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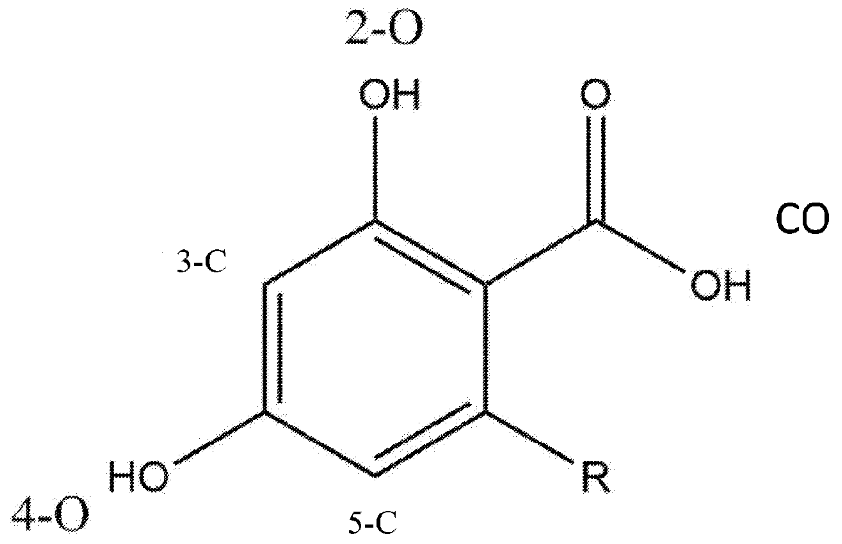
(54) Titre : COMPOSITIONS ET PROCEDES D'UTILISATION D'ENZYMES GENETIQUEMENT MODIFIEES
(54) Title: COMPOSITIONS AND METHODS FOR USING GENETICALLY MODIFIED ENZYMES

FIG. 49: Alkylresorcyclic Acid (i.e. ORA, DVA, OA, etc) prenylation site numbering



(57) Abrégé/Abstract:
The disclosure relates to the biosynthesis of cannabinoids and related prenylated phenolic compounds using recombinant enzymes. In particular, the disclosure provides recombinant prenyltransferase enzymes engineered to produce a greater amount of a desired product, or to have a greater ability to catalyze a reaction using a desired substrate, as compared to the wild type prenyltransferase. The disclosure also provides methods of preparing such recombinant enzymes; as well as methods of use thereof in improving the biosynthesis of cannabinoids and related prenylated phenolic compounds.

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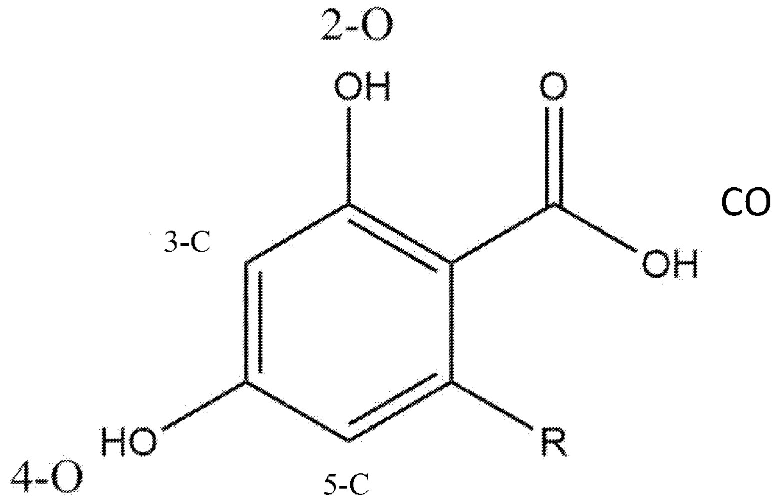
(54) Title: COMPOSITIONS AND METHODS FOR USING GENETICALLY MODIFIED ENZYMES

FIG. 49: Alkylresorcyclic Acid (i.e. ORA, DVA, OA, etc) prenylation site numbering



(57) **Abstract:** The disclosure relates to the biosynthesis of cannabinoids and related prenylated phenolic compounds using recombinant enzymes. In particular, the disclosure provides recombinant prenyltransferase enzymes engineered to produce a greater amount of a desired product, or to have a greater ability to catalyze a reaction using a desired substrate, as compared to the wild type prenyltransferase. The disclosure also provides methods of preparing such recombinant enzymes; as well as methods of use thereof in improving the biosynthesis of cannabinoids and related prenylated phenolic compounds.

[Continued on next page]

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COMPOSITIONS AND METHODS FOR USING GENETICALLY MODIFIED ENZYMES

CROSS-REFERENCE

[001] This application claims the benefit of U.S. Provisional Application No. 62/833,449, filed April 12, 2019, which application is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[002] The present disclosure is generally related to the biosynthesis of organic compounds, such as cannabinoids, using recombinant enzymes, such as recombinant aromatic prenyltransferases.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[003] The contents of the text file named “REBI_002_00US_SeqList_ST25.txt”, which was created on April 12, 2019 and is 1.19 megabytes in size, are hereby incorporated by reference in its entirety.

BACKGROUND

[004] Cannabinoids include a group of more than 100 chemical compounds mainly found in the plant *Cannabis sativa L.* Due to the unique interaction of cannabinoids with the human endocannabinoid system, many of these compounds are potential therapeutic agents for the treatment of several medical conditions. For instance, the psychoactive compound Δ^9-tetrahydrocannabinol (Δ^9-THC) has been used in the treatment of pain and other medical conditions. Several synthetic *Cannabis*-based preparations have been used in the USA, Canada and other countries as an authorized treatment for nausea and vomiting in cancer chemotherapy, appetite loss in acquired immune deficiency syndrome and symptomatic relief of neuropathic pain in multiple sclerosis.

[005] Cannabinoids are terpenophenolic compounds, produced from fatty acids and isoprenoid precursors as part of the secondary metabolism of *Cannabis*. The main cannabinoids produced by *Cannabis* are Δ^9-tetrahydrocannabidiol (THC), cannabidiol (CBD) and cannabinol (CBN), followed by cannabigerol (CBG), cannabichromene (CBC) and other minor constituents. Currently, Δ^9-THC and CBD are either extracted from the plant or chemically synthesized. However, agricultural production of cannabinoids faces challenges such as plant susceptibility to climate and diseases, low content of less-abundant cannabinoids, and need for extraction of cannabinoids by chemical processing. Furthermore, chemical synthesis of cannabinoids has failed to be a cost-effective alternative mainly because of complex synthesis leading to high production cost and low yields.

[006] Therefore, there is a pressing need for biotechnology-based synthetic biology approaches which can enable the synthesis of high-quality cannabinoids in a cost-effective and environmentally friendly manner. Further, there is also a need for the synthesis of a diverse group of chemical compounds including not limited to cannabinoids using similar synthetic biology approaches.

SUMMARY

[007] The disclosure provides recombinant polypeptides comprising an amino acid sequence with at least 80% identity to the amino acid sequence of a prenyltransferase, wherein the recombinant polypeptide comprises at least one amino acid substitution compared to the amino acid sequence of the prenyltransferase, wherein said recombinant polypeptide converts a substrate and a prenyl donor to at least one prenylated product, and wherein the recombinant polypeptide produces a ratio of an amount of the at least one prenylated product to an amount of total prenylated products that is higher than the prenyltransferase under the same condition.

[008] In some aspects, the recombinant polypeptide comprises an amino acid sequence with at least 95% identity to the amino acid sequence of the prenyltransferase. In some aspects, the amino acid sequence has at least 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of the prenyltransferase. In some aspects, the at least one amino acid substitution comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions to the amino acid sequence of the prenyltransferase.

[009] In some aspects, the prenyltransferase is selected from the group consisting of ORF2, HypSc, PB002, PB005, PB064, PB065, and Atapt (interchangeably referred to herein as “PBJ”). In some aspects, the prenyl donor is selected from Dimethylallyl diphosphate (DMAPP), geranyl diphosphate (GPP), farnesyl diphosphate (FPP), geranylgeranyl pyrophosphate (GGPP), or any combination thereof. In some aspects, the prenyl donor is not a naturally occurring donor of the prenyltransferase. In some aspects, the substrate is selected from olivetolic acid (OA), divarinolic acid (DVA), olivetol (O), divarinol (DV), orsellinic acid (ORA), dihydroxybenzoic acid (DHBA), apigenin, naringenin and resveratrol. In some aspects, the substrate is not a naturally occurring substrate of the prenyltransferase.

[010] In some aspects, the at least one prenylated product comprises a prenyl group attached to any position on an aromatic ring of the substrate. In some aspects, the at least one prenylated product is selected from the group consisting of UNK1, UNK2, UNK3, RBI-08, 5-DOA, RBI-05, RBI-06, 4-O-GOA, RBI-02 (CBGA - cannabigerolic acid), RBI-04 (5-GOA), UNK4, RBI-56, UNK5, RBI-14 (CBFA), RBI-16 (5-FOA), RBI-24, RBI-28, RBI-26 (CBGVA - cannabigerovarinic acid), RBI-27, RBI-38, RBI-39, RBI-09, RBI-10, RBI-03 (5-GO), RBI-20, RBI-01 (CBG - cannabigerol), RBI-15, RBI-34, RBI-32, RBI-33, RBI-07, RBI-29, RBI-30, RBI-12, and RBI-11.

[011] In some aspects, the prenyltransferase is ORF2. In some aspects, the substrate is OA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; 5-C; or 5-C and 3-C on the aromatic ring of OA. In some aspects, the at least one prenylated product comprises UNK1, UNK2, UNK3, RBI-08, RBI-17, or RBI-18.

[012] In some aspects, the substrate is OA and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; 5-C; or 3-C and 5-C on the aromatic ring of OA. In some aspects, the at least one prenylated product comprises RBI-05, RBI-06, UNK-4, RBI-02 (CBGA), RBI-04 (5-GOA) or RBI-07.

[013] In some aspects, the substrate is OA and the prenyl donor is FPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 2-O; 4-O; 3-C; and 5-C on the aromatic ring of OA. In some aspects, the at least one prenylated product comprises RBI-56, UNK5, RBI-14 (CBFA), or RBI-16 (5-FOA).

[014] In some aspects, the substrate is DVA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; and 5-C on the aromatic ring of DVA.

[015] In some aspects, the substrate is DVA and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; 5-C; 3-C and 5-C; or 5-C and 2-O on the aromatic ring of DVA. In some aspects, the at least one prenylated product comprises RBI-24, RBI-28, UNK11, RBI-26, RBI-27, RBI-29, or RBI-30.

[016] In some aspects, the substrate is DVA and the prenyl donor is FPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; and 5-C on the aromatic ring of DVA. In some aspects, the at least one prenylated product comprises UNK12, UNK13, UNK14, RBI-38, or RBI-39.

[017] In some aspects, the substrate is O and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C/5-C; 2-O/4-O; or 3-C on the aromatic ring of O. In some aspects, the at least one prenylated product comprises RBI-10, UNK16, or RBI-09.

[018] In some aspects, the prenyltransferase is HypSc. In some aspects, the substrate is O and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C/5-C; 2-O/4-O; or 3-C on the aromatic ring of O. In some aspects, the at least one prenylated product comprises RBI-10, UNK16 or RBI-09.

[019] In some aspects, the prenyltransferase is PB005. In some aspects, the substrate is O and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C/5-C; 2-O/4-O; 3-C; 1-C and 5-C; or 1-C and 3-C on the aromatic ring of O. In some aspects, the at least one prenylated product comprises RBI-10, UNK16, RBI-09, RBI-11 or RBI-12.

[020] In some aspects, the substrate is O and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C/5-C; 2-O/4-O; or 3-C on the aromatic ring of O. In some aspects, the at least one prenylated product comprises RBI-20, RBI-01 (CBG), or RBI-03 (5-GO).

[021] In some aspects, the substrate is O and the prenyl donor is FPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C/5-C; 2-O/4-O; 4-O/2-O; or 3-C on the aromatic ring of O. In some aspects, the at least one prenylated product comprises RBI-15, UNK18 or UNK19.

[022] In some aspects, the substrate is DV and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C/5-C; 2-O/4-O; or 3-C on the aromatic ring of DV. In some aspects, the at least one prenylated product comprises UNK54, UNK55 or UNK56.

[023] In some aspects, the substrate is ORA and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, 4-O, 3-C, 5-C, or 5-C and 3-C on the aromatic ring of ORA.

[024] In some aspects, the substrate is ORA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, or 5-C on the aromatic ring of ORA.

[025] In some aspects, the substrate is ORA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, or 4-O on the aromatic ring of ORA.

[026] In some aspects, the substrate is ORA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, or 3-C on the aromatic ring of ORA.

[027] In some aspects, the prenyltransferase is PB064. In some aspects, the substrate is ORA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O or 3-C on the aromatic ring of ORA.

[028] In some aspects, the prenyltransferase is PB065. In some aspects, the substrate is ORA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, or 2-O on the aromatic ring of ORA.

[029] In some aspects, the prenyltransferase is PB002. In some aspects, the substrate is ORA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position CO on the aromatic ring of ORA.

[030] In some aspects, the prenyltransferase is Atapt. In some aspects, the substrate is ORA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position 4-O on the aromatic ring of ORA.

[031] In some aspects, the substrate is ORA and the prenyl donor is FPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, 4-O, 3-C, or 5-C on the aromatic ring of ORA.

[032] In some aspects, the substrate is DHBA and the prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, 4-O, 3-C, or 5-C on the aromatic ring of DHBA.

[033] In some aspects, the substrate is DV and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to positions 5-C and 1-C; or 3-C and 5-C on the aromatic ring of DV. In some aspects, the at least one prenylated product comprises RBI-36, or UNK35.

[034] In some aspects, the substrate is OA and the prenyl donor is GPP, DMAPP or both. In some aspects, the at least one prenylated product comprises a prenyl group attached to positions 5-C and 3-C; or CO and 3-C on the aromatic ring of OA.

[035] In some aspects, the substrate is OA and the prenyl donor is GPP, FPP or both. In some aspects, the at least one prenylated product comprises a prenyl group attached to positions 5-C and 3-C on the aromatic ring of OA.

[036] In some aspects, the substrate is O and the prenyl donor is GPP, FPP or both. In some aspects, the at least one prenylated product comprises a prenyl group attached to positions 5-C and 3-C on the aromatic ring of O.

[037] In some aspects, the substrate is apigenin and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from C-13; C-15; C-3; C-12; C-16; C-9; or C-5 on the aromatic ring of apigenin. In some aspects, the at least one prenylated product comprises UNK47, UNK48, UNK49, UNK50, or UNK51. In some aspects, the substrate is naringenin and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from C-3; or C-5 on the aromatic ring of naringenin. In some aspects, the at least one prenylated product comprises RBI-41 or RBI-42. In some aspects, the substrate is resveratrol and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from C-11; C-13; C-3; C-10; C-14; or C-1/5 on the aromatic ring of resveratrol. In some aspects, the at least one prenylated product comprises RBI-48 or RBI-49.

[038] In some aspects, the substrate comprises olivetolic acid (OA), divarinolic acid (DVA), olivetol (O), resveratrol, piceattanol and related stilbenes, naringenin, apigenin and related flavanones and flavones, respectively, Isoliquiritigenin, 2'-O-methylisoliquiritigenin and related chalcones, catechins and epi-catechins of all possible stereoisomers, biphenyl compounds such as 3,5-dihydroxy-biphenyl, benzophenones such as phlorobenzophenone, isoflavones such as biochanin A, genistein, daidzein, 2,4-dihydroxybenzoic acid, 1,3-benzenediol, 2,4-dihydroxy-6-methylbenzoic acid; 1,3-Dihydroxy-5-methylbenzene; 2,4-Dihydroxy-6-aethyl-benzoesaeure; 5-ethylbenzene-1,3-diol 2,4-dihydroxy-6-propylbenzoic acid; 5-propylbenzene-1,3-diol; 2-butyl-4,6-dihydroxybenzoic acid; 5-butylbenzene-1,3-diol; 2,4-dihydroxy-6-pentyl-benzoic acid; 5-pentylbenzene-1,3-diol; 5-hexylbenzene-1,3-diol; 2-heptyl-4,6-dihydroxy-benzoic acid; 5-heptylbenzene-1,3-diol; 5-Dodecylbenzene-1,3-diol; 5-nonadecylbenzene-1,3-diol; 1,3-Benzenediol; 3,4',5-Trihydroxystilbene; 4'5-Tetrahydroxystilbene; 1,2-Diphenylethylene; 2-Phenylbenzopyran-4-one; 2-Phenylchroman-4-one; 1,3-benzenediol; 5,7,4'-Trihydroxyflavone; (E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one; 4,4'-dihydroxy-2'-methoxychalcone; 1,3-Diphenylpropenone; (2R,3S)-2-(3,4-Dihydroxyphenyl)chroman-3,5,7-triol; (2R,3R)-2-(3,4-

Dihydroxyphenyl)-3,5,7-chromanetriol; Phenylbenzene; 5-Phenylresorcinol; diphenylmethanone; 3-phenyl-4H-chromen-4-one; 5,7-Dihydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one; 4',5,7-Trihydroxyisoflavone; 4',7-Dihydroxyisoflavone; 4-Hydroxy-6-methyl-2H-pyran-2-one; 1,6-DHN; or any combination thereof.

[039] In some aspects, the substrate is a prenylated molecule. In some aspects, the prenylated molecule is selected from the group consisting of UNK1, UNK2, UNK3, RBI-08, 5-DOA, RBI-05, RBI-06, 4-O-GOA, RBI-02 (CBGA), RBI-04 (5-GOA), UNK4, RBI-56, UNK5, RBI-14 (CBFA), RBI-16 (5-FOA), RBI-24, RBI-28, RBI-26, RBI-27, RBI-38, RBI-39, RBI-09, RBI-10, RBI-03 (5-GO), RBI-20, RBI-01 (CBG), RBI-15, RBI-34, RBI-32, RBI-33, RBI-07, RBI-29, RBI-30, RBI-12, and RBI-11.

[040] In some aspects, the amino acid sequence of ORF2 comprises SEQ ID NO: 1, and the at least one amino acid substitution comprises at least one amino acid substitution in SEQ ID NO: 1 on a position chosen from the group consisting of amino acid positions 17, 25, 38, 49, 53, 106, 108, 112, 118, 119, 121, 123, 161, 162, 166, 173, 174, 177, 205, 209, 213, 214, 216, 219, 227, 228, 230, 232, 271, 274, 283, 286, 288, 294, 295, and 298. In some aspects, the at least one amino acid substitution is located on a position chosen from the group consisting of amino acid positions 17, 25, 38, 49, 53, 106, 108, 112, 118, 119, 162, 166, 173, 174, 205, 209, 213, 219, 227, 228, 230, 232, 271, 274, 283, 286, 288, and 298. In some aspects, the amino acid sequence of ORF2 comprises SEQ ID NO: 1, and the at least one amino acid substitution is chosen from the group consisting of A17T, C25V, Q38G, V49A, V49L, V49S, A53C, A53D, A53E, A53F, A53G, A53H, A53I, A53K, A53L, A53M, A53N, A53P, A53Q, A53R, A53S, A53T, A53V, A53W, A53Y, M106E, A108G, E112D, E112G, K118N, K118Q, K119A, K119D, Y121W, F123A, F123H, F123W, Q161A, Q161C, Q161D, Q161E, Q161F, Q161G, Q161H, Q161I, Q161K, Q161L, Q161M, Q161N, Q161P, Q161R, Q161S, Q161T, Q161V, Q161W, Q161Y, M162A, M162F, D166E, N173D, L174V, S177E, S177W, S177Y, G205L, G205M, C209G, F213M, S214A, S214C, S214D, S214E, S214F, S214G, S214H, S214I, S214K, S214L, S214M, S214N, S214P, S214Q, S214R, S214T, S214V, S214W, S214Y, Y216A, L219F, D227E, R228E, R228Q, C230N, C230S, A232S, V271E, L274V, Y283L, G286E, Y288A, Y288C, Y288D, Y288E, Y288F, Y288G, Y288H, Y288I, Y288K, Y288L, Y288M, Y288N, Y288P, Y288Q, Y288R, Y288S, Y288T, Y288V, Y288W, V294A, V294F, V294N, Q295A, Q295C, Q295D, Q295E, Q295F, Q295G, Q295H, Q295I, Q295K, Q295L, Q295M, Q295N, Q295P, Q295R, Q295S, Q295T, Q295V, Q295W, Q295Y, L298A, L298Q, and L298W.

[041] In some aspects, the amino acid sequence of ORF2 comprises SEQ ID NO: 1, and the at least one amino acid substitution to SEQ ID NO: 1 comprises two or more amino acid substitutions to SEQ ID NO: 1 selected from the group consisting of:

(a) A17T, C25V, Q38G, V49A, V49L, V49S, A53C, A53D, A53E, A53F, A53G, A53H, A53I, A53K, A53L, A53M, A53N, A53P, A53Q, A53R, A53S, A53T, A53V, A53W, A53Y, M106E, A108G, E112D, E112G, K118N, K118Q, K119A, K119D, Y121W, F123A, F123H, F123W, Q161A, Q161C, Q161D, Q161E, Q161F, Q161G, Q161H, Q161I, Q161K, Q161L, Q161M, Q161N, Q161P, Q161R, Q161S, Q161T, Q161V, Q161W, Q161Y, M162A, M162F, D166E, N173D, L174V, S177E, S177W, S177Y, G205L, G205M, C209G, F213M, S214A, S214C, S214D, S214E, S214F, S214G, S214H, S214I, S214K, S214L, S214M, S214N, S214P, S214Q, S214R, S214T, S214V, S214W, S214Y, Y216A, L219F, D227E, R228E, R228Q, C230N, C230S, A232S, V271E, L274V, Y283L, G286E, Y288A, Y288C, Y288D, Y288E, Y288F, Y288G, Y288H, Y288I, Y288K, Y288L, Y288M, Y288N, Y288P, Y288Q, Y288R, Y288S, Y288T, Y288V, Y288W, V294A, V294F, V294N, Q295A, Q295C, Q295D, Q295E, Q295F, Q295G, Q295H, Q295I, Q295K, Q295L, Q295M, Q295N, Q295P, Q295R, Q295S, Q295T, Q295V, Q295W, Q295Y, L298A, L298Q, and L298W;

OR

(b) A53T and S214R; S177W and Q295A; S214R and Q295F; Q161S and S214R; S177W and S214R; Q161S and Q295L; Q161S and Q295F; V49A and S214R; A53T and Q295F; Q161S and S177W; Q161S, V294A and Q295W; A53T, Q161S and Q295W; A53T and S177W; A53T, Q161S, V294A and Q295W; A53T, V294A and Q295A; V49A and Q295L; A53T, Q161S, V294N and Q295W; A53T and Q295A; Q161S, V294A and Q295A; A53T and Q295W; A53T, V294A and Q295W; A53T, Q161S and Q295A; A53T, Q161S, V294A and Q295A; and A53T, Q161S, V294N and Q295A.

[042] In some aspects, the at least one prenylated product comprises UNK6, UNK7, UNK8, UNK9, or UNK10. In some aspects, the at least one prenylated product comprises UNK20, UNK21, UNK22, UNK23, UNK24, or UNK59. In some aspects, the at least one prenylated product comprises UNK25, UNK26, or UNK29. In some aspects, the at least one prenylated product comprises UNK25, UNK26 or UNK27. In some aspects, the at least one prenylated product comprises UNK25 or UNK28. In some aspects, the at least one prenylated product comprises UNK25, UNK26 or UNK28. In some aspects, the at least one prenylated product comprises UNK25 or UNK26. In some aspects, the at least one prenylated product comprises UNK25. In some aspects, the at least one prenylated product comprises UNK27. In some aspects, the at least one prenylated product comprises UNK30, UNK31, UNK32, UNK33, or UNK34. In some aspects, the at least one prenylated product comprises UNK36, UNK38, or RBI-22. In some aspects, the at least one prenylated product comprises UNK42. In some aspects, the at least one prenylated product comprises UNK46.

[043] In some aspects, the substrate is DV and the prenyl donor is GPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, 1-C, or 5-C on the aromatic ring of DV. In some aspects, the at least one prenylated product comprises RBI-32 or RBI-33.

[044] In some aspects, the substrate is OA and the prenyl donor is GGPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, or 5-C on the aromatic ring of OA. In some aspects, the at least one prenylated product comprises UNK60 or UNK61.

[045] In some aspects, the substrate is ORA and the prenyl donor is GGPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, or 5-C on the aromatic ring of ORA. In some aspects, the at least one prenylated product comprises UNK62 or UNK63.

[046] In some aspects, the substrate is DVA and the prenyl donor is GGPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, or 5-C on the aromatic ring of DVA. In some aspects, the at least one prenylated product comprises UNK64 or UNK65.

[047] The disclosure further provides nucleic acid molecules, comprising a nucleotide sequence encoding any one of the recombinant polypeptides disclosed herein, or a codon degenerate nucleotide sequence thereof. In some aspects, the nucleotide sequence comprises at least 500, 600, 700, 800, or 900 nucleotides. In some aspects, the nucleic acid molecule is isolated and purified.

[048] The disclosure provides a cell vector, construct or expression system comprising any one of the nucleic acid molecules disclosed herein; and a cell, comprising any one of the cell vectors, constructs or expression systems disclosed herein. In some aspects, the cell is a bacteria, yeast, insect, mammalian, fungi, vascular plant, or non-vascular plant cell. In some aspects, the cell is a microalgae cell. In some aspects, the cell is an E. coli cell.

[049] The disclosure provides a plant, comprising any one of the cells disclosed herein. In some aspects, the plant is a terrestrial plant.

[050] The disclosure provides methods of producing at least one prenylated product, comprising, contacting any one of the recombinant polypeptides disclosed herein with a substrate and a prenyl donor, thereby producing at least one prenylated product. In some aspects, the recombinant polypeptide is the recombinant polypeptide of any one of claims 13, 16, 19, 22, 24, 27, 30, 34, 38, 41, 44, 47, 50, 52, 54, 56, 59, 62, 65, 68, 70, 72, 74, 77, 79, and 81.

[051] The disclosure provides methods of producing at least one prenylated product, comprising, a) contacting a first recombinant polypeptide with a substrate and a first prenyl donor, wherein the first recombinant polypeptide is any of the recombinant polypeptides disclosed herein, thereby producing a first prenylated product; and b) contacting the first prenylated product and a second prenyl donor with a second recombinant polypeptide, thereby producing a second prenylated product. In some aspects, the first recombinant polypeptide and the second recombinant polypeptide are selected from the recombinant polypeptide of any one of claims 13, 16, 19, 22, 24, 27, 30, 34, 38, 41, 44, 47, 50, 52, 54, 56, 59, 62, 65, 68, 70, 72, 74, 77, 79, and 81.

[052] In some aspects, the first recombinant polypeptide is the same as the second recombinant polypeptide. In some aspects, the first recombinant polypeptide is different from the second recombinant polypeptide. In some aspects, the first prenyl donor is the same as the second prenyl donor. In some aspects, the first prenyl donor is different from the second prenyl donor. In some aspects, the first prenylated product is the same as the second prenylated product. In some aspects, the first prenylated product is different from the second prenylated product.

[053] In some aspects, (a) the first recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is ORF2, and the second recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is PB005; or the first recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is PB005 and the second recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is ORF2; (b) the first prenyl donor is GPP and the second prenyl donor is DMAPP; or the first prenyl donor is DMAPP, and the second prenyl donor is GPP; and (c) the substrate is O. In some aspects, the first prenylated product or the second prenylated product comprises a prenyl group attached to positions of 5-C and 3-C; 5-C and 1-C; and 5-C, 1-C and 3-C on the aromatic ring of O.

[054] In some aspects, (a) the first recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is ORF2, and the second recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is PB005; or the first recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is PB005 and the second recombinant polypeptide is a recombinant polypeptide wherein the prenyltransferase is ORF2; (b) the first prenyl donor is FPP and the second prenyl donor is DMAPP; or the first prenyl donor is DMAPP, and the second prenyl donor is FPP; and (c) the substrate is O. In some aspects, the first prenylated product or the second prenylated product comprises a prenyl group attached to positions 5-C and 3-C; or 5-C and 1-C on the aromatic ring of O.

[055] In some aspects, the second recombinant polypeptide is a cyclase. In some aspects, the cyclase comprises cannabidiolic acid synthase (CBDAS) or tetrahydrocannabinolic acid synthase (THCAS). Further details on CBDAS and THCAS are provided in "Cannabidiolic - acid synthase, the chemotype - determining enzyme in the fiber - type Cannabis sativa" Taura et al., Volume 581, Issue 16, June 26, 2007, Pages 2929-2934; and "The Gene Controlling Marijuana Psychoactivity. Molecular Cloning and Heterologous Expression of Δ1-Tetrahydrocannabinolic acid synthase from Cannabis sativa L." Sirikantaramas et al. The Journal of Biological Chemistry, Vol. 279, No. 38, Issue of September 17, pp. 39767–39774, 2004, respectively, each of which is incorporated herein by reference in their entireties for all purposes.

[056] In some aspects, the cyclase is derived from a plant belonging to the *Rhododendron* genus and wherein the cyclase cyclizes an FPP moiety. In some aspects, the cyclase is Daurichromenic Acid Synthase (DCAS). Further details on DCAS is provided in "Identification and Characterization of Daurichromenic Acid Synthase Active in Anti-HIV Biosynthesis" Iijima et al. Plant Physiology Aug 2017, 174 (4) 2213-2230, the contents of which are incorporated herein by reference in its entirety.

[057] In some aspects, the secondary enzyme is a methyltransferase. In some cases, the methyltransferase is a histone methyltransferase, N-terminal methyltransferase, DNA/RNA methyltransferase, natural product methyltransferase, or non-SAM dependent methyltransferases.

[058] In some aspects, the at least one prenylated product comprises UNK40, UNK41, UNK66 or UNK67. In some aspects, the at least one prenylated product comprises UNK44 or UNK45.

