lucigen, Middleton, WI) expression vector and were expressed in a *Pseudomonas fluorescens* strain. pRANGER™ is a broad host commercially available plasmid vector containing the pBBR1 replicon, Kanamycin resistance and an pBAD promoter for inducible expression of genes. For the enzyme assay a modification of the semicarbazide assay for the quantification of alpha keto acid was used to calculate the activity of each enzyme (Kim, S.; Lee, S.B. *Biochem J.* 2005, 387, 271). SEQ ID NOs: 30-32 and 33-35 show the

amino acid and nucleotide sequences, respectively, of the gluconate dehydratases #0385, #0336, and E3HJU7.

Example 2 – Step 3 - 3-dehydro-gluconic acid (DHG) to (4S)-4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH)

[0089] Enzymes of the family (EC 1.1.1.127) can be used to perform this step. Two examples are 2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase and DHG dehydrogenases. Five enzymes from this family were cloned as shown in Table 2 below. pRANGER™ vector was used in every case.

Table 2: Cloned of DHG oxidoreductase (or 2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase)

Organism	pSGI (Vector)	Gene ID	WT/SYN	Expression Host
Agrobacterium sp (SGI)	374	#9041	WT	<i>P. fluorescens</i>
Agrobacterium tumefaciens (SGI)	375	#8939	WT	<i>P. fluorescens</i>
E. coli	376	P37769	WT	<i>P. fluorescens</i>
Sphingomonas (SGI)	395	#5112	WT	<i>P. fluorescens</i>
Hoeflea phototrophica (SGI)	396	#7103	WT	<i>P. fluorescens</i>

[0090] The product prepared from the dehydration of gluconate in Step 2 was used as substrate for assaying the lysates of Table 2. As shown in the following Table 3, enzymes were identified showing activity for the oxidation of DHG in assays measuring NADH formation (absorbance increase at 340 nm).

Table 3: Activity calculations for oxidation of DHG to 2,5-DDH using DHG oxidoreductase.A unit = $\mu\text{mole/min}$ of NADH

ENZ	U/mg (100 mM DHG)		
	pH=7.5	pH=8.5 (10 mM DHG)	pH=9.5
pSGI_395	0.012	0.070 (0.02)	0.120
pSGI_396	0.033	0.139 (0.018)	0.418
pSGI_374	0.007	0.043 (0.012)	0.091
pSGI_376	0.007	0.121 (0.01)	1.610

[0091] Further verification of the formation of 2,5-DDH by these enzymes was shown in Step 16 where the reduction of 2,5-DDH (made from the dehydration of 5KGA) with pSGI-395 at acidic pH was shown.

Example 3 – Steps 7 and 7B - Conversion of guluronic acid to D-glucaric acid (7) and conversion of L-Iduronic acid to Idaric acid (7B).

[0092] To demonstrate Steps 7 and 7B the following study was performed. Uronate dehydrogenases (EC 1.1.1.203) are enzymes that oxidize glucuronic and galacturonic acid. Three enzymes with sequence similarity to the known uronate dehydrogenase (Expasy: Q7CRQ0; Prather, K.J, et al., *J. Bacteriol.* 2009, 191, 1565) were cloned from bacterial strains as shown in Tables 4 & 5.

Table 4 – Cloned Uronate Dehydrogenases

Organism	pSGI (pET28)	Gene ID	Expression
Agrobacterium	474	#8807	BL21DE3
Rhizobium	475	#8958	BL21DE3
Pseudomonas	476	#1770	BL21DE3

Table 5 – Sequence Identity

	475	476	Q7CRQ0
474_Agrobacterium	73	49	90
475_Rhizobium		51	74
476_Pseudomonas			50

[0093] Each protein was expressed with a His tag from pET28 and was purified prior to their screening. Protein gels of the crude lysates and purified enzymes are shown in the gel of Fig. 1. After purification all enzymes were tested for activity against glucuronate, as well as against guluronate and iduronate. Kinetic measurements at different substrate concentrations were performed and the calculated activities and Km values for each enzyme are shown in Table 6. All enzymes showed good activity for glucuronate, and also for L-iduronate and guluronate.

Table 6: Activity and Km value for purified uronate dehydrogenases.

Enzyme	Vmax ($\mu\text{M}/\text{min}/\text{mg}$); and Km (mM)		
	Glucuronate	Iduronate	Guluronate (Vm only)
474	128.2 ; 0.37	0.96 ; 29.8	0.017
475	47.4 ; 0.22	0.59 ; 42.1	0.016
476	90.9 ; 0.34	1.36 ; 29.6	0.014

[0094] Each plasmid shown in Table 4 was transformed in BL21DE3 *E. coli* cells. Clarified lysates were mixed with equal volume of (25 mL) of equilibration buffer and purified on an Ni NTA column. Activity of each purified enzyme was measured in by mixing 0.050 mL of various dilutions of each purified enzyme with 0.95 mL of reaction buffer (100 mM TrisHCl, pH 8.0, 50 mM NaCl, 0.75 mM NAD⁺). The reaction progress was measured by monitoring of the formation of NADH at 340 nm. Figures 6a and 6b provide Lineweaver-Burk plots for the oxidation of glucuronate and iduronate, with all three enzymes shown in Figure 6. Clear positive slopes were obtained with all enzymes giving the activities shown in the table above. Protein sequences of the uronate dehydrogenases are shown as SEQ ID NOs: 1-3 and the genes as SEQ ID NO: 4-6.

Example 4 - Step-15: Conversion of 5-ketogluconate (5-KGA) to L-Iduronic acid (15) or guluronic acid (15A).

[0095] This example illustrates the identification of an enzyme capable of isomerizing 5-KGA to iduronic acid (Step 15) or guluronic acid (Step 15A). Thirteen enzymes from three different isomerase families were cloned as shown in Table 7, while their % sequence identity is shown in Table 8.

Table 7: Isomerases cloned

EC	Organism	pSGI (pET28)	Gene ID Archetype® or Expasy	WT/SYN
5.3.1.17	Rhizobium	433	#8938	WT
5.3.1.17	E. coli	434	Q46938 (Expasy)	WT
5.3.1.17	Rhizobium	435	#3891	WT
5.3.1.17	Pannonibacter	436	#7102	WT
5.3.1.n1	Lactobacillus	458	A5YBJ4 (Expasy)	SYN
5.3.1.n1	Acidophilum	440	F0J748 (Expasy)	SYN
5.3.1.n1	Bacillus	437	#9209	WT
5.3.1.n1	Ochrobactrum	438	#9732	WT
5.3.1.n1	Halomonas	439	#7403	WT
5.3.1.12	Sphingobacteria	478	#1874	WT
5.3.1.12	Thermotoga	479	Q9WXR9	SYN
5.3.1.12	Bacillus	480	Q9KFI6	SYN
5.3.1.12	Bacillus	481	O34808	SYN

Table 8: % Identities of isomerases

	EC	436	434	435	458	440	437	438	439	481	480	479	478
433	5.3.1.17	<u>65</u>	<u>44</u>	<u>43</u>	16	13	18	11	14	6	11	11	7
436	5.3.1.17		<u>45</u>	<u>46</u>	18	14	15	12	13	5	10	11	7
434	5.3.1.17			<u>46</u>	17	10	15	10	13	6	10	12	7
435	5.3.1.17				18	16	18	14	16	9	11	13	7
458	5.3.1.n1					<u>37</u>	<u>57</u>	<u>41</u>	<u>44</u>	6	7	9	5
440	5.3.1.n1						<u>40</u>	<u>67</u>	<u>50</u>	6	6	6	5
437	5.3.1.n1							<u>46</u>	<u>51</u>	8	7	10	6
438	5.3.1.n1								<u>52</u>	5	5	6	4

439	5.3.1.n1									6	7	8	5
481	5.3.1.12										<u>7</u>	<u>36</u>	<u>54</u>
480	5.3.1.12											<u>7</u>	<u>7</u>
479	5.3.1.12												<u>37</u>
478	5.3.1.12												

[0096] As shown in Table 8, enzymes with medium homology (underlined) within each family were selected for cloning. The data demonstrated that enzymes from all families showed activity for the isomerization of 5-KGA giving L-iduronate as the main product. Two enzymes from the 5.3.1.17 family (433 & 434) were also used in the example showing the formation of DDG from 5-ketogluconate (5KGA).

[0097] Activity for the isomerization of 5KGA and iduronate using enzymes from Table 7 was measured using an enzymatic method that detected the formation of products by their activity against two different enzymes. For example, isomerization of 5KGA was detected by measuring the activity of the product iduronate using uronate dehydrogenase (pSGI-476). Isomerization of iduronate was detected by measuring the activity 5KGA reductase (pSGI-383, EC 1.1.1.69) of the product 5KGA. Presence of the products was also detected by GC-MS.

[0098] Enzymes from all families showed varying activity for the isomerization of 5KGA and iduronate. Two enzymes from EC 5.3.1.12 were used in a cell free reaction to isomerize 5KGA and ultimately produce DDG as described in the example. The enzymes were also purified by gel electrophoresis and showed a single band. The purified isomerases were used in reactions using lysate and buffer containing 5KGA or Iduronate. Product formation was demonstrating using both HPLC and the previously described enzymatic methods. Results for 17h of incubation using both HPLC and enzyme assays are shown in Figure 7a. All enzymes showed good activity for the isomerization of both 5KGA and iduronate. Yields for iduronate isomerization by pSGI433, pSGI 434, pSGI 435, and p SGI 436 were 56%, 48% 42%, (436 not measured), respectively when measured enzymatically and 78.8%, 78.5%, 73.3% and 76.6%, respectively when measured by HPLC assay. Yields after 16h for 5KGA isomerization by the same enzymes were 18%, 17%, and 19% respectively (436 not measured) when measured by enzymatic assay, and 16.6%, 17.8%, 16.3%, and 16.9%, respectively, when measured by HPLC assay.

EC 5.3.1.12 enzymes

[0099] Enzymes from the EC 5.3.1.12 family (glucuronate isomerases) were also purified by gel electrophoresis, isolated, and used to prepare reactions by mixing with buffer (50 mM HEPES, 1 mM ZnCl₂, pH 8.0) that contained 5 mM of 5KGA or Iduronate. The reactions were incubated at

30 °C and analyzed for product formation using both HPLC and enzymatic methods. Results are shown in Figure 7b.

5.3.1.17 Enzymes

[0100] Enzymes pSGI-478 and pSGI-479 (5-dehydro-4-deoxy-D-glucuronate isomerases) showed isomerization activity for both 5KGA and iduronate. This activity was also confirmed with the enzymatic assays as above. Yields for isomerization of iduronate by pSGI-478 and -479 were 50% and 37%, respectively, when measured enzymatically, and 20% and 18% when measured by HPLC. Yields for 5KGA isomerization were 23% and 26%, respectively, when measured enzymatically, and 24% and 16%, respectively when measured by HPLC. Results are shown in Figure 7a.

5.3.1.n1 Enzymes

[0101] Enzymes in this family were purified by gel electrophoresis. Product formation was measured using enzymatic assays as described above and the results are shown in Figure 8. All enzymes cloned in this family were shown to have activity for the isomerization of 5KGA and iduronate.

[0102] In each case plasmids were transformed in BL21DE3 and proteins purified on a Ni NTA column.

Example 5: Step 16 – 5-keto-gluconate (5KGA) to (4S)-4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH)

[0103] The three gluconate dehydratases described in Step 2 (Example 1) were expressed as described in Example 1, along with a purified glucarate dehydratase from Step 8. Enzymatic reactions for activity were performed and HPLC-MS analysis showed the formation of 2,5-DDH (Figure 9), which was also confirmed by the fact that formation of the new product was accompanied by the reduction of 5-KGA only in the samples containing gluconate dehydratases, as well as by enzymatic assays with DHG dehydratase (pSGI-395). Good slopes at 340 nm indicating large enzyme activity were obtained when NADH, pSGI-395 lysate and aliquots of the previous reactions were mixed (data not shown). This result in combination with the HPLC analysis prove that the gluconate dehydratases examined dehydrate 5KGA to 2,5-DDH.

Example 6: Step 19 – Conversion of 1,5-gluconolactone to guluronic acid δ -lactone.

[0104] 1,5-gluconolactone oxidation is a side activity of enzymes from the alditol oxidases (EC 1.1.3.41) family. These enzymes oxidize various alditols such as sorbitol, xylitol, glycerol and others. Enzymes were identified having activity for the oxidation of 1,5-gluconolactone, as shown in Table 6 below.

Table 6. Alditol oxidases with activity on 1,5-gluconolactone.

Enzyme	Enzyme Source	Sorbitol U/mg	1,5- Gluconolactone			
			U/mg	Reaction Setup		
				Enzyme mg	Substrate mg/mM	Yield
AO#13	<i>Terriglobuds roseus</i>	0.23	0.02	5.3	15 / 85	7%
AO#22	<i>Granulicella mallensis</i>	0.27	0.015	7.6	15 / 85	9%
AO#28	<i>Streptomyces acidiscabies</i>	1.30	0.010	15	15 / 85	8%
AO#36	<i>Actinomycetales (SGI)</i>	1.83	0.102	25	90 / 35	46%
AO#51	<i>Frankia sp</i>	0.59	0.019	NT	NT	NT
AO#57	<i>Propionibacteriacaeae (SGI)</i>	1.47	0.051	40	70 / 57	6%
AO#76	<i>Streptomyces sp.</i>	1.45	0.045	8.2	15 / 85	23%
AO#251*	<i>Paenibacillus sp.</i>	0.47	0.003	24	15 8.5	~2%

*crude lysate

[0105] Reactions were prepared using lysates of all the purified enzymes shown on Table 6. Reactions were prepared in 50 mM K-phosphate buffer, pH 7.0 with 0.5 mg/mL catalase and incubated at 30 °C. A new product was observed by HPLC-MS analysis showing the same retention time as guluronate after comparison with authentic standards (Figure 10). This was confirmed by GC-MS, where the product also had the same MS fingerprint as guluronate. It is therefore clear that all the alditol oxidases described in the Table oxidize the 6-OH of 1,5-gluconolactone to produce the guluronic acid lactone. All alditol oxidases were cloned in pET28a with a HisTag and were expressed in BL21DE3 and purified on a Ni NTA column.

Example 7 – Synthesis of FDCA and Other Intermediates

[0106] Purified DDG mono potassium salt was used for the dehydration to 2,5-FDCA. Sulfuric acid was added to DDG and the reaction stirred at 60°C. The in situ yield was calculated (by HPLC-MS) to be ~24% and ~27%.

[0107] The reaction solutions were combined and then diluted by pouring into ice (to neutralize the heat). Approximately equivalent volume of THF was added, and the solution transferred to a separation funnel. Sodium chloride salt was added until separation was achieved. The solution was agitated between additions for best possible dissolution. The aqueous layer was removed, and the THF layer washed 3x more with sat. NaCl solution. Sodium sulfate was added and the solution left sitting overnight. Two layers formed again overnight. The aqueous layer was

discarded and then silica gel was added to the solution. It was then concentrated down to solids via rotovap. The solids were loaded into a silica flash column and then separated via chromatographically. The fraction was concentrated and dried. The isolated yield was 173.9mg. Corrected yield: 24.9%. ^1H and ^{13}C NMR and HPLC-MS analysis confirmed the product

Dehydration of DDG Dibutyl-2,5-FDCA in BuOH/H₂SO₄

[0108] Dehydration of un-derivitized lyophilized DDG containing the dehydration salts in BuOH was done using a Dean-Stark apparatus. Under these conditions, DDG was added to BuOH, and then H₂SO₄ was added and the reaction heated at 140 °C. After stirring for 4 h HPLC-MS analysis shows the disappearance of DDG and the formation of dibutyl-2,5-FDCA. The in situ yield was calculated (by HPLC-MS) to be 36.5%.

[0109] The mixture was extracted with water, 1% NaOH, and again with water. Then the organic layer was concentrated to a final mass of 37.21g. A portion of this mass (3.4423g) was removed and 0.34 g of dibutyl-2,5-FDCA was purified using HPLC. Extrapolating the yield of the isolated product to the total amount of compound isolated from the reaction (37.21g) and taking into account the amount of salts present in the original DDG (~60% pure by weight) the reaction yield was calculated to be 42%. ^1H and ^{13}C NMR and HPLC-MS analysis confirmed the product

Synthesis of dibutyl DDG

[0110] In another aspect the invention provides a method for synthesizing a derivative of DDG. The method involves contacting DDG with an alcohol, an inorganic acid, and optionally a co-solvent to produce a derivative of DDG. Optionally the derivative of DDG can be purified. The reaction can have a yield of the derivative of DDG of at least 10% molar yield or at least 15% molar yield or at least 20% molar yield or at least 25% or at least 30% or at least 35% molar yield or at least 40% molar yield. The inorganic acid can be sulfuric acid and the alcohol can be methanol, ethanol, propanol, butanol, isobutanol, or any C1-C20 alcohol. In various embodiments the co-solvent can be any of THF, acetone, acetonitrile, an ether, butyl acetate, an dioxane, chloroform, methylene chloride, 1,2-dichloroethane, a hexane, toluene, and a xylene. When the alcohol is ethanol the DDG derivative will be DDG mono-ethyl ester and/or DDG diethyl ester. When the alcohol is butanol the DDG derivative will be DDG mono-butyl ester and/or DDG dibutyl ester.

[0111] DDG mono-potassium salt was used for derivatization according to the following protocol. In a 1L Morton type indented reaction vessel equipped with a mechanical stirrer and heating mantle was charged with 60:40 DDG:KCl (31.2 mmol), BuOH, and heptane. In a separate vial, sulfuric acid was added to water, and allowed to cool after dissolution. The solution was then added to the flask. The solution was kept at 30°C.

[0112] The precipitate was filtered off concentrated. The remaining gel was dissolved in EtOAc, and then TLC plates were spotted with the solutions and the plates were sprayed with a phosphomolybdic acid mixture, and then heated to at least 150°C on a hot plate to identify the DDG-

DBE fraction. Isolated yield: 4.62 g (15.2 mmol, 47% yield), > 98% purity. ^1H and ^{13}C NMR and HPLC-MS analysis confirmed the product.

[0113] Different solvents can be used in the synthesis of DDG esters, such as mixtures of BuOH (5%-95% v/v) with co-solvents such as THF, acetone, acetonitrile, ethers (dibutyl, ditheyl etc), esters such as Butyl-acetate, 1,6-dioxane, chloroform, methylene chloride, 1,2-dichloroethane, hexanes, toluene, and xylenes may be used as cosolvents. Reaction catalysts such as acids (sulfuric, hydrochloric, polyphosphoric or immobilized acids such as DOWEX) or bases (pyridine, ethyl-amine, diethyl-amine, boron trifluoride) or other catalysts commonly used for the esterification of carboxylic acids.

Dehydration of dibutyl-DDG to dibutyl-FDCA in n-BuOH/H₂SO₄

[0114] A stock solution of DDG-DBE (di-butyl ester) was made in butanol and transferred to a clean, dry 100mL round-bottomed flask equipped with a stir bar. To the flask, 25mL of conc. sulfuric acid was added. The flask was sealed and then stirred at 60°C for 2hrs. The in situ yield was calculated to be ~56%. The reaction solution was concentrated and the residue was dissolved in MTBE and transferred to a separation funnel, and then washed with water. The recovered organic layer was concentrated and then separated via HPLC for an isolated yield: 250.7 mg (~90% purity) and 35% isolated yield (corrected for purity). ^1C and ^{13}C NMR and HPLC-MS analysis confirmed the product.

Example 8 – Cell free synthesis of DDG and FDCA and derivatives from 5-KGA (Route 2A)

[0115] This example illustrates the enzymatic conversion of 5KGA to DDG using purified enzymes according to Scheme 6 (a sub-Scheme of 2B), and also illustrates the DDG produced being dehydrated to FDCA using chemical steps. The Scheme involves the steps of isomerization of 5KGA (Step 15) and the subsequent oxidation to idaric acid (Step 7B). DDG was also dehydrated under differing chemical conditions to FDCA. The last step (Step-8A) was performed using glucarate dehydratase from *E. coli*.

[0116] Scheme 6 is illustrated in Figure 11. The scheme was performed using a cell free enzymatic synthesis of DDG from 5-KGA. The Scheme involves the performance of steps 15, 7B and 8A. Two additional proteins were used to complete the reaction path, the first being NADH-oxidase (Step A) that is recycling the NAD⁺ cofactor in the presence of oxygen, and catalase (Step B) that decomposes the peroxide produced from the action of NADH oxidase. The enzymes are shown in the following Table 7. All enzymes contained a HisTag and were purified using an Ni-NTA column. Yields for this synthesis of DDG were calculated to be at least 88-97%.

[0117]

Table

<i>STEP</i>	<i>Enzyme</i>	<i>EC</i>	<i>Organism</i>
15	pSGI-433 (DTHU_IS)	5.3.1.17	<i>Rhizobium</i> (SGI)
15	pSGI-434 (DTHU_IS)	5.3.1.17	<i>E. coli</i>
7B	pSGI-476 (UroDH)	1.1.1.203	<i>Pseudomonas</i> (SGI)
8A	pSGI-353 (GlucDH)	4.2.1.40	<i>E. coli</i>
A	pSGI-431 (NADH_OX)	1.6.3.1	<i>Thermus thermophilus</i>
B	Catalase	1.11.1.6	<i>Corynebacterium</i>

7:

[0118] 500 mL of liquid culture was purified for each isomerase for the reaction. Besides the enzymes shown on Table 7, each reaction contained 50 mM TrisHCl (pH 8.0), 50 mM NaCl, 1 mM ZnCl₂ and 2 mM MgCl₂, 1 mM MnCl₂ and 1 mM NAD⁺. Reactions were analyzed by HPLC after 16 h of incubation and Figure 12 presents the chromatograms.

[0119] For dehydration to FDCA, the reaction mixtures of both samples were combined and lyophilized into a white powder, which was split into two samples and each dissolved in AcOH with 0.25M H₂SO₄ or in 4.5 mL BuOH with 0.25M H₂SO₄. Both reactions were heated in sealed vials for 2-4 h at 120 °C. Reaction products are shown in Figure 13.

[0120] Samples 1 and 2 represent authentic standard and the 3h time point from the reaction in AcOH/ H₂SO₄, respectively. Spiking of sample 2 with sample 1 gave a single peak further verifying the FDCA product. Samples 1 and 3 (Figure 13) represent authentic standard and the 4h time point from the reaction in BuOH/ H₂SO₄, respectively. The formation of FDCA from the enzymatic reactions further confirms the presence of DDG in these samples.

Example 9 – Synthesis of DDG from Glucose and Gluconate

[0121] This example shows the enzymatic conversion of glucose and gluconate to DDG. The reaction was conducted with purified enzymes, and crude lysates as a catalyst. Enzymes and substrates were combined in a bio-reactor as shown in the Table below:

	Substrate	ST-1	ST-14 pSGI-504	ST-15 pSGI-434	ST-7B pSGI-476	ST-8A pSGI-353	ST-A pSGI-431	ST-B
Rxn-1	Glucose 600 mg	2 mg	7 mL ¹	50 mL ²	7.5 mL ¹	1 mL ³	4 mL ⁴	2 mg
Rxn-2	Gluconate 700 mg	-	7 mL	50 mL	7.5 mL	1 mL	4 mL	2 mg

1. Lysate from 500 mL liquid culture of recombinant *E. coli* with plasmid
2. Lysate from 2L liquid culture of BL21DE3/pSGI-434
3. Purified enzyme, ~30 Units of activity (or 3 mg of purified GlucD)
4. Lysate from 250 mL of culture

[0122] The reaction was incubated at 35 oC and dissolved oxygen and pH were kept at 20% and 8 respectively. Time points were analyzed by HPLC-MS and the results are shown in Figure 17b. Extracted chromatograms verified the DDG mass (not shown) and corresponding MS fragmentation. The results clearly showed production of DDG during incubation of the enzymes with either glucose or gluconate.

Example 10 - Construction of expression cassettes for recombinant glucarate dehydratases.

[0123] The following example describes the creation of recombinant nucleic acid constructs that contained coding sequence of a D-glucarate dehydratase activity (GDH, EC 4.2.1.40) for heterologous expression in *E. coli* cells.

[0124] Genes encoding D-Glucarate dehydratase from *E. coli* (Expasy: P0AES2;), *Acinetobacter ADP1* (Expasy: P0AES2), as well as a proprietary *Pseudomonas* bacterial strain (BP1MICT2128114) were PCR-amplified from genomic DNA.

[0125] Each of the PCR-amplified genes was subsequently cloned into the bacterial transformation vector pET24a(+), in which the expression of each of the GDH genes was placed under control of a T7 promoter. The nucleotide sequences of each of the PCR-amplified inserts were also verified by sequencing confirmation.

Example 11 - *E. coli* strains expressing recombinant glucarate dehydratases.

[0126] Each of the expression vectors constructed as described in Example 9 was introduced into NovaBlue(DE3) *E. coli* by heat shock-mediated transformation. Putative transformants were selected on LB agar supplemented with Kanamycin (50 µg/ml). Appropriate PCR primers were used in colony-PCR assays to confirm positive clones that contained each of the expression vectors.

[0127] For each expression vector, a bacterial colony was picked from transformation plates and allowed to grow at 30°C in liquid LB media supplemented with Kanamycin (50 µg/ml) for two days. The culture was then transferred into vials containing 15% glycerol and stored at -80°C as a frozen pure culture.

Example 11 - Demonstration of *in vitro* synthesis of DDG by using cell lysate of recombinant *E. coli* cells expressing a GDH enzyme

[0128] This Example describes how *in vitro* synthesis of DDG intermediate was achieved using recombinant GDH enzymes produced in *E. coli* cells.

[0129] Preparation of cell lysates: Recombinant bacterial strains constructed as described previously in Example 2 were grown individually in 3 mL of liquid LB media supplemented with Kanamycin (50 µg/ml) at 30°C on a rotating shaker with rotation speed pre-set at 250 rpm for 1 day. This preculture was used to inoculate 100 mL of TB media containing Kanamycin (50 µg/ml), followed by incubation at 30°C on a rotating shaker pre-set at 250 rpm for 2-3 hour until early log phase ($OD_{600} \sim 0.5-0.6$) before isopropyl D-1 thiogalactopyranoside (IPTG; 0.25 mM final concentration) was added to induce protein expression. Cells were allowed to grow for another 18 hours at 30°C before they were harvested by centrifugation, resuspended in 15 mL of lysis buffer (10 mM phosphate buffer, pH 7.8, 2 mM $MgCl_2$) and were lysed by sonication. The production of recombinant enzymes in *E. coli* cells was quantified using standard pre-cast SDS-PAGE gels system (BioRad), and specific activity was measured according to a procedure described by Gulick *et al.* (*Biochemistry* 39, 4590-4602, 2000). Cell lysates were then tested for the ability to convert gram amounts of glucarate to DDG as described in greater details below.

[0130] Enzymatic dehydration of glucarate: Five grams of mono-potassium glucarate (~0.02 moles) were added to 85 mL of 5 mM potassium phosphate buffer containing 10 mM $MgCl_2$. The substrate glucarate was found slowly dissolved following the addition of ~2 mL of 5M NaOH. The pH of the reaction was adjusted to about 7.8. Subsequently, 15 mL of a cell lysate containing each of the three recombinant dehydratases in 10 mM phosphate buffer, pH 7.8, as described in Example 3. After incubation with gentle stirring at 30°C for 1-2

hours, the reactions were analyzed using HPLC-MS techniques. HPLC-MS results indicated a new peak as the only major product with a molecular weight corresponding to predicted product DDG, and trace amounts of the mono-potassium glucarate substrate. No other byproducts were detected by HPLC-MS analysis, indicating that the conversion reaction catalyzed by each of the recombinant enzymes was very efficient and highly specific.

Purification of DDG product from enzymatic reactions:

[0131] DDG produced via enzymatic dehydration was purified by using either of the two following techniques.

[0132] The enzymatic dehydration reactions were acidified to pH~2.0 with 6M HCl, filtered to eliminate precipitated proteins, and subsequently lyophilized. Methanol (MeOH) was added to the lyophilized powders, followed by gentle stirring for 10-15 minutes to dissolve the DDG product but not the other salts in the dehydration reaction mixtures (such as KCl and phosphates). Substantially pure DDG acid was obtained following filtration of the suspensions and evaporation of MeOH.

[0133] In some instances, an alternative procedure was deployed for the purification of DDG salt, in which the first MeOH filtrate was condensed to a volume of ~15-25 mL, then mixed with an equal volume of MeOH containing 0.5M KOH. Potassium salt of DDG precipitated after addition of KOH was subsequently isolated by filtration.

[0134] Results of HPLC-MS analyses indicated that DDG product constituted at least 95% of the total products in the samples obtained from either of the two purification techniques.

Example 12 - Demonstration of *in vitro* synthesis of FDCA from DDG in one-step chemical reaction

[0135] Applicants have discovered that the synthesis of FDCA (*i.e.* the free acid form) could be achieved by a chemical conversion of DDG to FDCA in the presence of H₂SO₄. The reaction was performed as follows. Approximately 20 mg of DDG acid (crude lyophilized powder with salts previously purified as described in Example 3) and 0.25 M of H₂SO₄ were added into an air tight sealed tube containing 1 mL of water and 1 mL of DMSO. The DDG was found completely dissolved in this solution. The reaction was stirred at 105°C for 18 hours. Results of an HPLC-MS analysis performed on a crude reaction sample indicated the formation of FDCA free acid (FDCA: 2,5-furan dicarboxylic acid) as the major product, as well as insignificant amounts of some other unidentified byproducts. As a control in HPLC-MS analysis, a commercial FDCA was analyzed in the same conditions.

Example 13 - Demonstration of *in vitro* synthesis of FDCA-esters (dimethyl-, diethyl-, dibutyl-, and isopropyl- esters)

Synthesis of diethyl-2,5 FDCA from purified DDG:

[0136] In an air tight sealed tube, 18 mL of EtOH, 0.2 gram (1 mmole) of DDG acid, previously purified as described in Example 11, and 0.25 M of H₂SO₄ were added. The DDG acid was not completely dissolved in this solution. The reaction was gently stirred at 105°C for 18 hours. Results of a GC-MS analysis of a crude reaction sample indicated that the formation of diethyl-FDCA the major product. As a control, an authentic FDCA was chemically synthesized, esterified to diethyl-FDCA and analyzed in the same conditions.

Example 14 - Synthesis of dibutyl-2,5 FDCA from purified DDG

[0137] In an air tight sealed tube, 18 mL of n-BuOH, 0.2 gram (1 mmole) of DDG acid, previously purified as described in Example 11, and 0.25 M of H₂SO₄ were added. The DDG acid was not completely dissolved in this solution. The reaction was gently stirred at 105°C for 18 hours. As shown in FIGURE 15, results of the GC-MS analysis of a reaction sample indicated that diethyl-FDCA (FDCA: 2,5-furan dicarboxylic acid) was formed as the major product. As a control, an authentic FDCA was chemically synthesized, esterified to diethyl-FDCA, and analyzed in the same conditions.

Example 15 - Synthesis of dibutyl-2,5 FDCA from crude DDG (unpurified):

[0138] 0.2 gram (1 mmole) of crude DDG acid, which was an unpurified lyophilized powder obtained directly from the enzymatic dehydration of glucarate as described in Example 11, was added into an air tight sealed tube containing 18 mL of n-BuOH, followed by addition of 0.25 M of H₂SO₄. The crude DDG acid was not completely dissolved in this solution. The reaction was gently stirred at 105°C for 18 hours. Results of a GC-MS analysis of a crude reaction sample indicated that diethyl-FDCA (FDCA: 2,5-furan dicarboxylic acid) was formed as the major product. The GC-MS result indicated that the present of contaminant salts in crude/unpurified lyophilized powder did not significantly affect the reaction outcome. As a control, an authentic FDCA was chemically synthesized, esterified to diethyl-FDCA, and analyzed in the same conditions.

Example 16 - *In vitro* production of FDCA and/or esters using immobilized acids

[0139] In industrial practices, immobilized acids offer many advantages for performing dehydrations since they can typically operate in several types of solvent (aqueous, organic or mixed, etc.). In addition, they can be easily recycled and be re-used. Following some examples of the synthesis of esters of FDCA using immobilized AMBERLYST®15 (Rohm and Haas, Philadelphia, PA) and DOWEX®50 WX8 (Dow Chemical Co, Midland, MI).

Synthesis of dibutyl-FDCA from crude DDG by using DOWEX®50 WX8

[0140] In an air tight sealed tube, 2 mL of n-Butanol, 20 mg of crude DDG acid (unpurified lyophilized powder containing salts) and 200 mg of DOWEX®50 WX8 were combined. The DDG

was not completely dissolved in this solution. The reaction was gently stirred at 105°C for 18 hours. Results of the GC-MS analysis of a crude reaction sample indicated that diethyl-FDCA (FDCA: 2,5-furan dicarboxylic acid) was formed as the major product. This GC-MS result indicated that the present of contaminant salts (phosphate and NaCl) in crude/unpurified lyophilized powder did not significantly affect the reaction outcome. As a control, an authentic FDCA was chemically synthesized esterified to diethyl-FDCA and analyzed in the same conditions.

Synthesis of dibutyl-FDCA from crude DDG by using AMBERLYST®15

[0141] In an air tight sealed tube, 2 mL of n-Butanol, 20 mg of crude DDG acid (crude lyophilized powder with salts) and 200 mg of AMBERLYST®15 (Rohm and Haas, Philadelphia, PA) were combined. The DDG was not completely dissolved in this solution. The reaction was gently stirred at 105°C for 18 hours. Results of the GC-MS analysis of a crude reaction sample indicated that diethyl-FDCA (FDCA: 2,5-furan dicarboxylic acid) was formed as the major product. This GC-MS result indicated that the present of contaminant salts (phosphate and NaCl) in crude/unpurified lyophilized powder did not significantly affect the reaction outcome. As a control, an authentic FDCA was chemically synthesized esterified to diethyl-FDCA and analyzed in the same conditions.

Synthesis of ethyl-FDCA from crude DDG by using AMBERLYST®15

[0142] In an air tight sealed tube, 2 mL of ethanol, 20 mg of crude DDG acid (unpurified lyophilized powder containing salts) and 200 mg of AMBERLYST®15 (Rohm and Haas, Philadelphia, PA) were combined. The DDG was not completely dissolved in this solution. The reaction was gently stirred at 105°C for 18 hours. Results of the GC-MS analysis of a crude reaction sample indicated that diethyl-FDCA (FDCA: 2,5-furan dicarboxylic acid) was formed as the major product. This GC-MS result indicated that the present of contaminant salts (phosphate and NaCl) in crude/unpurified lyophilized powder did not significantly affect the reaction outcome. As a control, a commercial FDCA was chemically esterified to diethyl-FDCA and analyzed in the same conditions.

Synthesis of diethyl-FDCA from crude DDG by using DOWEX®50 WX8

[0143] In an air tight sealed tube, 2 mL of ethanol, 20 mg of crude DDG acid (unpurified lyophilized powder containing salts) and 200 mg of DOWEX®50 WX8 were combined. The DDG was not completely dissolved in this solution. The reaction was gently stirred at 105°C for 18 hours. Results of the GC-MS analysis of a crude reaction sample indicated that diethyl-FDCA (FDCA: 2,5-furan dicarboxylic acid) was formed as the major product. This GC-MS result indicated that the present of contaminant salts (phosphate and NaCl) in crude/unpurified lyophilized powder did not significantly affect the reaction outcome. As a control, a commercial FDCA was chemically esterified to diethyl-FDCA and analyzed in the same conditions.

Example 17 - Production of FDCA derivatives

[0144] The synthesis of a number of high-value FDCA derivatives is described in Figure 16 in which dehydration of DTHU produces furfural-5-carboxylic acid, *i.e.* FCA, which is then

chemically or enzymatically oxidized to FDCA, be reduced to FCH, or be transaminated (using chemical reductive amination or transaminase) to amino acid-AFC.

Example 18 – Production of di-butyl FDCA in a gas phase reaction

[0145] In this example the inlet of the GC was used as a high temperature reactor to catalyze the dehydration of di-butyl DDG to di-butyl FDCA. The resulting products were chromatographically separated detected by mass spectrometry. A solution of di-butyl DDG (10 mM) and sulfuric acid (100 mM) in butanol was placed in a GC vial. The vial was injected into a GC and FDCA Dibutyl ester was observed. The reaction occurred in the 300 °C inlet (residence time = 4 seconds). The average yield of 6 injections was 54%.