[059] In some aspects, the first recombinant polypeptide is PB005, and the second recombinant polypeptide is HypSc; or the first recombinant polypeptide is HypSc, and the second recombinant polypeptide is PB005. In some aspects, the substrate is DV; and the first prenyl donor and the second prenyl donor is DMAPP. In some aspects, the at least one prenylated product comprises a prenyl group attached to positions of 5C and 3C; or 5C and 1C on the aromatic ring of DV. In some aspects, the at least one prenylated product comprises UNK57 or UNK58.

[060] The disclosure further provides compositions comprising the at least one prenylated product produced by any one of the methods disclosed herein. The disclosure also provides compositions comprising the first prenylated product and/or the second prenylated product produced by any one of the methods disclosed herein.

[061] The disclosure provides a composition comprising a prenylated product, wherein the prenylated product comprises a substitution by a prenyl donor on an aromatic ring of a substrate, wherein the substrate is selected from the group consisting of olivetolic acid (OA), divarinolic acid (DVA), olivetol (O), divarinol (DV), orsellinic acid (ORA), dihydroxybenzoic acid (DHBA), apigenin, naringenin and resveratrol.

[062] In some aspects, the prenyl donor is selected from the group consisting of DMAPP, GPP, FPP, GGPP, and any combination thereof. In some aspects, the prenylated product is selected from any of the prenylated products in Table C. In some aspects, the prenylated product is selected from the group consisting of UNK1, UNK2, UNK3, RBI-08, RBI-17, RBI-05, RBI-06, UNK4, RBI-02 (CBGA), RBI-04 (5-GOA), RBI-56, UNK5, RBI-14 (CBFA), RBI-16 (5-FOA), UNK6, UNK7, UNK8, UNK9, UNK10, RBI-24, RBI-28, UNK11, RBI-26 (CBGVA), RBI-27, UNK12, UNK13, UNK14, RBI-38, RBI-39, RBI-10, UNK16, RBI-09, RBI-10, UNK16, RBI-09, RBI-10, UNK16, RBI-09, RBI-10, RBI-03 (5-GO), RBI-20, RBI-01 (CBG), RBI-03 (5-GO), RBI-15, UNK18, UNK19, RBI-15, UNK54, UNK55, UNK56, UNK54, UNK20, UNK21, UNK22, UNK23, UNK24, UNK25, UNK26, UNK27, UNK28, UNK29, RBI-32, RBI-33, UNK30, UNK31, UNK32, UNK33, UNK34, UNK60, UNK61, UNK62, UNK63, UNK64, UNK65, RBI-07, RBI-29, RBI-30, RBI-36, UNK35, UNK36, RBI-22, UNK38, RBI-18, UNK40, UNK41, UNK42, RBI-12, RBI-11, UNK44, UNK45, UNK46, UNK57, UNK58, UNK59, UNK66, and UNK67. In some aspects, the prenylated product is selected from the group consisting of RBI-01, RBI-02, RBI-03, RBI-04, RBI-05, RBI-07, RBI-08, RBI-09, RBI-10, RBI-11, and RBI-12. In some aspects, the prenylated product is RBI-29 or UNK59.

BRIEF DESCRIPTION OF THE FIGURES

[063] FIG. 1 shows a heatmap of prenylated products produced from Orf2 mutants when using OA as substrate and DMAPP as donor.

[064] FIG. 2 shows a heatmap of prenylated products produced from Orf2 mutants when using OA as substrate and GPP as donor.

[065] FIG. 3 shows a heatmap of prenylated products produced from Orf2 mutants when using OA as substrate and FPP as donor.

[066] FIG. 4 shows a heatmap of prenylated products produced from Orf2 mutants when using O as substrate and GPP as donor.

[067] FIG. 5 shows a heatmap of prenylated products produced from Orf2 mutants when using DVA as substrate and GPP as donor

[068] FIG. 6 shows a heatmap of prenylated products produced from Orf2 mutants when using DVA as substrate and FPP as donor.

[069] FIG. 7 shows a heatmap of prenylated products produced from selected Orf2 mutants when using ORA as substrate and GPP as donor.

[070] FIG. 8 shows a heatmap of prenylated products produced from selected Orf2 mutants when using Apigenin as substrate and GPP as donor.

[071] FIG. 9 shows a heatmap of prenylated products produced from selected Orf2 mutants when using Naringenin as substrate and GPP as donor.

[072] FIG. 10 shows a heatmap of prenylated products produced from selected Orf2 mutants when using Resveratrol as substrate and GPP as donor.

[073] FIG. 11 shows a heatmap of prenylated products produced from prenyltransferase enzymes when using ORA as substrate and DMAPP as donor.

[074] FIG. 12 shows a heatmap of prenylated products produced from prenyltransferase enzymes when using DV as substrate and DMAPP as donor.

[075] FIG. 13 shows a heatmap of prenylated products produced from prenyltransferase enzymes when using DV as substrate and GPP as donor.

[076] FIG. 14 shows a heatmap of prenylated products produced from prenyltransferase enzymes when using DVA as substrate and DMAPP as donor.

[077] FIG. 15 shows a heatmap of prenylated products produced from prenyltransferase enzymes when using O as substrate and DMAPP as donor.

[078] FIG. 16 shows the predicted prenylation products using OA as substrate and DMAPP as Donor.

[079] FIG. 17 shows the predicted prenylation products using OA as substrate and GPP as Donor.

[080] FIG. 18 shows the predicted prenylation products using OA as substrate and FPP as Donor.

[081] FIG. 19 shows the predicted prenylation products using O as substrate and GPP as Donor.

[082] FIG. 20 shows the predicted prenylation products using DVA as substrate and GPP as Donor.

[083] FIG. 21 shows the predicted prenylation products using DVA as substrate and FPP as Donor.

[084] FIG. 22 shows the predicted prenylation products using ORA as substrate and GPP as Donor.

[085] FIG. 23 shows the predicted prenylation products using Apigenin as substrate and GPP as Donor.

[086] FIG. 24 shows the predicted prenylation products using Naringenin as substrate and GPP as Donor.

[087] FIG. 25 shows the predicted prenylation products using Reservatrol as substrate and GPP as Donor.

[088] FIG. 26 shows the predicted prenylation products using ORA as substrate and DMAPP as Donor.

[089] FIG. 27 shows the predicted prenylation products using DV as substrate and DMAPP as Donor.

[090] FIG. 28 shows the predicted prenylation products using DV as substrate and GPP as Donor.

[091] FIG. 29 shows the predicted prenylation products using DVA as substrate and DMAPP as Donor.

[092] FIG. 30 shows the predicted prenylation products using O as substrate and DMAPP as Donor.

[093] FIG. 31 shows the predicted prenylation products using CBGA as substrate and DMAPP as Donor.

[094] FIG. 32 shows the predicted prenylation products using RBI-04 as substrate and DMAPP as Donor.

[095] FIG. 33 shows the predicted prenylation products using RBI-04 as substrate and FPP as Donor.

[096] FIG. 34 shows the predicted prenylation products using RBI-04 as substrate and GPP as Donor.

[097] FIG. 35 shows the predicted prenylation products using RBI-08 as substrate and DMAPP as Donor.

[098] FIG. 36 shows the predicted prenylation products using RBI-08 as substrate and GPP as Donor.

[099] FIG. 37 shows the predicted prenylation products using RBI-09 as substrate and GPP as Donor.

[0100] FIG. 38 shows the predicted prenylation products using RBI-10 as substrate and DMAPP as Donor.

[0101] FIG. 39 shows the predicted prenylation products using RBI-10 as substrate and FPP as Donor.

[0102] FIG. 40 shows the predicted prenylation products using RBI-10 as substrate and GPP as Donor.

[0103] FIG. 41 shows the predicted prenylation products using RBI-12 as substrate and GPP as Donor.

[0104] FIG. 42 shows the predicted prenylation products using RBI-03 as substrate and DMAPP as Donor.

[0105] FIG. 43 shows the predicted prenylation products using O as substrate and FPP as Donor.

[0106] FIG. 44 shows the predicted prenylation products using ORA as substrate and FPP as Donor.

[0107] FIG. 45 shows the predicted prenylation products using OA as substrate and GGPP as Donor.

[0108] FIG. 46 shows the predicted prenylation products using ORA as substrate and GGPP as Donor.

[0109] FIG. 47 shows the predicted prenylation products using DVA as substrate and GGPP as Donor.

[0110] FIG. 48 shows the prenylation site numbering for alkylresorcinol substrates (i.e. DV, O, etc).

[0111] FIG. 49 shows the prenylation site numbering for alkylresorcyclic acid substrates (i.e. ORA, DVA, OA, etc.)

[0112] FIG. 50 shows the Apigenin prenylation site numbering.

[0113] FIG. 51 shows the Naringenin prenylation site numbering.

[0114] FIG. 52 shows the Reservatrol prenylation site numbering.

[0115] FIG. 53 shows the total nMol of prenylated products produced by ORF2 triple mutants using OA as substrate and FPP as donor.

[0116] FIG.54 shows that % CBFA produced by ORF2 triple mutants using OA as substrate and FPP as donor

[0117] FIG. 55: % enzymatic activity of ORF2 triple mutants using OA as substrate and FPP as donor

[0118] FIG. 56: CBFA production potential of ORF2 triple mutants using OA as substrate and FPP as donor

[0119] FIG. 57: Cluster map of ORF2 triple mutants clustered based on CBFA production potential and %5-FOA produced, using OA as substrate and FPP as donor

[0120] FIG. 58: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone A04

[0121] FIG. 59: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone C05

[0122] FIG. 60: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone A09

[0123] FIG. 61: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant H02

[0124] FIG. 62: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone D04

[0125] FIG. 63: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone F09

[0126] FIG. 64: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone D11

[0127] FIG. 65: Analysis of ORF-2 enzymatic function of mutan70ts derived from the breakdown of ORF-2 triple mutant clone E09

[0128] FIG. 66: Analysis of enzymatic activity of site-saturated ORF2 mutants of Q295 using OA as substrate and FPP as donor.

[0129] FIG. 66C: 5-FOA production (using OA as substrate and FPP as donor) by ORF2 mutants carrying site saturation Q295 mutations

[0130] FIG. 67: Analysis of enzymatic activity of site-saturated ORF2 mutants of Q161 using OA as substrate and FPP as donor

[0131] FIG. 67C: 5-FOA production (using OA as substrate and FPP as donor) by ORF2 mutants carrying site saturation Q161 mutations

[0132] FIG. 68: Analysis of enzymatic activity of site-saturated ORF2 mutants of S214 using OA as substrate and FPP as donor

[0133] FIG. 68C: 5-FOA production (using OA as substrate and FPP as donor) by ORF2 mutants carrying site saturation S214 mutations

[0134] FIG. 69: ORF-2 activity (using OA as substrate and FPP as donor) of S214R-Q295F Stacking variant

[0135] FIG. 70: ORF-2 activity (using OA as substrate and FPP as donor) of S177W-Q295A Stacking variant

[0136] FIG. 71: ORF-2 activity (using OA as substrate and FPP as donor) of A53T-Q295F Stacking variant

[0137] FIG. 72: ORF-2 activity (using OA as substrate and FPP as donor) of S177W-Q295A Stacking variant

[0138] FIG. 73: Total nMol of prenylated products produced by ORF2 triple mutants using OA as substrate and DMAPP as donor

[0139] FIG. 74: % 3-DOA produced by ORF2 triple mutants using OA as substrate and DMAPP as donor

[0140] FIG. 75: % enzymatic activity of ORF2 triple mutants using OA as substrate and DMAPP as donor

[0141] FIG. 76: 3-DOA production potential of ORF2 triple mutants using OA as substrate and DMAPP as donor

[0142] FIG. 77: Cluster map of ORF2 triple mutants clustered based on 3-DOA production potential and %5-DOA produced, using OA as substrate and DMAPP as donor

[0143] FIG. 78: Complete amino acid replacement at position Q161 and S214 in Orf2 allows a structure function mechanism for CBGA production and regiospecific prenylation.

[0144] FIG. 79: Complete amino acid replacement at position Q295 in Orf2 allows a structure function mechanism for CBGA production and regiospecific prenylation.

[0145] FIG. 80: Carbon and proton NMR assignments for CBGVA.

[0146] FIG. 81: Carbon and proton NMR assignments for RBI-29.

[0147] FIG. 82: Carbon and proton NMR assignments for UNK-59.

[0148] FIG. 83: Carbon and proton NMR assignments for CBG.

[0149] FIGs. 84A-K: Proton NMR signals obtained in DMSO at 600MHz for the following compounds: RBI-01 (FIG. 84A); RBI-02 (FIG. 84B); RBI-03 (FIG. 84C); RBI-04 (FIG. 84D); RBI-05 (FIG. 84E); RBI-07 (FIG. 84F); RBI-08 (FIG. 84G); RBI-09 (FIG. 84H); RBI-10 (FIG. 84I); RBI-11 (FIG. 84J); and RBI-12 (FIG. 84K).

DETAILED DESCRIPTION

Definitions

[0150] As used herein, and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a protein” can refer to one protein or to mixtures of such protein, and reference to “the method” includes reference to equivalent steps and/or processes known to those skilled in the art, and so forth.

[0151] As used herein, the term “about” or “approximately” when preceding a numerical value indicates the value plus or minus a range of 10%. For example, “about 100” encompasses 90 and 110.

[0152] The term “wild type”, abbreviated as “WT”, is a term of the art understood by skilled persons and means the typical form of an organism, strain, gene, protein, or characteristic as it occurs in nature as distinguished from mutant or variant forms. For example, a WT protein is the typical form of that protein as it occurs in nature.

[0153] The term “mutant protein” is a term of the art understood by skilled persons and refers to a protein that is distinguished from the WT form of the protein on the basis of the presence of amino acid modifications, such as, for example, amino acid substitutions, insertions and/or deletions.

[0154] Amino acid modifications may be amino acid substitutions, amino acid deletions and/or amino acid insertions. Amino acid substitutions may be conservative amino acid substitutions or non-conservative amino acid substitutions. A conservative replacement (also called a conservative mutation, a conservative substitution or a conservative variation) is an amino acid replacement in a protein that changes a given amino acid to a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size). As used herein, “conservative variations” refer to the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another; or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Other illustrative examples of conservative substitutions include the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to praline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; valine to isoleucine or leucine, and the like.

[0155] Amino acid substitution, interchangeably referred to as amino acid replacement, at a specific position on the protein sequence is denoted herein in the following manner: "one letter code of the WT amino acid residue -amino acid position- one letter code of the amino acid residue that replaces this WT residue". For example, an ORF2 polypeptide which is a Q295F mutant refers to an ORF2 polypeptide in which the wild type residue at the 295th amino acid position (Q or glutamine) is replaced with F or phenylalanine. Some mutants have more than one amino acid substitutions, for example, mutant L174V_S177E refers to an ORF2 polypeptide in which the wild type residue at the 174th amino acid position (L or leucine) is replaced with V or valine; and the wild type residue at the 177th amino acid position (S or serine) is replaced with E or glutamic acid.

[0156] The modified peptides can be chemically synthesized, or the isolated gene can be site-directed mutagenized, or a synthetic gene can be synthesized and expressed in bacteria, yeast, baculovirus, tissue culture, and the like.

[0157] As used herein, "total prenylated products" produced refers to the sum of nMols of the various prenylated products produced by an enzyme in a set period of time. For instance, when OA is used as a substrate and GPP is used as a donor, then the "total prenylated products" refers to a sum of the nMol of CBGA and the nMol of 5-GOA produced by the prenyltranferase enzyme ORF2 in a set period of time.

[0158] As used herein, "%prenylated product 1" within total prenylated products is calculated using the equation: nMol of prenylated product 1/ [nMol of total prenylated products]. For example, "%CBGA" is calculated using the equation: nMol of CBGA / [nMol of CBGA+5-GOA]. Also, as an example, "%5-GOA" within prenylated products is calculated using the equation: nMol of 5-GOA / [nMol of CBGA + 5-GOA].

[0159] As used herein, % enzymatic activity of an ORF2 mutant is calculated using the equation: total prenylated products produced by a mutant/ total prenylated products produced by wild-type ORF2. For example, wild-type ORF2 has 100% enzyme activity.

[0160] As used herein, the production or production potential of a prenylated product 1 is calculated using the formula: %product 1 among total prenylated products * % enzymatic activity. For example, "CBGA production potential" (used interchangeably with "CBGA production") is calculated using the equation: %CBGA among total prenylated products * % enzymatic activity. Also, as an example, "5-GOA production potential" (used interchangeably with "5-GOA production") is calculated using the equation: %5-GOA among total prenylated products * % enzymatic activity.

[0161] A "vector" is used to transfer genetic material into a target cell. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g. circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g., retroviruses, adenoviruses, lentiviruses, and adeno-associated viruses). In embodiments, a viral vector may be replication incompetent. Viral vectors also include polynucleotides carried by a virus for transfection into a host cell. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors." Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

[0162] As used herein "sequence identity" refers to the extent to which two optimally aligned polynucleotides or polypeptide sequences are invariant throughout a window of alignment of components, e.g. nucleotides or amino acids. An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in the reference sequence segment, i.e. the entire reference sequence or a smaller defined part of the reference sequence. "Percent identity" is the identity fraction times 100. Comparison of sequences to determine percent identity can be accomplished by a number of well-known methods, including for example by using mathematical algorithms, such as, for example, those in the BLAST suite of sequence analysis programs.

[0163] As used herein, the code names refer to the chemical compounds described in the specification and drawing of the present application. For example, the code name "RBI-24" refers to the chemical compound (*E*)-3,7-dimethylocta-2,6-dien-1-yl 2,4-dihydroxy-6-propylbenzoate, the chemical structure of which is shown in FIG.20. Similarly, the code name

"UNK20" refers to the chemical compound (*E*)-3,7-dimethylocta-2,6-dien-1-yl 2,4-dihydroxy-6-methylbenzoate, the chemical structure of which is shown in FIG.22.

Cannabinoid synthesis

[0164] The biosynthesis of cannabinoids often starts with the short-chain fatty acid, hexanoic acid. Initially, the fatty acid is converted to its coenzyme A (CoA) form by the activity of an acyl activating enzyme. Subsequently, olivetolic acid (OA) is biosynthesized by the action of a type III polyketide synthase (PKS), and, in some cases, a polyketide cyclase (olivetolic acid cyclase [OAC]).

[0165] A geranyl diphosphate:olivetolate geranyltransferase, named cannabigerolic acid synthase (CBGAS), is responsible for the C-alkylation by geranyl diphosphate (GPP) to CBGA. Subsequently, the monoterpene moiety of CBGA is often stereoselectively cyclized by three different enzymes cannabichromenic acid synthase (CBCAS), cannabidiolic acid synthase (CBDAS) and tetrahydrocannabinolic acid synthase (THCAS) to synthesize cannabichromenic acid (CBCA), cannabidiolic acid (CBDA) and Δ^9-THCA, respectively.

[0166] The central precursor for cannabinoid biosynthesis, CBGA, is synthesized by the aromatic prenyltransferase CBGAS by the condensation of GPP and OA. In considering the biosynthesis of cannabinoids in a heterologous system, one major challenge is that CBGAS (e.g. CsPT1 and CsPT4) is an integral membrane protein, making high titer of functional expressed protein in *E. coli* and other heterologous systems unlikely. Besides the integral membrane prenyltransferases found in plants, soluble prenyltransferases are found in fungi and bacteria. For instance, *Streptomyces* sp. strain CL190 produces a soluble prenyltransferase NphB or ORF2, which is specific for GPP as a prenyl donor and exhibits broad substrate specificity towards aromatic substrates. When expressed in E. coli, ORF2 of SEQ ID NO:2 is as a 33kDa soluble, monomeric protein having 307 residues. Further details about ORF2 and other aromatic prenyltransferases may be found in U.S. Patent No. US 7,361,483; U.S. Patent No. 7,544,498; and U.S. Patent No. 8,124,390, each of which is incorporated herein by reference in its entirety for all purposes.

[0167] ORF2 is a potential alternative to replace the native CBGAS in a biotechnological production of cannabinoids and other prenylated aromatic compounds. However, the wild type ORF2 enzyme produces a large amount of 5-geranyl olivetolate (5-GOA) and only a minor amount of CBGA, the latter of which is the desired product for cannabinoid biosynthesis.

[0168] Further, other prenyltransferase homologues of ORF2 include HypSc, PB002, PB005, PB064, PB065, and Atapt.

[0169] This disclosure provides prenyltransferase mutants, engineered by the inventors to produce produces a ratio of an amount of at least one prenylated product to an amount of total prenylated products that is higher than that produced by the WT prenyltransferase under the same conditions. The disclosure also provides prenyltransferase mutants which have been engineered to catalyze reactions using a desired substrate and/or a desired donor and to produce higher amounts of a desired product, as compared to the WT prenyltransferase under the same conditions.

[0170] The production of cannabinoids at large industrial scale is made possible using microalgae and dark fermentation. Engineering into the chloroplast of the microalgae offers unique compartmentalization and environment. The *Cannabis* plant genes express in this single cell plant system and have the post-translational modifications. This dark fermentation process allows one to drive cell densities beyond 100g/per liter and has been scaled to 10,000 L.

Prenyltransferase mutants

[0171] The disclosure provides recombinant polypeptides comprising an amino acid sequence with at least about 70% identity to the amino acid sequence of WT prenyltransferase. In some aspects, the polypeptides disclosed herein may have a sequence identity of about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 99.5% identity to the amino acid sequence of WT prenyltransferase. In some aspects, the mutant recombinant polypeptides (interchangeably used with “recombinant polypeptides”) disclosed herein may comprise a modification at one or more amino acids, as compared to the WT prenyltransferase sequence. In some aspects, the mutant recombinant polypeptides disclosed herein may comprise a modification at 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 23 amino acids, 24 amino acids, 25 amino acids, 26 amino acids, 27 amino acids, 28 amino acids, 29 amino acids, 30 amino acids, 31 amino acids, 32 amino acids, 33 amino acids, 34 amino acids, 35 amino acids, or 36 amino acids, as compared to the WT prenyltransferase sequence.

[0172] In some aspects, the prenyltransferase is selected from the group consisting of ORF2, HypSc, PB002, PB005, PB064, PB065, and Atapt. The amino acid sequence of ORF2 is set forth in SEQ ID NO: 1. The amino acid sequence of PB005 is set forth in SEQ ID NO: 602. The amino acid sequence of PBJ or Atapt is set forth in SEQ ID NO: 604.

[0173] In some aspects, the prenyltransferase belongs to the ABBA family of prenyltransferases. In some aspects, the prenyltransferase comprises a protein fold with a central barrel comprising ten anti-parallel β-strands surrounded by α-helices giving rise to a repeated α-β-β-α (or "ABBA") motif. Further details of this family and examples of prenyltransferases that may be used are provided in "The ABBA family of aromatic prenyltransferases: broadening natural product diversity" Tello et al. Cell. Mol. Life Sci. 65 (2008) 1459 – 1463, the contents of which are incorporated herein by reference in its entirety for all purposes.

[0174] In some aspects, the prenyltransferase is ORF2 comprising an amino acid sequence set forth in SEQ ID NO: 1. In some aspects, mutant recombinant polypeptides disclosed herein comprise a modification in one or more amino acid residues selected from the group consisting of the following amino acid residues, A17, C25, Q38, V49, A53, M106, A108, E112, K118, K119, Y121, F123, Q161, M162, D166, N173, L174, S177, G205, C209, F213, S214, Y216, L219, D227, R228, C230, A232, V271, L274, Y283, G286, Y288, V294, Q295, and L298 of the WT ORF2 polypeptide. For instance, the mutant ORF2 polypeptides disclosed herein may comprise an amino acid modification at 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 23 amino acids, 24 amino acids, 25 amino acids, 26 amino acids, 27 amino acids, 28 amino acids, 29 amino acids, 30 amino acids, 31 amino acids, 32 amino acids, 33 amino acids, 34 amino acids, 35 amino acids, or 36 amino acids selected from the group consisting of the following amino acid residues, A17, C25, Q38, V49, A53, M106, A108, E112, K118, K119, Y121, F123, Q161, M162, D166, N173, L174, S177, G205, C209, F213, S214, Y216, L219, D227, R228, C230, A232, V271, L274, Y283, G286, Y288, V294, Q295, and L298 of the WT ORF2 polypeptide.

[0175] In some aspects, the mutant ORF2 polypeptides disclosed herein may comprise an amino acid substitution of at least one amino acid residue selected from the group consisting of A17, C25, Q38, V49, A53, M106, A108, E112, K118, K119, Y121, F123, Q161, M162, D166, N173, L174, S177, G205, C209, F213, S214, Y216, L219, D227, R228, C230, A232, V271, L274, Y283, G286, Y288, V294, Q295, and L298. For instance, the mutant ORF2 polypeptides disclosed herein may comprise an amino acid substitution of 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, 21 amino acids, 22 amino acids, 23 amino acids, 24 amino acids, 25 amino acids, 26 amino acids, 27 amino acids, 28 amino acids, 29 amino acids, 30 amino acids, 31 amino acids, 32 amino acids, 33 amino acids, 34 amino acids, 35 amino acids, or 36 amino acids selected from the group consisting of A17, C25, Q38, V49, A53, M106, A108, E112, K118, K119, Y121, F123, Q161, M162, D166, N173, L174, S177, G205, C209, F213, S214, Y216, L219, D227, R228, C230, A232, V271, L274, Y283, G286, Y288, V294, Q295, and L298.

[0176] In some aspects, the mutant ORF2 polypeptides disclosed herein comprise an amino acid sequence comprising at least one amino acid substitution, as compared to the amino acid sequence of WT ORF2, wherein the at least one amino acid substitution does not comprise an alanine substitution on an amino acid residue selected from the group consisting of 47, 64, 110, 121, 123, 126, 161, 175, 177, 214, 216, 288, 294 and 295.

[0177] In some aspects, the mutant ORF2 polypeptides disclosed herein comprise an amino acid sequence comprising at least one amino acid substitution, as compared to the amino acid sequence of WT ORF2, wherein at least one amino acid substitution is at a position selected from the group consisting of 1-46, 48-63, 65-109, 111-120, 122, 124, 125, 127-160, 162-174, 176, 178-213, 215, 217-287, 289-293, 296-307, on WT-ORF2.

[0178] In some aspects, the mutant ORF2 polypeptides disclosed herein comprise an amino acid sequence with at least about 70% identity (for instance, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 99.5% identity, inclusive of all values and subranges therebetween) to the amino acid sequence of SEQ ID Nos 2-300. In some aspects, the mutant ORF2 polypeptides disclosed herein comprise the amino acid sequence of SEQ ID Nos 2-300. In some aspects, the mutant ORF2 polypeptides disclosed herein consist of the amino acid sequence of SEQ ID Nos 2-300.

[0179] In some aspects, the mutant recombinant polypeptides disclosed herein catalyze a reaction using at least one prenyl donor. In some aspects, the at least one prenyl donor is DMAPP, GPP, FPP, or any combination thereof.

[0180] In some aspects, the mutant recombinant polypeptide uses a donor that is not a naturally occurring donor of the WT prenyltransferase. A “naturally-occurring donor” as used herein, refers to the donor that is used by the WT prenyltransferase to catalyze a prenylation reaction in nature (such as, in the organism that the WT prenyltransferase is found in nature). For instance, a naturally occurring donor of WT ORF2 is GPP; the disclosure provides ORF2 mutants that are able to use donors other than GPP (such as FPP) in the prenylation reaction.

[0181] In some aspects, the mutant recombinant polypeptides disclosed herein catalyze a reaction using any known substrate of a prenyltransferase such as ORF2, HypSc, PB002, PB005, PB064, PB065, and Atapt. In some aspects, the substrate is selected from the group consisting of OA, DVA, O, DV, ORA, DHBA, apigenin, naringenin and resveratrol.

[0182] In some aspects, the mutant recombinant polypeptide uses a substrate that is not a naturally occurring substrate of the WT prenyltransferase. A "naturally-occurring substrate" as used herein, refers to a substrate that is used by the WT prenyltransferase to catalyze a prenylation reaction in nature (such as, in the organism that the WT prenyltransferase is found in nature). For instance, a naturally occurring substrate of WT ORF2 is 1,3,6,8-tetrahydroxynaphthalene (THN); the disclosure provides ORF2 mutants that are able to use substrates other than THN (such as OA, apigenin, etc) in the prenylation reaction. Further details are provided in "Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products" Kuzuyama et al., *Nature* volume 435, pages 983–987 (2005), the contents of which are incorporated by reference in its entirety.

[0183] In some aspects, the substrate is any natural or synthetic phenolic acids with a 1, 3-dihydroxyl motif, alternatively a resorcinol ring including but not limited to resveratrol, piceattanol and related stilbenes, naringenin, apigenin and related flavanones and flavones, respectively, Isoliquiritigenin, 2'-O-methylisoliquiritigenin and related chalcones, catechins and epi-catechins of all possible stereoisomers, biphenyl compounds such as 3,5-dihydroxy-biphenyl, benzophenones such as phlorobenzophenone, isoflavones such as biochanin A, genistein, and daidzein. For instance, the substrate may be any substrate listed in Tables A and B; and FIGs. 117-119.