GC Settings: Direct liquid inject / MS detector

Inlet: 300° C, total flow 29.51 ml/min, split ratio 10:1, split flow 24.1 ml/min,
Septum Purge flow 3 mL/min.

GC liner: 4 mm, glass wool (P/N 5183-4647)

Column Flow: 2.41 ml/min He constant pressure control

Oven Program: At 40 °C hold for 2 min, then ramp 25 °C/min to 275 °C, then ramp 40 °C/min to 325 °C, hold for 2 min.

Column: HP-5MS, Agilent Technologies, 30m x 0.25mm x 0.25um.

Total Runtime: 14.65 minutes

MSD Transfer line: 290 °C

MS Source: 250 °C

MS Quad: 150 °C

Retention times:

2,3-FDCA Dibutyl ester: 9.3 min

2,5-FDCA Dibutyl ester: 9.7 min

[0146] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0147] No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0148] It should also be understood that the foregoing examples are offered to illustrate, but not limit, the invention.

<SEQ ID NO: 1> protein #474

MAMKRLLLVTGAAGQLGRVMRKRLASMAEIVRLADLAPLDPAGPNEECMQCDLAD
 ADAVDAMVAGCDGIVHLGGISVEKPFQILQGNIIGLYNLYEAARAHGQPRIIFASSN
 HTIGYYPQTERLGPDPVFRPDGLYGVSKCFGESLARMYFEKFGQETALVRIGSCTPEP
 LNYRMLSTWFSHDDFVSLIEAAFRAPVLGCPVWVGASANDASWWDNSHLGFIGWKP
 KDNAEAFRRKIAETTPQPDARDPIVRFQGGVFVDNPIFKET*

<SEQ ID NO: 2> protein #475

MKRLITGAAGALGRVMRERLAPMATILRLSDIPIGAARQNEEIVQCDLADAKAVH
 ALVEDCDGIVHLGGVSVERKFSQIVAGNIVGLYNLYEAARAHMPRIVFASSNHTIGF
 YPQTERLSVDHPYRPDGLYGVSKCFGESLAHMYHEKFGQETALVRIGSCVTEPVNH
 RMLSTWLSYDDFVSLIEAVFRAPKLGCPVIWVGASNNDAGWWDNSAAGFLGWKPKD
 NAEIFRSKIEAACERPGSDDPAARWQGGGLFTQDPIFPEDE*

<SEQ ID NO: 3> Protein #476

MTTAYTPFNRLLLTGAAGGLGKVLRESLRPYANVLRVSDIAAMSPATGAHEEVQVC
 DLADKAAVHQLVEGVDAIHLFGGVSVVERPFEEILGANICGVFHIYEARRHGVKRVI
 FASSNHVIGFYKQDETIDANCPRRPDSYYGLSKSYGEDMASFYFDRIYGIETVSIRIGSS
 FPEPHNRRMMSTWLSFADLTQLLERALYTPNVGHTVVYGMSANKNVWWDNHLAA
 HLGFPKDSSEVFRAQIDAQPMPAADDPAMVFQGGAFVAAGPFGDD*

SEQ ID NO: 4 pSGI-474-#8807-DNA

ATGGCAATGAAACGGCTTCTTGTTACCGGTGCTGCGGGCCAGCTTGGCCGCGTTA
 TGCGCAAACGCCTTGCATCGATGGCCGAGATCGTTCGCCTTGCCGATCTCGCCCC
 GCTCGATCCGGCAGGCCCCGAACGAGGAATGCATGCAATGCGACCTTGCGGATGC
 AGACGCCGTTGACGCCATGGTTGCCGGTTGCGACGGCATCGTTCACCTCGGCGGC
 ATATCGGTGGAGAAGCCTTTCGAACAAATCCTTCAGGGCAACATCATCGGGCTGT
 ATAATCTCTATGAGGCCGCCCGCGCCACGGCCAGCCGCGCATCATCTTCGCCAG
 TTCGAACCATACGATCGGTTATTACCCGCAGACGGAGAGGCTTGGACCGGATGTT
 CCCTTCCGCCCCGGATGGGCTTTACGGCGTCTCCAAATGTTTCGGCGAGAGCCTTG
 CCCGCATGTATTTTCGAGAAATTCGGCCAGGAGACCGCACTTGTCCGCATCGGCTC
 CTGCACGCCGGAACCCCTTAATTACCGCATGCTGTCCACCTGGTTTTTCGCATGAC
 GATTTTCGTCTCGCTGATCGAGGCGGCGTTCCGCGCCCCCGTGCTCGGCTGCCCA
 TCGTCTGGGGGGCGTCGGCCAACGATGCGAGCTGGTGGGACAATTTCGCATCTCG
 GCTTTATTGGATGGAAACCGAAGGACAATGCCGAGGCCTTCCGCCGGAAGATTG
 CCGAAACGACGCCGCAGCCGGACGCGCGCGACCCGATTGTCCGCTTTCAGGGTG
 GCGTGTTTGTGCGACAACCCGATCTTCAAGGAGACGTGA

SEQ ID NO: 5 pSGI-475-#7895- DNA

ATGAAGAGACTTCTGATTACCGGCGCAGCGGGTGCCTGGGCGCGTGATGCGG
GAAAGGCTCGCACCCATGGCAACGATTCTGCGCCTTTCCGATATCGCCCCGATTG
GAGCGGCCCCGCCAGAACGAGGAAATCGTCCAGTGCGATCTTGCCGATGCCAAAG
CAGTGCATGCTCTGGTCTGAAGATTGCGACGGGATCGTCCATCTCGGTGGCGTCTC
AGTAGAGCGCAAGTTCTCGCAGATCGTCGCCGGCAACATCGTCGGCCTTTACAAT
CTCTACGAAGCCGCACGCGCGCATCGGATGCCGCGCATCGTCTTTGCAAGTTCCA
ATCACACCATCGGCTTTTATCCGCAAACCGAACGGTTGTCGGTGGACCATCCCTA
TCGTCCGGACGGGCTCTACGGCGTATCGAAATGTTTCGGCGAGTCTCTGGCGCAT
ATGTACCATGAGAAGTTCGGGCAGGAGACGGCACTCGTGCGCATCGGGTCCTGC
GTGACCGAACCGGTCAACCATCGCATGCTTTCCACCTGGCTTTCTACGATGATT
TCGTCTCGCTTATCGAGGCCGTATTCGTGCGCCGAACTCGGCTGCCCCGTCAT
CTGGGGCGCGTCGAACAACGATGCAGGATGGTGGGACAATTCCGCCGCCGGCTT
TCTCGGCTGGAAGCCGAAAGACAATGCCGAAATCTTCCGTTCGAAGATCGAAGC
CGCTTGCGAACGCCCCGGTTCTGATGATCCGGCCGCCCGCTGGCAAGGCGGGCTC
TTCACGCAGGACCCGATCTTCCCAGAGGACGAGTAA

SEQ ID NO: 6 pSGI-476-#1770-DNA

ATGACCACAGCCTACACCCCCTTCAATCGCCTGCTACTCACCGGAGCGGCAGGCG
GCCTCGGCAAGGTCCTGCGCGAAAGCCTGCGACCTTATGCCAACGTCCTGCGCGT
CTCCGACATCGCGGCCATGAGCCCTGCCACAGGCGCCCATGAAGAAGTCCAGGT
CTGCGACCTCGCCGATAAAGCGGCGGTCCATCAACTGGTCGAAGGCGTCGACGC
AATCCTGCACTTCGGTGGCGTATCGGTGGAGCGGCCCTTCGAGGAAATCCTCGGG
GCCAATATCTGCGGCGTGTTTCATATCTATGAAGCGGCGCGCCGGCATGGCGTAA
AGCGGGTGATCTTCGCCAGCTCCAACCACGTCATCGGTTTTTTATAAGCAGGACGA
AACCATCGACGCCAACTGCCCCGCGCCGCCCCGACAGCTACTACGGTCTGTCCAA
GTCCTACGGCGAAGACATGGCCAGCTTCTACTTCGACCGCTACGGCATCGAGACC
GTGAGCATCCGCATCGGCTCCTCGTTCCCCGAGCCGCACAATCGCCGCATGATGA
GCACCTGGCTGAGCTTTGCCGACCTGACGCAGCTGCTCGAACGCGCGCTGTACAC
CCCCAACGTCGGCCACACCGTGGTCTACGGCATGTCCGCTAACAAGAACGTCTG
GTGGGACAACCACCTGGCCGCGCACCTGGGCTTCCAACCGAAGGACAGCTCCGA
GGTGTTCGTGCGCAGATCGATGCCAGCCGATGCCCGCCGCCGATGACCCGGC
GATGGTCTTTCAAGGCGGCGCCTTTGTGCGCAGCCGGGCGGCTTCGGCGACGACTGA

SEQ ID NO: 7 pSGI-433 #8938-Protein

MLNVETRHAVHADHARSLDTEGLRRHFLAQGLFAEGEIRLIYTHYDRFVMGGAVPD
 GAPLVLDHVEETKTPGFLDRREMGIIVNIGAEGSVHAGNESWSLNRGDVLYLGMGAG
 PVTFEGAGRIFYLVSAHAHRSNRLVTPADSKEVKLGALSTSNKRTINQFIHPLVMES
 CQLVLGYTTLEDGSVWNTMPAHVHDDRMEAYLYFGMDETSRVLHLMGEPQQTRH
 LFVANEEGAISPPWSIHAGAGIGSYTFIWAMAGDNVDYTDMEFIQPGDLR*

SEQ ID NO: 8 pSGI-434_Q46938-Protein

MDVRQSIHSAHAKTLDLTQGLRNEFLVEKVFADEYTMVYSHIDRIIVGGIMPITKTVS
 VGGEVKGQLGVSYFLERRELGVINIGGAGTITVDGQCYEIGHRDALYVGKGAKEVV
 FASIDTGTAKFYYNCAHAHTTYPTKKVTPDEVSPVTLGDNLTNNRRTINKYFVPDVL
 ETCQLSMGLTELAPGNLWNTMPCHTHERRMEVYFYFNMDDDACVFHMMGQPQET
 RHIVMHNEQAVISPSWSIHSGVGTKAYTFIWGMVGENQVFDDMDHVAVKDLR

SEQ ID NO: 9 pSGI-435; gene #3891-Protein

MTMKILYGAGPEDVKGYDTQRLRDAFLDDLFADDRVSFTYTHVDRILGGAVPVT
 TSLTFGSGTEIGTPYLLSAREMGIANLGGTGTIEVDGQRFTLENRDVLYVGRGARQM
 TASSLSAERPARFYMNVPAGADFPRLITRGEAKPLDLGDARRSNRRRLAMYIHPE
 VSPSCLLLMGITDLAEGSAWNTMPPHLHERRMEAYCYFDLSPEDRVIHMMGRPDET
 RHLVVADGEAVLSPAWSIHMGAGTGPYAFVWGMTGENQEYNDVAPVAVADLK*

SEQ ID NO: 10 pSGI-436; gene #7102-Protein

MLTVETRHAIQDTAKRMDTEELRKHFHMGSLFAAGEIRLVYTHYDRMIVGAAVPS
 GAPLVLDQVKECGTASILDRREMAVVNVGASGKVSAAGETYAMERGDVLYLPLGS
 GKVTFEGEGRFYILSAPAHAAYPARLIRIGEAKEVKLGSAETSNDRITTYQFVHPAVMT
 SCQLVVGYTQLHNGSVWNTMPAHVHDDRMEAYLYFDMKPEQRFVHFHMGEPQETR
 HLVMKNEDAVVSPWSIHCAGGTGSYTFIWAMAGDNVDYKDVEMVAMEDLR*

SEQ ID NO: 11 pSGI-437; gene #9209 -Protein

MSYLLRKQPSNEVSNGVKLVHEVTKSNSDLTYVEFKVLDLASGSSYAEELKKQEICI
 VAVTGNITVTDHESTFENIGTRESVFERKPTDSVYISNDRSFEITAVSDARVALCYSPS
 EKQLPTKLIKAEDNGIEHRGKFSNKRTVHNILPDS DPSANSLVVEVYTDSGNWSSYP
 PHKHDQDNLPEESFLEETYYHELDPGQGFVFQRVYTDDRSIDETMTVENENVVIVPA
 GYHPVGVPDGYTSYYLNVAMAGPTRKWKFHNDPAHEWILER*

SEQ ID NO: 12 pSGI-438; gene #9732 -Protein

MANLLRKPNGTHGKVHDITPENAKWGYVGFGLFRLKSGESVSEKTGSTEVILVLVE
 GKAKISASGEDFGEMGERLNVFEKLPPHCLYVPAESDWHATATTDVLA VCTAPGK
 PGRKAQKLGPESLTLEQRGKGANTRFIHNIAMESRDVADSLLVTEVFTPQGNWSSYP

PHRHDEDNFPDMTYLEETYYHRLNPAQGFGFQRVFTEDGSLDETMAVSDGDVVLVP
KGHHPCGAPYGYEMYYLNVMAGPLRKWRFKNHPDHDWIFKRDNP*

SEQ ID NO: 13 pSGI-439; gene #7403-Protein

MASLLVRPTAPDAQGTVIDVTPESAGWTHVGFVRVHKLAKGQRLEASSDDQEVCLVL
LTGRATVTCGEHRFEDIGQRMDIFEQIPPYAVYLPDHVSYAVEATTDLELAVCTAPG
HGNHAPRLIAPDNIKQSTRGQGTNTRHVHDILPETEPADSLLVVEVFTPAGNWSSYP
HKHDVDNLPHESHLEETYYHRINPEQGFAFQRVYTDDRSLDETMAVENGCCVLVPK
GYHPVGASHGYSLYLNVMAAGPKRAWKFHNDPDHEWLMNAG*

SEQ ID NO: 14 pSGI-440; gene F0J748-Protein

MPDLLRKPFGTHGKVHDITPAAAGWRHVGFGLYRLRAGEFAAEATGGNEVILVMV
EGKASIRAAGRWDGVLGERMSVFEKSPPHSLYVPNGAEWALVAETDCIVAVCSAPG
RGGHAARRIGPEGIVLTARGE GTNTRHINNIA MEAEDYCDALLVTEVFTPAGHWSSY
PSHRHDEDDDDPRITYLEETYYHRLNPASGFGVQRVYTDDRALDQTMASDGDVVLV
PRGHHPCAAPYGIEMYYLNVMAAGPLRKWRFLPDPELGIK

SEQ ID NO: 15 pSGI-458; gene A5YBJ4-Protein

MSLLYHKQNQELSSGVRLIQDVNASNSPMKYTAVKVLEFSADSSYEETLEAFEAGIV
VLEGKVTITADDQTFEDVGQRTSIFDKIPTDSVYVSTGLAFGIRAKQAAKILIA YAPT
N QTFP

VRLIRGNIHQVEHRGKYNNKRLVQNILPDNLPFADKLLLVEVYTDSANWSSYP
PHRH DHDDLPAESLLEEIYYHEMRPKQG FVFQRVYTDDL SLD ETMAVQ
NQD VVVV PKGY HPVGVPDGYDSY LNVMA GPTRVWHFHN
APEHAWIIDRQ

SEQ ID NO: 16 pSGI-478; gene #1874-Protein

MKKFMDENFLLQTETAQKLYHNHAANMPIDYHCHINPKDIAEDRMFKTITEIWLY
GDHYKWRAMRTNGVDERFCTGDASDWEKFEKWAETVPHTLRNPLYHWTHLELKK
FFGINEILSPKNAREIYDACNEKLQTPAYSCRNIIRMANVHTICTTDDPVD
TLEYHQQI KEDGFEVAVLP
AWRPDKAMMVEDPKFFNDYMDQLAEAAAGIHIESFEDLMEALDTR
HQYFHDNGCRLSDHGLDTVFAEDYTEEEIKAIFKKIRGGSRLSETEILKFKSCMLY
EY GVMDHSRGWTQQLHIGAQRNNNTRLFKKLGPDTGFDSIGDKPIAEPLAKLLDRLDQ
ENKLCKTVLYNLNPRDNELYATMLGNFQDGSVPGKIQYGSWWFLDQKDGMIKQ
MNALS NLGLLSRFVGM LTDSRSFLSYTRHEYFRRTL CNLLGNDVENGEIPADMELLG
SMVENICFNNAKNYFNF*

SEQ ID NO: 17 pSGI-479; gene Q9WXR9-Protein

MFLGEDYLLTNRAAVRLFNEVKDLPIVDPHNHLDAKDIVENKPWNDIWEVEGATDH
YVWELMRRCGVSEYITGSR SNKEKWLALAKVFPRFVGNPTYEWIHLDLWRRFNIK

KVISEETAEEIWEETKKKLPEMTPQKLLRDMKVEILCTDDPVSTLEHHRKAKEAVE
GVTILPTWRPDRAMNVDKEGWREYVEKMGERYGEDTSTLDGFLNALWKSHEHFKE
HGCVASDHALLEPSVYYVDENRARAVHEKAFSGEKLTQDEINDYKAFMMVQFGKM
NQETNWVTQLHIGALRDYRDSLFKTLGPDSGGDISTNFLRIAEGRLYFLNEFDGKLKI
VLYVLDPTHLP TIST IARAFPNVYVGAPWWFNDSPFGMEMHLKYLASVDLLYNLAG
MVTDSRKLLSFGSRTEMFRRVLSNVVGEMVEKGQIPIKEARELVKHVSYDGP KALFF
G

SEQ ID NO: 18 pSGI-480; gene Q9KFI6-Protein

MSINSREVLAEKVKNVNNQPV TDMH THLFSPNFGEILLWDIDELLTYHYLVAEVM
RWT DVSIEAFWAMSKREQADLIWEELFIKRSPVSEACRGVLTCLQGLGLDPATRD LQ
VYREYFAKKTSEEQVDTVLQLANVSDVVM TNDPFDDNERISWLEGKQPDSRFHAAL
RLDPLLNEYEQTKHRLRDWGYKVND EWN EGS IQEVKRFLTDWIERMDPVYMAVSL
PPTFSFPEESNRGRIIRDCLLPVAEKHNIPFAMMIGVKKRVHPALGDAGDFVGKASM
DGVEHLLREYPNNKFLVTMLSRENQH ELVVLARKFSNLMIFGCWWFMNNPEIINEM
TRMRMEMLGTSFIPQHSDARVLEQLIYKWHHSKSIIAEVLIDKYDDILQAGWEVTEE
EIKRDVADLFSRNFWR FVGRNDHVT SVKVEQQT

SEQ ID NO: 19 pSGI-481; gene O34808-Protein

MEPFMGKNFLLKNETAVSLYHNYAKDMP IIDYHCHLSPKEIYENKTFQNITEAWLYG
DHYKWRIMRANGIEETYITGDAPDEEEKFMAWAKTVPM AIGNPLYNWTHLELQRFFG
IYEILNEKSGSAIWKQTNKLLKGEGFGARDLIVKSNVKVVCTTDDPVDSLEYHLLK
EDKDFPVSVLPGRPD KGLEINREGFPEWVQALEDAA AISITTYDEFLKALEKRVRFF
HSAGGRVSDHAIDTMVFAETTKEEAGRIFSDRLQGTEVSC EDEKKFKTYTLQFLCGL
YAE LDWAMQFHINALRNTNTKMMKRLGPD TGYDSMNDEEIAKPLYKLLNSVEMKN
QLPKTILYSLNPNDNYVIASMINSFQDGITPGKIQFGTAWWFNDTKDGMLDQMKALS
NVGLFSRFIGMLTDSRSFLSYTRHEYFR RIVCNLIGEWVENGEVPRDMELLGSIVQGI
CYDNAKH YFQFQEEKANV

SEQ ID NO: 20 pSGI-433; gene #8938-DNA

ATGCTCAACGTGGAAACGAGGCACGCCGTT CACGCGGATCACGCGAGATCACTC
GACACAGAGGGCCTGCGCCGGCACTTCCTGGCCCAGGGCCTGTTTGCGGAGGGC
GAGATACGGCTGATCTATACGCATTATGATCGATTCGTCATGGGAGGCGCCGTGC
CGGACGGCGCGCCACTTGTGCTCGATCATGTCTGAGGAGACGAAAACGCCGGGCT
TTCTCGACCGACGGGAGATGGGAATCGTCAATATCGGTGCTGAGGGCAGCGTGC
ATGCCGGCAACGAAAGCTGGTCGCTGAACCGTGGTGACGTACTTTATCTCGGCAT
GGGGGCGGGACCGGTCACCTTCGAAGGGGCTGGGCGCTTCTACCTCGTCTCGGC

ACCGGCGCATCGCAGCCTGCCCAACCGGCTCGTCACGCCGGCCGACAGCAAGGA
GGTCAAGCTTGGCGCTCTCGAGACTTCCAACAAACGCACCATCAATCAGTTCATT
CATCCCCTGGTCATGGAAAGCTGCCAGCTCGTGCTGGGATATACCACGCTGGAGG
ACGGCTCGGTCTGGAATACCATGCCCCGCGCATGTGCACGACCGACGCATGGAGG
CCTATCTCTATTTTCGGCATGGATGAGACATCGCGGGTTCTGCATCTGATGGGCGA
GCCGCAGCAAACGAGGCATCTCTTCGTCGCCAATGAGGAAGGGGCGATCTCTCC
GCCGTGGTCCATCCATGCGGGAGCAGGCATTGGCAGCTATACCTTCATCTGGGCC
ATGGCGGGCGACAATGTCGATTATACCGACATGGAGTTCATCCAGCCGGGAGAT
CTTCGATGA

SEQ ID NO: 21 pSGL-434; gene Q46938-Protein

ATGGACGTAAGACAGAGCATCCACAGTGCGCACGCAAAAACGCTGGATACCCAA
GGGCTGCGCAATGAATTTTTTGGTTGAAAAGGTATTTGTGCGCGATGAGTACACCA
TGGTTTACAGCCACATTGACCGAATTATTGTTGGCGGCATTATGCCGATAACTAA
AACGGTTTCCGTTGGCGGGGAAGTTGGTAAACAACCTCGGCGTAAGCTATTTCTTT
GAACGTCGCGAGTTAGGTGTTATCAATATTGGCGGTGCCGGTACGATTACTGTGCG
ATGGCCAATGCTATGAAATCGGTCACCGCGACGCCCTGTATGTTGGTAAAGGTGC
AAAAGAAGTTGTCTTTGCCAGTATTGATACCGGCACTCCGGCGAAGTTTTATTAC
AATTGCGCACCCGCGCATAACGACGTATCCCACCAAAAAAGTCACACCGGACGAA
GTATCTCCAGTCACGTTAGGCGATAACCTCACCAGTAACCGTCGCACGATTAACA
AATATTTTGTCCCGGATGTACTGGAAACCTGCCAATTGAGTATGGGGCTGACGGA
GCTGGCTCCGGGTAACTTGTGGAACACCATGCCGTGTCACACCCACGAGCGCCG
GATGGAAGTTTATTTCTATTTCAATATGGATGATGACGCCTGCGTTTTCCACATGA
TGGGGCAGCCGCAAGAAACGCGTCATATTGTGATGCATAACGAGCAGGCGGTGA
TCTCCCCGAGCTGGTCGATCCATTCCGGTGTGCGGAACCAAAGCTTATACCTTTAT
CTGGGGCATGGTCGGTGAAAACCAGGTCTTTGATGATATGGACCATGTGGCCGTT
AAAGATTTGCGCTAG

SEQ ID NO: 22 pSGL-435; gene #3891-Protein

ATGACGATGAAGATACTCTACGGCGCCGGACCGGAGGATGTGAAAGGGTATGAC
ACGCAGCGCCTGCGCGACGCCTTCCTGCTGGACGACCTCTTCGCCGACGACCGGG
TCAGTTTTCACATATACCCATGTCGATCGCCTCATCCTCGGCGGGGCGTCCCGGT
GACGACGAGCCTCACCTTCGGCTCCGGCACGGAGATCGGAACGCCCTACCTGCTT
TCCGCCCCGCGAGATGGGGATCGCCAATCTCGGCGGCACGGGCACGATCGAGGTG
GATGGCCAGCGCTTCACGCTCGAAAACCGCGACGTGCTCTATGTCGGTCGCGGC
GCCCCGCGAGATGACCGCCTCCAGCCTGTGCGCGGAGAGGCCAGCCCGCTTCTAC

ATGAATTCCGTGCCCCGCCGGCGCCGATTTCCCGCACCGTCTGATCACCCGCGGAG
AGGCCAAGCCCCTCGATCTCGGGCGATGCGCGCCGCTCGAACAGGGCGCCGGCTCG
CAATGTACATCCATCCGGAGGTCTCGCCGTCCTGCCTGCTGCTCATGGGCATCAC
CGATCTTGCCGAGGGCAGCGCCTGGAACACCATGCCGCCGCATCTGCACGAGCG
GCGGATGGAGGCCTATTGCTACTTCGATCTCTCGCCCCGAGGACCGGGTCATCCAC
ATGATGGGTTCGGCCGGACGAAACCCGCCACCTTGTCGTGGCCGACGGCGAGGCG
GTCCTCTCTCCCGCCTGGTCGATCCATATGGGTGCCGGGACGGGGCCCTACGCCT
TCGTCTGGGGCATGACCGGCGAAAACCAGGAATACAACGACGTCGCTCCCGTAG
CCGTGGCTGATCTCAAATGA

SEQ ID NO: 23 pSGI-436; gene #7102-Protein

ATGCTGACCGTCGAAACCCGCCACGCCATTGATCCGCAGACCGCAAAGCGGATG
GACACGGAAGAGCTGCGCAAGCATTTCACATGGGCAGCCTGTTTGCTGCCGGT
GAAATCCGCCTCGTCTACACCCACTATGACCGCATGATCGTCGGCGCTGCCGTGC
CCTCGGGCGCGCCGCTGGTGCTGGATCAGGTCAAGGAATGCGGCACCGCCAGCA
TCCTCGACCGCCGCGAGATGGCTGTCGTCAACGTTCGGCGCCAGCGGCAAGGTCT
CTGCAGCAGGCGAAACCTACGCCATGGAACGCGGGCGACGTGCTCTATCTGCCGC
TGGGCTCCGGCAAGGTGACCTTCGAAGGCGAAGGCCGCTTCTACATTCTCTCCGC
TCCGGCCCACGCTGCTTACCCGGCCCCGCCTGATCCGCATCGGCGAGGCCGAGAA
GGTCAAGCTCGGCTCGGCCGAGACCTCCAACGACCGCACCATCTACCAGTTCGTG
CATCCGGCGGTGATGACTTCCTGCCAACTCGTCGTCGGCTACACCCAGCTGCACA
ACGGCTCTGTCTGGAACACCATGCCCCGCCACGTGCATGACCGGCGCATGGAGG
CCTATCTCTATTTGACATGAAGCCGGAGCAGCGCGTGTTCCACTTCATGGGCGA
GCCGCAGGAAACCCGCCATCTGGTCATGAAGAACGAGGATGCGGTGGTCTCCCC
GCCCTGGTCCATCCACTGCGGCGCAGGCACCGGCAGCTACACCTTCATCTGGGCC
ATGGCCGGCGACAACGTCGACTACAAGGACGTGGAAATGGTCGCCATGGAGGAT
CTGCGGTGA

SEQ ID NO: 24 pSGI-437; gene #9209 –DNA

ATGAGTTATTTGTTGCGTAAGCCGCAGTCGAATGAAGTGTCTAATGGGGTCAAAC
TGGTGACGAAGTAACGAAATCCAACCTCTGATCTCACCTATGTAGAGTTTAAAGT
GTTAGATCTCGCTTCCGGTTCCAGCTATGCAGAAGAATTGAAAAAACAGGAAAT
CTGTATTGTCGCGGTAACGGGAAACATTACAGTGACCGATCACGAGTCGACTTTT
GAGAATATCGGCACGCGTGAAAGCGTATTCGAACGAAAACCGACAGACAGCGTC
TATATTTCAAATGACCGTTCCTTTGAGATCACAGCGGTCAGCGACGCAAGAGTGG
CGCTTTGCTATTCTCCATCGGAAAAACAGCTTCCGACAAAGCTGATCAAAGCGGA

AGACAATGGCATTGAGCATCGCGGGAAGTTTTCAAACAAACGTACTGTTACAA
CATTCTTCCGGATTTCAGACCCTTCAGCTAACAGCCTATTAGTAGTTGAAGTCTAT
ACAGACAGCGGCAACTGGTCCAGCTATCCGCCTCATAAACATGATCAAGACAAT
TTGCCGGAGGAATCTTTTTTAGAAGAAACGTACTACCATGAGTTAGACCCGGGAC
AGGGCTTTGTGTTTCAGCGTGTATACACAGATGACCGCTCGATTGACGAGACAAT
GACTGTAGAAAATGAAAACGTTGTCATCGTTCCTGCAGGATACCACCCGGTAGG
CGTGCCGGACGGATACACATCCTACTATTTAAATGTCATGGCAGGGCCGACGCG
GAAATGGAAGTTTCATAATGACCCGGCGCATGAGTGGATTTTAGAACGTAA

SEQ ID NO: 25 pSGI-438; gene #9732 -DNA

ATGGCCAATTTGTTGCGCAAGCCCAACGGCACGCATGGCAAGGTCCACGACATC
ACTCCGGAAAACGCCAAATGGGGTTATGTCGGGTTTCGGGCTCTTTCGTCTCAAAT
CCGGCGAGAGTGTCTCCGAAAAGACCGGATCGACGGAGGTGATCCTTGTCTTGT
GGAAGGCAAGGCAAAGATTTCCGCTTCTGGCGAGGATTCGGCGAGATGGGTGA
ACGCTTAAACGTGTTTCGAGAACTGCCGCCACACTGCCTCTATGTGCCTGCTGAA
AGCGACTGGCATGCAACCGCCACGACAGATTGTGTTCTGGCTGTTTGCACCGCAC
CGGGCAAGCCAGGCCGCAAGGCACAGAAGCTTGGGCCGAAAGCTTGACACTTG
AACAACGCGGAAAAGGTGCCAATACCCGCTTTATCCATAATATCGCAATGGAAA
GCCGCGATGTTGCCGATAGCCTTCTTGTACCGAGGTATTCACACCGCAGGGAAA
CTGGTCGTCTATCCACCCACAGACACGACGAAGACAATTTTCCGGATATGACC
TATCTGGAAGAGACCTATTATCACCGTCTCAACCCGGCGCAGGGCTTCGGCTTCC
AGCGTGTTTTACCGAAGACGGAAGCCTTGATGAAACCATGGCGGTCTCTGACG
GAGACGTCGTGCTTGTACCAAAAGGCCACCATCCATGTGGCGCGCCCTATGGCTA
CGAGATGTATTATCTCAATGTGATGGCCGGTCCCTTGCGCAAATGGCGCTTCAAG
AACCATCCCGACCATGACTGGATTTTCAAACGCGACAATCCGTAA

SEQ ID NO: 26 pSGI-439; gene #7403-DNA

ATGGCTTCCCTACTGGTACGCCCCACCGCCCCAGATGCCAGGGCACCGTGATTG
ACGTTACCCCTGAATCTGCTGGCTGGACGCACGTTGGCTTTCGGGTGCATAAACT
CGCCAAGGGCCAGCGCCTGGAGGCCAGCAGCGATGATCAGGAAGTCTGCCTGGT
GCTGCTCACCGGTCGCGCCACGGTAACTTGCGGCGAGCACCGCTTTGAAGATATT
GGCCAGCGTATGGATATTTTTGAGCAGATCCCTCCCTATGCGGTTTACCTACCTG
ACCATGTTAGCTACGCGGTGGAAGCGACCACAGACTTAGAGCTAGCGGTGTGCA
CCGCCCTGGGCATGGCAACCATGCCCCACGGCTCATCGCGCCTGACAACATCA
AGCAAAGCACCCGTGGCCAGGGCACCAACACCCGCCATGTTACGATATTCTGC
CGGAAACCGAGCCCGCCGATAGCCTATTAGTAGTCGAAGTATTCACACCTGCGG

GTA ACTGGT C GAGCTACCCGCCCCACAAACACGATGTGGATAACTTACCCACG
AATCACATCTGGAAGAGACCTACTACCACCGCATTAACCCTGAACAAGGGTTCG
CCTTCCAGCGCGTTTACACCGATGACCGCAGCCTTGATGAAACCATGGCGGTGGA
AAACGGCTGCTGTGTGTTGGTTCCCAAGGGTTACCATCCGGTGGGCGCCTCCCAT
GGCTACTCGCTCTACTACTTAAATGTGATGGCGGGGCCCAAGCGGGCATGGAAA
TTTCACAACGACCCCGACCACGAATGGCTGATGAACGCTGGATAG

SEQ ID NO: 27 pSGI-440; gene F0J748-DNA

ATGCCGGACTTACTGAGAAAACCGTTTGGCACCCATGGCAAAGTGCACGATATT
ACCCAGCAGCAGCAGGTTGGAGACATGTTGGTTTTGGCTTATATCGCTTAAGAG
CGGGCGAATTTGCAGCAGAAGCGACAGGCGGCAATGAAGTTATTCTGGTGATGG
TTGAGGGCAAAGCGTCTATTAGAGCAGCAGGCAGAGATTGGGGCGTTTTAGGCG
AACGTATGAGCGTCTTCGAAAAAAGTCCACCACATTCCTGTATGTCCCGAATGG
TGCAGAATGGGCCTTAGTAGCCGAAACAGATTGCATTGTAGCAGTGTGTAGCGCT
CCGGGTAGAGGAGGTCATGCTGCAAGAAGAATTGGTCCTGAAGGTATTGTGTTA
ACCGCCAGAGGTGAAGGCACCAATACACGCCACATCAACAACATCGCCATGGAA
GCCGAAGATTATTGTGATGCCCTGTTAGTCACCGAAGTGTTACCCAGCCGGCC
ATTGGAGCTCTTATCCATCTCATCGTCATGATGAAGACGACGATCCGCGCATCAC
CTATTTAGAAGAGACCTACTATCATCGCTTAAATCCTGCCTCGGGCTTTGGCGTTC
AACGCGTCTATACCGATGATCGCGCCTTAGATCAAACCATGGCGGTTTCTGATGG
CGATGTTGTTTTAGTTCCTCGCGGCCATCATCCGTGTGCAGCCCCGTATGGTATTG
AAATGTATTACCTGAACGTCATGGCCGGCCCGTTACGTAAATGGCGCTTTTTACC
TGATCCTGAACCTTGGCATTGCGAAATAA

SEQ ID NO: 28 pSGI-458; gene A5YBJ4-DNA

ATGTCTCTGCTGTACCACAAGCAGAACCAGGAACTGAGTAGTGGTGTGCGCCTG
ATCCAAGATGTTAATGCCAGCAATAGCCCGATGAAATATACCGCCGTGAAAGTG
CTGGAGTTTAGCGCCGATAGCAGCTATGAGGAAACCTTAGAGGCCTTTGAAGCC
GGCATTGTTGTGTTAGAGGGCAAAGTGACCATCACCGCCGACGATCAAACCTTCG
AAGATGTGGGTCAAAGAACCTCGATCTTCGACAAAATCCCGACCGATAGCGTTT
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AATCGCGTATGCTCCGACCAATCAGACCTTCCCAGTTCGCTTAATTCGCGGCAAT
ATCCACCAGGTGGAACATCGCGGCAAGTACAACAACAAACGCTTAGTGCAGAAC
ATTCTCCCGGATAATCTCCCGTTCGCCGATAAATTACTGCTGGTTGAGGTGTACA
CCGATAGCGCCAATTGGAGCTCCTATCCGCCGCATAGACATGATCACGATGATTT
ACCGGCCGAAAGTCTGTTAGAGGAGATCTACTATCACGAAATGCGCCCGAAGCA

GGGCTTCGTCTTTCAACGCGTGTATACCGATGATCTGAGTCTGGATGAGACCATG
GCCGTTCAAATCAAGATGTTGTCGTTGTCCCGAAAGGCTATCATCCGGTTGGTG
TCCCGACGGCTATGATTCGTATTACCTGAACGTGATGGCCGGCCCGACAAGAGT
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SEQ ID NO: 29 pSGI-478; gene #1874-DNA