[0184] Table A: Examples of ORF2 substrates which are benzoic acids and benzenediols

IUPAC Chemical Name	Common Name	Tail Chain Length	CAS#
2,4-dihydroxybenzoic acid	β-Resorcylic acid	0-carbon	89-86-1
1,3-benzenediol	resorcinol	0-carbon	108-46-3
2,4-dihydroxy-6-methylbenzoic acid	o-orsellinic Acid	1-carbon	480-64-8
1,3-Dihydroxy-5-methylbenzene	Orcinol	1-carbon	504-15-4
2,4-Dihydroxy-6-aethyl-benzoesaeure		2-carbon	4299-73-4
5-ethylbenzene-1,3-diol		2-carbon	4299-72-3

2,4-dihydroxy-6-propylbenzoic acid	Divarinic Acid	3-carbon	4707-50-0
5-propylbenzene-1,3-diol	Divarin	3-carbon	500-49-2
2-butyl-4,6-dihydroxybenzoic acid		4-carbon	173324-41-9
5-butylbenzene-1,3-diol		4-carbon	46113-76-2
2,4-dihydroxy-6-pentyl-benzoic acid;	Olivetolic Acid	5-carbon	491-72-5
5-pentylbenzene-1,3-diol	Olivetol	5-carbon	500-66-3
5-hexylbenzene-1,3-diol		6-carbon	5465-20-3
2-heptyl-4,6-dihydroxy-benzoic acid	sphaerophorolcarboxylic acid	7-carbon	6121-76-2
5-heptylbenzene-1,3-diol	Sphaerophorol	7-carbon	500-67-4
5-Dodecylbenzene-1,3-diol		12-carbon	72707-60-9
5-nonadecylbenzene-1,3-diol		19-carbon	35176-46-6

[0185] Table B: Examples of other aromatic compounds which are ORF2 substrates

IUPAC Chemical Name	**Common Name**	**CAS#**
1,3-Benzenediol	resorcinol	108-46-3
3,4',5-Trihydroxystilbene	resveratrol	89-86-1
4'5-Tetrahydroxystilbene	Piceatannol	4339-71-3
1,2-Diphenylethylene	stilbene	103-30-0
2-Phenylbenzopyran-4-one	flavone	525-82-6
2-Phenylchroman-4-one	flavanone	487-26-3
1,3-benzenediol	naringenin	108-46-3
5,7,4'-Trihydroxyflavone	apigenin	8002-66-2
(E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	Isoliquiritigenin	961-29-5
4,4'-dihydroxy-2'-methoxychalcone	2'-O-Methylisoliquiritigenin	112408-67-0
1,3-Diphenylpropenone	chalcone	94-41-7
(2R,3S)-2-(3,4-Dihydroxyphenyl)chroman-3,5,7-triol	catechin	7295-85-4
(2R,3R)-2-(3,4-Dihydroxyphenyl)-3,5,7-chromanetriol	epi-catechin	7295-85-4
Phenylbenzene	biphenyl	92-52-4
5-Phenylresorcinol	3,5-Dihydroxybiphenyl	7028-41-3
diphenylmethanone	benzophenone	119-61-9
3-phenyl-4H-chromen-4-one	isoflavone	574-12-9
5,7-Dihydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one	biochanin A	491-80-5
4',5,7-Trihydroxyisoflavone	Genistein	690224-00-1

4',7-Dihydroxyisoflavone	Diadzein	486-66-8
4-Hydroxy-6-methyl-2H-pyran-2-one	Triacetic acid lactone	675-10-5
1,6-DHN		575-44-0

[0186] In some aspects, the products of ORF2 prenylation may further serve as substrates for ORF2. Therefore, the substrate may also be any product of an ORF2 prenylation reaction.

[0187] In some aspects, the mutant recombinant polypeptides disclosed herein produce a higher amount of total nMol of prenylated products than the WT prenyltransferase. In some aspects, the mutant recombinant polypeptides disclosed herein produce an amount of total nMol of prenylated products that is about 1% to about 1000% (for example, about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, or about 900%), inclusive all the values and subranges that lie therebetween, higher than the amount of total nMol of prenylated products produced by WT prenyltransferase.

[0188] In some aspects, the mutant recombinant polypeptides disclosed herein have an enzymatic activity higher than WT prenyltransferase. In some aspects, the mutant recombinant polypeptides disclosed herein have an activity that is about 1% to about 1000% (for example, about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, or about 900%), inclusive all the values and subranges that lie therebetween, higher than the enzymatic activity of WT prenyltransferase.

Mechanism of ORF2 function

[0189] The inventors have discovered a ratcheting mechanism of Orf2 mutants at Q161 and S214. WT enzyme contains an active site Q161 and S214 which both form a weak hydrogen bond with the carboxylate of olivetolic acid, resulting in a 1:5 ratio CBGA:5GOA. Mutagenesis at position Q161 to Q161H, creating a more permanent hydrogen bond donor results in almost 100% CBGA production. Mutation to Q161P loses the hydrogen bond donor, as well as modifying the secondary structure at this position. Here the olivetolic acid flips its binding position within the active site, resulting in 97% 5GOA. Similarly S214, which sits opposite in the pocket, can be mutated to S214H, which can also hydrogen bond to olivetolic acid carboxylate and also results in almost 100% CBGA production. Mutated to S214V also flips its binding position, resulting in 90% 5GOA. See FIG. 78.

[0190] The inventors have also discovered a ratcheting mechanism of Orf2 mutants at Q295. The Q295 can interact with both the hydrocarbon tail of olivetolic acid, as well as the hydrophobic terminus of the GPP substrate. Mutation Q295 to Q295F enhances these hydrophobic interations, leading to 98% CBGA. Alternatively mutating to Q295H forms a protonated residue, which can destabilize the hydrocarbon tail, resulting in the substrate ratcheting binding orientation. The resulting hydrogen bond with the carboxylate of olivetolic acid stabilizes the flipped binding orientation, resulting in 90% 5GOA. See FIG. 79.

Polynucleotides, Vectors and Methods

[0191] The disclosure provides isolated or purified polynucleotides that encode any one of the recombinant polypeptides disclosed herein. The disclosure provides polynucleotides comprising a nucleic acid sequence with at least about 80% identity (for instance, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99%, and inclusive of all values and subranges therebetween) to the nucleic acid sequence set forth in SEQ ID NO: 301 (ORF2); SEQ ID NO: 601 (PB005) and SEQ ID NO: 603 (PBJ).

[0192] The disclosure provides a vector comprising any one of the recombinant polynucleotide sequences disclosed herein.

[0193] The disclosure further provides a host cell comprising any one of the vectors disclosed herein; any one of the polynucleotides disclosed herein; or any one of the polynucleotides encoding the recombinant polypeptides disclosed herein. Non-limiting examples of host cells include microbial host cells, such as, for example, bacteria, *E. coli*, yeast, microalgae; non-microbial hosts, such as, for example, insect cells, mammalian cell culture, plant cultures; and whole terrestrial plants. In some aspects, expression of any one of the vectors disclosed herein; any one of the polynucleotides disclosed herein; or any one of the polynucleotides encoding the recombinant polynucleotides disclosed herein may be done *ex vivo* or *in vitro*. In some aspects, expression of any one of the vectors disclosed herein; any one of the polynucleotides disclosed herein; or any one of the recombinant polynucleotides disclosed herein may be done in cell-free systems.

[0194] The disclosure provides methods of producing any one of the recombinant polynucleotides disclosed herein, comprising culturing the host cell comprising any one of the vectors disclosed herein, in a medium permitting expression of the recombinant polynucleotide, and isolating or purifying the recombinant polynucleotide from the host cell.

[0195] It is to be understood that the description above as well as the examples that follow are intended to illustrate, and not limit, the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

[0196] All patents, patent applications, references, and journal articles cited in this disclosure are expressly incorporated herein by reference in their entireties for all purposes.

EXAMPLES

Example 1: Methods for generating and studying aromatic prenyltransferase variants

[0197] A. Construction of a synthesized gene library of n=96 Orf2 variants with select amino acid substitutions and other Orf2 varaints.

[0198] DNA plasmids encoding the 96 “tripleton” variants of orf2 (orf2 variants) were ordered and delivered in the background of the T5 expression vector pD441-SR from DNA2.0 (now ATUM, catalog pD441-SR). The sequences for the 96 variants are described as SEQ ID NO: DNA_150247-DNA_150342. Each Orf2 variant contains a unique combination of three amino acid substitutions relative to the base construct (SEQ ID NO: DNA_consensus).

[0199] All variants aside from the tripleton parental variants were created using site directed mutagenesis with QuikChange II Site-Directed Mutagenesis Kit (Agilent catalog #200523). Standard manufacturer protocols were employed.

[0200] B. Construction of synthesized prenyltransferase enzymes.

[0201] DNA plasmids encoding aromatic prenyltransferase enzymes (APTs) were ordered and delivered in the background of the T5 expression vector pD441-SR from DNA2.0 (now ATUM, catalog pD441-SR).

[0202] C. Expression and purification of proteins from the synthesized Orf2 gene library of Orf2 variants and prenyltransferase enzymes.

[0203] DNA plasmids containing each of the Orf2 variants or prenyltransferase enzymes were individually transformed into OneShot BL21(DE3) chemically competent E. coli cells (Invitrogen catalog C600003) according to the chemically competent cell transformation protocol provided by Invitrogen. This resulted in 96 individual E. coli cell lines, each containing one plasmid encoding an Orf2 variant.

[0204] To induce protein expression, individual cell lines encoding each of the “orf2 variants” or “APTs” was individually inoculated into 2 milliliters LB media with 50 micrrograms per milliliter of Kanamycin sulfate in 15 milliliter culture tubes and grown at 37 degrees Celsius for 16 hours with vigorous shaking. After 16 hours, each culture was diluted into 38 milliliters LB media with 50 micrograms per milliliter of Kanamycin sulfate for a total of 40 milliliters. The absorbance at 600nm (OD600) was monitored until it reached a value of 0.6 absorbance units. When the OD600 reached a value of 0.6, then IPTG was added to each culture to a final concentration of 500 micrograms per milliliter, resulting in an "induced culture." Each "induced culture" was grown at 20 degrees Celsius with vigorous shaking for 20 hours.

[0205] After the cultures were grown under protein induction conditions, the target protein was extracted following a standard protein purification protocol. Each "induced culture" was spun at 4,000G for 5 minutes. The supernatant was discarded, leaving only a cell pellet. Each individual cell pellet was resuspended in 25 milliliters of a solution containing 20 millimolar Tris-HCL, 500 millimolar sodium chloride, 5 millimolar imidazole, and 10% glycerol ("lysis buffer"), resulting in a "cell slurry." To each individual "cell slurry", 30 microliters of 25 units per microliter Benzonase (Millipore, Benzonase, catalog number 70664-1), as well as 300 microliters of phosphatase and protease inhibitor (Thermo-Fisher, Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free, catalog number 78441) was added. Each individual "cell slurry" was then subjected to 30 second pulses of sonication, 4 times each, for a total of 120 seconds, using the Fisher Scientific Sonic Dismembrator Model 500 under 30% amplitude conditions. In between each 30 second pulse of sonication, the "cell slurry" was placed on ice for 30 seconds. After sonication, each individual "cell slurry" was centrifuged for 45 minutes at 14,000 times gravity.

[0206] Protein purification columns (Bio-Rad, Econo-Pac Chromotography Columns, catalog number 7321010) were prepared by adding 1.5 milliliters His60 resin slurry (Takara, His60 nickel superflow resin, catalog number 635660). 5 milliliters deionized water was added to resin slurry, to agitate and rinse the resin. The columns were then uncapped and the resulting flow-through was discarded. Then, 5 milliliters deionized water was added a second time, and the resulting flow-through was discarded. Then, 10 milliliters "lysis buffer" was added to the resin, completely disturbing the resin bed, and the flow-through was discarded.

[0207] The protein purification columns were capped, and the supernatant from the "cell slurry" was added to the resin bed without disturbing the resin bed. The columns were uncapped, allowing the supernatant to pass over the resin bed. The resin was then washed 2 times with 10 milliliters of a solution containing 20 millimolar Tris-HCl, 500 millimolar sodium chloride, and 20 millimolar imidazole ("wash buffer"). The flow-through from the wash steps was discarded. The protein was then eluted off the column with 10 milliliters of a solution containing 20 millimolar Tris-HCl, 200 millimolar sodium chloride, and 250 millimolar imidazole. The eluted protein was collected and dialyzed overnight in 4 liters of a solution containing 200 millimolar Tris-HCl and 800 millimolar sodium chloride in 3.5-5.0 kilodalton dialysis tubing (Spectrum Labs, Spectra/Por dialysis tubing, catalog number 133198). After overnight dialysis, protein was concentrated to approximately 10 milligrams per milliliter using centrifugal protein filters (Millipore Amicon Ultra-15 Ultracel 10K, catalog number UFC901024).

[0208] C. Screening of the Orf2 protein variants and aromatic prenytransferase enzymes for protein activity and phenotypes.

[0209] The library of Orf2 variants and APTs were screened for protein expression by western blot with an anti-HIS antibody (Cell Signaling Technologies, anti-his monoclonal antibody, catalog number 23655) according to the protocol provided by Cell Signaling Technologies for the antibody. The enzymes that had detectable levels of protein expression as determined by western blot were used in a prenylation assay.

[0210] Proteins that exhibited detectable expression by Western blot were assayed for prenylation activity using a substrate (e.g. olivetolic acid, olivetol, divarinic acid, etc.) and a donor molecule (e.g. GPP, FPP, DMAPP, etc.).. Unless otherwise stated, each prenylation reaction assay was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 2 millimolar donor molecule (e.g. GPP), 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar substrate (e.g. olivetolic acid), and 20 micrograms Orf2 protein, Orf2 variant protein, or APT. These reactions were incubated for 16 hours at 30°C.

[0211] The prenylated products obtained from the various reactions described in these Examples is summarized in Table C below.

[0212] Table C - prenylated product summary

Name of prenylated product	Prenyl transferase	Substrate	Donor	Attachment Site of the prenyl group on the substrate
UNK1	Orf2	OA	DMAPP	CO
UNK2	Orf2	OA	DMAPP	2-O
UNK3	Orf2	OA	DMAPP	4-O
RBI-08	Orf2	OA	DMAPP	3-C
RBI-17	Orf2	OA	DMAPP	5-C
RBI-05	Orf2	OA	GPP	CO
RBI-06	Orf2	OA	GPP	2-O
UNK4	Orf2	OA	GPP	4-O
RBI-02 (CBGA)	Orf2	OA	GPP	3-C
RBI-04 (5-GOA)	Orf2	OA	GPP	5-C
RBI-56	Orf2	OA	FPP	2-O
UNK5	Orf2	OA	FPP	4-O

RBI-14 (CBFA)	Orf2	OA	FPP	3-C
RBI-16 (5-FOA)	Orf2	OA	FPP	5-C
UNK6	Orf2	DVA	DMAPP	CO
UNK7	Orf2	DVA	DMAPP	2-O
UNK8	Orf2	DVA	DMAPP	4-O
UNK9	Orf2	DVA	DMAPP	3-C
UNK10	Orf2	DVA	DMAPP	5-C
RBI-24	Orf2	DVA	GPP	CO
RBI-28	Orf2	DVA	GPP	2-O
UNK11	Orf2	DVA	GPP	4-O
RBI-26 (CBGVA)	Orf2	DVA	GPP	3-C
RBI-27	Orf2	DVA	GPP	5-C
UNK12	Orf2	DVA	FPP	CO
UNK13	Orf2	DVA	FPP	2-O
UNK14	Orf2	DVA	FPP	4-O
RBI-38	Orf2	DVA	FPP	3-C
RBI-39	Orf2	DVA	FPP	5-C
RBI-10	Orf2	O	DMAPP	1-C or 5-C
UNK16	Orf2	O	DMAPP	2-O or 4-O
UNK16	Orf2	O	DMAPP	2-O or 4-O
RBI-09	Orf2	O	DMAPP	3-C
RBI-10	Orf2	O	DMAPP	1-C or 5-C
RBI-10	HypSc	O	DMAPP	1-C or 5-C
UNK16	HypSc	O	DMAPP	2-O or 4-O
UNK16	HypSc	O	DMAPP	2-O or 4-O
RBI-09	HypSc	O	DMAPP	3-C
RBI-10	HypSc	O	DMAPP	1-C or 5-C
RBI-10	PB005	O	DMAPP	1-C or 5-C
UNK16	PB005	O	DMAPP	2-O or 4-O
UNK16	PB005	O	DMAPP	2-O or 4-O
RBI-09	PB005	O	DMAPP	3-C
RBI-10	PB005	O	DMAPP	1-C or 5-C
RBI-03 (5-GO)	Orf2	O	GPP	1-C or 5-C
RBI-20	Orf2	O	GPP	2-O or 4-O
RBI-20	Orf2	O	GPP	2-O or 4-O
RBI-01 (CBG)	Orf2	O	GPP	3-C
RBI-03 (5-GO)	Orf2	O	GPP	1-C or 5-C
RBI-15	Orf2	O	FPP	1-C or 5-C
UNK18	Orf2	O	FPP	2-O or 4-O
UNK18	Orf2	O	FPP	4-O or 2-O
UNK19	Orf2	O	FPP	3-C
RBI-15	Orf2	O	FPP	1-C or 5-C
UNK54	PB005	DV	DMAPP	1-C or 5-C
UNK55	PB005	DV	DMAPP	2-O or 4-O
UNK55	PB005	DV	DMAPP	2-O or 4-O
UNK56	PB005	DV	DMAPP	3-C

UNK54	PB005	DV	DMAPP	1-C or 5-C
UNK20	Orf2	ORA	GPP	CO
UNK21	Orf2	ORA	GPP	2-O
UNK22	Orf2	ORA	GPP	4-O
UNK23	Orf2	ORA	GPP	3-C
UNK24	Orf2	ORA	GPP	5-C
UNK25	Hypsc, 064, 065, orf2, 002, 005	ORA	DMAPP	CO
UNK26	Hypsc, 064, 065, orf2	ORA	DMAPP	2-O
UNK27	hypsc, Atapt	ORA	DMAPP	4-O
UNK28	064, 005	ORA	DMAPP	3-C
UNK29	orf2	ORA	DMAPP	5-C
RBI-32	PB005	DV	GPP	3C
RBI-33	PB005	DV	GPP	1-C or 5-C
UNK30	Orf2	ORA	FPP	CO
UNK31	Orf2	ORA	FPP	2-O
UNK32	Orf2	ORA	FPP	4-O
UNK33	Orf2	ORA	FPP	3-C
UNK34	Orf2	ORA	FPP	5-C
UNK60	Orf2	OA	GGPP	3C
UNK61	Orf2	OA	GGPP	5-C
UNK62	Orf2	ORA	GGPP	3C
UNK63	Orf2	ORA	GGPP	5-C
UNK64	Orf2	DVA	GGPP	3C
UNK65	Orf2	DVA	GGPP	5-C
RBI-07	Orf2	OA	GPP	3-C + 5-C
RBI-29	Orf2	DVA	GPP	3-C + 5-C
RBI-30	Orf2	DVA	GPP	5-C + 2-O
RBI-36	Orf2	DV	GPP	3-C + 5-C
UNK35	Orf2	DV	GPP	5-C + 1-C
UNK36	Orf2	OA	GPP, DMAPP	5-C (GPP) + 3-C (DMAPP)
RBI-22	Orf2	OA	GPP, DMAPP	5-C (DMAPP) + 3-C (GPP)
UNK38	Orf2	OA	GPP, DMAPP	CO (GPP) + 3-C (DMAPP)
RBI-18	Orf2	OA	DMAPP	5-C + 3-C
UNK40	005 +Orf2	O	GPP, DMAPP	5-C (GPP) + 3-C (DMAPP)
UNK41	005+Orf2	O	GPP, DMAPP	5-C (DMAPP) + 3-C (GPP)
UNK42	Orf2	OA	GPP, FPP	5-C (GPP) + 3-C (FPP)
RBI-12	PB005	O	DMAPP	1-C+5-C
RBI-11	PB005	O	DMAPP	1-C+3-C
UNK44	005+Orf2	O	FPP, DMAPP	5-C (DMAPP) + 3-C (FPP)

UNK45	005+Orf2	O	FPP, DMAPP	5-C (DMAPP) + 1-C(FPP)
UNK46	Orf2	O	GPP, FPP	5-C (GPP) + 3-C (FPP)
UNK57	PB005/HypSc	DV	DMAPP	5-C + 3-C
UNK58	PB005/HypSc	DV	DMAPP	5-C + 1-C
UNK59	Orf2	ORA	GPP	5-C + 3-C
UNK66	005+Orf2	O	GPP, DMAPP	5-C (DMAPP) + 1-C (GPP)
UNK67	005+Orf2	O	GPP, DMAPP	5-C (DMAPP) + 1-C (DMAPP)+ 3-C (GPP)

Example 2: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using OA as substrate and DMAPP as donor.

[0213] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to “breakdown” analysis. “Breakdown” analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. “Breakdown” variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position.

[0214] The wild type Orf2 prenylation reaction using OA as substrate and DMAPP as donor produces 5 products as detected by HPLC. The respective retention times of these products are approximately 3.9, 5.44, 5.57, 6.29, and 6.66 minutes.

[0215] Table 1 provides a summary of the prenylation products produced from OA and DMAPP, their retention times, and the hypothesized prenylation site on OA. FIG. 16 shows the predicted chemical structures of the respective prenylation products.

Table 1: Predicted prenylation products of Orf2 or Orf2 Mutants when using OA as substrate and DMAPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK1	OA	DMAPP	CO	3.9
UNK2	OA	DMAPP	2-O	6.66
UNK3	OA	DMAPP	4-O	6.29
RBI-08	OA	DMAPP	3-C	5.44

RBI-17	OA	DMAPP	5-C	5.57

[0216] Table 2 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using Olivetolic Acid (OA) as substrate and Dimethylallyl pyrophosphate (DMAPP) as donor. Table 2 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 2: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using OA as substrate and DMAPP as donor

ID#	**Mutations**	**3.9**	**5.44**	**5.57**	**6.29**	**6.66**
1	WT	0.0055	0.0809	0.058	0.0052	0.0193
2	V9_Q38G_E112D_F123H	0.0021	0.0901	0.1688	0.0124	0.0045
3	V17_V49L_F123A_Y283L	0.0043	0.0365	0.0163	0.0001	0.0026
4	V25_L219F_V294N_Q295A	0.0102	0.3034	0.0456	0.0004	0.0986
5	V33_A17T_C25V_E112G	0.0028	0.0471	0.0501	0.0007	0.0075
6	V49_G205L_R228E_C230N	0.0038	0.0245	0.0185	0.0008	0.0074
7	V57_C25V_A232S_V271E	0.0031	0.0192	0.0163	0.0002	0.0055
8	V65_V49A_Q161S_V294A	0.0125	0.3382	0.1002	0.0006	0.1914
9	V73_V49S_K118Q_S177E	0.0093	0.028	0.0213	0.0002	0.0089
10	V81_V49L_D166E_L274V	0.0037	0.0287	0.0221	0.001	0.004
11	V89_Y121W_S177Y_G286E	0.0009	0.0308	0.0208	0.0002	0.0067
12	V10_V49A_S177Y_C209G	0.0039	0.0203	0.0112	0.001	0.0086
13	V26_A53E_A108G_K118N	0.0031	0.0224	0.0276	0.0001	0.0055
14	V34_A53Q_Y121W_A232S	0.0034	0.0194	0.0162	0.0005	0.0074
15	V42_D166E_S177Y_S214F	0.0018	0.0235	0.011	0.0011	0.0061
16	V58_K118Q_L174V_R228Q	0.0036	0.0213	0.0115	0.0001	0.008
17	V66_C25V_F213M_Y216A	0.0019	0.0236	0.0107	0.0001	0.0077
18	V74_M106E_Y121W_D166E	0.0022	0.02	0.0075	0.0008	0.01
19	V82_V49S_K119D_F213M	0.0022	0.0215	0.0078	0.0003	0.007
20	V90_A17T_F123W_L298A	0.0026	0.0361	0.0189	0.001	0.008
21	V3_V49S_M162A_Y283L	0.0036	0.0354	0.0755	0.0073	0.0093
22	V11_K118N_K119A_V271E	0.003	0.0168	0.0076	0.001	0.0072
23	V19_V49L_S214R_V271E	0.0046	0.0233	0.0092	0.0001	0.0072
24	V35_A53Q_S177Y_Y288H	0.0088	0.0993	0.0948	0.0151	0.0379
25	V43_Q161A_M162F_Q295A	0.0149	0.7629	0.0088	0.0002	0.4698
26	V51_V49L_K119D_G205M	0.0042	0.0263	0.0104	0.0004	0.0113
27	V59_V49S_S214G_V294A	0.0067	0.0323	0.0351	0.0002	0.0048
28	V67_A108G_K119D_L298A	0.0026	0.0239	0.0083	0.001	0.0046
29	V75_A53Q_L274V_Q295A	0.004	0.0268	0.0095	0.0002	0.0101
30	V83_E112D_L219F_V294F	0.0066	0.0762	0.0657	0.0079	0.0132
31	V91_N173D_F213M_V294F	0.0014	0.0206	0.0205	0.001	0.0077
32	V4_K118Q_Q161W_S214F	0.0029	0.023	0.0193	0.0001	0.0086
33	V20_D227E_C230N_Q295W	0.0025	0.0281	0.0237	0.0001	0.0073

34	V28_A53T_D166E_Q295W	0.0066	0.095	0.0939	0.0214	0.0219
35	V44_A53E_Q161A_V294N	0.0054	0.1369	0.0624	0.001	0.0241
36	WT	0.001	0.101	0.066	0.001	0.013
37	V52_K119A_S214G_L298A	0.001	0.021	0.006	0.001	0.005
38	V60_E112D_K119A_N173D	0.001	0.019	0.007	0.001	0.006
39	V68_K118N_C209G_R228Q	0.001	0.02	0.007	0.001	0.008
40	V76_V49A_F123A_Y288H	0.001	0.021	0.008	0.001	0.007
41	V84_F123H_L174V_S177E	0.001	0.104	0.057	0.002	0.011
42	V92_A53T_E112D_G205M	0.003	0.122	0.141	0.019	0.028
43	V69_A53T_M106E_Q161S	0.001	0.106	0.056	0.001	0.014
44	V60_E112D_K119A_N173D	0.001	0.019	0.003	0.001	0.009
45	V62_A53T_N173D_S214R	0.001	0.024	0.004	0.001	0.008
46	V70_Q38G_D166E_Q295A	0.001	0.14	0.08	0.002	0.009
47	V78_K119D_Q161W_L298Q	0.001	0.021	0.006	0.001	0.007
48	V94_A17T_V49A_C230N	0.001	0.017	0.004	0.001	0.007
49	V15_A53E_F213M_R228Q	0.001	0.02	0.005	0.001	0.007
50	V23_L219F_Y283L_L298W	0.001	0.029	0.043	0.001	0.01
51	V31_D227E_R228E_L298Q	0.001	0.015	0.003	0.001	0.007
52	V39_A53T_K118N_S214F	0.001	0.026	0.087	0.001	0.007
53	V47_K118Q_F123A_R228E	0.001	0.016	0.004	0.001	0.004
54	V55_V49S_Y216A_V294N	0.001	0.017	0.005	0.001	0.007
55	V63_F123W_M162F_C209G	0.001	0.021	0.005	0.001	0.007
56	V79_V49A_Y121W_C230S	0.001	0.023	0.005	0.001	0.005
57	V87_S177W_Y288H_V294N	0.001	0.027	0.005	0.001	0.006
58	V95_A17T_Q161W_A232S	0.001	0.194	0.067	0.001	0.015
59	V8_K119A_Q161A_R228Q	0.001	0.029	0.005	0.001	0.01
60	V16_A53Q_S177W_L219F	0.002	0.093	0.069	0.003	0.007
61	V32_M162A_C209G_Y288H	0.001	0.035	0.007	0.001	0.008
62	V40_S177E_S214R_R228E	0.001	0.031	0.007	0.001	0.009
63	V48_V49L_E112D_G286E	0.001	0.024	0.006	0.001	0.007
64	V56_F123A_M162F_S214G	0.002	0.038	0.046	0.005	0.01
65	V72_E112G_G205M_L298W	0.001	0.061	0.163	0.033	0.007
66	V80_M162A_N173D_S214F	0.002	0.028	0.012	0.001	0.007
67	V88_A108G_Q161S_G205M	0.001	0.04	0.087	0.001	0.007
68	WT	0.001	0.076	0.047	0.002	0.017
69	Q38G_D166E	0.001	0.039	0.031	0.001	0.009
70	Q38G_Q295A	0.001	0.1	0.062	0.004	0.02
71	D166E_Q295A	0.001	0.049	0.011	0.001	0.018
72	L219F_V294N	0.002	0.147	0.074	0.003	0.034
73	L219F_Q295A	0.003	0.114	0.013	0.001	0.048
74	V294N_Q295A	0.003	0.257	0.111	0.009	0.057
75	A53Q_S177W	0.001	0.149	0.059	0.001	0.017
76	A53Q_L219F	0.001	0.069	0.056	0.003	0.017
77	S177W_L219F	0.001	0.068	0.062	0.001	0.009

78	A108G_Q161S	0.001	0.038	0.123	0.001	0.007
79	A108G_G205M	0.001	0.031	0.031	0.001	0.006
80	Q161S_G205M	0.001	0.089	0.028	0.001	0.021
81	F123H_L174V	0.002	0.101	0.113	0.006	0.007
82	F123H_S177E	0.001	0.188	0.106	0.001	0.007
83	L174V_S177E	0.002	0.096	0.046	0.001	0.012
84	A53T_D166E	0.001	0.051	0.061	0.004	0.01
85	A53T_Q295W	0.008	0.459	0.307	0.104	0.09
86	D166E_Q295W	0.002	0.107	0.064	0.007	0.021
87	A53Q_S177Y	0.001	0.059	0.05	0.004	0.002
88	A53Q_Y288H	0.013	0.2	0.099	0.018	0.13
89	S177Y_Y288H	0.002	0.059	0.033	0.003	0.024
90	V49A_Q161S	0.003	0.146	0.045	0.001	0.065
91	V49A_V294A	0.002	0.094	0.04	0.003	0.059
92	Q161S_V294A	0.009	0.479	0.103	0.001	0.091
93	A53T_M106E	0.001	0.077	0.073	0.007	0.014
94	A53T_Q161S	0.005	0.348	0.116	0.002	0.06
95	M106E_Q161S	0.001	0.06	0.028	0.001	0.011
96	A53T_K118N	0.001	0.023	0.018	0.001	0.002
97	A53T_S214F	0.001	0.18	0.296	0.024	0.01
98	K118N_S214F	0.001	0.024	0.047	0.001	0.01
99	WT	0.002	0.082	0.056	0.001	0.018
100	A108G	0.001	0.035	0.162	0.001	0.007
101	A53Q	0.001	0.072	0.056	0.002	0.017
102	A53T	0.004	0.183	0.16	0.02	0.031
103	D166E	0.001	0.05	0.051	0.001	0.007
104	F123H	0.002	0.106	0.153	0.01	0.006
105	G205M	0.001	0.072	0.046	0.003	0.014
106	K118N	0.001	0.027	0.03	0.001	0.005
107	L219F	0.001	0.07	0.059	0.001	0.015
108	M106E	0.001	0.051	0.036	0.001	0.008
109	Q161S	0.003	0.204	0.076	0.001	0.03
110	Q295A	0.01	0.308	0.029	0.002	0.128
111	Q295W	0.017	0.894	0.361	0.069	0.171
112	Q38G	0.001	0.064	0.047	0.001	0.014
113	S177E	0.002	0.13	0.066	0.001	0.016
114	S177W	0.001	0.089	0.059	0.001	0.013
115	S177Y	0.001	0.069	0.06	0.001	0.012
116	S214F	0.001	0.049	0.072	0.001	0.005
117	V294A	0.006	0.218	0.104	0.006	0.051
118	V294N	0.003	0.171	0.071	0.003	0.039
119	V49A	0.003	0.05	0.025	0.001	0.017
120	Y288H	0.005	0.095	0.034	0.001	0.053
121	Q161D	0.002	0.093	0.038	0.001	0.013