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ACCCCAAAGACATCGCGGAAGACCGGATGTTTAAAACCATCACCGAAATCTGGT
TGTACGGCGATCATTATAAATGGCGCGCCATGCGTACAAACGGCGTTGACGAGC
GCTTTTGCACCGGCGATGCAAGCGATTGGGAAAAGTTTGAAAAGTGGGCCGAAA
CGGTTTCCTCATACCCTGCGTAATCCGCTTTATCACTGGACACACCTGGAGCTAAA
GAAATTTTTCGGGATTAACGAGATCCTGAGTCCGAAAAATGCCCGGGAAATTTAT
GATGCCTGTAACGAAAACTGCAAACGCCCCGCGTATAGTTGCCGCAACATCATC
CGGATGGCCAATGTGCATACAATCTGTACCACCGACGACCCGGTTGACACACTG
GAATATCATCAGCAAATTAAGAAGACGGCTTTGAAGTGGCGGTTTTACCTGCCT
GGCGTCCGATAAAGCGATGATGGTGGAAAGACCCGAAGTTCTTTAACGACTATA
TGGACCAGTTGGCCGAAGCTGCCGGTATCCATATCGAATCGTTTGAGGATTTGAT
GGAAGCCTTGGATACGCGTCACCAGTATTTTCATGATAATGGTTGCCGTTTGTCC
GACCACGGGCTGGATACCGTTTTTGTCTGAAGATTATACGGAGGAAGAAATTA
GCGATCTTCAAAAAAATCCGTGGCGGCAGCAGGCTTAGCGAAACGGAAATCCTG
AAATTCAAGTCCTGCATGTTGTACGAATATGGGGTGATGGACCATTTCGCGCGGCT
GGACACAACAATTGCACATTGGCGCACAAACGCAACAACAACACCCGTTTGTTC
AAAAATTAGGTCCCGACACTGGTTTTCGATTTCGATTGGCGATAAGCCGATCGCTGA
ACCATTTGGCCAAATTGCTCGACCGCCTGGATCAGGAAAACAAATTGTGCAAAAC
GGTTTTGTATAATCTGAATCCGCGTGATAACGAGTTGTACGCTACCATGTTGGGC
AACTTTCAGGACGGATCGGTTCCCGGGAAAATTCAATACGGCTCGGGTTGGTGGT
TTCTCGATCAGAAAGACGGCATGATTAAACAGATGAATGCCCTTTCCAATCTGGG
TTTGCTGAGCCGTTTCGTAGGCATGCTGACCGACTCAAGGAGCTTCCTTTCGTAC
ACCCGTCACGAATATTTCCGTCTGACCTTTGCAACCTGCTTGGGAATGATGTTG
AAAACGGGGGAGATTCCGGCAGATATGGAGCTTTTGGGCAGTATGGTTGAGAATA
TTTGTTTTAATAACGCGAAGAACTATTTTAATTTTAG

SEQ ID NO: 30 pSGI-479; gene Q9WXR9-DNA

ATGTTTCTGGGCGAAGACTATCTGCTGACCAATCGTGCGGCAGTTCGTCTGTTCA
ACGAAGTGAAAGATCTGCCGATCGTTGATCCGCATAACCACCTGGATGCGAAAG

ATATCGTGGAACAAACCGTGGAACGACATCTGGGAAGTGGAAGGTGCGACCG
ATCACTATGTGTGGGAAGTATGCGTCGTTGTGGTGTAGCGAAGAATATATTAC
CGGCTCTCGTAGCAACAAAGAAAAATGGCTGGCGCTGGCGAAAGTGTTTCCGCG
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CAAAAAAAACTGCCGGAGATGACCCCGCAGAACTGCTGCGCGACATGAAAGT
GGAAATCCTGTGCACCACCGATGATCCGGTGTCTACCCTGGAACATCACCGTAAA
GCGAAAGAAGCCGTGGAAGGCGTGACCATTTTACCGACCTGGCGTCCGGATCGT
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GCCACGAACACTTCAAAGAACACGGCTGTGTGGCGAGCGATCATGCGCTGCTGG
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CATTTTCTGGTGAAAAACTGACTCAAGATGAAATCAACGACTATAAAGCGTTCAT
GATGGTGCAGTTCGGCAAAATGAACCAGGAAACCAACTGGGTGACCCAGCTGCA
CATTTGGTGCCCTGCGCGATTACCGCGATAGCCTGTTCAAACCCCTGGGCCCCGGAT
TCTGGTGGCGATATCAGCACCAACTTTCTGCGTATTGCTGAAGGTCTGCGTTATTT
TCTGAACGAATTTGATGGTAAACTGAAAATTGTGCTGTACGTGCTGGATCCGACC
CATTTACCGACCATTTTCGACCATTTGCACGTGCGTTCCCGAACGTGTATGTGGGTG
CACCGTGGTGGTTCAACGATAGCCCGTTTCGGCATGGAAATGCACCTGAAATACCT
GGCGAGCGTTGATCTGCTGTACAATCTGGCTGGTATGGTTACCGATTCACGTAAA
TTACTGAGTTTTGGTTCTCGTACCGAAATGTTTCGTCGCGTTCTGTCTAATGTGGT
TGCGGAAATGGTGGAAAAAGGCCAGATCCCGATCAAAGAAGCGCGCGAACTGGT
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SEQ ID NO: 31 pSGL-480; gene Q9KFI6-DNA

ATGAGCATCAACAGCCGTGAAGTTCTGGCGGAAAAAGTGAAAAACGCGGTGAAC
AACCAGCCGGTTACCGATATGCATACCCACCTGTTTAGCCCGAACTTTGGCGAAA
TTCTGCTGTGGGACATCGATGAACTGCTGACCTATCACTACCTGGTTGCGGAAGT
TATGCGTTGGACCGATGTGAGCATTGAAGCGTTTTGGGCAATGAGCAAACGTGA
ACAGGCCGATCTGATTTGGGAAGAACTGTTTCATCAAACGCAGCCCGGTGAGCGA
AGCATGTCGTGGCGTTCTGACCTGTTTACAAGGTTTAGGTCTGGATCCGGCAACT
CGTGATTTACAGGTGTATCGTGAATACTTCGCCAAAAAAACCAGCGAGGAACAG
GTGGATACCGTTCTGCAGCTGGCAAATGTGAGCGATGTGGTGTATGACCAATGATC
CGTTCGATGATAATGAACGCATCAGCTGGCTGGAAGGCAAACAGCCGGATAGCC
GCTTTCATGCAGCGTTACGTCTGGATCCGCTGCTGAATGAATATGAACAGACCAA

ACATCGTCTGCGTGATTGGGGTTATAAAGTGAACGACGAATGGAACGAAGGCAG
CATCCAGGAAGTGAAACGCTTTCTGACCGACTGGATTGAACGTATGGATCCGGTG
TATATGGCGGTGAGCTTACCGCCGACCTTCAGCTTTCCGGAAGAATCGAACCGTG
GCCGCATTATCCGTGATTGTCTGTTACCGGTTGCAGAAAAACATAACATCCCGTT
TGCAATGATGATTGGCGTGAAAAACGCGTGCATCCGGCGTTAGGTGATGCAGG
CGATTTTGTGGGTAAAGCAAGTATGGATGGCGTTGAACACCTGCTGCGCGAATAC
CCGAACAACAAATTCCTGGTGACCATGCTGAGCCGCGAAAACCAGCACGAACTG
GTGGTTCTGGCGCGTAAATTTAGTAACCTGATGATTTTTGGTTGTTGGTGGTTTAT
GAACAACCCGGAGATCATCAACGAAATGACCCGCATGCGCATGGAAATGCTGGG
TACCAGCTTTATCCCGCAGCACAGCGATGCCCGTGTTCTGGAACAGCTGATCTAT
AAATGGCACCACAGCAAAAGCATCATCGCGGAAGTCCTGATCGACAAATACGAC
GACATCCTGCAAGCAGGTTGGGAAGTTACCGAAGAAGAAATCAAACGTGATGTG
GCAGATCTGTTTAGCCGCAACTTTTGGCGCTTTGTGGGCCGTAACGATCACGTGA
CCAGCGTGAAAGTGGAACAGCAGACCTGA

SEQ ID NO: 32 pSGL-481; gene O34808-DNA

ATGGAACCGTTTATGGGCAAAAACCTTCCTGCTGAAAAACGAGACCGCGGTGAGC
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GCCCCGAAAGAAATCTACGAGAACAAAACCTTCCAGAACATCACCGAAGCGTGGC
TGTACGGCGATCACTACAAATGGCGCATCATGCGTGCGAATGGCATCGAAGAAA
CCTATATTACCGGTGATGCACCGGACGAAGAAAAATTCATGGCGTGGGCGAAAA
CCGTGCCGATGGCCATTGGTAATCCGCTGTATAACTGGACCCATCTGGAAGTGA
ACGTTTTTTTTGGCATCTACGAAATCCTGAACGAAAAAAGCGGCAGCGCGATCTGG
AAACAGACCAACAACTGCTGAAAGGCGAAGGCTTTGGTGCGCGTGATCTGATC
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GAATACCATCTGCTGCTGAAAGAAGACAAAGACTTCCCGGTTAGCGTTTTACCGG
GTTTTTCGTCCGGATAAAGGTCTGGAAATCAACCGTGAAGGCTTTCCGGAATGGGT
TCAAGCCCTGGAAGATGCGGCCGCAATTAGCATTACGACCTATGATGAATTTCTG
AAAGCGCTGGAAAAACGCGTGCGCTTCTTCCATAGTGCGGGTGGTCGTGTTAGCG
ATCATGCAATCGATACCATGGTTTTTCGCCGAAACCACCAAAGAAGAAGCGGGTC
GCATTTTTTAGTGATCGTCTGCAAGGCACCGAAGTTAGCTGCGAAGACGAGAAAA
AATTCAAAACCTACACCCTGCAGTTTCTGTGTGGCCTGTATGCCGAAGTGGACTG
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ACGCCTGGGTCCGGATACCGGTTATGATAGCATGAACGATGAAGAAATCGCGAA
ACCGCTGTACAACTGCTGAACAGCGTGGAATGAAAAACCAACTGCCGAAAAC

CATCCTGTACAGCCTGAACCCGAACGACAACTACGTGATCGCGAGCATGATCAA
CAGCTTCCAGGATGGCATCACCCCGGGCAAAATTCAGTTTGGCACCGCATGGTGG
TTCAACGATACCAAAGATGGTATGCTGGATCAGATGAAAGCACTGAGCAATGTG
GGCCTGTTTAGCCGTTTTATTGGCATGCTGACCGATAGCCGTAGCTTTCTGAGCTA
TACCCGTCACGAATACTTTCGCCGCATTGTGTGTAACCTGATCGGCGAATGGGTG
GAAAACGGCGAAGTTCCGCGCGATATGGAAGTCTGGGTAGTATTGTGCAAGGT
ATTTGCTACGATAACGCGAAACATTACTTCCAGTTCAGGAGGAAAAAGCGAAC
GTGTGA

SEQ ID NO: 33 pSGI-359-0385-Protein

MSQTPRKLRSQKWFDDPAHADMTAIYVERYLNYGLTRQELQSGRPIIGIAQTGSDLAPCNRH
HLALAERVKAGIRDAGGIPMEFPVHPLAEQGRRPTAALDRNLAYLGLVEILHGYPLDGVVLT
TGCDKTTPACLMAAATVDLPAIVLSGGPMLDGWHDGQRVSGTVIWHARNLMAAGKLDY
EGFMTLATASSPSVGH CNTMGTALSMNSLAEALGMSLPTCASIPAPYRERAQMAYATGMRI
CDMVREDLRPSHILTRQAFENAIVVASALGASTNCPPHLIAMARHAGIDLSLDDWQRLGEDV
PLLVNCVPAGEHLGEGFHRAGGVPAVMHELFAAGRLHPDCPTVSGKTIGDIAAGAKTRDAD
VIRSCAAPLKHRAGFIVLSGNFFDSAIKMSVVGEAFRRAYLSEPGSENAFEARAIVFEGPEDY
HARIEDPALNIDEHCILVIRGAGTVGYPGSAEVVNMAPPSHLIKRGVDSLPCLDGRQSGTSG
SPSILNMSPEAAVGGGLALLRTGDKIRVDLNQRSVTALVDDAEMARRKQEPYQAPASQTP
WQELYRQLVGQLSTGGCLEPATLYLKVIETRGDPRHSH

SEQ ID NO: 34 pSGI-360-0336-Protein

MSERIKKMNDQNKRIFLRSQEWFDDEHADMTALYVERYMNYGLTRAEQSGRPIIGIAQTG
SDLTPCNRHHKELAERVKAGIRDAGGIPMEFPVHPIAEQTRRPTAALDRNLAYLGLVEILHGY
PLDGVVLTGTGCDKTTPACLMAAATTDIPAIVLSGGPMLDGHFKEGELIGSGTVLWHARNLLAT
GEIDYEGFMEMTTASPSVGH CNTMGTALSMNALAEALGMSLPTCASIPAPYRERGQMAYM
TGKRICEMVLEDLRPSKIMNKQSFENAIIVASALGASSNCPPHLIAIARHMGIELSLEDWQRV
GENIPLIVNCPAGKYLGEFHRAGGVPAVLHELQKASVLHEGCASVSGKTMGEIAKNAKT
SNVDVIFPYEQPLKHGAGFIVLSGNFFDSAIMKMSVVGEAFKKTYLSDPNGENSFEARAIVFE
GPEDYHARINDPALDIDEHCILVIRGAGTVGYPGSAEVVNMAPPAELIKKGIDSLPCLDGRQ
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TPWQEMYRNMVGQLSTGGCLEPATLYMRVINQDNLPRHSH

SEQ ID NO: 35 pSGI-365 E3HJU7-Protein

MSQTPRKLRSQKWFDDPAHADMTAIYVERYLNYGLTRQELQSGRPIIGIAQTGSDLAPCNRH
HLALAERIKAGIRDAGGIPMEFPVHPLAEQGRRPTAALDRNLAYLGLVEILHGYPLDGVVLT
GCDKTTPACLMAAATVDIPAIVLSGGPMLDGWHDGQRVSGTVIWHARNLMAAGKLDYEG
FMTLATASSPSIGH CNTMGTALSMNSLAEALGMSLPTCASIPAPYRERGQMAYATGLRICDM
VREDLRPSHVLTRQAFENAIVVASALGASSNCPPHLIAMARHAGIDLSLDDWQRLGEDVPLL

VNCVPAGEHLGEGFHRAGGVPAVLHELAAAGRLHMDCATVSGKTIGEIAAAAKTNNADVIR
SCDAPLKHRAGFIVLSGNFFDSAIHKMSVVGEAFRRAYLSEPGSENAFEARAIVFEGPEDYHAR
IEDPTLNIDEHCILVIRGAGTVGYPGSAEVVNMAPPShLLKRGIDSLPCLGDGRQSGTSASPSIL
NMSPEAAVGGGLALLRTGDRIRVDLNQRSVIALVDQTEMERRKLEPPYQAPESQTPWQELY
RQLVGQLSTGGCLEPATLYLKVVETRGRDPRHSH

SEQ ID NO: 36 pSGI-359-0385-DNA

ATGTCTCAGACACCCCGCAAGTTGCGCAGCCAGAAATGGTTCGACGACCCTGCGCATGC
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ACCGCCATCACCTGGCGCTGGCCGAGCGCGTCAAAGCGGGCATCCGGGACGCGGGCGGC
ATCCCGATGGAGTTCCCGTGACCCGCTGGCCGAACAAGGCCGGCGGCCACGGCCGC
GCTGGACCGCAACCTGGCCTATCTGGGCCTGGTCGAAATCCTGCACGGCTACCCCTTGGA
CGGGGTGGTGCTGACGACTGGCTGCGACAAGACCACGCCTGCCTGCCTGATGGCCGCCG
CCACGGTCGACCTGCCC GCCATCGTGCTGTCCGGCGGCCCATGCTGGACGGCTGGCACG
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CGCGGCGTGATTCCCTGCCGTGCCTGGGGGATGGCAGGCAAAGCGGCACTTCCGGCAG
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ACGCGGAAATGGCAAGACGGAAGCAAGAACCGCCCTACCAGGCACCGGCCTCGCAAAC
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GA

SEQ ID NO: 37 pSGI-360-0336-DNA

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AACTGGCAGTGATTAACTCCATGTAACCGTCACCACAAAGAACTTGCTGAACGGGTAA
AGCAGGTATTTCGAGATGCGGGAGGTATTCCTCATGGAATTCCTCCGTTACCCGATTGCAGA
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AATATTGCATGGTTATCCGCTTGATGGTGTGGTGCTAACCACAGGTTGTGACAAAACACTAC
ACCTGCTTGTTTAATGGCTGCCGCAACGACAGATATACCAGCCATTGTGTTGTCTGGTGG
ACCAATGCTAGATGGTCATTTTAAAGGTGAGTTAATTGGTTCTGGGACTGTGCTTTGGCA
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CTTCAGCATCGCCTTCGGTCGGACATTGCAACACCATGGGCACTGCACTTTCTATGAATG
CCTTGGCAGAAGCTTTGGGCATGTCTTTACCGACATGTGCAAGTATTCCAGCGCCGTATC
GCGAACGAGGGCAAATGGCCTATATGACAGGCAAAGAATTTGTGAAATGGTTTTAGAA
GATTTACGCCCTTCTAAAATCATGAACAAACAATCATTTGAAAATGCCATCGCGGTAGCT
TCAGCATTAGGGGCATCAAGTAATTGCCCTCCTCACCTCATTGCAATTGCCCGTCATATG
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AACTGTATGCCTGCGGGTAAATATTTAGGTGAAGGTTTTACCGTGCTGGCGGTGTTCTT
GCTGTTTTGCATGAATTACAAAAGGCCAGCGTTTTACATGAAGGCTGTGCATCAGTCAGC
GGTAAAACGATGGGAGAAATTGCTAAAAATGCTAAAACCTCCAATGTAGATGTTATTTTT
CCATATGAACAACCATTAACATGGTGCAGGTTTTATTGTGCTTAGTGGCAATTTCTTC
GACAGCGCCATTATGAAAATGTCTGTTGTGGGTGAAGCATTTAAGAAAACCTATTTATCT
GACCCAAATGGGGAAAATAGCTTTGAAGCACGGGCAATCGTTTTTTGAAGGGCCAGAGGA
CTACCATGCACGAATTAATGATCCAGCCTTAGACATTGATGAACATTGTATTTTGGTCAT
TCGTGGCGCTGGAACAGTGGGCTATCCAGGTAGTGCAGAAGTTGTAAATATGGCTCCAC
CCGCAGAGTTAATTAAGGAGGATCGATTCACTGCCTTGCTTAGGAGATGGCCGCCAA
AGTGGTACGTCTGCCAGCCCTTCTATTTTAAATATGTCACCCGAAGCGGCGGTAGGCGGT
GGAATTGCATTATTAAGACCAATGACCGTTTACGCATTGATCTCAATAAACGCTCCGTC
AACGTAATCATTTCTGACGAAGAGTTAGAACAACGCCGCCGTGAGTGGAAACCGACGGT
CTCTTCATCTCAAACACCTTGGAAGAAATGTATCGCAACATGGTGGGTCAATTATCCAC
TGGCGGTTGTTTGGAACCTGCAACTTTATATATGCGAGTCATAAATCAAGACAACCTTCC
AAGACACTCTCATTA

SEQ ID NO: 38 pSGI-365 E3HJU7-DNA

ATGAGCCAAACACCGCGTAAATTACGCAGCCAGAAGTGGTTTGACGATCCTGCACATGC
CGATATGACCGCCATCTATGTTGAACGCTACCTGAACTATGGCTTAACCCGCCAAGAACT
GCAAAGTGGTCGCCCCGATTATTGGTATTGCCCAAACCGGCAGCGATTTAGCCCCGTGTAA
TCGCCATCATTTAGCCTTAGCCGAACGCATTAAAGCAGGCATTAGAGATGCAGGCGGCA

TTCCTATGGAATTTCCCGTTCATCCGCTGGCCGAACAAGGTAGACGTCCTACAGCAGCAT
TAGATCGCAATTTAGCCTATTTAGGCCTGGTGGAAATTTTACACGGCTATCCCCTGGACG
GTGTGGTGCTGACAACCGGTTGCGATAAAACAACACCGGCGTGTTTAATGGCAGCTGCA
ACAGTTGATATTCCGGCGATCGTGTTATCAGGTGGTCCGATGTTAGATGGCTGGCATGAT
GGCCAAAGAGTTGGCAGTGGTACCGTGATTTGGCATGCACGCAATTTAATGGCAGCAGG
CAAACCTGGATTATGAAGGCTTCATGACCCTGGCGACAGCCTCTTCTCCGAGTATTGGACA
CTGTAATACCATGGGCACAGCCTTAAGCATGAATAGTCTGGCAGAAGCCCTGGGTATGTC
TTTACCGACCTGTGCGTCTATTCCAGCCCCGTATAGAGAACGCGGTCAAATGGCGTATGC
TACTGGTTTACGCATTTGCGATATGGTGCGCGAAGATTTACGCCCCGTCACATGTTTTAAC
CCGCCAAGCCTTCGAAAATGCCATTGTTGTTGCCTCAGCCTTAGGTGCAAGCTCTAATTG
TCCCCCTCATTTAATTGCCATGGCCCCGTATGCCGGTATCGACTTAAGCCTGGATGACTG
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AGGTGAAGGATTTTCATCGCGCGGGTGGTGTTTCTGCTGTTTTACATGAATTAGCTGCCGC
AGGTGCTTTACATATGGATTGTGCTACCGTTTCTGGCAAGACCATCGGCGAAATTGCAGC
TGCCGCAAAAACCAACAACGCAGACGTGATTCGCTCGTGATGCCCCGTAAAACATA
GAGCCGGCTTTATTGTGTTAAGCGGCAATTTCTTCGACTCCGCCATCATCAAGATGTCCG
TTGTGGGTGAAGCCTTTCGCAGAGCCTATTTAAGTGAACCTGGCAGCGAAAATGCCTTTG
AAGCCCGTGCCATCGTGTTTGAAGGCCCGGAAGACTATCATGCCCGCATTGAAGATCCG
ACCCTGAATATTGATGAACACTGCATTCTGGTGATTTCGCGGCGCAGGTACCGTTGGTTAT
CCTGGTAGTGCTGAAGTTGTGAATATGGCCCCGCCGAGCCATTTATTAACGCGGTATT
GATTCATTACCTTGCCTGGGAGATGGCCGCCAAAGTGGTACCTCAGCTAGTCCGTCTATC
CTGAATATGAGCCCTGAAGCCGCCGTTGGAGGAGGTTTAGCATTATTAAGAACCGGTGA
TCGCATTTCGCGTCGATCTGAATCAACGCTCAGTCATTGCATTAGTCGACCAGACCGAAAT
GGAACGCCGCAAATTAGAACCACCGTATCAAGCACCTGAAAGCCAAACCCCGTGGCAAG
AACTGTATCGCCAATTAGTCGGTCAACTGTCAACAGGCGGCTGCCTGGAACCAGCCACCT
TATATTTAAAAGTCGTGGAAACCCGTGGAGATCCTCGTCATAGCCATTAA

SEQ ID NO: 39 - AO#13-0573

MDRRELLKTSALLMAAAPLARAANVPEDHANVPRTNWSKNFHYSTSRVYAPTTPEEVPAIV
LENGHLKGLGSRHCFNNIADSQY AQISMREVKG IQIDEAAQTVTVGAGIAYGELAPVLDKAG
FALANLASLPHISVGGTIATATHGSGVGNKNLSSATRAIEIVKADGSILRLSRDTDGERFRMA
VVHLGALGVLT KVTLDIVPRFDMSQVVYRNLSFDQLEHNLD TILSSGYSVSLFTDWQRNRVN
QVWIKDKATADAPQKPLPPMFYGATLQTAKLHPIDDHPADACTEQMGSVGPWYLRLPHFK
MEFTPSSGEELQTEYFVAR KDGYRAIRAVEKLRDKITPHLFITEIRTIAADDLPMSMAYQRDS
MAIHFTWKPEEPTVRKLLPEIEAALAPFGVRPHWGKIFEIPPSYLHKQYPALPRFRAMAQALD
PGGKFRNAYLDRNIFGA

SEQ ID NO: 40 - AO#22-8001

MDKRDFLKGSATTAVALLMMGLNESKAFADDSVPRTNWSGNYHYSTNKVLQPASVAETQD
AVRSVAGVRLGTRHSFNGIADSQIAQISTLKLKDVSLDAKSSTVTVGAGIRYGD LAVQLDA
KGFALHNLASLPHISVGGACATATHGSGMGNGNLATAVKAVEFVAADGSVHTLSRDRDGD
RFAGSVVGLGALGVVTHLTLQVQPRFEMTQVVYRDLPFSELEHHLPEIMGAGYSVSLFTDW
QNGRAGEVWIKRRVDQGGASAPPARFFNATLATTKLHPILDHPAEACTDQLNTVGPWYERL
PHFKLNFTPSSGQELQTEFFVPFDRGYDAIRAVETLRDVITPHLYITELRAVAADDLWMSMAY
QRPSLAIHFTWKPETDAVLKLLPQIEAKLAPFGARPHWAKVFTMKSSHVAPLYPRLKDFLVL
AKSFDPKGKFQNAFLQDHVDIA

SEQ ID NO: 41 - AO#28-9635.1

MTASVTNWAGNISFVAKDVVRPGGVEALRKVVAGNDRVRVLGSGHSFNRIAEPGADGVLV
SLDALPQVIDVDTERRTVRVGGGVKYAELARHVNESGLALPNMASLPHISVAGSVATGTHGS
GVNNGPLATPVREVELLTADGSLVTIGKDDARFPGAVTSLGALGVVVALTLDLEPAYGVEQ
YTFTELPLEGLDFEAVASAAYSVSLFTDWREAGFRQVWVKRRIDEPYAGFPWAAPATEKLHP
VPGMPAENCTDQFGAAGPWHERELPHFKAFTPSGDELQSEYLLPREHALAALDAVGNVRE
TVSTVLQICEVRTIAADTQWLSPAYGRDSVALHFTWTDDMDAVLPAVRAVESALDGFGARP
HWGKVFTTAPAALRERYPRLDDFRTLRLDELDPAGKFTNAFVRDVLEG

SEQ ID NO: 42 - AO#36-7049

MTLERNWAGHTHTFAAPRIVNATSIDEVRALVAEAARTGTRVRALGTRHSFTDLADSDGTLIT
VLDIPADPVFDEAAGSVTIGAGTRYGIAAAWLAEHGLAFHNMGSLPHISVGGAIATGTHGSG
NDNGILSSAVSGLEYVDATGELVHVRRGDPGFDGLVVGLGAYGIVVRVTVDVQPAYRVRQD
VYRDVPWDAVLADFEGVTGGAYSVSIFTNWLGDTVEQIWWKTRLVAGDDELVPVPESWLG
VQRDSLTAGNLVETDPDNLTLQGGVPGDWWERLPHFRLESTPSNGDEIQTEYFIDRADGPAA
ITALRALGDRIAPLLLVTTELRTAAPDKLWLSGAYHREMLAVHFTWRNLPEEVRAVLPAIEEA
LAPFDARPHWGKLNLLTAERIAEVVPRRLADARDLFEELDPAGTFSNAHLERIGVRLPR

SEQ ID NO: 43 - AO#51-9823

MRDAAAANWAGNVRFGAARVVAPESVGELQEIVAGSRKARALGTGHSFSRIADTDGTLIAT
ARLPRRIQIDDGSVTVSGGIRYGD LARELAPNGWALRNLGSLPHISVAGACATGTHGSGDRN
GSLATSVALELVTASGELVSVRRGDEDFDGHVIALGALGVTVAVTLDLVPGFQVRQLVYE
GLTRDTLLESVQEIFAASYSVSVFTGWDPESSQLWLKQRVDGPGDDGEPPAERFGARLATRP
LHPVPGIDPTHHTTQQLGVPGPWHERELPHFRLDFTPSAGDELQTEYFVAREHAAAAIEALFAIG
AVVRPALQISEIRTVAADALWLSPAYRRDVMALHFTWISAEGTVMPAVA AVERALAPFDPV
PHWGKVFALPPAAVRAGYPRAAEFLALAARRDPEAVFRNQYLDAYLPAA

SEQ ID NO: 44 - AO#57-0794

MTQRNWAGNVSYSSSRVAEPASVDDLTA LVESEPRVRPLGSRHCFNDIADTPGVHVS LARLR
GEEPRLTAPGTLRTPAWLRYGDLVPVLR EAGAALANLASLPHISVAGAVQTGTHGSGDRIGT
LATQVSALELVTGTGEVRLRLERGEPDFDGAVVGLGALGVLTHVELDVSPARDVAQH VYEGV

RLDDVLADLGAVTGAGDSVSMFTHWQDPAVVSQVWVKSGGDVDDAAIRDAGGRPADGPR
HPIAGIDPTPCTPQLGEPGPWYDRLPHFRLEFTPSVGEELQSEYLVD RDDA VDAIRAVQDLAP
RIAPLLFVCEIRTMASDGLWLSPAQGRDTVGLHFTWRPDESAVRQLLPEIERALPASARPHW
GKVFTLPGHDVAARYPRWADFVALRRRLDPERRFANAYLERLGL

SEQ ID NO: 45 - AO#76-BAA19135

MTPAEKNWAGNITFGAKRLCVPRSVRELRETVAASGAVRPLGTRHSFNTVADTSGDHVSLA
GLPRVVDIDVPGRAVSLSAGLRFGEFAAELHARGLALANLGS LPHISVAGAVATGTHGSGVG
NRS LAGAVRALSLVTADGETRTLRR TDEDFAGAVVSLGALGVVTSLELDLVP AFEVRQWVY
EDLPEATLAARFDEVMSAAYSVS VFTDWRPGPVGQVWLKQRVGDEGARSVM PAEWLGAR
LADGPRHPVPGMPAGNCTAQQGVPGP WHERLPHFRMEFTPSNGDELQSEYFVARADAVAA
YEALARLRDRIAPVLQVSELRTVAADDLWLSPA HGRDSVAFHFTWVPDAAAVAPVAGAIEE
ALAPFGARPHWGKVFSTAPEVLRTL YPRYADFEELVGRHDPEGTFRNAFLDRYFRR

SEQ ID NO: 46 - AO#251-F3MC79

MGDKLNWAGNYRYSMELEPKSLEEVKDLVVSRTSIRVLG SCHSFNGIADTGGSHLSLRK
MNRVIDLDRVQRTVTVEGGIRYGDLCRYLNDHGYALHN LASLPHISVAGAVATATHGSGDL
NASLASSVRAIELMKSDGEVTVLTRGTDPEFDGAVVGLGGLGVVTKLKL DLVPSFQVSQTVY
DRLPFSALDHGIDEILSSAYSVSLFTDWA EPIFNQVWVKRKVGINGEDETSPDFFGALPAPEKR
HMLVGLQSVVNCSEQMGDPGPWYERLPHFRMEFTPSAGNELQSEYFVPRRHAVEAMRALGK
LRDRIAPLLFISEIRTIASDTFWMSPCYRQDSVGLHFTWKPDWERVRQLLPLIERELEPFAARP
HWAKLFTMESEMIQARYERLADFRQLLLRYDPIGKFRNTFLDHYIMH

SEQ ID NO: 47 - AO#13-0573-DNA

ATGGATCGTCGTGAACTGCTGAAAACCTCTGCACTGCTGATGGCAGCAGCACCGTTAGCA
CGTGCAGCAAATGTTCCGGAAGATCATGCAAATGTTCCGCGTACCAATTGGAGCAAAAA
CTTCCACTATAGCACCAGCCGCGTTTATGCACCGACTACCCCGGAAGAAGTTCCGGCAAT
TGTTCTGGAAAATGGTCATCTGAAAGGTCTGGGTTCTCGTCACTGCTTCAACAACATCGC
CGATAGCCAGTATGCGCAGATCAGCATGCGCGAAGTTAAAGGCATTCAGATCGATGAAG
CCGCACAAACCGTTACCGTGGGTGCAGGTATTGCGTATGGTGAATTAGCACCGGTGCTGG
ATAAAGCGGGTTTTGCACTGGCAAATTTAGCAAGTTTACCGCATATCAGCGTGGGTGGCA
CCATTGCAACCGCAACACATGGCTCTGGCGTTGGTAACAAAAACCTGTCTTCTGCAACCC
GTGCAATTGAAATCGTGAAAGCGGATGGCAGCATTCTGCGTCTGTGCGGTGATACTGATG
GTGAACGTTTTCTGATGGCGGTGGTTCATCTGGGTGCATTAGGTGTTTTAACCAAAGTTA
CCCTGGATATCGTGCCGCGCTTCGATATGTCTCAGGTGGTGTATCGCAACCTGTCCTTTGA
TCAGCTGGAACACAACCTGGATACCATTCTGAGCTCTGGCTATAGCGTTAGCCTGTTTAC
CGACTGGCAGCGTAATCGTGTTAATCAGGTGTGGATCAAAGATAAAGCGACCGCGGATG
CACCGCAAAAACCGTTACCTCCGATGTTTTATGGTGCGACCCTGCAAACCGCAAAACTGC
ATCCGATCGATGATCATCCGGCAGATGCATGTACCGAACAAATGGGTAGTGTTGGTCCGT
GGTATTTACGTCTGCCGCATTTCAA AATGGAGTTTACCCCGAGCAGCGGTGAAGAATTAC

AGACCGAATACTTCGTGGCGCGCAAAGATGGCTATCGCGCAATTCGTGCCGTGGAAAAA
CTGCGCGATAAAATTACCCCGCACCTGTTTATCACCGAAATCCGCACCATTGCAGCAGAT
GATCTGCCGATGAGCATGGCATATCAACGTGACAGTATGGCGATTTCATTTTACCTGGAAA
CCGGAAGAACCGACCGTGCGTAAATTACTGCCGGAATCGAAGCAGCACTGGCGCCGTT
TGGTGTTCGTCCGCATTGGGGCAAATTTTGAATTCGCGCGAGCTATCTGCATAAACA
GTATCCGGCACTGCCGCGTTTTTCGCGCAATGGCACAGGCATTAGATCCTGGTGGCAAATT
TCGTAATGCATATCTGGATCGTAACATCTTTGGCGCGTAG

SEQ ID NO: 48 - AO#22-8001-DNA

ATGGACAAACGCGATTTCTGAAAGGTAGCGCAACCACCGCAGTTGCACTGATGATGGG
TCTGAATGAAAGCAAAGCGTTTTCGGGATGATAGCGTTCCGCGTACCAATTGGAGCGGCA
ACTACCATTATAGCACCAACAAAGTGCTGCAGCCGGCAAGTGTTGCAGAAACCCAAGAT
GCAGTTCGTAGTGTTGCAGGTGTTTCGTGCATTAGGTACTCGTCATAGCTTTAACGGCATC
GCGGATAGCCAGATTGCCAGATTAGTACCCTGAAACTGAAAGATGTGAGCCTGGATGC
GAAAAGCTCGACCGTGACCGTTGGTGCAGGTATTCGTTATGGTGATCTGGCGGTTTCAGCT
GGATGCGAAAGGTTTTGCTCTGCATAATCTGGCAAGTCTGCCGCATATTTCTGTTGGTGG
TGCATGTGCAACTGCGACCCATGGTTCAGGTATGGGTAATGGTAATTTAGCAACCGCAGT
TAAAGCGGTGGAATTTGTTGCGGCGGATGGTAGCGTGCATACCCTGTCTCGTGATCGTGA
TGGTGATCGTTTTTTCGGGGCTCTGTTGTTGGTCTGGGTGCATTAGGTGTTGTTACCCATTTA
ACCCTGCAAGTTCAGCCACGTTTCGAAATGACCCAGGTGGTGTACCGTGATCTGCCATTT
AGTGAACCTGGAACATCATCTGCCGGAATTTATGGGTGCCGGTTATAGCGTGTCCCTGTTT
ACCGATTGGCAGAATGGTCGTGCAGGTGAAGTGTGGATCAAACGTCGCGTGGATCAAGG
TGGTGCAAGTGCTCCTCCAGCTCGTTTTTTTAATGCAACCTTAGCAACCACCAAAGTGA
CCCGATCCTGGATCATCCTGCTGAAGCATGTACCGATCAGTTAAATACCGTAGGTCCGTG
GTATGAACGTTTACCGCACTTCAAAGTGAAGTTCACCCCGAGCAGTGGCCAAGAATTACA
GACCGAGTTTTTTCGTGCCGTTTCGATCGCGGCTATGACGCCATTCGTGCCGTTGAAACTTT
ACGTGATGTGATTACCCCGCACCTGTATATCACCGAACTGCGTGCAGTTGCAGCTGATGA
TTTATGGATGAGCATGGCATATCAACGTCCGAGTCTGGCAATCCATTTTACCTGGAAACC
GGAAACCGATGCAGTGCTGAAATTAAGTCCGCGAGATTGAAGCGAAACTGGCCCCGTTTG
GTGCTCGTCCGCATTGGGCAAAGTTTTTACCATGAAAAGCAGCCATGTGGCACCGCTGT
ATCCGCGCCTGAAAGATTTTCTGGTTCTGGCAAATCCTTTGATCCGAAAGGCAAATTCC
AAAACGCGTTTCTGCAGGACCATGTGGACATCGCATAG