122	Q161P	0.001	0.046	0.036	0.001	0.011
123	Q161W	0.001	0.055	0.061	0.001	0.008
124	A53I	0.002	0.072	0.045	0.001	0.008
125	A53R	0.002	0.04	0.03	0.001	0.007
126	A53T	0.003	0.188	0.169	0.021	0.031
127	A53W	0.001	0.024	0.013	0.001	0.005
128	V64_M106E_M162A_Y216A	0.001	0.017	0.008	0.001	0.006
129	WT	0.001	0.092	0.067	0.003	0.014
130	WT	0.002	0.079	0.051	0.003	0.018
131	Q295Q	0.002	0.079	0.051	0.003	0.018
132	Q295C	0.018	0.855	0.03	0.019	0.543
133	Q295E	0.001	0.064	0.018	0.001	0.01
134	Q295F	0.074	3.511	0.096	0.016	1.113
135	Q295G	0.007	0.381	0.086	0.002	0.131
136	Q295H	0.007	0.208	0.162	0.025	0.054
137	Q295I	0.025	1.125	0.033	0.002	0.671
138	Q295L	0.033	1.618	0.039	0.005	0.616
139	Q295M	0.043	2.088	0.087	0.015	0.592
140	Q295N	0.002	0.143	0.029	0.001	0.041
141	Q295P	0.001	0.049	0.013	0.001	0.012
142	Q295R	0.001	0.011	0.008	0.001	0.005
143	Q295S	0.003	0.173	0.031	0.001	0.049
144	Q295T	0.002	0.094	0.016	0.001	0.032
145	Q295V	0.019	0.739	0.036	0.003	0.269
146	Q295W	0.014	0.889	0.329	0.107	0.21
147	A53T_V294A	0.009	0.663	0.489	0.081	0.141
148	A53T_Q161S_V294A	0.013	1.132	0.306	0.005	0.188
149	A53T_Q161S_V294N	0.009	0.903	0.244	0.004	0.15
150	A53T_Q295A	0.01	0.344	0.06	0.009	0.141
151	Q161S_V294A_Q295A	0.052	2.369	0.223	0.006	0.539
152	A53T_Q161S_Q295A	0.022	1.181	0.136	0.004	0.33
153	A53T_V294A_Q295A	0.045	1.216	0.161	0.052	0.402
154	A53T_Q161S_V294A_Q295A	0.056	2.603	0.308	0.011	0.539
155	A53T_Q161S_V294N_Q295A	0.03	2.286	0.351	0.009	0.377
156	A53T_Q295W	0.015	0.831	0.543	0.171	0.166
157	Q161S_V294A_Q295W	0.026	1.165	0.307	0.016	0.246
158	A53T_Q161S_Q295W	0.024	1.157	0.33	0.028	0.208
159	A53T_V294A_Q295W	0.014	0.716	0.455	0.117	0.141
160	A53T_Q161S_V294A_Q295W	0.021	1.042	0.332	0.026	0.19
161	A53T_Q161S_V294N_Q295W	0.024	1.173	0.365	0.018	0.215
162	WT	0.001	0.094	0.066	0.004	0.018
163	S214K	0.001	0.078	0.05	0.001	0.01
164	Q161A	0.001	0.101	0.053	0.003	0.021
165	Q161H	0.028	1.693	0.06	0.001	0.507

166	Q161K	0.001	0.043	0.05	0.011	0.005
167	A53F	0.001	0.015	0.006	0.001	0.007
168	S177W_Q295A	0.03	6.53	0.024	0.001	1.194
169	S177W_S214R	0.001	0.166	0.01	0.001	0.052
170	Q161S_S177W	0.001	0.143	0.028	0.001	0.019
171	A53T_S177W	0.001	0.157	0.108	0.004	0.02
172	V49A_Q295L	0.006	0.093	0.009	0.001	0.025
173	V49A_S214R	0.001	0.08	0.008	0.001	0.04
174	A53T_Q295F	0.078	2.46	0.113	0.035	0.864
175	A53T_S214R	0.007	1.158	0.042	0.001	0.306
176	A53T_A161S	0.008	0.524	0.2	0.004	0.085
177	Q161S_Q295F	0.086	3.918	0.096	0.003	1.178
178	Q161S_Q295L	0.088	4.011	0.086	0.025	1.18
179	Q16S_S214R	0.001	0.236	0.035	0.001	0.064
180	S214R_Q295F	0.126	5.266	0.02	0.002	3.086
181	WT	0.001	0.064	0.043	0.003	0.016
182	WT	0.001	0.064	0.043	0.003	0.016
183	S214D	0.002	0.079	0.035	0.001	0.013
184	S214E	0.001	0.224	0.291	0.003	0.009
185	S214F	0.001	0.042	0.067	0.002	0.009
186	S214H	0.003	0.651	0.022	0.001	0.204
187	S214I	0.001	0.043	0.051	0.001	0.012
188	S214L	0.001	0.024	0.049	0.001	0.004
189	S214M	0.001	0.047	0.071	0.002	0.008
190	S214N	0.001	0.026	0.022	0.001	0.005
191	S214R	0.001	0.292	0.018	0.001	0.086
192	S214T	0.001	0.06	0.039	0.001	0.018
193	S214V	0.001	0.044	0.031	0.001	0.016
194	S214W	0.001	0.075	0.044	0.001	0.007
195	S214Y	0.001	0.062	0.169	0.003	0.011
196	Q161G	0.001	0.048	0.035	0.001	0.01
197	Q161N	0.001	0.047	0.038	0.001	0.013
198	Q161Q	0.001	0.053	0.036	0.002	0.016
199	A53M	0.002	0.083	0.058	0.006	0.022
200	A53N	0.001	0.025	0.017	0.001	0.009
201	A53S	0.001	0.078	0.059	0.004	0.001
202	A53V	0.005	0.178	0.091	0.006	0.036
203	V24_A17T_F213M_S214R	0.001	0.111	0.005	0.001	0.035
204	A53G	0.001	0.029	0.026	0.001	0.005
205	R228E	0.001	0.01	0.004	0.001	0.005
206	WT	0.001	0.073	0.053	0.002	0.019
207	Q161C	0.001	0.138	0.095	0.002	0.025
208	Q161F	0.001	0.18	0.108	0.004	0.045
209	Q161I	0.002	0.115	0.076	0.005	0.034

210	Q161L	0.001	0.17	0.088	0.009	0.048
211	Q161L	0.001	0.128	0.067	0.004	0.037
212	Q161M	0.003	0.13	0.099	0.002	0.044
213	Q161R	0.001	0.335	0.033	0.001	0.04
214	Q161S	0.002	0.124	0.05	0.001	0.024
215	Q161T	0.001	0.116	0.05	0.001	0.025
216	Q161Y	0.16	1.608	0.262	0.003	0.258
217	A53D	0.001	0.039	0.033	0.001	0.011
218	A53E	0.001	0.011	0.007	0.001	0.005
219	A53K	0.001	0.073	0.063	0.007	0.016
220	A53L	0.005	0.13	0.078	0.015	0.029
221	A53Q	0.001	0.068	0.059	0.005	0.017
222	A53Y	0.001	0.016	0.006	0.001	0.008
223	WT	0.001	0.069	0.049	0.002	0.017
224	V36_F123H_L274V_L298A	0.001	0.015	0.017	0.001	0.006
225	Q295D	0.013	0.547	0.086	0.002	0.142
226	Q295K	0.001	0.082	0.032	0.001	0.02
227	S214P	0.001	0.012	0.005	0.001	0.007
228	A53P	0.001	0.011	0.011	0.001	0.007
229	WT	0.031	0.066	0.048	0.004	0.012
230	K118Q	0.074	0.027	0.064	0.004	0.008
231	K119Q	0.029	0.012	0.005	0.001	0.003
232	M162A	0.025	0.191	1.105	0.284	0.033
233	K119D	0.035	0.091	0.064	0.003	0.02
234	F123A	0.023	0.148	0.12	0.017	0.006
235	K118N	0.02	0.018	0.038	0.001	0.003
236	Q161W	0.096	0.052	0.072	0.001	0.003
237	D227E	0.034	0.052	0.056	0.004	0.008
238	L274V	0.029	0.02	0.013	0.001	0.009
239	S214G	0.033	0.041	0.265	0.048	0.006
240	Y216A	0.033	0.01	0.005	0.001	0.003
241	F123W	0.031	0.011	0.006	0.001	0.001
242	V271E	0.034	0.01	0.004	0.001	0.001
243	N173D	0.041	0.01	0.004	0.001	0.001
244	R228Q	0.024	0.01	0.005	0.001	0.001
245	M162F	0.028	0.044	0.018	0.001	0.01
246	A232S	0.03	0.385	0.054	0.001	0.115
247	C230S	0.021	0.024	0.018	0.001	0.005
248	V294F	0.032	0.052	0.039	0.006	0.009
249	Y283L	0.027	0.057	0.031	0.003	0.008
250	S214R	0.026	0.513	0.03	0.001	0.148
251	G286E	0.033	0.012	0.002	0.001	0.009
252	S214A	0.001	0.03	0.046	0.006	0.009
253	S214A	0.001	0.038	0.053	0.01	0.021

254	S214G	0.0009	0.0428	0.2804	0.0536	0.007
255	S214Q	0.0023	0.1456	0.1448	0.0018	0.0052
256	Q161E	0.0062	0.0477	0.032	0.0009	0.0134
257	Q161V	0.0011	0.0754	0.0588	0.0019	0.0188
258	A53C	0.0031	0.0791	0.0544	0.0007	0.0183
259	WT	0.001	0.065	0.047	0.005	0.016

[0217] The amount of each prenylation product was measured by HPLC. FIG. 1 shows a heatmap of the HPLC areas of each prenylation product generated using OA as substrate and DMAPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 2.

Example 3: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using OA as substrate and GPP as donor

[0218] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to “breakdown” analysis. “Breakdown” analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. “Breakdown” variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position.

[0219] The wild type Orf2 prenylation reaction using OA as substrate and GPP as donor produces 6 products as detected by HPLC. The respective retention times of these products are approximately 6.14, 7.03 [CBGA], 7.27 [5-GOA], 8.17, 8.77, and 11.6 minutes.

[0220] Table 3 provides a summary of the prenylation products produced from OA and GPP, their retention times, and the hypothesized prenylation site on OA. FIG. 17 shows the predicted chemical structures of the respective prenylation products.

Table 3: Predicted prenylation products of Orf2 or Orf2 Mutants when using OA as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time

RBI-05	OA	GPP	CO	6.14
RBI-06	OA	GPP	2-O	8.77
UNK4	OA	GPP	4-O	8.17
RBI-02 (CBGA)	OA	GPP	3-C	7.03
RBI-04 (5-GOA)	OA	GPP	5-C	7.27
RBI-07	OA	GPP	3-C + 5-C	11.6

[0221] Table 4 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using OA as substrate and GPP as donor. Table 4 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 4: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using OA as substrate and GPP as donor

ID#	**Mutations**	**6.14**	**CBGA**	**5-GOA**	**8.17**	**8.77**	**11.6**
1	WT	0.2794	7.9349	13.7212	1.0323	0.4271	1.9618
2	V9_Q38G_E112D_F123H	0.1061	1.0302	5.2532	0.1011	0.073	0.2181
3	V17_V49L_F123A_Y283L	0.07	0.1966	0.076	0.0238	0.0048	0.0002
4	V25_L219F_V294N_Q295A	0.3916	12.2815	1.9643	1.4293	0.7139	0.4415
5	V33_A17T_C25V_E112G	0.2338	3.6625	10.4026	0.641	1.8779	0.4371
6	V49_G205L_R228E_C230N	0.044	0.0786	0.0978	0.0086	0.0205	0.011
7	V57_C25V_A232S_V271E	0.0533	0.1055	0.034	0.0244	0.005	0.0005
8	V65_V49A_Q161S_V294A	0.9607	12.1374	7.434	1.8802	1.6359	0.6581
9	V73_V49S_K118Q_S177E	2.4814	1.454	0.7051	0.0547	0.8276	0.0316
10	V81_V49L_D166E_L274V	0.0656	0.1064	0.0287	0.0092	0.0079	0.0012
11	V89_Y121W_S177Y_G286E	0.0507	0.0455	0.0225	0.0049	0.0018	0.0008
12	WT	0.2572	6.3536	10.0533	0.7506	0.2991	1.4653
13	V52_K119A_S214G_L298A	0.0832	0.1415	10.2648	0.0255	0.0235	0.1171
14	V60_E112D_K119A_N173D	0.0392	0.0151	0.0781	0.0009	0.0001	0.0023
15	V68_K118N_C209G_R228Q	0.0709	0.034	0.0426	0.0009	0.001	0.0003
16	V76_V49A_F123A_Y288H	0.062	0.0381	0.0229	0.0021	0.0018	0.0023
17	V84_F123H_L174V_S177E	0.3055	2.1758	0.6027	0.1708	0.0502	0.0747
18	V92_A53T_E112D_G205M	0.3547	5.2677	35.928	1.2267	0.5641	3.3962
19	V69_A53T_M106E_Q161S	0.6502	19.6975	7.6006	1.7073	0.3979	2.796
20	V60_E112D_K119A_N173D	0.0561	0.253	0.1639	0.0251	0.039	0.0315
21	V62_A53T_N173D_S214R	0.1688	2.6452	0.0297	0.9909	0.0003	0.0071
22	V70_Q38G_D166E_Q295A	0.4737	3.4776	0.7322	0.2353	0.0732	0.1125
23	WT	0.2827	7.1705	11.5331	0.8652	0.3439	1.3876
24	Q295A	1.45	30.5523	5.1674	3.4945	0.5593	2.9359

25	V10_V49A_S177Y_C209G	0.0758	0.096	0.0479	0.0079	0.08	0.0294
26	V26_A53E_A108G_K118N	0.0828	0.0789	0.08	0.0073	0.0056	0.005
27	V34_A53Q_Y121W_A232S	0.0836	0.074	0.0259	0.0057	0.0026	0.0009
28	V42_D166E_S177Y_S214F	0.0795	0.0941	0.0515	0.01	0.0055	0.0012
29	V58_K118Q_L174V_R228Q	0.0903	0.1705	0.2533	0.0174	0.01	0.0003
30	V66_C25V_F213M_Y216A	0.0811	0.3019	0.3944	0.056	0.0145	0.0043
31	V74_M106E_Y121W_D166E	0.0881	0.1227	0.0352	0.013	0.0097	0.0005
32	V82_V49S_K119D_F213M	0.076	0.1102	0.0306	0.0102	0.0053	0.0002
33	V90_A17T_F123W_L298A	0.0817	0.4756	0.9124	0.1185	0.0793	0.0155
34	V3_V49S_M162A_Y283L	0.1636	0.3405	4.5126	0.0373	0.0566	0.1002
35	V11_K118N_K119A_V271E	0.0805	0.1113	0.0375	0.0128	0.0126	0.0053
36	V19_V49L_S214R_V271E	0.0788	0.1846	0.037	0.0157	0.0098	0.0023
37	V35_A53Q_S177Y_Y288H	1.633	8.8464	2.5998	1.1577	1.0822	0.1161
38	V43_Q161A_M162F_Q295A	0.2118	3.5161	1.2921	0.8034	0.1313	0.045
39	V51_V49L_K119D_G205M	0.0824	0.1206	0.0388	0.0144	0.0043	0.0013
40	V59_V49S_S214G_V294A	3.2839	1.4838	4.583	0.0931	0.3677	0.1361
41	V67_A108G_K119D_L298A	0.1131	0.1369	0.1136	0.013	0.0139	0.001
42	V75_A53Q_L274V_Q295A	0.0825	0.597	0.1642	0.0681	0.0231	0.0037
43	V83_E112D_L219F_V294F	0.2227	3.6877	11.4492	0.4814	0.2136	0.7145
44	V91_N173D_F213M_V294F	0.0663	0.1974	10.3487	0.0444	0.0166	0.2421
45	V4_K118Q_Q161W_S214F	0.0797	0.363	0.3916	0.0553	0.0124	0.002
46	V20_D227E_C230N_Q295W	0.1509	1.0926	0.3784	0.3591	0.0298	0.01
47	V28_A53T_D166E_Q295W	0.8082	10.436	6.5108	1.9787	0.2202	0.9405
48	V44_A53E_Q161A_V294N	0.0887	1.723	25.4591	0.4753	0.1107	1.1284
49	WT	0.2425	6.4286	10.4623	0.6951	0.2566	0.5593
50	WT	0.2499	5.874	8.9833	0.6112	0.2655	0.6241
51	V78_K119D_Q161W_L298Q	0.0685	0.1699	0.0603	0.0033	0.0131	0.0136
52	V94_A17T_V49A_C230N	0.0987	0.1648	0.1333	0.0023	0.1625	0.0055
53	V15_A53E_F213M_R228Q	0.0718	0.2147	4.5314	0.0244	0.0191	0.0586
54	V23_L219F_Y283L_L298W	0.0866	1.0864	8.9357	0.1104	0.0763	0.2369
55	V31_D227E_R228E_L298Q	0.0556	0.0592	0.0855	0.0872	0.02	0.0069
56	V39_A53T_K118N_S214F	0.0526	2.2095	3.9318	0.0648	0.0048	0.0547
57	V47_K118Q_F123A_R228E	0.0604	0.0776	0.078	0.0067	0.007	0.0001
58	V55_V49S_Y216A_V294N	0.4959	1.9114	0.4928	0.1476	0.1559	0.0087
59	V71_M106E_G205L_C209G	0.0518	0.0997	0.0249	0.0092	0.0086	0.0033
60	V79_V49A_Y121W_C230S	0.0694	0.0708	0.0208	0.0033	0.0074	0.0026
61	V87_S177W_Y288H_V294N	0.0725	0.5522	0.0445	0.0868	0.0123	0.0062
62	V95_A17T_Q161W_A232S	0.4328	23.1993	0.9315	1.8941	0.9875	0.0966
63	V8_K119A_Q161A_R228Q	0.0647	0.2165	0.1833	0.0196	0.0156	0.0033
64	V16_A53Q_S177W_L219F	0.2639	12.9917	1.637	0.3433	0.1857	0.3446
65	V32_M162A_C209G_Y288H	0.0692	0.2351	0.2343	0.0444	0.0204	0.0111
66	V40_S177E_S214R_R228E	0.071	0.1508	0.0335	0.0153	0.0086	0.0041
67	V48_V49L_E112D_G286E	0.0628	0.2671	0.0386	0.0575	0.0892	0.0026
68	V56_F123A_M162F_S214G	0.0895	0.1889	2.8827	0.0324	0.022	0.0303

69	V72_E112G_G205M_L298W	0.1442	1.6029	20.1789	0.174	0.248	0.6997
70	V80_M162A_N173D_S214F	0.0491	0.7197	5.9863	0.3816	0.0261	0.0878
71	V88_A108G_Q161S_G205M	0.35	7.8534	4.4162	1.0133	0.4621	0.549
72	WT	0.2595	7.5193	13.3225	0.8722	0.3068	0.6495
73	Q38G_D166E	0.1125	1.696	3.3192	0.135	0.0809	0.0863
74	Q38G_Q295A	0.3453	8.3585	11.1794	0.8498	0.3854	1.5188
75	D166E_Q295A	0.3403	5.9791	1.1668	0.5835	0.4446	0.1339
76	L219F_V294N	0.3331	9.5132	23.3479	1.7313	0.5213	1.7665
77	L219F_Q295A	0.3374	8.5459	0.9632	0.7676	0.4075	0.1568
78	L219F_Q295A	0.3491	10.339	0.9624	0.9641	0.4572	0.1088
79	V294N_Q295A	0.3448	9.491	25.3286	1.8217	0.6272	2.3726
80	A53Q_S177W	0.267	16.0111	1.9004	0.581	0.274	0.8811
81	A53Q_S177W	0.2679	18.1078	2.2106	0.6227	0.248	0.5122
82	A53Q_L219F	0.2547	7.0862	15.0794	0.6211	0.2459	0.8256
83	WT	0.2166	5.7052	10.3837	0.6679	0.326	0.4558
84	WT	0.1964	4.9344	8.3046	0.5323	0.2672	0.5161
85	A108G_Q161S	0.2656	4.0905	2.095	0.498	0.2241	0.554
86	A108G_G205M	0.1069	0.7184	1.7257	0.1012	0.0519	0.1179
87	Q161S_G205M	0.2449	10.3718	10.2265	1.315	0.3328	1.2632
88	F123H_L174V	0.1403	0.6711	1.7437	0.0771	0.0465	0.1729
89	F123H_S177E	0.3403	1.9731	0.5717	0.15	0.0774	0.153
90	L174V_S177E	0.3898	16.4952	2.7406	0.7724	0.2891	1.2376
91	A53T_D166E	0.242	3.1403	18.5969	0.4713	0.3019	1.3883
92	A53T_Q295W	1.6781	22.1195	6.0823	1.6555	0.4152	3.7797
93	D166E_Q295W	0.7739	13.0528	2.9087	1.6617	0.2638	1.1289
94	A53Q_S177Y	0.1722	1.6822	6.6658	0.1745	0.1247	0.3941
95	A53Q_Y288H	2.0851	13.2602	2.0825	1.4116	1.8522	0.2549
96	S177Y_Y288H	0.7662	4.8269	0.8808	0.7668	0.6572	0.0963
97	V49A_Q161S	0.5978	6.6391	3.2987	0.7232	0.7494	0.2188
98	V49A_V294A	0.741	2.9734	4.071	0.3087	0.8879	0.1941
99	Q161S_V294A	0.2907	18.5112	19.4499	2.4585	0.549	3.238
100	A53T_M106E	0.4607	8.5722	13.3998	0.6753	0.2034	1.1296
101	A53T_K118N	0.1698	1.0746	6.1515	0.1137	0.0954	0.311
102	A53T_S214F	0.1244	14.0659	19.3815	0.5432	0.0211	0.3179
103	A53T_S214F	0.0534	5.7351	7.2164	0.3014	0.0485	0.1489
104	K118N_S214F	0.0788	0.5533	0.5112	0.0412	0.0184	0.0479
105	WT	0.4287	10.433	16.3978	1.2802	0.4668	1.1985
106	Q295W	0.683	17.6777	1.7024	1.9224	1.0897	0.8575
107	Q295C	0.6718	21.8175	1.785	1.8402	2.0448	1.7573
108	Q295E	0.2404	7.3647	0.5962	0.2611	0.1293	0.111
109	Q295F	0.9554	62.6583	0.6746	2.5003	1.2552	0.9292
110	Q295G	0.6592	19.6614	3.352	2.3502	0.8261	1.7693
111	Q295H	0.6702	16.0317	34.4247	2.4852	0.3933	1.5102
112	Q295I	0.7531	24.5172	0.6814	0.6973	1.2208	0.2052

113	Q295L	1.017	42.3189	0.8181	1.9052	3.3264	0.6838
114	Q295M	1.0329	50.0921	1.7649	2.497	1.7455	1.4423
115	Q295N	0.3461	5.4797	4.0139	0.6466	0.6109	0.1501
116	WT	0.2794	7.7755	13.2073	0.9478	0.3935	0.7294
117	A108G	0.1028	1.0247	1.9316	0.1598	0.0929	0.0583
118	A53Q	0.2373	6.8076	17.9665	0.8513	0.2734	1.2782
119	A53T	0.4698	9.639	33.3605	1.6065	0.7544	4.0906
120	D166E	0.1719	3.5491	7.1374	0.371	0.2443	0.4411
121	F123H	0.095	1.0763	3.4321	0.1215	0.0978	0.1436
122	G205M	0.2882	7.6703	16.3875	1.0934	0.4238	1.2809
123	K118N	0.1028	1.0956	1.879	0.0971	0.0929	0.0493
124	L219F	0.1908	5.9595	8.0826	0.6165	0.2318	0.3464
125	L219F	0.246	7.3438	9.5117	0.6977	0.2841	0.3849
126	M106E	0.1691	4.3079	3.2674	0.2687	0.0997	0.1292
127	WT	0.2721	7.8954	12.4886	0.751	0.3353	0.4043
128	Q161S	0.3172	22.413	17.1289	2.607	0.6246	3.1877
129	Q295A	0.4619	13.257	1.5994	0.9306	0.6536	0.5911
130	Q295W	1.8373	43.6399	5.4222	2.3826	0.5376	0.9611
131	Q38G	0.2139	4.1646	6.3441	0.4349	0.1855	0.3908
132	S177E	0.5335	24.3551	3.2656	1.5548	0.4375	1.645
133	S177W	0.2431	13.5221	1.0317	0.4704	0.3223	0.4572
134	S177Y	0.1585	2.0079	4.2248	0.181	0.1149	0.1737
135	S214F	0.0648	4.2346	3.1597	0.161	0.0091	0.0686
136	V294A	0.3317	9.1221	24.672	1.4785	0.5044	2.0348
137	V294N	0.297	7.5944	19.5151	1.3176	0.4402	0.8056
138	V49A	0.563	2.9941	2.673	0.248	0.8594	0.1493
139	Y288H	1.0891	8.1857	0.9592	1.2335	0.9156	0.0611
140	Q161D	0.1486	5.9897	0.9657	0.5883	0.1173	0.0344
141	Q161P	0.1031	1.5397	22.6152	0.3745	0.2025	0.6503
142	Q161W	0.1348	1.4308	2.4821	0.2116	0.1461	0.0576
143	A53I	0.8859	12.3261	26.2444	0.7359	1.4753	0.4959
144	A53R	0.2385	3.2831	8.8328	0.2998	0.3083	0.2622
145	A53T	0.4372	9.0726	30.1103	1.2665	0.5775	2.3975
146	A53W	0.1326	1.9501	7.8002	0.2677	0.135	0.2937
147	V64_M106E_M162A_Y216A	0.0707	0.2105	0.3622	0.0191	0.014	0.0326
148	WT	0.3951	6.4459	10.029	0.5996	0.2187	0.5594
149	K118Q	0.2773	2.9905	10.2832	0.1687	0.1305	0.3055
150	K119Q	0.1461	0.2304	0.874	0.0355	0.0174	0.0167
151	M162A	0.1766	0.476	16.0271	0.0655	0.0107	0.4676
152	Q161A	0.2113	4.4385	36.2776	1.2967	0.3311	2.6936
153	K119D	0.4193	7.7581	10.6118	0.8274	0.4077	2.0115
154	G205L	0.2478	2.1074	6.6107	0.3247	0.0956	0.1912
155	F123A	0.268	1.9874	5.053	0.2065	0.1143	0.4062
156	K118N	0.2261	1.7015	2.9776	0.1282	0.0962	0.0571

157	Q161W	0.2608	1.9803	3.5027	0.362	0.1793	0.0972
158	D227E	0.3836	5.9881	11.523	0.6316	0.2788	0.6984
159	WT	0.5656	10.3883	16.1129	1.304	0.5864	1.8709
160	WT	0.4649	8.0525	11.5233	1.0342	0.4325	1.7098
161	Q295W	1.9421	40.163	4.5826	3.0238	0.7556	6.6166
162	Q295P	0.4679	4.9878	1.6758	0.5541	0.792	0.3127
163	Q295R	0.3226	0.3891	6.9755	0.0748	0.0444	0.1745
164	Q295S	0.4731	6.0574	2.4658	0.8139	0.5717	0.2357
165	Q295T	0.4314	2.2987	0.5716	0.1575	0.2201	0.0178
166	Q295V	1.2494	19.6029	0.5385	0.6364	3.0718	0.2259
167	A53T_V294A	0.4167	5.8761	36.6497	1.3877	0.4617	3.3157
168	A53T_Q161S_V294A	0.5039	15.381	33.5956	2.8747	0.5372	5.0464
169	A53T_Q161S_V294N	0.3568	11.9604	27.5382	2.4274	0.4483	3.6533
170	A53T_Q295A	1.4841	26.0366	3.7553	2.131	2.1193	6.2522
171	Q161S_V294A_Q295A	0.8397	46.9066	9.5266	3.9359	1.4569	6.8713
172	A53T_Q161S_Q295A	0.9326	34.1016	14.121	3.9918	1.3472	7.7645
173	A53T_V294A_Q295A	1.9935	37.8163	4.0888	2.503	2.968	10.274
174	A53T_Q161S_V294A_Q295A	1.0662	36.8247	18.7595	4.0408	1.4274	10.6352
175	A53T_Q161S_V294N_Q295A	0.8243	28.9549	15.8073	3.9841	1.2173	9.6389
176	A53T_Q295W	2.8333	41.0901	9.6799	3.1369	0.8036	10.3205
177	Q161S_V294A_Q295W	2.5294	68.3285	2.8122	3.5179	1.0696	4.4695
178	A53T_Q161S_Q295W	3.1489	68.7659	4.4902	3.7534	1.0874	7.7376
179	A53T_V294A_Q295W	2.3271	38.5309	12.362	3.4467	0.7316	9.2623
180	A53T_Q161S_V294A_Q295W	2.7241	63.9702	4.908	3.5416	0.8621	6.4643
181	A53T_Q161S_V294N_Q295W	2.4544	58.018	7.059	3.6741	0.9941	7.4983
182	WT	0.3273	7.5303	13.0854	0.9789	0.429	1.3818
183	L274V	0.18	1.6769	4.0405	0.3029	0.0859	0.1306
184	S214G	0.5101	0.9282	30.7747	0.222	0.4255	0.8022
185	Y216A	0.1704	0.4385	0.554	0.1316	0.0326	0.0097
186	F123W	0.0596	0.0333	0.0779	0.006	0.003	0.0051
187	V271E	0.0803	0.0522	0.0307	0.0087	0.0006	0.0057
188	N173D	0.1069	0.7167	1.8555	0.1497	0.0369	0.0522
189	R228Q	0.0909	0.8429	1.7305	0.074	0.036	0.0219
190	M162F	0.2485	4.4581	0.6972	0.5871	0.0533	0.0933
191	A232S	0.6408	36.2083	2.6149	5.1383	1.7018	1.9619
192	C230S	0.2263	3.5449	5.7749	0.6643	0.1284	0.4746
193	V294F	0.2697	3.8771	10.1682	0.6331	0.2769	1.1748
194	Y283L	0.2493	5.3759	12.915	0.7704	0.2779	0.5191
195	S214R	1.2478	50.9997	0.0411	4.4719	0.0638	0.0995
196	G286E	0.0983	0.206	0.1239	0.1018	0.0026	0.01
197	V63_F123W_M162F_C209G	0.0443	0.012	0.0502	0.002	0.0014	0.0134
198	WT	0.1295	3.9794	7.4058	0.5023	0.2259	0.2396
199	S177W_L219F	0.1351	5.9191	0.618	0.1856	0.0846	0.0683

200	S214C	0.0291	0.3974	1.582	0.1749	0.0029	0.0154
201	S214D	0.0839	1.7316	1.2328	0.3774	0.0072	0.0518
202	S214E	0.1331	3.514	0.1887	0.1044	0.0117	0.002
203	S214F	0.0212	1.8923	1.6135	0.0784	0.0012	0.0024
204	S214H	0.3828	42.8471	0.035	3.0202	0.0109	0.0176
205	S214I	0.0255	2.1462	0.6227	0.3675	0.001	0.0035
206	S214L	0.0207	0.3664	0.147	0.0065	0.0039	0.0006
207	S214M	0.025	1.2355	0.2679	0.0664	0.0013	0.0022
208	S214N	0.5202	2.582	1.2494	0.1251	0.0113	0.0002
209	S214R	0.5724	18.2997	0.0387	2.6847	0.0327	0.0064
210	S214K	0.1002	1.3288	0.2202	0.4215	0.0024	0.0076
211	Q161A	0.1296	3.5758	19.5936	0.727	0.1917	1.4337
212	Q161H	0.6716	81.4919	0.1983	3.5414	0.1028	0.7037
213	Q161K	0.1422	6.6077	2.1052	0.8148	0.0439	0.1206
214	A53F	0.0774	0.557	0.1938	0.0262	0.0074	0.0029
215	A53H	0.0706	0.3996	0.4786	0.0307	0.0123	0.0055
216	S177W_Q295A	0.2927	56.035	0.1016	2.1226	0.2866	0.1206
217	S177W_S214R	0.2153	14.1529	0.0913	2.2588	0.1406	0.0075
218	Q161S_S177W	0.1678	21.9926	0.6705	0.6861	0.2034	0.1344
219	A53T_S177W	0.5864	25.6741	1.8121	0.9362	0.5536	2.4301
220	V49A_Q295L	0.395	2.3805	0.277	0.1062	0.6176	0.001
221	V49A_S214R	0.2034	3.4446	0.0741	1.7704	0.0072	0.0053
222	A53T_Q295F	1.1064	52.6928	1.1825	1.8096	0.9711	0.9881
223	A53T_S214R	1.1626	62.6579	0.1069	2.9573	0.068	0.0177
224	A53T_A161S	0.3052	16.0001	24.5577	2.6147	0.535	6.7362
225	Q161S_Q295F	0.6414	55.4403	0.6309	2.1875	0.7435	0.0564
226	Q161S_Q295L	0.7049	57.0803	0.4619	2.0677	0.6818	0.2445
227	Q16S_S214R	0.6373	24.2694	0.1169	1.989	0.0414	0.0071
228	S214R_Q295F	0.8804	34.6447	0.1255	2.5773	0.0884	0.001
229	WT	0.2208	5.5566	8.7128	0.4774	0.2105	0.0567
230	WT	0.2019	6.6574	11.2225	0.8057	0.3334	0.4059
231	L274V	0.0826	1.6646	3.9537	0.2627	0.0688	0.0329
232	S214T	0.2083	6.712	10.2212	0.9388	0.2872	0.2863
233	S214V	0.1755	5.0328	8.8147	0.6174	0.2149	0.0792
234	S214W	0.0449	0.1535	0.6665	0.0326	0.0087	0.0005
235	S214Y	0.0496	0.5011	0.4133	0.0955	0.0054	0.0088
236	Q161G	0.1208	3.8872	7.4013	0.5613	0.3219	0.0963
237	Q161N	0.221	5.6957	7.523	1.2476	0.4097	0.2463
238	Q161Q	0.2016	5.4929	8.742	0.6879	0.234	0.1869
239	A53M	0.311	9.7583	19.2442	1.1438	0.4805	2.0646
240	A53N	0.2218	2.4624	10.3493	0.3211	0.3024	0.0897
241	A53S	0.3224	8.1922	18.0214	1.0041	0.4861	0.6177
242	A53V	0.7299	14.7985	22.9622	1.3494	1.3611	1.3562
243	V24_A17T_F213M_S214R	0.3521	16.6698	1.1314	4.1319	0.0629	0.1537

244	Q295D	0.5733	18.3969	11.5976	2.4133	1.5527	0.6172
245	Q295K	0.0819	1.6736	2.1622	0.2654	0.1629	0.0108
246	Q295Y	0.2237	7.6066	12.2165	0.8911	0.3371	0.1724
247	A53G	0.1547	2.7595	5.9764	0.24	0.1403	0.0229
248	R228E	0.0515	0.2099	0.1217	0.0622	0.0373	0.0004
249	V36_F123H_L274V_L298A	0.051	0.1485	0.8637	0.0289	0.0137	0.0018
250	A53T_Q161S	0.3657	19.2281	31.4494	3.5463	0.8091	7.6038
251	M106E_Q161S	0.1744	7.49	2.589	0.6149	0.1254	0.0924
252	Q161H	0.9829	109.9146	0.227	5.9319	0.1264	1.1306
253	WT	0.1954	4.6359	7.4486	0.3732	0.1468	0.0272
254	Q161F	0.128	27.5673	7.257	1.5873	0.1279	0.04
255	Q161C	0.158	4.7623	17.4493	0.8952	0.6105	0.0815
256	Q161I	0.2042	9.7125	13.328	1.9642	0.4285	0.1821
257	Q161L	0.2876	18.4053	14.7978	2.3238	0.598	0.1327
258	Q161L	0.2246	10.9114	7.7533	1.1244	0.2879	0.1269
259	Q161M	0.382	7.7445	4.7748	1.1765	0.1278	0.0187
260	Q161R	0.2666	46.6768	1.2868	2.4397	0.1476	0.3194
261	Q161S	0.2517	16.4399	12.1391	1.6485	0.3805	0.3996
262	Q161T	0.1981	13.056	13.825	1.2124	0.39	0.23
263	Q161Y	0.4703	63.2878	1.2931	3.2096	0.0907	0.4055
264	A53D	0.0871	2.9572	4.5759	0.5434	0.0472	0.0281
265	A53E	0.0379	0.1118	0.2432	0.0218	0.0042	0.0004
266	A53K	0.3449	7.4579	20.1422	0.8075	0.6095	0.179
267	A53L	0.3036	13.0793	22.6841	1.2092	0.4786	0.2762
268	A53Q	0.2069	6.3683	16.0499	0.6179	0.2693	0.2291
269	A53Y	0.0732	0.7478	1.257	0.0585	0.0426	0.0032
270	Q295A	1.45	30.5523	5.1674	3.4945	0.5593	2.9359
271	Q295W	0.683	17.6777	1.7024	1.9224	1.0897	0.8575
272	WT	0.4649	8.0525	11.5233	1.0342	0.4325	1.7098
273	L174V	0.339	7.2679	9.5109	0.6455	0.2795	0.1771
274	S214G	0.4628	0.9812	34.2622	0.211	0.3627	0.0795
275	S214P	0.0645	0.0151	0.1079	0.0008	0.0023	0.0053
276	S214Q	0.3381	37.0271	0.2656	0.1828	0.0046	0.0036
277	Q161E	0.1599	2.703	1.7568	0.4425	0.1704	0.0228
278	Q161V	0.129	4.6063	10.6973	1.195	0.4385	0.1816
279	A53C	0.334	9.5731	16.0387	1.0506	0.5481	0.4817
280	A53P	0.0747	0.0451	0.39	0.0083	0.0036	0.0052
281	Y288A	1.2332	70.5504	0.122	5.152	1.3043	0.4672
282	Y288C	0.8582	59.513	0.1853	5.4251	1.0554	0.154
283	Y288D	0.0662	3.2022	0.0347	1.7484	0.0233	0.0039
284	Y288E	0.0559	2.6166	0.0307	1.4904	0.0141	0.0049
285	Y288F	1.0143	67.0312	0.0858	4.7424	0.0819	0.0079
286	Y288G	0.1738	11.8688	0.0676	2.6629	0.0994	0.0016
287	Y288H	1.0257	6.1445	0.7417	0.9226	0.4448	0.0117

288	Y288I	0.9064	71.5931	0.3191	4.4341	0.4007	0.0446
289	Y288K	0.0245	0.6425	0.029	0.3762	0.002	0.0003
290	Y288L	0.7057	84.6669	0.2346	4.7892	0.5323	0.1376
291	Y288M	0.9983	54.3471	0.2693	4.862	0.3085	0.0364
292	Y288P	0.7331	77.4833	0.104	5.5638	0.5515	0.1371
293	Y288R	0.0229	1.1367	0.0766	0.7247	0.0043	0.0032
294	Y288S	0.3611	12.8468	0.0977	3.6178	0.2047	0.0046
295	Y288T	0.6419	54.0312	0.3235	4.2209	0.8107	0.0219
296	Y288W	0.3844	16.3538	0.1631	1.9368	0.0849	0.0016
297	A232S	0.4929	33.1432	2.3783	4.1203	1.2447	0.3794
298	N173D	0.0836	1.9762	0.0376	1.0538	0.005	0.0006
299	N173D	0.0236	0.2661	0.6775	0.0489	0.0074	0.0029
300	M162F	0.1961	3.5943	0.6082	0.4251	0.0244	0.0037
301	WT	0.2123	7.0619	10.2794	0.8529	0.3416	0.7319
302	A17T	0.1242	4.0412	7.8405	0.628	0.5977	0.1111
303	A232S	0.0591	1.9577	8.8043	0.5397	0.0842	0.0704
304	M162F	0.2146	3.7911	0.256	0.6318	0.0476	0.0124
305	WT	0.282	9.093	15.161	1.181	0.452	0.88
306	A232S	0.431	32.214	2.462	4.182	3.258	0.477
307	A232S	0.393	30.338	2.061	3.897	3.301	0.713
308	S214A	0.305	0.96	15.595	0.525	0.216	0.317
309	S214A	0.36	1.376	18.837	0.706	0.272	0.143
310	S214Q	0.375	36.474	0.344	0.248	0.006	0.039
311	S214Q	0.33	30.356	0.229	0.176	0.016	0.024
312	Q161E	0.246	3.219	2.183	0.636	0.3	0.117
313	Y288N	0.217	4.42	0.16	1.786	0.078	0.003

[0222] The amount of each prenylation product was measured by HPLC. FIG. 2 shows a heatmap of the HPLC areas of each prenylation product generated using OA as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time with the exception of CBGA and 5-GOA which are labeled by molecule name. Enzyme variants are labeled by ID# as listed in Table 4.

Example 4: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using OA as substrate and FPP as donor

[0223] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to "breakdown" analysis. "Breakdown" analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. "Breakdown" variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position.

[0224] The wild type Orf2 prenylation reaction using OA as substrate and FPP as donor produces 4 products as detected by HPLC. The respective retention times of these products are approximately 8.4 [CBFA], 8.8 [5-FOA], 9.9, and 11.1 minutes.

[0225] Table 5 provides a summary of the prenylation products produced from OA and FPP, their retention times, and the hypothesized prenylation site on OA. FIG. 18 shows the predicted chemical structures of the respective prenylation products.

Table 5: Predicted prenylation products of Orf2 or Orf2 Mutants when using OA as substrate and FPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
RBI-56	OA	FPP	2-O	11.127
UNK5	OA	FPP	4-O	9.912
RBI-14 (CBFA)	OA	FPP	3-C	8.362
RBI-16 (5-FOA)	OA	FPP	5-C	8.805

[0226] Table 6 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using OA as substrate and FPP as donor. Table 6 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 6: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using OA as substrate and FPP as donor

ID#	Mutations	CBFA (8.362)	5-FOA (8.805)	9.912	11.127
0	WT	0.1254	0.3451	0.0109	0.0086
1	V9_Q38G_E112D_F123H	0.0981	1.2392	0.0095	0.0064
2	V17_V49L_F123A_Y283L	0.0211	0.0112	0.0014	0.001
3	V25_L219F_V294N_Q295A	0.4785	0.0627	0.0942	0.0289
4	V33_A17T_C25V_E112G	0.0685	0.1632	0.0101	0.0225
5	V49_G205L_R228E_C230N	0.0203	0.0046	0.001	0.0003

6	V57_C25V_A232S_V271E	0.0203	0.0046	0.0009	0.0001
7	V65_V49A_Q161S_V294A	0.1861	0.0386	0.0164	0.0253
8	V73_V49S_K118Q_S177E	0.0188	0.0373	0.0011	0.0016
9	V81_V49L_D166E_L274V	0.0115	0.0013	0.0006	0.0002
10	V89_Y121W_S177Y_G286E	0.012	0.0008	0.001	0.0005
11	V10_V49A_S177Y_C209G	0.0135	0.005	0.0004	0.0002
12	V26_A53E_A108G_K118N	0.0159	0.0038	0.0012	0.0008
13	V34_A53Q_Y121W_A232S	0.01	0.0021	0.001	0.0009
14	V42_D166E_S177Y_S214F	0.0123	0.0029	0.0005	0.0003
15	V58_K118Q_L174V_R228Q	0.0188	0.0034	0.0002	0.0005
16	V66_C25V_F213M_Y216A	0.0056	0.0015	0.0001	0.0008
17	V74_M106E_Y121W_D166E	0.0176	0.0034	0.0019	0.0003
18	V82_V49S_K119D_F213M	0.0097	0.0016	0.0006	0.0003
19	V90_A17T_F123W_L298A	0.0425	0.0707	0.0096	0.0042
20	V3_V49S_M162A_Y283L	0.0114	0.1739	0.0003	0.0024
21	V11_K118N_K119A_V271E	0.0089	0.0008	0.0005	0.0014
22	V19_V49L_S214R_V271E	0.0105	0.002	0.0008	0.0005
23	V35_A53Q_S177Y_Y288H	0.2502	0.0845	0.0394	0.0183
24	V43_Q161A_M162F_Q295A	0.2689	0.0092	0.021	0.003
25	V51_V49L_K119D_G205M	0.0093	0.0018	0.0003	0.0009
26	V59_V49S_S214G_V294A	0.0174	0.0507	0.0008	0.0033
27	V67_A108G_K119D_L298A	0.0059	0.0014	0.0008	0.0004
28	V75_A53Q_L274V_Q295A	0.0132	0.0047	0.0006	0.001
29	V83_E112D_L219F_V294F	0.1103	1.0019	0.0147	0.0045
30	V91_N173D_F213M_V294F	0.0055	0.01	0.0007	0.0004
31	V4_K118Q_Q161W_S214F	0.0081	0.0014	0.0022	0.0004
32	V20_D227E_C230N_Q295W	0.0115	0.007	0.0007	0.0002
33	V28_A53T_D166E_Q295W	0.101	0.1975	0.0129	0.0021

34	V44_A53E_Q161A_V294N	0.0159	0.0285	0.0015	0.0009
35	WT	0.3691	0.815	0.0637	0.0307
36	WT	0.3563	0.746	0.0509	0.0303
37	V52_K119A_S214G_L298A	0.0227	0.0155	0.0021	0.0008
38	V60_E112D_K119A_N173D	0.036	0.0026	0.0003	0.0012
39	V68_K118N_C209G_R228Q	0.0296	0.0031	0.0002	0.0004
40	V76_V49A_F123A_Y288H	0.0225	0.0012	0.0014	0.0011
41	V84_F123H_L174V_S177E	0.1191	0.1545	0.0127	0.0057
42	V92_A53T_E112D_G205M	0.2532	2.6287	0.0476	0.0352
43	V69_A53T_M106E_Q161S	0.1155	0.1727	0.0134	0.0045
44	V60_E112D_K119A_N173D	0.0278	0.0034	0.002	0.0003
45	V62_A53T_N173D_S214R	0.0281	0.0004	0.0096	0.0014
46	V70_Q38G_D166E_Q295A	0.1879	0.2481	0.0211	0.0131
47	V78_K119D_Q161W_L298Q	0.0334	0.0077	0.0005	0.0002
48	V94_A17T_V49A_C230N	0.023	0.0018	0.001	0.0005
49	V15_A53E_F213M_R228Q	0.0235	0.0153	0.0001	0.0002
50	V23_L219F_Y283L_L298W	0.1093	1.4518	0.0013	0.0044
51	V31_D227E_R228E_L298Q	0.01	0.0044	0.0008	0.0012
52	V39_A53T_K118N_S214F	0.0369	0.0042	0.0008	0.0017
53	V47_K118Q_F123A_R228E	0.008	0.0025	0.0007	0.0005
54	V55_V49S_Y216A_V294N	0.021	0.004	0.0007	0.0005
55	V71_M106E_G205L_C209G	0.0572	0.0039	0.0014	0.0012
56	V79_V49A_Y121W_C230S	0.0212	0.003	0.0023	0.0006
57	V87_S177W_Y288H_V294N	0.0575	0.004	0.0083	0.0017
58	V95_A17T_Q161W_A232S	0.2039	0.0213	0.0124	0.0076
59	V8_K119A_Q161A_R228Q	0.0231	0.0012	0.0012	0.0011
60	V16_A53Q_S177W_L219F	0.2665	0.1223	0.035	0.0001
61	V32_M162A_C209G_Y288H	0.0407	0.0049	0.0017	0.0007

62	V40_S177E_S214R_R228E	0.0542	0.0002	0.0028	0.0021
63	V48_V49L_E112D_G286E	0.0326	0.0023	0.0003	0.0162
64	V56_F123A_M162F_S214G	0.0396	0.4291	0.002	0.0004
65	V72_E112G_G205M_L298W	0.2705	3.1689	0.0161	0.0122
66	V80_M162A_N173D_S214F	0.0213	0.0972	0.0016	0.0006
67	V88_A108G_Q161S_G205M	0.0208	0.0167	0.0003	0.003
68	V64_M106E_M162A_Y216A	0.0266	0.0067	0.001	0.0012
69	V63_F123W_M162F_C209G	0.0281	0.003	0.001	0.001
70	V24_A17T_F213M_S214R	0.6667	0.0121	0.166	0.001
71	V36_F123H_L274V_L298A	0.0126	0.0325	0.0008	0.0004
72	WT	0.182	0.337	0.0244	0.0158
73	Q38G_D166E	0.0299	0.0877	0.0024	0.0028
74	Q38G_Q295A	0.2205	0.546	0.0438	0.0287
75	D166E_Q295A	0.1585	0.0333	0.0338	0.0208
76	L219F_V294N	0.2322	0.2744	0.0459	0.0256
77	L219F_Q295A	0.2943	0.0308	0.056	0.0297
78	V294N_Q295A	0.5592	0.6994	0.1025	0.0584
79	A53Q_S177W	0.1762	0.059	0.0164	0.0009
80	A53Q_L219F	0.129	0.4877	0.022	0.0113
81	S177W_L219F	0.1792	0.0469	0.0312	0.001
82	A108G_Q161S	0.0175	0.0087	0.0033	0.0012
83	A108G_G205M	0.0263	0.1237	0.0035	0.0033
84	Q161S_G205M	0.0697	0.0405	0.0074	0.0042
85	F123H_L174V	0.1042	0.6771	0.0176	0.0066
86	F123H_S177E	0.1582	0.2375	0.0296	0.013
87	L174V_S177E	0.3606	1.3093	0.075	0.0057
88	A53T_D166E	0.0895	0.8308	0.0134	0.0086
89	A53T_Q295W	0.8241	1.2303	0.1612	0.0259
90	D166E_Q295W	0.1797	0.1318	0.0345	0.0045

91	A53Q_S177Y	0.0386	0.2353	0.0008	0.001
92	A53Q_Y288H	1.1458	0.1285	0.2604	0.0705
93	S177Y_Y288H	0.2683	0.0491	0.0629	0.0326
94	V49A_Q161S	0.0848	0.0242	0.0043	0.0136
95	V49A_V294A	0.1831	0.1548	0.0187	0.1053
96	Q161S_V294A	0.3405	0.0888	0.0409	0.017
97	A53T_M106E	0.1477	1.1549	0.0278	0.0164
98	A53T_Q161S	0.2004	0.2315	0.0309	0.0102
99	M106E_Q161S	0.0351	0.0166	0.0018	0.0003
100	A53T_K118N	0.0219	0.0473	0.0011	0.0015
101	A53T_S214F	0.419	0.0873	0.0203	0.0021
102	A53T_S214F	0.2654	0.0578	0.0172	0.0003
103	K118N_S214F	0.0175	0.0049	0.0019	0.0005
104	A108G	0.0599	0.1243	0.0055	0.0072
105	A53Q	0.2319	0.6862	0.0317	0.0245
106	A53T	0.3639	1.6305	0.0657	0.0512
107	D166E	0.1258	0.3017	0.0142	0.0142
108	F123H	0.1956	1.2205	0.0267	0.0182
109	G205M	0.1938	0.4822	0.028	0.0239
110	K118N	0.0428	0.0311	0.0033	0.0041
111	L219F	0.238	0.3455	0.0294	0.0182
112	M106E	0.1225	0.22	0.016	0.009
113	Q161S	0.2429	0.0598	0.018	0.0124
114	Q295A	0.8382	0.0761	0.1166	0.0875
115	Q295W	1.9456	0.8959	0.3114	0.0499
116	Q38G	0.1711	0.2818	0.0205	0.0148
117	S177E	0.4291	0.7748	0.0814	0.0097
118	S177W	0.413	0.063	0.0516	0.0068

119	S177Y	0.1073	0.3639	0.0116	0.0073
120	S214F	0.1109	0.0123	0.0049	0.0003
121	V294A	0.6188	0.7227	0.116	0.0796
122	V294N	0.4098	0.4108	0.0658	0.0468
123	V49A	0.1007	0.1018	0.0078	0.0547
124	Y288H	0.8326	0.0421	0.2104	0.0651
125	L174V	0.1059	0.2303	0.0054	0.0001
126	K118Q	0.0552	0.4075	0.0026	0.0059
127	K119Q	0.0324	0.0065	0.0002	0.0009
128	M162A	0.2073	1.955	0.0047	0.0002
129	Q161A	0.1357	0.275	0.018	0.0002
130	K119D	0.4031	0.9068	0.0716	0.0345
131	G205L	0.0817	0.1663	0.0084	0.0028
132	F123A	0.2341	0.691	0.0132	0.0055
133	K118N	0.0586	0.0546	0.0038	0.0052
134	Q161W	0.0338	0.0509	0.0005	0.0004
135	D227E	0.1383	0.4327	0.0148	0.0085
136	L274V	0.0556	0.097	0.0057	0.0038
137	S214G	0.1263	1.6669	0.0083	0.0591
138	Y216A	0.0268	0.0101	0.0003	0.0016
139	F123W	0.0141	0.0016	0.0006	0.0005
140	V271E	0.0421	0.0026	0.003	0.0001
141	N173D	0.021	0.0092	0.0001	0.0008
142	R228Q	0.024	0.0132	0.0022	0.001
143	M162F	0.1353	0.0125	0.0066	0.0009
144	A232S	0.5723	0.1803	0.1545	0.0491
145	C230S	0.0757	0.1728	0.0066	0.0021
146	V294F	0.4803	2.0674	0.0981	0.0128

147	Y283L	0.0723	0.2549	0.0074	0.0055
148	S214R	2.6729	0.0111	1.0301	0.0001
149	G286E	0.0452	0.0018	0.0113	0.001
150	R228E	0.0207	0.0028	0.0007	0.0015
151	A53T_V294A	1.2801	4.4539	0.3203	0.1968
152	A53T_Q161S_V294A	0.6708	0.4255	0.0842	0.0324
153	A53T_Q161S_V294N	0.4581	0.2995	0.061	0.0189
154	A53T_Q295A	1.5217	0.4336	0.2762	0.1661
155	Q161S_V294A_Q295A	2.5023	0.1045	0.3414	0.1399
156	A53T_Q161S_Q295A	1.3626	0.1371	0.2047	0.105
157	A53T_V294A_Q295A	4.3273	1.3268	0.6703	0.4987
158	A53T_Q161S_V294A_Q295A	2.8853	0.3387	0.4617	0.1904
159	A53T_Q161S_V294N_Q295A	1.4672	0.2062	0.1978	0.0576
160	A53T_Q295W	1.6479	2.2176	0.3642	0.0765
161	Q161S_V294A_Q295W	1.2893	0.2403	0.1614	0.0301
162	A53T_Q161S_Q295W	1.4412	0.6035	0.1903	0.0435
163	A53T_V294A_Q295W	1.2563	2.3283	0.3211	0.045
164	A53T_Q161S_V294A_Q295W	1.1775	0.5735	0.1538	0.0295
165	A53T_Q161S_V294N_Q295W	1.444	0.6805	0.2147	0.0557
166	Q295A	1.2973	0.1366	0.2239	0.1282
167	Q295C	2.4432	0.2588	0.3477	0.6523
168	Q295E	0.1742	0.0291	0.0165	0.0091
169	Q295F	9.5776	0.161	0.9022	0.3048
170	Q295G	0.5974	0.154	0.0941	0.0493
171	Q295H	0.9041	0.8249	0.1998	0.0832
172	Q295I	1.6234	0.0823	0.4239	0.0799
173	Q295L	4.7247	0.1617	0.7663	0.1983

174	Q295M	5.4574	0.357	0.9295	0.2639
175	Q295N	0.4216	0.2727	0.0595	0.0407
176	Q295P	0.352	0.096	0.0509	0.0497
177	Q295R	0.0571	0.0472	0.0006	0.0008
178	Q295S	0.3584	0.1364	0.049	0.0364
179	Q295T	0.1858	0.0365	0.0178	0.0117
180	Q295V	3.1982	0.1284	0.5856	0.2998
181	Q295W	2.2854	1.119	0.4268	0.0829
182	Q295Q	0.3695	0.6915	0.0572	0.0353
183	Q295D	0.5936	0.6559	0.0506	0.0265
184	Q295K	0.043	0.0377	0.0026	0.0021
185	Q295Y	0.2928	0.6636	0.0299	0.0143
186	S214K	0.0621	0.0164	0.005	0.001
187	S214D	0.1715	0.3347	0.0508	0.0009
188	S214E	0.1067	0.0137	0.0037	0.0002
189	S214F	0.143	0.0128	0.0042	0.001
190	S214H	1.2012	0.0141	0.2169	0.0007
191	S214I	0.2546	0.1171	0.0358	0.0019
192	S214L	0.0477	0.0039	0.0007	0.0003
193	S214M	0.0765	0.0092	0.0046	0.0007
194	S214N	0.1199	0.2288	0.0049	0.0016
195	S214R	2.4199	0.0085	0.8583	0.0006
196	S214T	0.3093	0.6422	0.0376	0.007
197	S214V	0.2486	0.5062	0.0275	0.0116
198	S214W	0.0202	0.0153	0.0013	0.0005
199	S214Y	0.0297	0.0058	0.0024	0.001
200	S214C	97.6105	0.0363	0.0584	0.0036

201	S214P	100.4364	0.0068	0.0005	0.0002
202	Q161D	0.0711	0.0036	0.0065	0.0036
203	Q161P	0.0752	0.0658	0.0056	0.0031
204	Q161W	0.0553	0.0372	0.0027	0.0023
205	Q161A	0.1471	0.346	0.0073	0.0015
206	Q161H	11.4099	0.1017	0.4454	0.0085
207	Q161K	0.3091	0.1306	0.0115	0.0005
208	Q161G	0.0685	0.0403	0.0067	0.0003
209	Q161N	0.1186	0.232	0.0126	0.0044
210	Q161Q	0.2108	0.3526	0.0156	0.0107
211	Q161C	0.0424	0.0787	0.009	0.0016
212	Q161F	0.3662	0.0404	0.1285	0.001
213	Q161I	0.0683	0.1596	0.0195	0.001
214	Q161L	0.16	0.1715	0.0323	0.0027
215	Q161L	0.1361	0.1589	0.024	0.0024
216	Q161M	0.1041	0.0444	0.0587	0.001
217	Q161R	0.5209	0.0589	0.013	0.0005
218	Q161S	0.0787	0.0319	0.0053	0.0007
219	Q161T	0.0924	0.1156	0.0088	0.0001
220	Q161Y	0.5214	0.0721	0.0747	0.0006
221	A53I	0.16	0.2559	0.0183	0.0403
222	A53R	0.0876	0.2113	0.0131	0.0157
223	A53T	0.373	2.0303	0.0699	0.0515
224	A53W	0.05	0.0607	0.0023	0.0033
225	A53F	0.0628	0.0091	0.0006	0.0006
226	A53H	0.0284	0.0202	0.001	0.0004
227	A53M	0.2911	0.9775	0.0241	0.0108
228	A53N	0.0364	0.1413	0.0025	0.0029

229	A53S	0.2729	0.8235	0.0326	0.0168
230	A53V	0.6655	1.0265	0.0983	0.0886
231	A53G	0.0926	0.2434	0.008	0.0037
232	A53D	0.0183	0.1077	0.0019	0.0007
233	A53E	0.0084	0.0033	0.0038	0.0001
234	A53K	0.0685	0.3496	0.0066	0.0013
235	A53L	0.1834	0.7254	0.0157	0.007
236	A53Q	0.0863	0.467	0.0096	0.0023
237	A53Y	0.0061	0.0079	0.0011	0.0006
238	A53P	95.3201	0.0071	0.0022	0.001
239	S177W_Q295A	10.3347	0.0119	0.4254	0.018
240	S177W_S214R	1.0699	0.006	0.2282	0.0008
241	Q161S_S177W	1.1284	0.0491	0.0608	0.0008
242	A53T_S177W	0.6999	0.4495	0.0652	0.0016
243	V49A_Q295L	0.0897	0.0156	0.0022	0.0027
244	V49A_S214R	0.9325	0.0111	0.1636	0.0004
245	A53T_Q295F	6.8272	0.4389	0.7712	0.0424
246	A53T_S214R	3.1427	0.0235	0.8942	0.001
247	A53T_A161S	0.1628	0.2227	0.0092	0.0024
248	Q161S_Q295F	5.0185	0.0458	0.2117	0.0855
249	Q161S_Q295L	5.2287	0.0436	0.2094	0.0662
250	Q16S_S214R	0.2075	0.0096	0.0381	0.0002
251	S214R_Q295F	10.6601	0.0249	0.8303	0.0009
252	WT	0.2877	0.5108	0.0499	0.0352
253	WT	0.3659	0.8081	0.0581	0.0309
254	WT	0.1106	0.2415	0.0156	0.0072
255	WT	0.2593	0.5299	0.0243	0.0071

256	WT	0.2069	0.4128	0.017	0.005
257	WT	0.1014	0.2634	0.0143	0.0028

[0227] The amount of each prenylation product was measured by HPLC. FIG. 3 shows a heatmap of the HPLC areas of each prenylation product generated using OA as substrate and FPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 6.