SEQ ID NO: 49 - AO#28-9635-DNA

ATGACCGCATCTGTGACCAATTGGGCGGGTAACATCAGCTTTGTGGCGAAAGATGTTGTT
CGTCCGGGTGGTGTGTAAGCACTGCGTAAAGTTGTTGCGGGTAATGATCGTGTTTCGTGTT
CTGGGTTCTGGTCATAGCTTTAACCGTATCGCTGAACCGGGTGCTGATGGTGTCTGGTT
AGCCTGGATGCATTACCGCAAGTGATTGATGTTGATACCGAACGTCGTACCGTGCGTGTT
GGTGGTGGTGTTAAATACGCGGAAGTGGCTCGTCATGTGAATGAATCTGGTCTGGCACTG

CCGAATATGGCATCTCTGCCGCATATTTCTGTTGCAGGTTCTGTTGCAACTGGTACCCATG
GTTCTGGTGTGAATAATGGCCCGTTAGCAACCCCGGTTTCGTGAAGTTGAATTATTAACCG
CGGATGGCTCTCTGGTGACCATCGGTAAAGATGATGCGCGTTTTCCGGGTGCAGTTACTT
CTCTGGGTGCGCTGGGTGTTGTTGTTGCACTGACCTTAGATTTAGAACCGGCGTATGGTG
TTGAACAGTATACCTTTACCGAATTACCGCTGGAAGGTCTGGACTTCGAAGCAGTTGCGA
GTGCAGCATATTCTGTTAGCCTGTTACCGGATTGGCGTGAAGCTGGTTTTTCGCCAAGTTTG
GGTGAAACGCCGCATTGATGAACCGTACGCGGGCTTTCCGTGGGCAGCACCGGCAACTG
AAAAATTACATCCGGTTCCGGGTATGCCAGCAGAAAATTGTACTGATCAATTTGGTGAG
CAGGTCCATGGCATGAACGTTTACCGCATTTTAAAGCGGAATTTACCCCGTCTAGCGGTG
ATGAATTACAGAGCGAATATCTGCTGCCGCGTGAACATGCACTGGCGGCACTGGATGCA
GTGGGCAACGTGCGTGAAACCGTTTCTACCGTGCTGCAGATTTGCGAAGTTCGTACCATT
GCAGCAGATACCCAGTGGTTAAGTCCGGCTTATGGTCGTGATAGTGTTGCATTACATTTT
ACTTGGACCGATGATATGGATGCAGTTTTACCTGCAGTTCGTGCCGTTGAAAGCGCGCTG
GATGGCTTTGGTGCTCGCCCGCATTGGGGTAAAGTGTTTACCACCGCACCGGCAGCATT
CGTGAACGTTATCCGCGTCTGGATGATTTTCGTACCCTGCGTGATGAATTAGATCCGGCA
GGCAAATTTACTAATGCATTTGTTTCGTGATGTTCTGGAAGGTTAG

SEQ ID NO: 50 - AO#36-7049-DNA

ATGACCCTGGAACGTAATTGGGCAGGTACCCATACCTTTGCAGCACCGCGTATTGTTAAT
GCAACCAGCATCGATGAAGTTCGTGCGTTAGTGGCAGAAGCAGCACGTACCGGTACCCG
TGTTTCGTGCATTAGGTACTCGTCATTCTTTTACCGATCTGGCAGATAGCGATGGTACCCTG
ATTACCGTGCTGGATATTCCGGCAGATCCAGTTTTTCGATGAAGCAGCAGGTAGCGTTACC
ATTGGTGCAGGTACCCGTTATGGTATTGCAGCAGCATGGTTAGCAGAACATGGTCTGGCG
TTTCAACATGGGTAGCCTGCCGCATATTAGCGTTGGTGGTGCAATTGCAACCGGTACC
CATGGTAGTGGTAATGATAACGGCATTCTGAGTAGCGCAGTTAGTGGTCTGGAATATGTT
GATGCGACCGGTGAACTGGTTCATGTGCGTCGTGGTGATCCTGGTTTTTGATGGTCTGGTT
GTTGGTTTAGGCGCGTATGGTATTGTGGTTCGTGTGACGGTGGATGTTCAACCGGCATAT
CGTGTTTCGCCAGGATGTGTATCGTGATGTTCCGTGGGATGCAGTTCTGGCAGATTTTGAA
GGTGTTACAGGTGGTGCGTATAGCGTTAGCATCTTTACCAACTGGCTGGGTGATACGGTG
GAACAGATTTGGTGGAACCCCGTCTGGTTGCAGGTGATGATGAACTGCCGGTGGTTCC
GGAAAGCTGGCTGGGTGTTCAACGTGATTCTTTAACCGCAGGTAATCTGGTTGAAACCGA
TCCGGATAATTTAACCCCTGCAAGGTGGTGTTCGGGGTGATTGGTGGAACGTTTACCGCA
TTTTCGTCTGGAAAGTACCCCGTCTAATGGTGATGAAATCCAGACCGAATACTTCATCGA
TCGCGCGGATGGTCCGGCGGCAATTACCGCACTGCGTGCAATTAGGTGATCGTATTGCTCC
GTTACTGTTAGTTACCGAATTACGTACCGCAGCTCCAGATAAACTGTGGCTGAGTGGCGC
ATATCATCGCGAAATGTTAGCGGTCCATTTTACCTGGCGTAATTTACCGGAAGAAGTGCG
TGCAGTTTTACCAGCGATCGAAGAAGCCCTGGCGCCGTTTGATGCTCGTCCGCATTGGGG
TAAACTGAATCTGTTAACCGCAGAACGTATTGCAGAAGTTGTTCCGCGTCTGGCTGATGC

ACGTGATCTGTTTGAAGAACTGGACCCGGCTGGTACCTTTTCTAATGCTCATCTGGAACG
TATTGGTGTTCGTTTACCGCGTTAG

SEQ ID NO: 51 - AO#51-9823-DNA

ATGCGTGATGCAGCAGCAGCAAATTGGGCAGGTAATGTGCGTTTTGGTGCAGCACGTGTT
GTTGCACCGGAAAGTGTTGGTGAAGTGCAGGAAATTGTTGCAGGTAGCCGTAAAGCACG
TGATTAGGTACCGGTCATAGCTTTAGCCGTATTGCAGATACCGATGGTACCCTGATTGC
TACCGCACGTTTACCACGTCGTATTAGATCGATGATGGCAGCGTTACCGTTTCTGGTGG
TATCCGTTATGGCGATCTGGCCCGTGAATTAGCACCGAATGGTTGGGCATTACGTAATCT
GGGTTCTTTACCGCACATTTAGTTGCAGGTGCATGTGCAACCGGTACCCATGGTTCAGG
TGATCGTAATGGTAGTCTGGCAACCTCTGTTGCAGCGTTAGAATTAGTTACCGCGTCTGG
TGAATTAGTGAGCGTTCGTCTGGCGATGAAGATTTTCGATGGCCATGTGATTGCGCTGGG
TGCACTGGGTGTTACTGTTGCAGTTACCCTGGATTTAGTTCCGGGTTTTAGGTTTCGTCAG
CTGGTGTATGAAGGTCTGACCCGTGATACCTTACTGGAAAGTGTGCAGGAAATCTTTGCT
GCGAGCTATAGTGTTAGCGTGTTTACCGGTTGGGACCCGGAAAGTTCTCAACTGTGGCTG
AAACAGCGCGTTGATGGTCCGGGCGATGATGGTGAACCACCGGCAGAACGTTTTGGTGC
ACGTTTAGCAACTCGTCCGTTACATCCAGTTCCGGGTATTGATCCGACTCATACTACTCA
ACAATTAGGTGTTCCAGGTCCGTGGCATGAACGTTTACCGCATTTTCGTCTGGATTTTACC
CCTTCTGCAGGTGATGAAGTGCAGAACCGAATACTTCGTGGCCCGCGAACATGCAGCGGC
GGCGATTGAAGCACTGTTTGCATTGGTGCGGTTGTTTCGTCCGGCATTACAAATTAGCGA
AATTCGTACCGTTGCAGCTGATGCATTATGGCTGTCTCCGGCATATCGTCGTGATGTTATG
GCGTTACATTTTACCTGGATTAGCGCAGAAGGTACCGTTATGCCAGCAGTTGCAGCAGTG
GAACGTGCACTGGCGCCGTTTGATCCGGTTCCTCATTGGGGTAAAGTTTTTGCGCTGCCG
CCAGCAGCAGTTCGTGCTGGTTATCCTCGTGCAGCAGAATTTTATGATTAGCAGCTCGT
CGTGATCCGGAAGCAGTTTTTCGTAATCAGTATTTAGATGCATATTTACCGGCAGCATAG

SEQ ID NO: 52 - AO#57-0794-DNA

ATGACCCAGCGTAATTGGGCGGGTAATGTGAGCTATAGTAGCAGCCGTGTTGCAGAACCC
AGCAAGTGTGGATGATTTAACCGCACTGGTTGAAAGTGAACCGCGTGTTTCGTCCGTTAGG
TAGTCGTCATTGCTTCAACGATATCGCCGATACCCCAGGTGTTTCATGTTTCTCTGGCACGT
CTGCGTGGTGAAGAACCGCGTTTAAACAGCACCGGGTACCTTACGTAATCCAGCTTGGTTA
CGTTATGGTGATTTAGTTCCGGTTCGTGCGTGAAGCAGGTGCAGCATTAGCAAATTTAGCA
TCTCTGCCGCATATTAGCGTTGCAGGTGCAGTTCAAACCGGTACCCATGGTTCAGGTGAT
CGTATTGGCACTCTGGCAACCCAAGTTAGCGCCCTGGAATTAGTGACCGGCACCGGTGA
AGTTTTACGCTTAGAACGTGGTGAACCTGATTTTATGATGGTGCGGTTGTTGGTTTAGGTGC
GTTAGGTGTTCTGACTCATGTGGAATTAGATGTTAGTCCGGCGCGTGATGTTGCACAGCA
CGTGTATGAAGGTGTTTCGTCTGGATGATGTTCTGGCGGATTTAGGCGCGGTTACTGGCGC
AGGTGATTCGGTGAGCATGTTTACCCATTGGCAAGATCCGGCAGTTGTTAGTCAGGTTTG
GGTTAAAAGTGGCGGTGATGTGGATGATGCAGCAATTCGTGATGCAGGTGGTTCGTCCGG

CAGATGGTCCGCGTCATCCAATTGCAGGTATTGATCCGACTCCATGTACTCCACAATTAG
GTGAACCAGGTCCGTGGTATGATCGTCTGCCGCATTTTCGTCTGGAATTTACCCCGAGTG
TTGGTGAAGAACTGCAAAGTGAATATCTGGTTGATCGCGATGATGCCGTTGATGCAATTC
GTGCGGTGCAGGATTTAGCCCCGCGTATTGCGCCGCTGCTGTTTGTTCGCGAAATTCGTA
CCATGGCAAGTGATGGTTTATGGCTGAGCCCGGCACAAGGTCGTGATACCGTTGGTCTGC
ATTTTACCTGGCGTCCTGATGAATCTGCAGTTCGTCAATTATTACCGGAAATTGAACGTG
CTTTACCGGCAAGTGCTCGTCCGCATTGGGGTAAAGTGTTTACCCTGCCGGGCCATGATG
TTGCAGCACGTTATCCGCGTTGGGCAGATTTTGTTCATTACGTCGTCGTTTAGATCCGGA
ACGTCGTTTCGCGAATGCATACCTGGAACGTTTAGGTCTGTAG

SEQ ID NO: 53 - AO#76-BAA19135-DNA

ATGACTCCGGCGGAAAAAAATTGGGCGGGCAACATCACCTTTGGTGCAAAACGTCTGTG
TGTTCCGCGTTCTGTTCTGTAAGTGCCTGAAACCGTTGCAGCATCTGGTGCAGTTCGTCC
GTTAGGTACTCGTCATAGCTTTAATACCGTTGCAGATACCAAGTGGTGATCATGTTAGTCT
GGCAGGTTTACCGCGTGTTGTGGACATCGATGTTCCGGGTCGTGCAGTTTCTCTGTCTGCT
GGTCTGCGTTTTTGGTGAATTTGCGGCTGAATTACATGCACGTGGTCTGGCGCTGGCAAAT
TTAGGTTCTCTGCCGCATATTAGCGTTGCAGGTGCAGTTGCAACCGGTACTCATGGTTCT
GGTGTGGTAATCGTTCTTTAGCAGGTGCAGTTCGTGCTTTATCTCTGGTAACCGCCGATG
GTGAAACCCGTACCTTACGTCGTACCGATGAAGATTTTGCAGGTGCAGTGGTTTCTCTGG
GTGCACTGGGTGTTGTTACTTCTCTGGAAGTGGATTTAGTTCCGGCGTTTGAAGTGCGTC
AGTGGGTGTACGAAGATCTGCCGGAAGCAACTTTAGCAGCTCGTTTTGATGAAGTTATGT
CAGCAGCGTATAGCGTGTCCTGTTTACCGATTGGCGTCCGGGTCCTGTTGGTCAAGTTT
GGCTGAAACAACGTGTTGGTGATGAAGGTGCTCGTAGTGTTATGCCAGCAGAATGGTTA
GGTGCACGTTTAGCAGATGGTCCGCGTCATCCAGTTCCAGGTATGCCTGCAGGTAATTGT
ACAGCACAACAAGGTGTTCCAGGTCCGTGGCATGAACGTTTACCGCATTTTCGCATGGAA
TTTACCCCGTCTAACGGCGATGAACTGCAAAGCGAATATTTTGTGGCGCGTGAGATGCA
GTTGCAGCGTATGAAGCATTAGCACGTCTGCGTGATCGTATTGCGCCGGTTCTGCAAGTT
AGCGAATTACGTACCGTTGCAGCAGATGATCTGTGGCTGAGTCCGGCACATGGTCGTGAT
AGTGTTGCGTTTTCATTTTACCTGGGTTCCGGATGCAGCAGCAGTTGCACCGGTTGCAGGT
GCTATTGAAGAAGCATTAGCACCGTTTGGTGCACGTCCACATTGGGGTAAAGTTTTTAGC
ACCGCACCGGAAGTTTTACGTACCTTATATCCGCGTTATGCCGATTTTGAAGAACTGGTG
GGCCGCCATGATCCGGAAGGCACCTTTTGTAAATGCATTTTATAGATCGCTACTTTTCGTCGCT
AG

SEQ ID NO: 54 - AO#251-F3MC79-DNA

ATGGGCGATAAACTGAATTGGGCGGGCAACTATCGTTATCGCAGCATGGAAGTCTGGA
ACCGAAAAGCCTGGAAGAAGTGAAAGATCTGGTGGTTAGCCGTACCAGCATTCGTGTTC
TGGGTAGCTGTCATAGCTTTAACGGCATTGCGGATACCGGTGGTAGTCATCTGAGTCTGC
GCAAAATGAACCGCGTGATTGATCTGGATCGTGTTACGCGTACCGTTACCGTTGAAGGTG

GTATTCGTTACGGTGATCTGTGCCGCTATCTGAACGATCATGGTTATGCCCTGCATAATCT
GGCAAGCTTACCGCACATCAGCGTTGCAGGTGCAGTTGCAACCGCAACCCATGGTTCTGG
TGATCTGAATGCAAGTCTGGCAAGCTCTGTTCTGTGCAATTGAACTGATGAAAAGCGATGG
CGAAGTTACGGTTCTGACCCGTGGTACCGATCCGGAATTTGATGGTGCAGTTGTTGGTCT
GGGTGGTTTAGGTGTTGTGACCAAACCTGAACTGGATCTGGTTCCGAGCTTTCAGGTGTC
GCAGACCGTGTATGATCGTCTGCCGTTTAGCGCACTGGATCATGGCATCGATGAAATTCT
GAGTAGTGCATATAGCGTTAGCCTGTTACCGATTGGGCGGAACCGATCTTTAATCAGGT
GTGGGTGAAACGCAAAGTGGGCATTAACGGCGAAGATGAAACAGTCCGGATTTTTTTTG
GCGCATTACCGGCACCGGAAAAACGCCACATGGTTCTGGGTCAGAGCGTGGTGAATTGC
AGCGAACAAATGGGTGATCCTGGTCCGTGGTATGAACGTTTACCGCATTTTCGCATGGAA
TTTACCCCGAGTGCAGGCAATGAATTACAGAGCGAATATTTTGTGCCGCGTCGTCATGCG
GTTGAAGCAATGCGTGCGTTAGGTAAACTGCGTGATCGTATTGCACCACTGCTGTTTCATC
AGCGAAATCCGCACCATTGCGAGCGATACCTTCTGGATGAGCCCGTGTTATCGTCAGGAT
TCTGTTGGTCTGCATTTTACCTGGAAACCGGATTGGGAACGTGTTTCGTCAGTTATTACCGC
TGATTGAACGTGAACTGGAACCGTTTGCGGCACGTCCGCATTGGGCGAAACTGTTTACCA
TGGAAGCGAAATGATTCAGGCGCGCTATGAACGTCTGGCGGATTTTCGTCAGCTGCTGC
TGCGTTATGATCCGATTGGCAAATTCCGTAACACCTTTCTGGATCACTACATCATGCACT
AA

SEQ ID NO: 55 pSGI-431 Q72LK2-Protein

MEATLPVLDAKTAALKRRSIRRYRKDPVPEGLLREILEAALRAPSAWNLQPWRIVVVRDPAT
KRALREAAFGQAHVEEAPVVLVLYADLEDALAHLEDEVIHPGVQGERREAQKQAIQRAFAA
MGQEARKAWASGQSYILLGYLLLLLEAYGLGSVPMLGFDPERVKAILGLPSHAAIPALVALG
YPAEEGYPSHRLPLERVVLWR