Example 5: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using O as substrate and GPP as donor

[0228] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to “breakdown” analysis. “Breakdown” analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. “Breakdown” variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position.

[0229] The wild type Orf2 prenylation reaction using O as substrate and GPP as donor produces 3 products as detected by HPLC. The respective retention times of these products are approximately 7.095 [CBG], 7.745 [5-GO], and 8.563 minutes.

[0230] Table 7A provides a summary of the prenylation products produced from O and GPP, their retention times, and the hypothesized prenylation site on O. FIG. 19 shows the predicted chemical structures of the respective prenylation products.

Table 7A: Predicted prenylation products of Orf2 or Orf2 Mutants when using O as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
RBI-03 (5-GO)	O	GPP	1-C/5-C	7.745
RBI-20	O	GPP	2-O/4-O	8.563
RBI-01 (CBG)	O	GPP	3-C	7.095

[0231] Tables 7B-7D provide NMR data of proton and carbon chemical shifts for CBG with (a) HSQC, (b) HMBC correlation and (c) final carbon and proton NMR assignments. The carbon and proton NMR assignments for CBG are shown in Figure 83.

Table 7B: Proton NMR assignments for CBG

PROTON					MULTIPLICITY	
Shift	Area	Protons	C Assignment	HSQC-DEPT	Options	Actual
0.861	3.3	3	C5"	0.85	CH1 or CH3	CH3
1.245	2.09	2	C3" Or C4"	1.23	CH2	CH2
1.288	1.97	2	C3" Or C4"	1.27	CH2	CH2
1.474	2.08	2	C2"	1.46	CH2	CH2
1.535	2.76	3	C10	1.52	CH1 or CH3	CH3
1.608	2.99	3	C9	X	X	CH3
1.695	2.74	3	C8	1.68	CH1 or CH3	CH3
1.887	1.86	2	C5	1.88	CH2	CH2
1.988	1.87	2	C4	1.98	CH2	CH2
2.324	2.01	2	C1"	2.31	CH2	CH2
3.13	1.88	2	C1	3.12	CH2	CH2
5.051	1	1	C6	5.04	CH1 or CH3	CH
5.167	1.09	1	C2	5.16	CH1 or CH3	CH
6.084	2.12	2	C1' + C5'	6.08	CH1 or CH3	CH2
8.857	2.01	2	C2' + C4'		X	X
	H Sum:	32				

Table 7C: Carbon NMR assignments for CBG

CARBON			
Shift	Assignment	Carbon ct.	NMR Predictions
14.39	C5"	1	14.1
16.37	C8	1	16.4
18	C9	1	18.6
22.26	C1	1	21.9
22.47	C4"	1	22.7
25.95	C10	1	24.6
26.73	C5	1	26.4
30.96	C2"	1	30.9
31.36	C3"	1	31.4
35.48	C1"	1	36.3
38.543	C4	1	39.7
106.7	C1' + C5'	2	107.5
111.89	C3'	1	113.4
124.09	C2	1	122.3
124.68	C6	1	123.5
131.04	C7	1	132
133.08	C3	1	136.5
140.637	C6'	1	143.2
147.7	C4' Or C2'	1	155.9
156.14	C4' Or C2'	1	155.9
	SUM	21	

Table 7D: HMBC for sample CBG

1D C							
C Shift	Assignment	Associated Proton Shifts			Proton List		
14.39	C5"	0.75			C3"		
16.37	C8	1.89	5.16		C5	C2	
18	C9	1.42	5.05		C2"	C6	
22.26	C1	X			X		
22.47	C4"	0.86			C3"		
25.95	C10	X			X		
26.73	C5	1.88			C5		
30.96	C2"	X			X		
31.36	C3"	1.47	1.29	2.32	C2"	C3" Or C4"	C1"
35.48	C1"	1.47	6.08		C2"	C1' + C5'	
38.543	C4	1.77	5.16		C8	C2	
106.7	C1' + C5'	8.86	2.33	6.08	C2' + C4'	C1"	C1' + C5'
111.89	C3'	3.12	8.86	6.08	C1	C2' + C4'	C1' + C5'
124.06	C2	3.12			C1		
124.68	C6	1.6	1.89		C9		
131.04	C7	1.53			C10		
133.08	C3	1.69	3.12	1.87	C8	C1	C5
140.637	C6'	2.32	1.46		C1"	C2"	
154.7	C4' Or C2'	8.86			C2' + C4'		
156.14	C4' Or C2'	3.12	8.86		C1	C2' + C4'	

[0232] Table 8 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using O as substrate and GPP as donor. Table 8 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 8: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using O as substrate and GPP as donor

ID#	**Mutations**	**CBG (7.095)**	**5GO (7.745)**	**8.563**
1	V9_Q38G_E112D_F123H	0.3065	0.4033	0.2568
2	V17_V49L_F123A_Y283L	0.1942	0.2095	0.1733
3	V25_L219F_V294N_Q295A	0.5735	0.4173	0.1966
4	V33_A17T_C25V_E112G	0.3182	0.3457	0.2034
5	V49_G205L_R228E_C230N	0.194	0.2399	0.1871
6	V57_C25V_A232S_V271E	0.1891	0.2273	0.1895
7	V65_V49A_Q161S_V294A	0.703	0.8977	0.2565
8	V73_V49S_K118Q_S177E	0.2141	0.2994	0.2057
9	V81_V49L_D166E_L274V	0.2202	0.2631	0.2112

10	V89_Y121W_S177Y_G286E	0.2499	0.3016	0.243
11	V10_V49A_S177Y_C209G	0.2202	0.2682	0.2271
12	V26_A53E_A108G_K118N	0.2397	0.2981	0.2248
13	V34_A53Q_Y121W_A232S	0.2661	0.3326	0.2679
14	V42_D166E_S177Y_S214F	0.2696	0.3306	0.2763
15	V58_K118Q_L174V_R228Q	0.3098	0.3717	0.3178
16	V66_C25V_F213M_Y216A	0.2775	0.3398	0.2835
17	V74_M106E_Y121W_D166E	0.2878	0.3451	0.2929
18	V82_V49S_K119D_F213M	0.2217	0.2841	0.235
19	V90_A17T_F123W_L298A	0.2115	0.2931	0.1939
20	V3_V49S_M162A_Y283L	0.2213	0.7384	0.2139
21	V11_K118N_K119A_V271E	0.2744	0.3159	0.2583
22	V19_V49L_S214R_V271E	0.2545	0.3185	0.258
23	V35_A53Q_S177Y_Y288H	0.371	0.703	0.2559
24	V43_Q161A_M162F_Q295A	1.8681	0.787	0.3027
25	V51_V49L_K119D_G205M	0.2333	0.3044	0.2386
26	V59_V49S_S214G_V294A	0.2284	0.4829	0.2326
27	V67_A108G_K119D_L298A	0.211	0.2503	0.1988
28	V75_A53Q_L274V_Q295A	0.2286	0.298	0.2172
29	V83_E112D_L219F_V294F	0.8983	0.8995	0.3051
30	V91_N173D_F213M_V294F	0.2854	0.6328	0.2284
31	V4_K118Q_Q161W_S214F	0.2761	0.3493	0.235
32	V20_D227E_C230N_Q295W	0.2291	0.2973	0.2118
33	V28_A53T_D166E_Q295W	0.405	0.6084	0.2292
34	V44_A53E_Q161A_V294N	0.5894	0.7298	0.2042
35	V52_K119A_S214G_L298A	0.1708	0.2959	0.1305
36	V60_E112D_K119A_N173D	0.1903	0.2403	0.1585
37	V68_K118N_C209G_R228Q	0.2002	0.2477	0.1604
38	V76_V49A_F123A_Y288H	0.136	0.1827	0.1209
39	V84_F123H_L174V_S177E	0.2886	0.3135	0.1886
40	V92_A53T_E112D_G205M	1.5896	1.2489	0.204
41	V69_A53T_M106E_Q161S	3.1916	1.3656	0.1869
42	V60_E112D_K119A_N173D	0.2314	0.2803	0.1361
43	V62_A53T_N173D_S214R	0.2207	0.2818	0.1661
44	V70_Q38G_D166E_Q295A	0.3134	0.3094	0.1762
45	V78_K119D_Q161W_L298Q	0.2054	0.2715	0.1388
46	V94_A17T_V49A_C230N	0.2159	0.2812	0.1529
47	V15_A53E_F213M_R228Q	0.2077	0.302	0.1532
48	V23_L219F_Y283L_L298W	0.2448	0.4232	0.143
49	V31_D227E_R228E_L298Q	0.1989	0.2764	0.1624
50	V39_A53T_K118N_S214F	0.2765	0.3188	0.1231
51	V47_K118Q_F123A_R228E	0.2329	0.3136	0.153
52	V55_V49S_Y216A_V294N	0.2206	0.3124	0.147
53	V71_M106E_G205L_C209G	0.2391	0.323	0.164

54	V79_V49A_Y121W_C230S	0.2207	0.299	0.1552
55	V87_S177W_Y288H_V294N	0.2266	0.3002	0.1614
56	V95_A17T_Q161W_A232S	1.0678	0.4634	0.1861
57	V8_K119A_Q161A_R228Q	0.24	0.3273	0.1598
58	V16_A53Q_S177W_L219F	0.4683	0.4481	0.2006
59	V32_M162A_C209G_Y288H	0.1947	0.2801	0.1537
60	V40_S177E_S214R_R228E	0.2652	0.3543	0.2028
61	V48_V49L_E112D_G286E	0.3004	0.3258	0.1862
62	V56_F123A_M162F_S214G	0.2201	0.3228	0.1673
63	V72_E112G_G205M_L298W	0.355	0.6902	0.1787
64	V80_M162A_N173D_S214F	0.3072	0.5322	0.1732
65	V88_A108G_Q161S_G205M	0.4996	0.4828	0.2088
66	V64_M106E_M162A_Y216A	0.1974	0.246	0.1603
67	V63_F123W_M162F_C209G	0.0917	0.1395	0.1304
68	V24_A17T_F213M_S214R	0.3021	0.3802	0.2112
69	V36_F123H_L274V_L298A	0.1982	0.2554	0.1354
70	Q38G_D166E	0.2704	0.3073	0.1579
71	Q38G_Q295A	0.8428	0.6827	0.2238
72	D166E_Q295A	0.5788	0.4059	0.1779
73	L219F_V294N	1.186	0.9075	0.2028
74	L219F_Q295A	0.5993	0.4027	0.1356
75	V294N_Q295A	1.9865	1.1733	0.2227
76	A53Q_S177W	0.4935	0.3688	0.1697
77	A53Q_L219F	0.4909	0.5052	0.1725
78	S177W_L219F	0.4067	0.3348	0.1599
79	A108G_Q161S	0.4665	0.4112	0.2023
80	A108G_G205M	0.3021	0.3478	0.181
81	Q161S_G205M	0.9204	0.5004	0.1039
82	F123H_L174V	0.2572	0.3425	0.1635
83	F123H_S177E	0.3424	0.3082	0.1772
84	L174V_S177E	0.7942	0.6381	0.2163
85	A53T_D166E	0.6316	0.6992	0.2206
86	A53T_Q295W	1.3244	1.2364	0.1855
87	D166E_Q295W	0.3642	0.5063	0.1428
88	A53Q_S177Y	0.5035	0.607	0.189
89	A53Q_Y288H	0.4187	1.1803	0.1699
90	S177Y_Y288H	0.3168	0.4557	0.1558
91	V49A_Q161S	0.7008	1.0062	0.2164
92	V49A_V294A	0.4574	0.6907	0.1735
93	Q161S_V294A	2.8501	1.1301	0.1967
94	A53T_M106E	2.0177	1.5187	0.237
95	A53T_Q161S	3.0733	1.3385	0.2506
96	M106E_Q161S	0.951	0.5947	0.1947
97	A53T_K118N	0.2334	0.3517	0.1228

98	A53T_S214F	6.4229	1.4309	0.4131
99	A53T_S214F	4.1685	1.0642	0.3362
100	K118N_S214F	0.2231	0.2519	0.1262
101	A108G	0.1192	0.1475	0.1146
102	A53Q	0.51	0.4795	0.1649
103	A53T	1.4988	1.0189	0.1734
104	D166E	0.3514	0.3681	0.1763
105	F123H	0.1357	0.1856	0.1306
106	G205M	0.6559	0.4994	0.1613
107	K118N	0.1983	0.2496	0.1537
108	L219F	0.4095	0.3989	0.1777
109	M106E	0.5112	0.435	0.1682
110	Q161S	1.4626	0.7537	0.1814
111	Q295A	1.0116	0.4067	0.1371
112	Q295W	0.8401	0.7437	0.1526
113	Q38G	0.336	0.3076	0.1473
114	S177E	0.5987	0.4703	0.1895
115	S177W	0.3765	0.2756	0.1434
116	S177Y	0.3691	0.3892	0.1566
117	S214F	1.6238	0.4704	0.1941
118	V294A	1.3204	0.8556	0.198
119	V294N	1.1311	0.7239	0.159
120	Y288H	0.2888	0.4703	0.1331
121	V49A	0.3386	0.4876	0.1878
122	Q295A	1.2977	0.5914	0.2119
123	Q295W	1.1485	1.066	0.259
124	L174V	0.2755	0.1437	0.0296
125	K118Q	0.1393	0.3647	0.1061
126	K119Q	0.063	0.0895	0.0623
127	M162A	0.0977	0.564	0.1246
128	Q161A	0.7044	0.5595	0.1193
129	K119D	0.7113	0.533	0.1274
130	G205L	0.1302	0.1256	0.0665
131	F123A	0.146	0.2765	0.1032
132	K118N	0.1298	0.2326	0.1285
133	Q161W	1.4229	0.329	0.1344
134	D227E	0.3969	0.3413	0.1133
135	L274V	0.1867	0.1766	0.1077
136	S214G	0.171	0.7571	0.1514
137	Y216A	0.1428	0.1533	0.1115
138	F123W	0.0811	0.1105	0.0873
139	V271E	0.1035	0.1322	0.1266
140	N173D	0.1867	0.1776	0.112
141	R228Q	0.1531	0.1972	0.1241

142	M162F	0.6655	0.3168	0.1161
143	A232S	1.6761	0.6551	0.1652
144	C230S	0.186	0.1798	0.1093
145	V294F	0.8439	0.6396	0.1292
146	Y283L	0.3707	0.3754	0.12
147	S214R	0.18	0.1577	0.1146
148	G286E	0.0963	0.1359	0.114
149	R228E	0.5308	0.4217	0.2098
150	A53T_V294A	4.3154	2.3259	0.3126
151	A53T_Q161S_V294A	5.3751	1.7353	0.2743
152	A53T_Q161S_V294N	4.8641	1.667	0.2765
153	A53T_Q295A	2.4689	0.8374	0.2766
154	Q161S_V294A_Q295A	5.1846	1.1046	0.314
155	A53T_Q161S_Q295A	6.5383	1.0823	0.3038
156	A53T_V294A_Q295A	4.2878	1.2019	0.288
157	A53T_Q161S_V294A_Q295A	6.8655	1.0392	0.3564
158	A53T_Q161S_V294N_Q295A	5.4091	1.0492	0.2815
159	A53T_Q295W	2.0002	1.6157	0.2086
160	Q161S_V294A_Q295W	2.6247	1.1964	0.2493
161	A53T_Q161S_Q295W	4.2451	1.5899	0.2071
162	A53T_V294A_Q295W	2.1217	1.2914	0.2998
163	A53T_Q161S_V294A_Q295W	4.1157	1.3136	0.2515
164	A53T_Q161S_V294N_Q295W	4.1445	1.2834	0.2092
165	Q295C	1.1112	0.6108	0.2639
166	Q295E	0.3485	0.5615	0.2689
167	Q295F	1.8946	1.0029	0.2393
168	Q295G	2.1139	0.7158	0.2253
169	Q295H	6.6017	2.9599	0.2678
170	Q295I	0.3872	0.4097	0.2505
171	Q295L	0.8165	0.5339	0.279
172	Q295M	2.2673	0.8435	0.253
173	Q295N	0.6222	0.5431	0.21
174	Q295P	0.3436	0.3472	0.1892
175	Q295R	0.2535	0.2964	0.2125
176	Q295S	0.6678	0.5267	0.2261
177	Q295T	0.5404	0.5097	0.2766
178	Q295V	0.4045	0.3997	0.2359
179	Q295D	0.7086	0.6476	0.187
180	Q295K	0.3478	0.418	0.2129
181	Q295Y	0.7029	0.6132	0.1873
182	Q295A	1.2977	0.5914	0.2119
183	Q295W	1.1485	1.066	0.259
184	S214K	0.268	0.1726	0.0856
185	S214C	0.1316	0.1527	0.0315

186	S214D	0.5941	0.4307	0.1566
187	S214E	4.3929	0.724	0.1754
188	S214F	1.7481	0.5769	0.2026
189	S214H	7.3615	0.3826	0.1521
190	S214I	1.1748	0.6441	0.222
191	S214L	1.0532	0.5453	0.1967
192	S214M	1.0082	0.5658	0.2189
193	S214N	1.9276	0.5276	0.2475
194	S214R	0.3476	0.3536	0.1495
195	S214T	0.6615	0.6016	0.198
196	S214V	0.5789	0.5238	0.1768
197	S214W	0.4247	0.3808	0.209
198	S214Y	0.487	0.4005	0.2027
200	S214G	0.0512	0.409	0.0463
201	S214P	0.0252	0.0391	0.0291
202	S214Q	8.4779	0.3014	0.0477
203	Q161D	1.0399	0.4872	0.1899
204	Q161P	0.1064	0.1022	0.0569
205	Q161W	0.7525	0.2667	0.154
206	Q161A	0.3657	0.343	0.0542
207	Q161H	5.7816	0.6558	0.2085
208	Q161K	0.2086	0.2366	0.0705
209	Q161G	1.2012	0.7311	0.1936
210	Q161N	0.8334	0.6653	0.1671
211	Q161Q	0.6143	0.5772	0.202
212	Q161C	1.8896	0.8687	0.2114
213	Q161F	7.2278	0.9128	0.1821
214	Q161I	3.4013	0.9068	0.2392
215	Q161L	5.3283	1.0625	0.1908
216	Q161L	4.9128	1.0446	0.2139
217	Q161M	3.4716	0.6675	0.205
218	Q161R	0.5188	0.5031	0.2032
219	Q161S	0.9388	0.5037	0.1905
220	Q161T	0.9365	0.6197	0.1915
221	Q161Y	5.467	0.9157	0.1691
222	Q161E	0.3212	0.3575	0.04
223	Q161V	0.9976	0.3447	0.054
224	A53I	1.0741	1.236	0.178
225	A53R	0.3302	0.3478	0.1714
226	A53T	1.6163	1.1007	0.2002
227	A53W	0.3676	0.3636	0.1472
228	A53F	0.142	0.1558	0.0545
229	A53H	0.1611	0.1991	0.0889
230	A53M	1.1404	0.9129	0.2386

231	A53N	0.3815	0.4335	0.2113
232	A53S	0.8135	0.696	0.198
233	A53V	1.5411	1.495	0.2286
234	A53G	0.443	0.5263	0.2207
235	A53D	0.3125	0.3139	0.1717
236	A53E	0.1933	0.2199	0.1851
237	A53K	0.5889	0.4933	0.1855
238	A53L	1.9059	1.3577	0.2164
239	A53Q	0.6045	0.5595	0.2097
240	A53Y	0.2169	0.284	0.161
241	A53C	0.415	0.308	0.0351
242	A53P	0.0561	0.0768	0.0527
243	S177W_Q295A	0.694	0.4575	0.0959
244	S177W_S214R	0.1776	0.2114	0.0831
245	Q161S_S177W	0.5912	0.4139	0.1082
246	A53T_S177W	0.9678	0.4316	0.0989
247	V49A_Q295L	0.2342	0.2992	0.0941
248	V49A_S214R	0.2154	0.2196	0.0938
249	A53T_Q295F	2.3515	0.773	0.1202
250	A53T_S214R	0.3473	0.2767	0.077
251	A53T_A161S	3.0213	1.1637	0.1421
252	Q161S_Q295F	2.6242	0.9004	0.1022
253	Q161S_Q295L	3.2538	1.0628	0.1334
254	Q16S_S214R	0.2947	0.2578	0.1119
255	S214R_Q295F	0.371	0.309	0.1276
256	WT	0.4172	0.3183	0.0367
258	WT	0.6835	0.606	0.2548
259	WT	0.7681	0.6793	0.2426
260	WT	0.6153	0.5887	0.2075
261	WT	0.6898	0.5861	0.2092
262	WT	0.5434	0.4288	0.152
263	WT	1.0129	0.8677	0.4139
264	WT	0.7708	0.6776	0.2865
265	WT	0.5786	0.4687	0.1302
266	WT	0.7036	0.5877	0.2007
267	WT	0.4344	0.3771	0.138
268	WT	0.6026	0.3457	0.0419
269	Y288A	1.0046	0.1104	0.152
270	Y288C	1.2257	0.2055	0.0993
271	Y288D	0.0238	0.0267	0.0221
272	Y288E	0.0181	0.0277	0.0216
273	Y288F	4.0602	0.9402	0.0843
274	Y288G	0.0974	0.0319	0.0176
275	Y288H	0.0747	0.2353	0.0297

276	Y288I	2.3134	0.4259	0.0745
277	Y288K	0.0334	0.0392	0.0242
278	Y288L	3.3977	0.5406	0.1476
279	Y288M	1.904	0.4272	0.053
280	Y288P	1.2987	0.238	0.1338
281	Y288R	0.0087	0.0048	0.0061
282	Y288S	0.1344	0.0574	0.0208
283	Y288T	1.3149	0.2483	0.0461
284	Y288W	0.6476	0.1843	0.031
285	A232S	1.3557	0.4728	0.0589
286	N173D-S214R	0.0034	0.006	0.0057
287	N173D	0.0309	0.0329	0.0145
288	M162F	0.427	0.1507	0.0417
289	Y288Y	0.3693	0.2484	0.0316
290	A17T	0.2115	0.1411	0.0301
291	A232S	1.2313	0.4976	0.0603
292	M162F-Q295A	1.4625	0.5356	0.0731
293	WT	0.203	0.179	0.036
294	A232S	0.195	0.123	0.056
295	A232S	0.192	0.119	0.05
296	S214A	0.128	0.196	0.047
297	S214A	0.144	0.229	0.047
298	S214Q	9.114	0.347	0.041
299	S214Q	8.816	0.41	0.057
300	Q161E	0.235	0.262	0.046
301	Y288N	0.203	0.197	0.158

[0233] The amount of each prenylation product was measured by HPLC. FIG. 4 shows a heatmap of the HPLC areas of each prenylation product generated using O as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 8.

Example 6: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using DVA as substrate and GPP as donor

[0234] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 .

Clones that exhibited improved function relative to the wild type enzyme were subjected to "breakdown" analysis. "Breakdown" analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. "Breakdown" variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position.

[0235] The wild type Orf2 prenylation reaction using DVA as substrate and GPP as donor produces 6 products as detected by HPLC. The respective retention times of these products are approximately 5.28, 6.39, 6.46, 7.31, 7.85, and 10.79 minutes.

[0236] Table 9A provides a summary of the prenylation products produced from DVA and GPP, their retention times, and the hypothesized prenylation site on DVA. FIG. 20 shows the predicted chemical structures of the respective prenylation products.

Table 9A: Predicted prenylation products of Orf2 or Orf2 Mutants when using DVA as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
RBI-24	DVA	GPP	CO	5.28
RBI-28	DVA	GPP	2-O	7.847
UNK11	DVA	GPP	4-O	7.313
RBI-26	DVA	GPP	3-C	6.39
RBI-27	DVA	GPP	5-C	6.46
RBI-29	DVA	GPP	3-C + 5-C	10.187

[0237] Tables 9B-9D provide NMR data of proton and carbon chemical shifts for CBGVA with (a) HSQC, (b) HMBC correlation and (c) final carbon and proton NMR assignments (the HMBC "Proton list" column in all NMR assignment tables displays protons which are J-Coupled to and within 1-4 carbons of the corresponding carbon in the row). The carbon and proton NMR assignments for CBGVA are shown in Figure 80.

Table 9B: Proton NMR Assignments for CBGVA

PROTON						
Shift	Area	Protons	C Assignment	HSQC-DEPT	Options	Actual
0.89	3.16	3	C3"	.89-.91	CH or CH3	CH3
1.501	2.09	2	C2"	1.5	CH2	CH2
1.52	3.19	3	C9	1.52	CH or CH3	CH3
1.587	2.9	3	C10	1.59	CH or CH3	CH3
1.708	3.12	3	C8	1.71	CH or CH3	CH3
1.897	2.08	2	C4	1.89	CH2	CH2
1.989	2.08	2	C5	2	CH2	CH2
2.755	1.9	2	C1"	2.75	CH2	CH2
3.183	1.97	2	C1	3.19	CH2	CH2
5.03	1	1	C6	5.03	CH or CH3	CH
5.149	1.04	1	C2	5.15	CH or CH3	CH
6.24	0.955	1	C5'	6.24	CH or CH3	CH
10.014	0.906	1	4'OH?	X	X	X
12.597	0.879	1	2'OH?	X	X	X
13.518	0.859	1	COOH?	X	X	X
	H Sum:	28				

Table 9C: Carbon NMR Assignments for CBGVA

CARBON			
Shift	Assignment	Carbon ct.	NMR Predictions
14.62	C3"	1	13.7
16.37	C8	1	16.4
17.98	C9	1	18.6
22.01	C1	1	21.9
25.09	C2"	1	24.1
25.91	C10	1	24.6
26.63	C5	1	26.4
38.35	C1"	1	38.7
39.77	C4	1	39.7
103.58	C1'	1	109.6
110.37	C5'	1	111.9
112.65	C3'	1	113.4
123.04	C2	1	122.3
124.58	C6	1	123.5
131.06	C7	1	132
134.01	C3	1	136.5
144.87	C6'	1	145.6
160.03	C2'	1	160.1
163.27	C4'	1	161.4
174.4	COOH	1	175.9
	C Sum:	20	

Table 9D: HMBC for sample CBGVA

1D C								
C Shift	Assignment	Associated Proton Shifts				Proton List		
14.62	C3"	0.98	0.77	1.49	2.74	C3"	C2"	C1"
16.37	C8	5.14				C2		
17.98	C9	1.41	1.58	1.61		C9	C10	C8
22.01	C1	X						
25.09	C2"	0.88	2.74			C3"	C1"	
25.91	C10	1.47				C9		
26.63	C5	X						
38.35	C1"	0.88	6.23	1.48		C3"	C2"	C5'
39.77	C4	5.14	1.7			C8	C2	
103.58	C1'	6.24	2.73			C1"	C5'	
110.37	C5'	2.75	2.74			C1"		
112.65	C3'	3.17	6.23	10.01		C1	C5'	4'OH?
123.04	C2	1.7	3.17	1.88		C8	C4	C1
124.58	C6	1.9				C5		
131.06	C7	1.99	1.58	1.51		C9	C10	C5
134.01	C3	3.17				C1		
144.87	C6'	2.75				C1"		
160.03	C2'	6.23	10.01	3.17		C1	C5'	4'OH?
163.27	C4'	3.17	3.17			C1		
174.4	COOH	X						

[0238] Tables 9E-9G provide NMR data of proton and carbon chemical shifts for RBI-29 with (a) HSQC, (b) HMBC correlation and (c) final carbon and proton NMR assignments. The carbon and proton NMR assignments for RBI-29 are shown in Figure 81.

Table 9E: Proton NMR assignments for RBI-29.

PROTON					MULTIPLICITY	
Shift	Area	Protons	C Assignment	HSQC-DEPT	Options	Actual
0.926	3.16	3	C3"	0.91	CH or CH3	CH3
1.455	2.23	2	C2"	1.44	CH2	CH2
1.521	3.19	3	C9	1.51	CH or CH3	CH3
1.535	3.19	3	C9'''	1.51	CH or CH3	CH3
1.587	3.11	3	C10	1.58	CH or CH3	CH3
1.602	3.16	3	C10'''	1.58	CH or CH3	CH3
1.717	6.13	6	C8 + C8'''	1.7	CH or CH3	CH3
1.904	2.21	2	C4'''	1.89	CH2	CH2
1.941	2.06	2	C4	1.94	CH2	CH2
2.007	4.25	4	C5+C5'''	2	CH2	CH2
2.752	1.99	2	C1"	2.74	CH2	CH2
3.283	4.09	4	C1 +C1'''	3.26-3.28	CH2	CH2
4.953	1	1	C6'''	4.94	CH or CH3	CH
5.034	2.11	2	C6+ C2'''	5.02	CH or CH3	CH
5.1	1.09	1	C2	5.1	CH or CH3	CH
8.829	1.06	1	4' OH?	X	X	X
12.027	0.829	1	2' OH?	X	X	X
13.508	0.779	1	COOH?	X	X	X
	H Sum:	44				

Table 9F: Carbon NMR assignments for RBI-29.

CARBON			
Shift	Assignment	Carbon ct.	NMR Predictions
15.23	C3"	1	13.7
16.48	C8	1	16.4
16.38	C8'''	1	16.4
17.97	C9	1	18.6
17.99	C9'''	1	18.6
22.52	C1	1	22.2
24.8	C2"	1	24.4
25.1	C1'''	1	25.1
25.91	C10	1	24.6
25.94	C10'''	1	24.6
26.53	C5	1	26.4
26.62	C5'''	1	26.4
32.95	C1"	1	33.6
39.66	C4'''	1	39.7
39.77	C4	1	39.7
106.12	C1'	1	106.3
113.63	C3'	1	113.3
123.11	C2	1	122.3
120.12	C2'''	1	122.3
124.53	C6	1	123.5
124.58	C6'''	1	123.5
124.61	C5'	1	125.1
131.08	C7''' + C7?	2	132
133.64	C3	1	136.5
134.26	C3'''	1	136.5
142.07	C6'	1	140.7
157.69	C2'	1	157.1
159.94	C4'	1	158.5
174.3	COOH	1	173.2
	C SUM:	30	

Table 9G: HMBC for sample RBI-29.

1D C									
C Shift	Assignment	Associated Proton Shifts				Proton List			
15.23	C3''	2.76	0.82	1.03	1.45	C3''	C2''	C1''	
16.38	C8'''	4.95	1.61			C6'''	C10'''		
16.48	C8	5.1				C2			
17.97	C9	5.03				C6+ C2'''			
17.99	C9'''	5.03				C6+ C2'''			
24.8	C2''	2.77	0.93	2.74		C3''	C1''		
25.91	C10	5.04				C6+ C2'''			
25.94	C10'''	5.04				C6+ C2'''			
26.53	C5	1.94				C4			
32.95	C1''	1.45	0.92			C3''	C2''		
39.68	C4'''	2.02	4.95	1.72		C8'''	C5'''	C6'''	
39.77	C4	5.1				C2			
106.12	C1'	2.76	2.75	2.74		C1''			
113.63	C3'	8.83	3.29			C1 +C1'''	4' OH		
120.12	C2'''	2.77	3.27	8.83	4.96	C1''	C1'''	C6'''	4' OH
123.11	C2	3.29	1.91	1.72		C8	C4	C1	
124.58	C6'''	1.6				C10'''			
124.61	C5'	3.27				C1 +C1'''			
131.05	C7	2.02				C5+C5'''			
131.07	C7'''	1.53				C9'''			
133.64	C3	3.27				C1			
134.26	C3'''	1.91	1.99			C4'''	C5'''		
142.07	C6'	2.77	3.27			C1''	C1 +C1'''		
157.69	C2'	8.83	3.29			4' OH	C1 +C1'''		
159.94	C4'	3.29				C1 +C1'''			
174.3	COOH	X							

[0239] Table 10 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using DVA as substrate and GPP as donor. Table 10 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 10: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using DVA as substrate and GPP as donor

ID#	Mutations	5.28	RBI-26 (6.39)	RBI-27 (6.46)	7.313	7.847	10.187
1	V9_Q38G_E112D_F123H	0.0116	0.2029	0.2594	0.0497	0.1237	0.0647
2	V17_V49L_F123A_Y283L	0.0157	0.1418	2.4804	0.1067	0.0802	0.0894
3	V17_V49L_F123A_Y283L	0.0139	0.2044	0.2668	0.0436	0.1284	0.1542
4	V25_L219F_V294N_Q295A	0.0601	1.6865	13.135	0.1194	0.2705	1.6922
5	V33_A17T_C25V_E112G	0.1202	1.6759	26.2413	0.1208	0.5823	1.1526
6	V49_G205L_R228E_C230N	0.0031	0.0047	0.4097	0.0818	0.014	0.0257
7	V57_C25V_A232S_V271E	0.0027	0.0414	0.1129	0.0885	0.0254	0.0108
8	V65_V49A_Q161S_V294A	0.3155	34.3128	9.7853	0.2417	1.1597	1.8023
9	V73_V49S_K118Q_S177E	4.4335	2.102	3.771	0.127	2.0094	0.6548
10	V81_V49L_D166E_L274V	0.0166	0.0117	0.0741	0.0819	0.0083	0.003
11	V89_Y121W_S177Y_G286E	0.0024	0.0012	0.1278	0.088	0.0215	0.0022
12	V10_V49A_S177Y_C209G	0.0002	0.0028	0.1592	0.0895	0.0462	0.0007
13	V26_A53E_A108G_K118N	0.0058	0.0096	0.1707	0.0999	0.0253	0.0008

14	V34_A53Q_Y121W_A232S	0.0016	0.0036	0.1282	0.1032	0.0234	0.0009
15	V42_D166E_S177Y_S214F	0.0014	0.0036	0.1247	0.1017	0.0526	0.0006
16	V58_K118Q_L174V_R228Q	0.0153	0.1069	2.2884	0.0987	0.0628	0.0304
17	V66_C25V_F213M_Y216A	0.033	0.4296	1.2759	0.0878	0.11	0.0223
18	V74_M106E_Y121W_D166E	0.0024	0.0021	0.1125	0.1051	0.0206	0.001
19	V82_V49S_K119D_F213M	0.002	0.002	0.1162	0.0957	0.017	0.0005
20	V3_V49S_M162A_Y283L	0.0439	0.3596	5.6085	0.0991	0.4092	0.2363
21	V11_K118N_K119A_V271E	0.0016	0.0003	0.0757	0.0918	0.0114	0.0005
22	V19_V49L_S214R_V271E	0.0091	0.0042	0.1222	0.0938	0.0161	0.0017
23	V35_A53Q_S177Y_Y288H	0.4867	7.087	1.851	0.1799	0.6778	0.1085
24	V43_Q161A_M162F_Q295A	0.0469	1.9058	3.0942	0.1386	0.1927	0.3506
25	V51_V49L_K119D_G205M	0.0049	0.0065	0.1274	0.0986	0.0177	0.0004
26	V59_V49S_S214G_V294A	1.346	1.4137	3.1464	0.1286	0.4483	0.1492
27	V67_A108G_K119D_L298A	0.0087	0.0009	0.1421	0.1074	0.0245	0.0012
28	V75_A53Q_L274V_Q295A	0.0017	0.0095	0.7593	0.1047	0.0231	0.0106
29	V83_E112D_L219F_V294F	0.1046	1.9929	22.6533	0.1317	0.4242	1.5442
30	V91_N173D_F213M_V294F	0.0221	0.2818	24.9336	0.0941	0.2283	0.7472
31	V4_K118Q_Q161W_S214F	0.0034	0.0183	1.8559	0.0908	0.0238	0.032
32	V20_D227E_C230N_Q295W	0.0447	0.2064	0.1871	0.0993	0.0301	0.0041
33	V28_A53T_D166E_Q295W	0.8331	5.0092	9.989	0.1365	0.4405	2.8021
34	V44_A53E_Q161A_V294N	0.0638	2.7024	12.5126	0.1655	0.2401	0.8576
35	V52_K119A_S214G_L298A	0.0438	0.3317	3.2222	0.0437	0.1041	0.1821
36	V60_E112D_K119A_N173D	0.002	0.0247	0.2694	0.0334	0.0163	0.07
37	V68_K118N_C209G_R228Q	0.0015	0.0619	0.0619	0.034	0.018	0.0329
38	V76_V49A_F123A_Y288H	0.0046	0.0409	0.0409	0.0308	0.0134	0.0077
39	V84_F123H_L174V_S177E	0.0692	0.5558	1.707	0.0307	0.0562	0.0889
40	V92_A53T_E112D_G205M	0.152	1.4182	46.3544	0.0583	0.3993	4.3169
41	V36_F123H_L274V_L298A	0.0113	0.0259	0.3661	0.0936	0.0279	0.0265
42	V69_A53T_M106E_Q161S	0.7098	7.8315	28.6444	0.08	1.0245	7.7325
43	V60_E112D_K119A_N173D	0.0118	0.1075	0.6999	0.0245	0.0269	0.2583
44	V62_A53T_N173D_S214R	0.1673	6.4563	6.4563	0.1349	0.1015	0.4075
45	V70_Q38G_D166E_Q295A	0.0959	0.7644	2.0051	0.0329	0.0894	0.2967
46	V78_K119D_Q161W_L298Q	0.0062	0.0157	0.1319	0.0299	0.0207	0.0362
47	V94_A17T_V49A_C230N	0.0076	0.0678	0.3399	0.038	0.0262	0.0205
48	V15_A53E_F213M_R228Q	0.0175	0.1647	12.1818	0.041	0.0742	0.0908
49	V23_L219F_Y283L_L298W	0.0107	0.3286	5.095	0.0347	0.0381	0.0508
50	V31_D227E_R228E_L298Q	0.0009	0.166	2.0097	0.0405	0.0338	0.0061
51	V39_A53T_K118N_S214F	0.0071	0.83	3.0304	0.0318	0.0326	0.0108
52	V47_K118Q_F123A_R228E	0.0079	0.0085	0.1104	0.0303	0.0387	0.0004
53	V55_V49S_Y216A_V294N	0.3685	2.3208	0.5932	0.0451	0.1893	0.2569
54	V63_F123W_M162F_C209G	0.0044	0.0131	0.0645	0.025	0.0185	0.0017
55	V63_F123W_M162F_C209G	0.0118	0.0046	0.1423	0.1068	0.0469	0.045
56	V71_M106E_G205L_C209G	0.006	0.0101	0.045	0.033	0.0215	0.0006
57	V79_V49A_Y121W_C230S	0.0073	0.0103	0.0448	0.0264	0.0218	0.0002
58	V87_S177W_Y288H_V294N	0.0074	0.0245	0.0336	0.0273	0.0197	0.0007
59	V95_A17T_Q161W_A232S	0.1967	39.9177	7.2044	0.0955	0.561	0.2573
60	V8_K119A_Q161A_R228Q	0.0055	0.3249	0.2954	0.0283	0.0291	0.0012
61	V16_A53Q_S177W_L219F	0.0805	8.2799	8.4137	0.0381	0.2414	2.9411
62	V24_A17T_F213M_S214R	0.2644	10.6799	1.9755	0.2939	0.2397	1.415
63	V32_M162A_C209G_Y288H	0.0022	0.008	0.0584	0.0283	0.0258	0.1209
64	V40_S177E_S214R_R228E	0.0105	0.0159	0.0344	0.0318	0.0221	0.0589
65	V48_V49L_E112D_G286E	0.0009	0.0161	0.0279	0.0318	0.1506	0.0259
66	V56_F123A_M162F_S214G	0.0134	0.0183	0.1865	0.0372	0.0267	0.0181
67	V64_M106E_M162A_Y216A	0.0099	1.9865	0.9067	0.0439	0.0528	0.11
68	V72_E112G_G205M_L298W	0.0478	0.8602	15.2104	0.0331	0.1345	0.3888
69	V80_M162A_N173D_S214F	0.0085	1.1313	4.8355	0.0179	0.0224	0.0462
70	V88_A108G_Q161S_G205M	0.404	5.3223	9.3605	0.1202	0.5826	4.5881
71	WT	0.1534	3.2939	25.5522	0.143	0.4528	4.6432

72	Q38G_D166E	0.0531	0.967	8.8512	0.0324	0.1771	0.2033
73	Q38G_Q295A	0.1662	3.8883	26.6189	0.0642	0.403	2.4124
74	D166E_Q295A	0.0571	1.1776	8.5988	0.0486	0.1606	0.5462
75	L219F_V294N	0.1025	3.3033	32.2708	0.0772	0.3164	2.1744
76	L219F_Q295A	0.0501	1.3315	8.1492	0.0456	0.1575	0.7358
77	V294N_Q295A	0.1248	4.0841	38.653	0.0985	0.4325	3.184
78	A53Q_S177W	0.071	8.75	8.2973	0.0366	0.2612	2.996
79	A53Q_L219F	0.1107	2.4675	30.8418	0.0499	0.3968	2.6169
80	S177W_L219F	0.0623	6.3564	5.7238	0.0375	0.2132	0.7634
81	A108G_Q161S	0.3131	5.0592	10.7488	0.1281	0.5627	2.8129
82	A108G_G205M	0.0726	0.7464	5.3991	0.0368	0.143	0.1928
83	Q161S_G205M	0.314	10.5475	26.7975	0.1626	0.6334	3.1132
84	F123H_L174V	0.0256	0.1954	1.872	0.0335	0.0404	0.1361
85	F123H_S177E	0.0978	0.634	2.0459	0.027	0.0731	0.1378
86	L174V_S177E	1.0119	23.9032	6.0703	0.1476	0.5944	1.0057
87	A53T_D166E	0.1264	1.2216	36.1931	0.0431	0.454	1.9745
88	A53T_Q295W	1.9159	13.8016	9.1083	0.0821	1.0984	14.3127
89	D166E_Q295W	0.5863	5.4552	4.8899	0.0814	0.2909	0.9001
90	A53Q_S177Y	0.0776	1.6255	12.1489	0.0345	0.3286	0.5968
91	A53Q_Y288H	1.0686	8.2035	2.5167	0.1246	1.1723	0.4187
92	S177Y_Y288H	0.2957	4.9997	0.9936	0.0474	0.3503	0.0887
93	V49A_Q161S	0.3787	30.2063	7.8094	0.1781	1.1448	1.372
94	V49A_V294A	0.2397	12.4846	7.9125	0.1001	0.6664	0.3137
95	Q161S_V294A	0.3123	16.8091	28.9812	0.1123	0.7715	9.659
96	A53T_M106E	0.4232	3.4372	28.2614	0.045	0.7028	2.1552
97	A53T_Q161S	0.3862	9.1042	29.1511	0.0457	0.611	6.006
98	M106E_Q161S	0.1518	3.3319	8.0635	0.0645	0.3214	0.5736
99	A53T_K118N	0.0959	0.712	16.7461	0.0318	0.3167	0.5034
100	A53T_S214F	0.0216	5.5146	18.8046	0.0328	0.0812	0.318
101	A53T_S214F	0.015	3.4108	10.2036	0.027	0.065	0.1592
102	K118N_S214F	0.0076	0.2044	0.3947	0.0339	0.0135	0.0195
103	A108G	0.045	0.5806	4.0899	0.0283	0.1501	0.172
104	A53Q	0.112	2.7407	33.1809	0.0494	0.4284	3.3236
105	A53T	0.2183	2.7698	45.2434	0.0583	0.6592	7.8943
106	D166E	0.1007	1.8957	19.0241	0.0375	0.3512	1.1227
107	F123H	0.0121	0.1307	1.4159	0.0235	0.0493	0.1171
108	G205M	0.1536	2.7465	26.3236	0.0674	0.5014	2.5218
109	K118N	0.0722	0.7924	5.849	0.036	0.2064	0.1193
110	L219F	0.1085	2.7357	19.9335	0.0515	0.3193	1.5967
111	M106E	0.0633	1.0405	3.9416	0.0237	0.1446	0.1373
112	Q161S	0.395	14.6696	21.3891	0.1376	0.6734	9.3316
113	Q295A	0.0969	2.7008	13.0209	0.0717	0.3548	2.7174
114	Q295W	0.7155	9.1763	3.9763	0.0596	0.3475	2.3076
115	Q38G	0.0984	2.0856	15.2255	0.0748	0.3309	1.076
116	S177E	1.1527	27.1399	5.6145	0.1559	0.5382	1.2392
117	S177W	0.0751	8.167	4.4896	0.033	0.2196	1.4872
118	S177Y	0.0624	1.3322	6.2469	0.0646	0.2523	0.2511
119	S214F	0.0045	1.0522	1.5619	0.0258	0.0143	0.0196
120	V294A	0.1405	4.4199	33.8137	0.1149	0.5394	6.0928
121	V294N	0.1121	3.429	31.862	0.1161	0.4903	4.3912
122	V49A	0.1905	6.5165	5.5114	0.0626	0.536	0.3822
123	Y288H	0.4036	4.1096	0.9622	0.1256	0.6521	0.1301
124	WT	0.1249	2.9334	25.2343	0.0646	0.3691	2.0163
125	L174V	0.1836	3.5358	22.2837	0.1427	0.4617	1.0333
126	K118N	0.1039	1.2611	8.1699	0.09	0.2522	0.1398
127	K118Q	0.0908	1.0934	27.4257	0.0867	0.3585	0.6408
128	Q161W	0.1011	0.6768	24.7827	0.0439	0.2526	0.3439
129	D227E	0.1421	2.6654	26.3001	0.1179	0.412	2.237

130	L274V	0.0397	1.0169	11.4671	0.1093	0.1642	0.385
131	S214G	0.7171	2.9071	14.6756	0.1489	0.9039	0.773
132	Y216A	0.144	0.9803	1.518	0.094	0.1158	0.0251
133	F123W	0.0094	0.0062	0.4912	0.0845	0.0258	0.0056
134	V271E	0.0129	0.0081	0.1683	0.0953	0.0335	0.0041
135	N173D	0.0347	0.6192	10.7673	0.0987	0.108	0.1021
136	R228Q	0.0471	0.7775	7.254	0.0904	0.1312	0.099
137	M162F	0.0819	2.1009	5.5282	0.1229	0.1237	1.8452
138	A232S	0.459	23.8334	8.9096	0.1803	1.3915	8.5504
139	C230S	0.1007	2.75	13.0536	0.1706	0.2211	1.0476
140	K119Q	0.0211	0.2784	5.2924	0.0804	0.0616	0.0512
141	R228E	0.0077	0.0623	0.2293	0.0883	0.1772	0.022
142	V294F	0.0812	1.7554	11.9659	0.1205	0.2965	0.614
143	Y283L	0.1071	2.7344	30.2377	0.1604	0.3776	0.9687
144	S214R	2.1392	53.1149	0.001	0.3194	0.3743	2.9412
145	G286E	0.0231	0.2041	0.7931	0.0914	0.0312	0.1842
146	M162A	0.0172	1.6258	23.0237	0.1002	0.329	0.7178
147	Q161A	0.1576	5.7143	17.0891	0.1445	0.5691	6.6368
148	K119D	0.1571	3.75	26.6466	0.1292	0.5189	6.1367
149	G205L	0.0559	1.2833	14.9855	0.1033	0.1442	0.542
150	F123A	0.0277	0.4359	2.4494	0.0963	0.3385	0.1685
151	A53T_V294A	0.1041	2.2627	34.0135	0.1159	0.5625	8.1547
152	A53T_Q161S_V294A	0.1718	5.7154	18.9083	0.0862	0.4181	5.9171
153	A53T_Q161S_V294N	0.1402	4.6934	17.5207	0.0946	0.4483	12.7291
154	A53T_Q295A	0.1197	1.7119	12.918	0.0969	0.549	11.3355
155	Q161S_V294A_Q295A	0.2124	11.5893	6.1801	0.1186	0.7545	20.6506
156	A53T_Q161S_Q295A	0.2399	6.9677	7.6228	0.0948	0.4729	4.3162
157	A53T_V294A_Q295A	0.1229	1.874	10.6083	0.0728	0.5437	10.7687
158	A53T_Q161S_V294A_Q295A	0.2802	8.3752	9.5435	0.1148	0.7828	28.0859
159	A53T_Q161S_V294N_Q295A	0.2565	7.7662	7.1111	0.1063	0.7522	34.9884
160	A53T_Q295W	1.6373	12.1532	7.1918	0.0977	1.1129	18.0539
161	Q161S_V294A_Q295W	0.3101	5.3676	3.451	0.0915	0.2333	1.1289
162	A53T_Q161S_Q295W	0.8058	10.4226	5.6942	0.0891	0.7716	9.9418
163	A53T_V294A_Q295W	1.8691	14.5967	8.5727	0.1099	1.1368	13.3037
164	A53T_Q161S_V294A_Q295W	1.1331	13.4626	11.7614	0.1854	0.7765	4.6893
165	A53T_Q161S_V294N_Q295W	0.7591	11.3653	13.5299	0.1746	0.7557	5.5845
166	Q295A	0.0655	2.0038	10.0405	0.1114	0.2956	2.1275
167	Q295W	1.065	11.8066	6.4685	0.1496	0.6682	4.8907
168	Q295C	0.0932	2.9121	9.6139	0.101	0.3937	4.292
169	Q295E	0.0207	1.7651	1.9432	0.0915	0.0506	0.2618
170	Q295F	1.3708	35.0794	1.1483	0.1637	2.5545	7.2897
171	Q295G	0.0519	1.8187	18.0005	0.1061	0.3483	6.4509
172	Q295H	0.4211	9.1506	19.1755	0.1779	0.5401	2.406
173	Q295I	0.2681	8.79	1.0036	0.0943	1.4647	0.4464
174	Q295L	0.2114	5.4162	4.0394	0.1077	1.0723	4.5794
175	Q295M	0.2618	8.7509	6.4515	0.1294	1.3546	11.8377
176	Q295N	0.0543	1.3219	20.4817	0.1028	0.4125	2.7856
177	Q295P	0.0724	1.4972	3.6145	0.0874	0.219	0.6531
178	Q295R	0.0043	0.1006	7.1948	0.0854	0.0554	0.1834
179	Q295S	0.0398	1.2416	15.8511	0.1131	0.248	1.1444
180	Q295T	0.0359	0.8869	5.8313	0.1032	0.1714	0.3931
181	Q295V	0.1485	1.9045	1.0598	0.037	0.7391	0.1365
182	Q295D	0.1064	3.3375	37.8092	0.1467	0.4666	1.3742
183	Q295K	0.0289	0.6459	10.0193	0.1022	0.1236	0.1361
184	Q295Y	0.15	3.8799	25.7461	0.1398	0.5447	1.0768
185	S214D	0.1248	4.8212	6.7036	0.2283	0.1557	0.824
186	S214E	0.1683	4.6655	1.5194	0.0982	0.2325	0.0637
187	S214F	0.0103	1.0741	1.4762	0.0999	0.0186	0.0194

188	S214H	0.3732	26.4158	0.001	0.239	0.3085	0.1902
189	S214I	0.0101	1.2463	1.409	0.1022	0.0197	0.0404
190	S214K	0.0846	4.8782	1.2723	0.0859	0.0344	0.1634
191	S214L	0.0083	0.14	0.0875	0.0713	0.0158	0.0247
192	S214M	0.0105	0.5869	0.4293	0.0776	0.0234	0.0243
193	S214N	0.973	4.2798	7.5619	0.1179	0.2841	0.0931
194	S214R	1.2573	34.1019	0.001	0.2668	0.2598	0.6229
195	S214T	0.133	3.5464	21.1803	0.1153	0.5028	1.2146
196	S214V	0.0875	2.2093	13.6844	0.0957	0.2688	0.6449
197	S214W	0.0088	0.0426	0.4008	0.0834	0.0188	0.0247
198	S214Y	0.0097	0.2006	0.2144	0.0762	0.0201	0.0209
199	S214C	0.0267	0.6854	21.995	0.1065	0.1374	0.3795
200	S214G	0.7307	3.0559	14.47	0.1147	0.622	0.622
201	S214P	0.0153	0.0393	1.1774	0.1058	0.0181	0.0233
202	S214Q	0.1706	3.5611	1.6229	0.0556	0.3723	0.3723
203	Q161C	0.0509	0.8844	43.2089	0.0634	0.4215	1.7517
204	Q161F	0.0837	10.0552	24.9092	0.0516	0.207	0.6356
205	Q161I	0.0759	1.2956	24.5569	0.0657	0.2488	1.1875
206	Q161L	0.0726	2.1623	26.0984	0.0651	0.2465	0.8572
207	Q161L	0.0631	1.8682	22.0069	0.0548	0.1889	0.8935
208	Q161M	0.1765	1.3606	41.9419	0.084	0.2606	0.4447
209	Q161R	0.1619	24.3846	3.7695	0.1052	0.2852	1.6835
210	Q161S	0.3461	12.437	19.8886	0.1486	0.4548	3.6143
211	Q161T	0.1657	6.9786	28.8877	0.1024	0.4342	4.0442
212	Q161Y	0.5964	21.0425	1.9789	0.1203	0.7872	12.6215
213	Q161A	0.1379	4.5896	19.6231	0.1788	0.4642	1.3495
214	Q161D	0.3729	3.1314	5.1056	0.0832	0.2034	0.2178
215	Q161H	0.8347	81.2454	0.001	0.3104	0.445	16.3332
216	Q161G	0.1213	2.5843	10.8548	0.1269	0.4907	0.4119
217	Q161K	0.1291	13.0135	2.8762	0.1408	0.222	3.5705
218	Q161N	0.202	2.5658	18.1028	0.1182	0.3937	1.678
219	Q161P	0.0658	2.0253	8.7803	0.0835	0.4269	0.4919
220	Q161Q	0.1189	3.3057	19.7637	0.1042	0.3368	1.5511
221	Q161W	0.0682	0.5008	17.8487	0.0535	0.2562	0.2668
222	Q161E	0.9022	4.3213	5.024	0.1677	0.1626	0.1626
223	Q161V	0.0896	1.536	13.4263	0.0714	0.3855	0.3855
224	A53G	0.1102	1.7457	13.7584	0.0992	0.322	0.1536
225	A53D	0.0652	1.2423	8.8984	0.0619	0.1081	0.3608
226	A53E	0.0073	0.0831	0.6345	0.0603	0.0119	0.0338
227	A53K	0.2531	3.2961	35.4059	0.073	0.6218	0.9172
228	A53L	0.153	5.5397	37.2614	0.1084	0.6553	1.6309
229	A53Q	0.126	2.7874	29.2018	0.0628	0.3578	0.9998
230	A53Y	0.099	1.2745	6.2225	0.0606	0.2013	0.0401
231	A53F	0.0288	1.2169	0.9987	0.0954	0.0365	0.0241
232	A53H	0.0219	0.4324	1.2156	0.1273	0.0624	0.0298
233	A53I	1.3589	7.3364	24.356	0.0701	2.5205	3.7819
234	A53M	0.1491	4.0903	33.0822	0.1398	0.5534	3.036
235	A53N	0.1752	1.396	18.8247	0.1036	0.3446	0.1973
236	A53R	0.1818	1.8241	20.7965	0.0455	0.5287	0.7574
237	A53S	0.1777	3.4592	30.3708	0.0809	0.477	1.8365
238	A53T	0.2181	2.7784	43.7465	0.0753	0.6791	6.1406
239	A53V	0.4721	6.5503	32.1044	0.1195	1.3511	1.936
240	A53W	0.0714	1.0017	20.3356	0.0499	0.283	0.7266
241	A53C	0.1836	4.5342	28.5658	0.1141	0.5567	0.5567
242	A53P	0.0069	0.0015	0.0887	0.086	0.0148	0.018
243	S177W_Q295A	0.2879	49.6105	0.001	0.1433	0.3429	0.4855
244	S177W_S214R	0.1756	8.898	0.001	0.2141	0.1526	0.0678
245	Q161S_S177W	0.1464	32.4331	2.5717	0.1568	0.399	1.061

246	A53T_S177W	0.2366	15.4625	8.8346	0.103	0.5306	2.9941
247	V49A_Q295L	0.1181	1.4388	1.2094	0.0596	0.281	0.0278
248	V49A_S214R	0.0702	3.7232	0.2083	0.1387	0.0551	0.0302
249	A53T_Q295F	2.8922	43.9523	2.8376	0.1994	3.5196	15.4435
250	A53T_S214R	2.2629	63.8414	0.001	0.2668	0.4	5.0836
251	A53T_A161S	0.4045	10.4118	26.798	0.2378	0.7315	15.9102
252	Q161S_Q295F	1.0875	46.2151	1.8605	0.2074	1.9207	3.6257
253	Q161S_Q295L	1.281	54.3225	1.5682	0.2466	2.4871	6.6647
254	Q16S_S214R	0.7657	29.2403	0.001	0.2615	0.2614	1.3356
255	S214R_Q295F	1.6437	35.9686	0.001	0.3189	0.2922	0.1282
256	WT	0.1334	2.8081	19.6766	0.0771	0.251	0.5108
257	WT	0.1817	3.9098	28.3319	0.0648	0.4219	5.6093
258	WT	0.156	3.609	29.5527	0.0726	0.4801	1.789
259	WT	0.1583	4.3295	30.5886	0.1363	0.6265	3.8492
260	WT	0.1405	3.4382	28.8822	0.142	0.4674	2.9774
261	WT	0.1464	4.0581	28.4161	0.1555	0.4595	0.8362
262	WT	0.1253	3.2069	22.7076	0.131	0.393	1.3584
263	WT	0.118	3.0373	20.2262	0.104	0.5182	4.06
264	WT	0.1345	3.7682	27.6547	0.0935	0.2818	0.2818
265	Y288A	1.026	13.8232	0.001	0.1892	0.6869	0.6869
266	Y288C	0.8557	17.3203	0.001	0.2429	0.6133	0.6133
267	Y288D	0.0498	0.9269	0.08	0.0898	0.1998	0.1998
268	Y288E	0.0304	0.361	0.0704	0.0691	0.0958	0.0958
269	Y288F	1.0675	86.6372	0.59	0.2631	0.346	0.346
270	Y288G	0.1955	13.5962	0.4393	0.2508	0.336	0.336
271	Y288H	0.3568	3.1893	0.827	0.139	0.298	0.298
272	Y288I	4.5539	64.9223	0.56	0.2809	0.4633	0.4633
273	Y288K	0.1383	2.2135	2.2135	0.1465	0.0263	0.0263
274	Y288L	5.7168	58.2768	1.3166	0.2538	0.916	0.916
275	Y288M	4.2171	55.2958	0.5908	0.2665	0.522	0.522
276	Y288P	1.4933	32.5754	0.2131	0.2457	0.9623	0.9623
277	Y288R	0.0204	0.4052	0.0521	0.0635	0.1646	0.1646
278	Y288S	0.2467	3.0757	0.1073	0.1676	0.3944	0.3944
279	Y288T	1.9406	25.6881	0.5724	0.2588	0.5747	0.5747
280	Y288W	0.1608	22.3033	0.616	0.2711	0.1796	0.1796
281	A232S	0.4997	25.0127	9.2312	0.1277	1.252	1.252
282	N173D-S214R	0.1009	3.6399	0.0187	0.1293	0.067	0.067
283	N173D	0.0255	0.898	7.2816	0.0873	0.0594	0.0594
284	M162F	0.0724	2.1125	5.0272	0.0838	0.0857	0.0857
285	WT	0.1586	4.6108	26.8708	0.1271	0.4956	0.4956
286	A17T	0.0646	2.1419	21.1073	0.1513	0.2712	0.2712
287	A232S	0.0548	2.0224	6.0788	0.12	0.1662	0.1662
288	M162F-Q295A	0.0449	2.123	1.8141	0.0849	0.1038	0.1038
289	WT	0.159	3.898	27.497	0.092	0.344	1.381
290	A232S-1	0.357	24.056	13.24	0.169	1.074	6.912
291	A232S-2	0.378	25.952	13.808	0.198	1.201	3.129
292	S214A-1	0.365	0.638	21.548	0.06	0.199	0.145
293	S214A-2	0.444	0.92	27.662	0.083	0.394	0.256
294	S214Q-1	0.188	4.662	1.743	0.044	0.206	0.547
295	S214Q-2	0.146	4.776	1.223	0.039	0.247	0.876
296	Q161E-2	1.351	5.319	5.769	0.125	0.204	0.342
297	Y288N	0.186	2.309	0.246	0.087	0.208	0.032

[0240] The amount of each prenylation product was measured by HPLC. FIG. 5 shows a heatmap of the HPLC areas of each prenylation product generated using DVA as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time with the exception of RBI-26 and RBI-27. Enzyme variants are labeled by ID# as listed in Table 10.