SEQ ID NO: 56 pSGI-431 Q72LK2-DNA

ATGGAAGCAACCTTACCGGTGTTAGACGCGAAAACCGCAGCACTGAAACGTCGTAGCAT
TCGCCGTTATCGCAAAGATCCAGTTCCGGAAGGTTTACTGCGCGAAATTCTGGAAGCAGC
ATTACGTGCACCGTCTGCATGGAATTTACAACCGTGCGCTATTGTGGTGGTTTCGTGATCC
GGCAACTAAACGTGCATTACGTGAAGCAGCATTTGGTCAAGCCCATGTGGAAGAAGCAC
CGGTTGTTCTGGTTCTGTACGCAGATCTGGAAGATGCACTGGCACATCTGGATGAAGTGA
TTCATCCGGGCGTTCAAGGTGAACGTCTGTAAGCGCAGAAACAAGCAATTCAGCGTGCA
TTTGCAGCAATGGGTCAGGAAGCTCGTAAAGCTTGGGCAAGCGGTCAAAGTTATATTCTG
CTGGGTTATCTGCTGCTGCTGCTGGAAGCATATGGTCTGGGTTCTGTTCCGATGCTGGGTT
TTGATCCTGAACGTGTTAAAGCGATTCTGGGCCTGCCGTCACATGCAGCGATTCCGGCAT
TAGTTGCACTGGGTTATCCGGCTGAAGAAGGTTATCCGAGTCATCGTTTACCGCTGGAAC
GTGTTGTTTTATGGCGTTGA

SEQ ID NO: 57: pSGI-374 #9041 Protein

MKNPFSLQGRKALVTGANTGLGQAIHAVGLAAAGAEVVCAARRAPDETLEMIASDGGKASA
LSIDFADPLAAKDSFAGAGFDILVNNAGIIRRADSVEFSELDWDEVMDVNLKALFFTTQAFAK
ELLAKGRSGKVVNIAASLLSFQGGIRVPSYTAACHGVAGLTKLLANEWAAGKINVNIAIPGYI
ETNNTALRADAARNKAILERIPAGRWGRSEDIAGAAVFLSSAAADYVHGAILNVDGGWLA
R

SEQ ID NO: 58 pSGI-375 #8939 Protein

MIAGVGGEARELALDLSDPMAAKDVFAGAYDLLINNAGIIRRADAVDFSEDDWDAVMDV
NLKAVFFTSQAFARALMSRNASGKIVNIAASLLSFQGGIRVASYTAACHGVAGITRLLANEWA
SRGINVNIAIPGYIATNNTALRADEERNAAILARIPAGRWGRAEDIAGTAVYLCSPAADYV
HGAILNVDGGWLAR

SEQ ID NO: 59 pSGI-376 P37769-Protein

MILSAFSLEGKVAVVTGCDTGLGQGMALGLAQAGCDIVGINIVEPTETIEQVTALGRRFLSLT
ADLRKIDGIPALLDRAVAEFGHIDILVNNAGLIRREDALEFSEKDWDVMDVNLKSVFFMSQA
AAKHFIQAQNGGKIINIASMLSFQGGIRVPSYTASKSGVMGVTRLMANEWAKHNINVNIAIP
GYMATNNTQQLRADEQRS AEILDRIPAGRWGLPSDLMGPVFLASSASDYVNGYTIADVGG
WLAR

SEQ ID NO: 60 pSGI-395 #5112 Protein

MPGMTTPFDLHGKTAIVTGANTGIGQAIASLAQAGADIAAVGRTPAQDTVQVRALGRRA
DIISADLSTIEPVQRVLDITLEKL GALDILVNNAGIIRRADSVDFTTEEDWDAVIDTNLKTTFFLC
QAAGRHMLAQGAGKIINIASLLSFQGGIRVPSYTASKSGVAGLTKLLANEWAAGKVNINIAIA
PGYIATNNTAALQADETRNRQIERIPAGRWGDPADIGGA VFLASSAADYIHGHTLAVDGG
WLAR

SEQ ID NO: 61 pSGI-396 #7103-Protein

MNPFSLEGKTALVTGANTGIGQAIAMALGRAGADVICA GRSSCAETVALIAGSKGKARELV
DFADPMAARDVFAAEPVDILVNNAGIIRRADAVDFTEADWDEVMDVNLKAVFFTCQAFGK
AVLGRGGNGKIVNIAASLLSFQGGIRVPSYTASKHGVAGITKLLANEWAAGKINVNIAIPGYIE
TNNTALRADPVRNKAILERIPAGRWGQASDIGEAAVFLASPAANYIHGAVLNVDGGWLAR

SEQ ID NO: 62 pSGI-374 #9041 DNA

ATGAAGAATCCCTTTTCGCTTCAGGGGCGTAAGGCGCTCGTCACCGGCGCGAATACGGGGCTTGGC
CAGGCGATTGCGGTTGGGCTCGCCGCGGCCGGTGCGGAGGTGGTCTGCGCCGCCCGCCGCGCGCC
GGATGAAACGCTGGAGATGATCGCCAGCGACGGCGGCAAGGCCAGCGCATTGTCCATCGATTTTG
CCGATCCGCTGGCGGCGAAGGACAGTTTTGCCGGCGCCGGTTTCGATATTCTCGTCAACAATGCCG
GTATCATCCGCCGTGCCGATTCCGTCGAGTTCTCCGAAC TCGACTGGGACGAGGTGATGGACGTCA
ATCTCAAGGCGCTGTTTTTACCACCCAGGCTTTTGCGAAAGAGCTGCTGGCGAAAGGCCGGTCCG
GCAAGGTGGTCAATATCGCTTCGCTCCTTTCTTTTCAGGGCGGTATTTCGCGTGCCGTCCTATACGGC
GGCGAAACATGGTGTCGCCGGCCTAACCAAACCTCTGGCGAATGAATGGGCCGCCAAGGGCATCA

ATGTGAATGCCATTGCGCCCGGTTATATCGAAACCAACAATACCGAGGCGCTACGCGCCGATGCG
GCTCGTAACAAGGCCATTCTCGAGCGCATCCCGGCCGGCCGCTGGGGGCGCTCGGAAGACATCGC
CGGGGCGGCGGTTTTCTGTTCATCTGCGGCGGCGGACTATGTGCATGGCGCCATTCTCAACGTCGA
TGGCGGCTGGCTGGCGCGCTGA

SEQ ID NO: 63 pSGI-375 #8939 DNA

ATGATCGCCGGCGTGGGGGGAGAAGCAAGGGAGCTGGCGCTCGATCTGTCCGATCCCATGGCGGC
AAAAGATGTTTTTGCTGAAGGCGCTTACGACCTCCTCATCAACAATGCCGGCATCATCCGCCGTGC
CGATGCAGTCGATTTCTCCGAGGATGACTGGGACGCGGTGATGGACGTGAACCTGAAAGCCGTCT
TCTTCACCTCGCAAGCCTTTGCGCGGGCTCTCATGTCCAGAAACGCAAGCGGAAAGATCGTTAACA
TTGCATCCCTTCTGTCTTTCAAGGCGGCATTGCGGTTGCCTCCTACACGGCCGCCAAGCACGGTGT
GGCAGGCATCACCAGACTGTTGGCAAACGAATGGGCGTCCCGCGGCATCAACGTCAATGCGATAG
CGCCCGGTTACATTGCCACGAACAACACGGAAGCGCTTCGAGCCGACGAGGAGCGCAACGCGGCG
ATCCTCGCACGCATTCCGGCTGGCCGCTGGGGGCGGGCGGAGGATATTGCGGGTACTGCTGTCTAT
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SEQ ID NO: 64 pSSI-376 P37769-DNA

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SEQ ID NO: 65 pSGI-395 #5112 DNA

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GATTGGGACGCGGTGATCGACACCAATCTCAAGACCACCTTCTTCTCTGTGACGGCCGCCGGTCGC
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SEQ ID NO: 66 pSGI-396 #7103-DNA

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GGAGGCTGGCTTGCCCGCTGA

SEQ ID NO: 67 pSGI-353 P0AES2

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IGKYLKGSLAYAEDPCGAEQGFSGREVMAEFRRATGLPTATNMIATDWRQMGHTLSLQSVD
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SEQ ID NO: 68 pSGI-244 #8114

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LGQHLEVPVAALLGEGQQRDEVEMLGYLFFIGDRNRTDLGYRDESNSDDAWFRVRNEEAM
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SVDIPLADPHFWTMAGSVRVAQMCADFGLTWGSHSNNHFDISLAMFTHVAAAAPGRVTAID
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SEQ ID NO: 69 pSGI-353 P0AES2-DNA

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SEQ ID NO: 70 pSGI-244 #8114

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SEQ ID NO: 71 pSGI-383 P50199

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VGQAVARHMIPRGRGKIVNICS VQSELARPGIAPYTATKGAVKNLTKGMATDWGRHGLQIN
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SEQ ID NO: 72 pSGI-383 P50199-DNA

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CLAIMS

What is claimed is:

1. A method for producing a product of an enzymatic or chemical pathway from a starting substrate, the pathway comprising one or more conversion steps selected from the group consisting of:
 - an enzymatic conversion of guluronic acid into D-glucarate (Step 7);
 - an enzymatic conversion of 5-ketogluconate (5-KGA) into L-Iduronic acid (Step 15);
 - an enzymatic conversion of L-Iduronic acid into Idaric acid (Step 7b); and
 - an enzymatic conversion of 5-ketogluconate into 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16);
 - an enzymatic conversion of 1,5-gluconolactone to gulurono-lactone (Step 19).
2. The method of claim 1 wherein the one or more conversion steps is the enzymatic conversion of guluronic acid into D-glucarate (Step 7).
3. The method of claim 1 wherein the one or more conversion steps is the enzymatic conversion of 5-ketogluconate (5-KGA) into L-Iduronic acid (Step 15).
4. The method of claim 1 wherein the one or more conversion steps is the enzymatic conversion of L-Iduronic acid into Idaric acid (Step 7b).
5. The method of claim 1 wherein the one or more conversion steps is the enzymatic conversion of 5-ketogluconate into 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16).
6. The method of claim 1 wherein the one or more conversion steps is the enzymatic conversion of 1,5-gluconolactone to gulurono-lactone (Step 19).
7. The method of claim 1 wherein the product of the enzymatic pathway is 5-dehydro-4-deoxy-glucarate (DDG).
8. The method of claim 1 wherein the substrate is glucose and the product is 5-dehydro-4-deoxy-glucarate (DDG), comprising the steps of:
 - the enzymatic conversion of D-glucose to 1,5-gluconolactone (Step 1);
 - the enzymatic conversion of 1,5-gluconolactone to gulurono-lactone (Step 19);
 - the enzymatic conversion of gulurono-lactone to guluronic acid (Step 1B);
 - the enzymatic conversion of guluronic acid to D-glucarate (Step 7);
 - the enzymatic conversion of D-glucarate to 5-dehydro-4-deoxy-glucarate (DDG) (Step 8).
9. The method of claim 1 wherein the substrate is glucose and the product is DDG, comprising the steps of:

the conversion of D-glucose to 1,5-gluconolactone (Step 1);
the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14);
the conversion of 5-ketogluconate (5-KGA) to L-Iduronic acid (Step 15);
the conversion of L-Iduronic acid to Idaric acid (Step 7b); and
the conversion of Idaric acid to DDG (Step 8a).

10. The method of claim 1 wherein the substrate is glucose and the product is DDG, comprising the steps of:

the conversion of D-glucose to 1,5-gluconolactone (Step 1);
the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14);
the conversion of 5- ketogluconate (5-KGA) to 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16);
the conversion of 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 4); and

the conversion of 4-deoxy-5-threo-hexosulose uronate (DTHU) to DDG (Step 5).

11. The method of claim 1 wherein the substrate is glucose and the product is DDG, comprising the steps of:

the conversion of D-glucose to 1,5-gluconolactone (Step 1);
the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14);
the conversion of 5-ketogluconate (5-KGA) to L-Iduronic acid (Step 15);
the conversion of L-Iduronic acid to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 7c); and
the conversion of 4-deoxy-5-threo-hexosulose uronate (DTHU) to DDG (Step 5).

12. The method of claim 8 further comprising the step of converting the DDG to 2,5-furan-dicarboxylic acid (FDCA).

13. The method of claim 9 further comprising the step of converting the DDG to 2,5-furan-dicarboxylic acid (FDCA).

14. The method of claim 10 further comprising the step of converting the DDG to 2,5-furan-dicarboxylic acid (FDCA).

15. The method of claim 11 further comprising the step of converting the DDG to 2,5-furan-dicarboxylic acid (FDCA).

16. The method of claim 12 wherein converting the DDG to FDCA comprises contacting DDG with acid to convert the DDG to FDCA.
17. The method of claim 13 wherein converting the DDG to FDCA comprises contacting DDG with acid to convert the DDG to FDCA.
18. The method of claim 14 wherein converting the DDG to FDCA comprises contacting DDG with acid to convert the DDG to FDCA.
19. The method of claim 15 wherein converting the DDG to FDCA comprises contacting DDG with acid to convert the DDG to FDCA.
20. A method for synthesizing a derivative of FDCA comprising:
 - contacting DDG with an alcohol, an inorganic acid at a temperature in excess of 60 C to form FDCA.
21. The method of claim 20 wherein the alcohol is butanol or ethanol.
22. The method of claim 20 having a yield of at least 25% molar.
23. A method of synthesizing a derivative of DDG comprising:
 - contacting DDG with an alcohol, an inorganic acid, and optionally a co-solvent to produce a derivative of DDG.
24. The method of claim 23 wherein:
 - a) the alcohol is ethanol or butanol;
 - b) the inorganic acid is sulfuric acid; and
 - c) the co-solvent is selected from the group consisting of: THF, acetone, acetonitrile, an ether, ethyl acetate, butyl acetate, an dioxane, chloroform, methylene chloride, 1,2-dichloroethane, a hexane, a heptane, toluene, carbon tetrachloride, petroleum ether, and a xylene.
25. A method for synthesizing derivative of FDCA comprising:
 - contacting a derivative of DDG with an inorganic acid to produce a derivative of FDCA.
26. The method of claim 25 having a yield of greater than 25% molar.
27. The method of claim 26 wherein the derivative is DDG is selected from the group consisting: methyl-DDG, ethyl-DDG, butyl-DDG, di-methyl DDG, di-ethyl-DDG, and di-butyl DDG.
28. The method of claim 25 further comprising that the derivative of FDCA is de-esterified to yield FDCA.
29. The method of claim 25 further comprising a step of polymerizing the derivative of FDCA.

30. A method for synthesizing FDCA comprising:
contacting DDG with an inorganic acid in a gas phase.
31. A method for synthesizing FDCA comprising:
contacting DDG with an inorganic acid at a temperature in excess of 120 C.
32. A method for synthesizing FDCA comprising:
contacting DDG with an inorganic acid under anhydrous reaction conditions.
33. A method for producing a product of an enzymatic or chemical pathway from a starting substrate, the pathway comprising one or more conversion steps selected from the group consisting of:
the conversion of DTHU to DDG (Step-5);
the conversion of gluconic acid to guluronic acid (Step-6);
the conversion of DEHU to DDH (Step 7A).
the conversion of guluronic acid to DEHU (Step 17A);
34. The method of claim 33 wherein the substrate is glucose and the product is DDG, comprising the steps of:
the conversion of D-glucose to 1,5-gluconolactone (Step 1);
the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
the conversion of gluconic acid to 3-dehydro-gluconic (DHG) (Step-2)
the conversion of 3-dehydro-gluconic (DHG) to 4,6-Dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step-3)
the conversion of 2,5 DDH to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 4)
the conversion of DTHU to DDG (Step-5).
35. The method of claim 33 wherein the substrate is glucose and the product is DDG, comprising the steps of:
the conversion of D-glucose to 1,5-gluconolactone (Step 1);
the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
the conversion of gluconic acid to guluronic acid (Step-6)
the conversion of guluronic to glucarate (Step-7)
the conversion of glucarate to DDG (Step-8)
36. The method of claim 33 wherein the substrate is glucose and the product is DDG, comprising the steps of:
the conversion of D-glucose to 1,5-gluconolactone (Step 1);
the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14);

- the conversion of 5- ketogluconate (5-KGA) to 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) (Step 16);
- the conversion of 4,6-dihydroxy 2,5-diketo hexanoate (2,5-DDH) to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 4); and
- the conversion of 4-deoxy-5-threo-hexosulose uronate (DTHU) to DDG (Step 5).
37. The method of claim 33 wherein the substrate is glucose and the product is DDG, comprising the steps of:
- the conversion of D-glucose to 1,5-gluconolactone (Step 1);
 - the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
 - the conversion of gluconic acid to 5-ketogluconate (5-KGA) (Step 14);
 - the conversion of 5-ketogluconate (5-KGA) to L-Iduronic acid (Step 15);
 - the conversion of L-Iduronic acid to 4-deoxy-5-threo-hexosulose uronate (DTHU) (Step 7B);
 - the conversion of 4-deoxy-5-threo-hexosulose uronate (DTHU) to DDG (Step 5).
38. The method of claim 33 wherein the substrate is glucose and the product is DDH, comprising the steps of:
- the conversion of D-glucose to 1,5-gluconolactone (Step 1);
 - the conversion of 1,5-gluconolactone to guluronic acid lactone (Step 19);
 - the conversion of guluronic acid lactone to guluronic acid (Step 1B);
 - the conversion of guluronic acid to DEHU (Step 17A);
 - the conversion of DEHU to DDH (Step 7A).
39. The method of claim 33 wherein the substrate is glucose and the product is DDH, comprising the steps of:
- the conversion of D-glucose to 1,5-gluconolactone (Step 1);
 - the conversion of 1,5-gluconolactone to gluconic acid (Step 1a);
 - the conversion of gluconic acid to guluronic acid (Step 6);
 - the conversion of guluronic acid to DEHU (Step 17A);
 - the conversion of DEHU to DDH (Step 7A).
40. The method of claim 33 wherein the one or more conversion steps is the conversion of DTHU to DDG (Step-5).
41. The method of claim 33 wherein the one or more conversion steps is the conversion of gluconic acid to guluronic acid (Step-6).

42. The method of claim 33 wherein the one or more conversion steps is the conversion of DEHU to DDH (Step 7A).
43. The method of claim 33 wherein the one or more conversion steps is the conversion of guluronic acid to DEHU (Step 17A).