Example 7: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using DVA as substrate and FPP as donor

[0241] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to “breakdown” analysis. “Breakdown” analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. “Breakdown” variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position.

[0242] The wild type Orf2 prenylation reaction using DVA as substrate and FPP as donor produces 5 products as detected by HPLC. The respective retention times of these products are approximately 7.05, 7.84, 8.03, 8.24, and 9.72 minutes.

[0243] Table 11 provides a summary of the prenylation products produced from DVA and FPP, their retention times, and the hypothesized prenylation site on DVA. FIG. 21 shows the predicted chemical structures of the respective prenylation products.

Table 11: Predicted prenylation products of Orf2 or Orf2 Mutants when using DVA as substrate and FPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK12	DVA	FPP	CO	7.05
UNK13	DVA	FPP	2-O	9.72
UNK14	DVA	FPP	4-O	8.24
RBI-38	DVA	FPP	3-C	7.84
RBI-39	DVA	FPP	5-C	8.03

[0244] Table 12 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using DVA as substrate and FPP as donor. Table 12 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 12: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using DVA as substrate and FPP as donor

ID#	**Mutations**	**7.05**	**7.84**	**8.03**	**8.24**	**9.72**
1	V9_Q38G_E112D_F123H	0.011	0.04	0.549	0.004	0.007
2	V17_V49L_F123A_Y283L	0.004	0.024	0.017	0.007	0.001
3	V25_L219F_V294N_Q295A	0.004	0.067	0.017	0.006	0.002
4	V33_A17T_C25V_E112G	0.015	0.06	0.121	0.006	0.006
5	V57_C25V_A232S_V271E	0.001	0.005	0.001	0.005	0.001
6	V65_V49A_Q161S_V294A	0.013	0.053	0.022	0.007	0.004
7	V73_V49S_K118Q_S177E	0.116	0.064	0.11	0.015	0.01
8	V10_V49A_S177Y_C209G	0.001	0.005	0.001	0.003	0.001
9	V26_A53E_A108G_K118N	0.001	0.001	0.001	0.005	0.001
10	V34_A53Q_Y121W_A232S	0.001	0.002	0.001	0.003	0.001
11	V42_D166E_S177Y_S214F	0.001	0.002	0.002	0.004	0.001
12	V58_K118Q_L174V_R228Q	0.001	0.002	0.002	0.003	0.001
13	V66_C25V_F213M_Y216A	0.001	0.003	0.001	0.004	0.001
14	V74_M106E_Y121W_D166E	0.001	0.002	0.001	0.004	0.001
15	V82_V49S_K119D_F213M	0.001	0.002	0.001	0.003	0.001
16	V3_V49S_M162A_Y283L	0.005	0.008	0.029	0.005	0.001
17	V11_K118N_K119A_V271E	0.001	0.002	0.001	0.003	0.001
18	V19_V49L_S214R_V271E	0.001	0.005	0.001	0.007	0.001
19	V35_A53Q_S177Y_Y288H	0.077	0.226	0.017	0.01	0.02
20	V43_Q161A_M162F_Q295A	0.004	0.076	0.016	0.005	0.001
21	V51_V49L_K119D_G205M	0.001	0.005	0.001	0.004	0.001
22	V67_A108G_K119D_L298A	0.001	0.006	0.001	0.003	0.001
23	V83_E112D_L219F_V294F	0.049	0.5	2.238	0.005	0.062
24	V91_N173D_F213M_V294F	0.001	0.028	0.049	0.003	0.001
25	V4_K118Q_Q161W_S214F	0.001	0.003	0.001	0.006	0.001
26	V28_A53T_D166E_Q295W	0.003	0.017	0.026	0.003	0.002
27	V44_A53E_Q161A_V294N	0.001	0.017	0.022	0.004	0.001
28	V52_K119A_S214G_L298A	0.001	0.008	0.001	0.005	0.001
29	V60_E112D_K119A_N173D	0.001	0.001	0.001	0.004	0.001
30	V68_K118N_C209G_R228Q	0.001	0.002	0.001	0.005	0.001
31	V84_F123H_L174V_S177E	0.02	0.051	0.157	0.005	0.001
32	V92_A53T_E112D_G205M	0.079	0.254	1.181	0.012	0.019
33	V36_F123H_L274V_L298A	0.0012	0.001	0.0007	0.0022	0.0003
34	V69_A53T_M106E_Q161S	0.013	0.027	0.493	0.006	0.006
35	V60_E112D_K119A_N173D	0.001	0.003	0.003	0.004	0.001
36	V62_A53T_N173D_S214R	0.001	0.025	0.002	0.003	0.001
37	V70_Q38G_D166E_Q295A	0.031	0.076	0.208	0.003	0.001
38	V78_K119D_Q161W_L298Q	0.001	0.004	0.005	0.002	0.001
39	V94_A17T_V49A_C230N	0.001	0.002	0.001	0.004	0.001
40	V15_A53E_F213M_R228Q	0.001	0.006	0.02	0.003	0.001

41	V23_L219F_Y283L_L298W	0.001	0.012	0.027	0.004	0.001
42	V31_D227E_R228E_L298Q	0.001	0.002	0.001	0.003	0.001
43	V39_A53T_K118N_S214F	0.001	0.015	0.001	0.004	0.001
44	V47_K118Q_F123A_R228E	0.001	0.003	0.002	0.003	0.001
45	V55_V49S_Y216A_V294N	0.002	0.007	0.001	0.003	0.001
46	V63_F123W_M162F_C209G	0.001	0.002	0.001	0.002	0.001
47	V71_M106E_G205L_C209G	0.0002	0.0035	0.0001	0.0049	0.0003
48	V79_V49A_Y121W_C230S	0.001	0.002	0.001	0.002	0.001
49	V87_S177W_Y288H_V294N	0.001	0.001	0.001	0.003	0.001
50	V95_A17T_Q161W_A232S	0.007	0.083	0.065	0.007	0.005
51	V8_K119A_Q161A_R228Q	0.001	0.004	0.001	0.004	0.001
52	V16_A53Q_S177W_L219F	0.002	0.128	0.144	0.005	0.001
53	V24_A17T_F213M_S214R	0.0123	0.1368	0.0087	0.0052	0.0001
54	V32_M162A_C209G_Y288H	0.001	0.004	0.001	0.005	0.001
55	V40_S177E_S214R_R228E	0.002	0.002	0.001	0.004	0.001
56	V48_V49L_E112D_G286E	0.001	0.003	0.001	0.003	0.004
57	V64_M106E_M162A_Y216A	0.001	0.002	0.001	0.001	0.001
58	V72_E112G_G205M_L298W	0.005	0.07	0.173	0.004	0.002
59	V80_M162A_N173D_S214F	0.001	0.008	0.008	0.002	0.001
60	V88_A108G_Q161S_G205M	0.001	0.005	0.012	0.003	0.001
61	Q38G_D166E	0.003	0.021	0.061	0.004	0.003
62	Q38G_Q295A	0.028	0.23	0.243	0.006	0.024
63	D166E_Q295A	0.002	0.037	0.012	0.005	0.002
64	L219F_V294N	0.012	0.184	0.1	0.003	0.007
65	L219F_Q295A	0.002	0.045	0.008	0.004	0.001
66	V294N_Q295A	0.017	0.203	0.112	0.004	0.016
67	A53Q_S177W	0.002	0.093	0.088	0.003	0.001
68	A53Q_L219F	0.007	0.061	0.156	0.003	0.002
69	S177W_L219F	0.001	0.045	0.026	0.002	0.001
70	A108G_Q161S	0.001	0.003	0.006	0.003	0.001
71	A108G_G205M	0.001	0.001	0.002	0.001	0.001
72	Q161S_G205M	0.003	0.021	0.071	0.004	0.001
73	F123H_L174V	0.006	0.016	0.163	0.003	0.001
74	F123H_S177E	0.024	0.045	0.132	0.003	0.001
75	L174V_S177E	0.028	0.236	0.131	0.004	0.002
76	A53T_D166E	0.016	0.055	0.262	0.003	0.003
77	A53T_Q295W	0.027	0.115	0.13	0.007	0.005
78	D166E_Q295W	0.001	0.009	0.003	0.001	0.001
79	A53Q_S177Y	0.003	0.013	0.073	0.004	0.001
80	A53Q_Y288H	0.12	0.566	0.018	0.01	0.043
81	S177Y_Y288H	0.043	0.149	0.004	0.003	0.01
82	V49A_Q161S	0.006	0.026	0.017	0.001	0.002
83	V49A_V294A	0.014	0.053	0.021	0.003	0.008
84	Q161S_V294A	0.008	0.087	0.069	0.003	0.003

85	A53T_M106E	0.022	0.044	0.312	0.005	0.005
86	A53T_Q161S	0.008	0.032	0.184	0.002	0.002
87	M106E_Q161S	0.001	0.007	0.041	0.003	0.001
88	A53T_K118N	0.001	0.001	0.001	0.001	0.001
89	A53T_S214F	0.001	0.004	0.001	0.001	0.001
90	K118N_S214F	0.001	0.003	0.001	0.002	0.001
91	A108G	0.001	0.001	0.002	0.001	0.001
92	A53Q	0.014	0.111	0.236	0.004	0.006
93	A53T	0.056	0.223	0.608	0.009	0.014
94	D166E	0.007	0.049	0.096	0.001	0.003
95	F123H	0.003	0.011	0.143	0.003	0.002
96	G205M	0.009	0.067	0.099	0.001	0.005
97	K118N	0.001	0.007	0.012	0.004	0.001
98	L219F	0.009	0.065	0.094	0.001	0.006
99	M106E	0.003	0.011	0.038	0.001	0.002
100	Q161S	0.01	0.075	0.153	0.001	0.002
101	Q295A	0.015	0.196	0.039	0.001	0.005
102	Q295W	0.011	0.09	0.039	0.002	0.002
103	Q38G	0.006	0.056	0.068	0.002	0.003
104	S177E	0.02	0.178	0.099	0.002	0.001
105	S177W	0.001	0.11	0.05	0.002	0.001
106	S177Y	0.002	0.01	0.034	0.002	0.001
107	S214F	0.001	0.018	0.002	0.001	0.001
108	V294A	0.012	0.228	0.086	0.001	0.006
109	V294N	0.008	0.129	0.059	0.001	0.002
110	V49A	0.01	0.029	0.028	0.001	0.004
111	Y288H	0.046	0.19	0.004	0.004	0.01
112	K118Q	0.0132	0.0342	0.3057	0.0054	0.0047
113	K119Q	0.0005	0.0052	0.0046	0.0062	0.001
114	M162A	0.0024	0.172	0.1925	0.0082	0.0023
115	Q161A	0.0044	0.0514	0.1017	0.0065	0.0039
116	K119D	0.0268	0.2098	0.2511	0.0056	0.0218
117	F123A	0.021	0.1354	1.3582	0.0061	0.0206
118	K118N	0.0071	0.0207	0.0373	0.0076	0.0009
119	Q161W	0.0015	0.0054	0.0783	0.0033	0.0014
120	D227E	0.0189	0.0974	0.1951	0.0074	0.0121
121	L274V	0.0014	0.0197	0.0241	0.005	0.0007
122	S214G	0.0992	0.062	0.0761	0.0088	0.0242
123	Y216A	0.0004	0.0034	0.0002	0.0054	0.0004
124	F123W	0.0001	0.001	0.0005	0.0034	0.0006
125	V271E	0.0003	0.0019	0.0002	0.0052	0.0002
126	N173D	0.0001	0.0054	0.0044	0.0037	0.0004
127	R228Q	0.0004	0.0037	0.007	0.002	0.001
128	M162F	0.0034	0.0838	0.0372	0.0042	0.0007

129	A232S	0.0736	0.3959	0.1775	0.0081	0.0705
130	C230S	0.0056	0.0453	0.0599	0.0056	0.0007
131	V294F	0.0367	0.2267	0.5666	0.0063	0.0568
132	Y283L	0.0157	0.103	0.1708	0.0038	0.0094
133	S214R	0.2092	1.5553	0.0287	0.02	0.0003
134	G286E	0.0005	0.0137	0.0012	0.004	0.0002
135	R228E	0.0003	0.0002	0.0002	0.0063	0.0003
136	A53T_V294A	0.1099	0.7571	0.8358	0.0107	0.024
137	A53T_Q161S_V294A	0.0457	0.237	0.5362	0.0062	0.0092
138	A53T_Q161S_V294N	0.0284	0.1637	0.3764	0.0072	0.0031
139	A53T_Q295A	0.0723	0.5523	0.2617	0.0069	0.0264
140	Q161S_V294A_Q295A	0.0267	0.2413	0.1134	0.0059	0.005
141	A53T_Q161S_Q295A	0.0526	0.2354	0.2785	0.0298	0.0083
142	A53T_V294A_Q295A	0.1679	1.3931	0.6261	0.018	0.0747
143	A53T_Q161S_V294A_Q295A	0.0987	0.438	0.529	0.0187	0.0239
144	A53T_Q161S_V294N_Q295A	0.0526	0.2073	0.2919	0.0085	0.0073
145	A53T_Q295W	0.0593	0.2272	0.2566	0.0073	0.0132
146	Q161S_V294A_Q295W	0.0083	0.0846	0.0528	0.0045	0.0006
147	A53T_Q161S_Q295W	0.0193	0.1301	0.2282	0.0069	0.0043
148	A53T_V294A_Q295W	0.0792	0.2985	0.3506	0.0113	0.0114
149	A53T_Q161S_V294A_Q295W	0.0273	0.15	0.2829	0.0054	0.0049
150	A53T_Q161S_V294N_Q295W	0.0243	0.1498	0.2751	0.0049	0.006
151	Q295C	0.0177	0.2424	0.0441	0.006	0.0343
152	Q295E	0.0001	0.0176	0.003	0.0052	0.0006
153	Q295F	0.0479	0.6113	0.0275	0.0077	0.0235
154	Q295G	0.003	0.049	0.0223	0.0037	0.0019
155	Q295H	0.0304	0.1238	0.0444	0.0056	0.0527
156	Q295I	0.0048	0.1541	0.0032	0.0016	0.0198
157	Q295L	0.0377	1.3192	0.0344	0.0072	0.1094
158	Q295M	0.0223	0.4255	0.0354	0.0046	0.0423
159	Q295N	0.0073	0.0733	0.0359	0.0041	0.0074
160	Q295D	0.0109	0.151	0.0783	0.0063	0.0033
161	Q295K	0.001	0.0006	0.0005	0.0023	0.0003
162	Q295P	0.0003	0.0118	0.0055	0.0049	0.0001
163	Q295R	0.0002	0.0037	0.0002	0.0009	0.0006
164	Q295S	0.0052	0.1048	0.0373	0.0047	0.0059
165	Q295T	0.0094	0.105	0.0199	0.005	0.0166
166	Q295V	0.0984	1.0999	0.0506	0.0123	0.5476
167	Q295Y	0.013	0.1182	0.1458	0.006	0.0136
168	Q295W	0.0007	0.0114	0.0014	0.0002	0.0004
169	WT Control	0.009	0.0742	0.0788	0.0027	0.006
170	S214D	0.004	0.0423	0.0623	0.0071	0.0007
171	S214E	0.0052	0.0214	0.0101	0.0054	0.0002
172	S214F	0.0002	0.0281	0.0019	0.0047	0.0001

173	S214H	0.0087	0.0832	0.0011	0.0067	0.0002
174	S214I	0.0003	0.0279	0.0127	0.0055	0.001
175	S214K	0.0012	0.0374	0.0225	0.0039	0.0001
176	S214L	0.0012	0.0091	0.0007	0.0046	0.0006
177	S214M	0.0006	0.0175	0.0008	0.0055	0.0001
178	S214N	0.0707	0.0405	0.0921	0.0127	0.0004
179	S214R	0.1858	2.5018	0.057	0.0175	0.0022
180	S214T	0.0152	0.1339	0.1388	0.0046	0.0115
181	S214V	0.0108	0.1068	0.1132	0.0046	0.0062
182	S214W	0.0007	0.0008	0.0014	0.0043	0.0016
183	S214Y	0.0007	0.0004	0.0004	0.0039	0.0002
184	Q161A	0.0078	0.0912	0.1146	0.0021	0.0122
185	Q161C	0.0054	0.0515	0.4969	0.0055	0.009
186	Q161D	0.001	0.006	0.005	0.001	0.001
187	Q161F	0.0014	0.3198	0.256	0.0064	0.0013
188	Q161G	0.0006	0.0155	0.0568	0.0066	0.001
189	Q161H	0.3945	19.8218	0.2343	0.0332	0.0283
190	Q161I	0.0058	0.0636	0.4341	0.0053	0.0095
191	Q161K	0.0095	0.2765	0.141	0.0036	0.0011
192	Q161L	0.0085	0.1492	0.5887	0.0075	0.0153
193	Q161M	0.015	0.0478	0.4349	0.006	0.0028
194	Q161N	0.0044	0.0422	0.1058	0.0051	0.0014
195	Q161P	0.001	0.01	0.023	0.001	0.001
196	Q161Q	0.0113	0.1271	0.1337	0.0047	0.0118
197	Q161R	0.0146	0.8334	0.4276	0.0062	0.0031
198	Q161S	0.0098	0.1224	0.2244	0.004	0.0055
199	Q161T	0.0085	0.214	0.4737	0.0055	0.0098
200	Q161W	0.001	0.004	0.045	0.002	0.001
201	Q161Y	0.0384	0.5159	0.2257	0.0045	0.0036
202	A53D	0.0041	0.0309	0.079	0.0044	0.0008
203	A53E	0.0007	0.0051	0.0024	0.0037	0.0004
204	A53F	0.001	0.0486	0.0016	0.0015	0.0001
205	A53G	0.0095	0.0276	0.0692	0.0073	0.0011
206	A53H	0.0164	0.0668	0.079	0.0089	0.0098
207	A53K	0.09	0.4495	0.973	0.0103	0.0542
208	A53L	0.1046	1.3768	1.9216	0.0108	0.0972
209	A53M	0.0238	0.2104	0.3487	0.0071	0.0198
210	A53N	0.0079	0.0336	0.0684	0.0054	0.0037
211	A53P	0.0004	0.0071	0.0069	0.0043	0.0002
212	A53Q	0.0285	0.2794	0.6075	0.0055	0.0178
213	A53R	0.008	0.04	0.077	0.002	0.003
214	A53S	0.0244	0.1586	0.2731	0.0069	0.0106
215	A53T	0.053	0.299	0.67	0.007	0.016
216	A53V	0.1704	0.7757	0.5053	0.0192	0.1256

217	A53W	0.002	0.013	0.038	0.002	0.001
218	A53Y	0.0063	0.0351	0.0357	0.0055	0.0059
219	S177W_Q295A	0.0489	5.7629	0.0051	0.0072	0.0116
220	S177W_S214R	0.0142	0.203	0.0024	0.0038	0.001
221	Q161S_S177W	0.0076	0.5362	0.0761	0.0017	0.0094
222	A53T_S177W	0.0148	0.4099	0.5618	0.0031	0.0085
223	V49A_Q295L	0.0023	0.0364	0.009	0.0351	0.0135
224	V49A_S214R	0.0263	0.6375	0.0121	0.0041	0.001
225	A53T_Q295F	0.1722	1.62	0.2003	0.0187	0.1032
226	A53T_S214R	0.2252	1.9636	0.0873	0.0226	0.0095
227	A53T_A161S	0.043	0.1852	0.8726	0.0054	0.0138
228	Q161S_Q295F	0.0266	0.4049	0.0432	0.0027	0.0339
229	Q161S_Q295L	0.0228	0.3622	0.0288	0.0039	0.025
230	Q16S_S214R	0.023	0.1759	0.0796	0.0028	0.0009
231	S214R_Q295F	0.576	6.1235	0.0155	0.0674	0.0111
232	WT	0.015	0.114	0.128	0.004	0.009
233	WT	0.019	0.129	0.15	0.004	0.012
234	WT	0.019	0.116	0.133	0.003	0.013
235	WT	0.016	0.157	0.143	0.002	0.011
236	WT	0.0118	0.0819	0.09	0.0048	0.0047
237	WT	0.0162	0.128	0.1362	0.0073	0.017
238	WT	0.0288	0.2778	0.2988	0.0051	0.0251
239	WT	0.0273	0.2258	0.2578	0.0069	0.0157
240	WT	0.0188	0.1259	0.1409	0.0034	0.0122
241	WT	0.0219	0.2037	0.2211	0.0077	0.0143

[0245] The amount of each prenylation product was measured by HPLC. FIG. 6 shows a heatmap of the HPLC areas of each prenylation product generated using DVA as substrate and FPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 12.

Example 8: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using ORA as substrate and GPP as donor

[0246] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 .

Clones that exhibited improved function relative to the wild type enzyme were subjected to "breakdown" analysis. "Breakdown" analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. "Breakdown" variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position. A subset of Orf2 Mutant enzymes were screened for prenylation when using Orsillenic Acid (ORA) as substrate and GPP as donor.

[0247] The wild type Orf2 prenylation reaction using ORA as substrate and GPP as donor produces 6 products as detected by HPLC. The respective retention times of these products are approximately 4.6, 5.7, 5.83, 6.35, 7.26, and 9.26 minutes.

[0248] Table 13A provides a summary of the prenylation products produced from ORA and GPP, their retention times, and the hypothesized prenylation site on ORA. FIG. 22 shows the predicted chemical structures of the respective prenylation products.

Table 13A: Predicted prenylation products of Orf2 or Orf2 Mutants when using ORA as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK20	ORA	GPP	CO	4.557
UNK21	ORA	GPP	2-O	7.258
UNK22	ORA	GPP	4-O	6.353
UNK23	ORA	GPP	3-C	5.707
UNK24	ORA	GPP	5-C	5.828
UNK59	ORA	GPP	5-C + 3-C	9.263

[0249] Tables 13B-13D provide NMR data of proton and carbon chemical shifts for UNK59 with (a) HSQC, (b) HMBC correlation and (c) final carbon and proton NMR assignments. The carbon and proton NMR assignments for UNK59 are shown in Figure 82.

Table 13B: Proton NMR assignments for UNK59

PROTON					MULTIPLICITY	
Shift	Area	Protons	C Assignment	HSQC-DEPT	Options	Actual
1.528	3.07	3	C9	1.52	CH3 or CH	CH3
1.53	3.07	3	C9'''	X	X	CH3
1.596	3.21	3	C10	1.58	CH3 or CH	CH3
1.6	2.92	3	C10'''	X	X	CH3
1.711	3.01	3	C8 or C8'''	1.7	CH3 or CH	CH3
1.715	2.96	3	C8 or C8'''	1.7	CH3 or CH	CH3
1.902	1.9	2	C4'''	1.9	CH2	CH2
1.938	2	2	C4	1.92	CH2	CH2
2.006	4.21	4	C5+C5'''	1.99	CH2	CH2
2.34	3.03	3	C1''?	2.33	CH3 or CH	CH3
3.287	2.05	2	C1 Or C1'''	3.28	CH2	CH2
3.298	2.35	2	C1 Or C1'''	3.28	CH2	CH2
4.921	1	1	C6'''	4.9	CH3 or CH	CH
5.026	1.02	1	C6 OR C2'''	5.02	CH3 or CH	CH
5.04	1.08	1	C6 OR C2'''	5.09	CH3 or CH	CH
5.101	1.09	1	C2	X	X	CH
8.857	0.968	1	4' OH?	X	X	X
11.95	0.994	1	2' OH?	X	X	X
13.5	1	1	COOH?	X	X	X
	H Sum:	40				

Table 13C: Carbon NMR assignments for UNK59

CARBON			
Shift	Assignment	Carbon ct.	NMR Predictions
16.43	C8	1	16.4
16.48	C8'''	1	16.4
17.98	C9	1	18.6
18	C9'''	1	18.6
18.4	C1"	1	14.2
22.48	C1	1	22.2
25.43	C1'''	1	24.8
25.91	C10	1	24.6
25.93	C10'''	1	24.6
26.56	C5	1	26.4
26.65	C5'''	1	26.4
39.7	C4 + C4'''	2	39.7
106.7	C1'	1	107.2
113.29	C3'	1	113
120.6	C2	1	122.3
123.15	C2'''	1	122.3
123.8	C6	1	123.5
124.55	C6'''	1	123.5
124.59	C5'	1	126
131.07	C7	1	132
131.1	C7'''	1	132
134.12	C3	1	136.5
134.26	C3'''	1	136.5
137.56	C6'	1	139.3
157.44	C2'	1	156.9
159.71	C4'	1	158.3
174.43	COOH	1	173.2
	C SUM:	28	

Table 13D: HMBC for sample UNK59

1D C Shift	Assignment	Associated Proton Shifts		Proton List	
16.43	C9'''	4.92		C6'''	
16.48	C8	5.1		C2	
17.98	C8'''	5.03		C2'''	
18	C9	1.59		C10	
18.4	C1"	X			
22.48	C1	X			
25.43	C1'''	X			
25.91	C10	1.52		C9	
25.93	C10'''	5.02		C2'''	
26.56	C5	1.94		C4	
26.65	C5'''	1.9		C4'''	
39.89	1.79	1.98		C8 or C8'''	C5+C5'''
106.7	C1'	2.34		C1"?	
113.29	C3'	8.86		4' OH?	
120.6	C2	3.29		C1 +C1'''	
123.15	C2'''	1.89	8.86	C4'''	
123.8	C6	3.29		C1 +C1'''	
124.55	C6'''	X			
124.59	C5'	1.52		C9	
131.07	C7	X			
131.1	C7'''	1.52		C9	
134.12	C3	X			
134.26	C3'''	1.71		C8 or C8'''	
137.56	C6'	3.29	1.99	C5+C5'''	C1 +C1'''
157.44	C2'	2.33		C1"?	
159.71	C4'	X			
174.43	COOH	X			

[0250] Table 14 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using ORA as substrate and GPP as donor. Table 14 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 14: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using ORA as substrate and GPP as donor

ID#	**Mutations**	**4.557**	**5.707**	**5.828**	**6.353**	**7.258**	**9.263**
1	A53Q+Y288H	0.3283	14.2943	0.5313	0.6722	2.6632	4.0885
2	Q161S+V294A	0.0102	26.4403	0.4963	0.1372	0.2948	0.4523
3	A53T	0.0335	61.3252	1.0407	0.7123	3.1675	1.3286

4	Q295A	0.0347	32.3728	0.4799	0.4833	0.8491	3.3298
5	Q295W	0.1928	15.2688	1.5169	1.1091	4.357	4.0242
6	V294A	0.0865	51.226	0.867	0.3911	1.2826	0.3834
7	Q295F	0.1585	13.9454	1.4399	0.9662	2.1466	2.3094
8	Q295H	0.0455	41.0933	0.8956	0.4223	0.9599	0.5652
9	S214R	0.0167	12.2428	0.1388	0.2801	0.1169	4.9605
10	WT	0.0284	50.6006	0.8257	0.2747	1.6682	1.6355

[0251] The amount of each prenylation product was measured by HPLC. FIG. 7 shows a heatmap of the HPLC areas of each prenylation product generated using ORA as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 14.

Example 9: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using Apigenin as substrate and GPP as donor

[0252] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to "breakdown" analysis. "Breakdown" analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. "Breakdown" variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position. A subset of Orf2 Mutant enzymes were screened for prenylation when using Apigenin as substrate and GPP as donor.

[0253] The wild type Orf2 prenylation reaction using Apigenin as substrate and GPP as donor produces 5 products as detected by HPLC. The respective retention times of these products are approximately 5.84, 6.77, 7.36, 7.68, and 8.19 minutes.

[0254] Table 15 provides a summary of the prenylation products produced from Apigenin and GPP, their retention times, and the hypothesized prenylation site on Apigenin. FIG. 23 shows the predicted chemical structures of the respective prenylation products.

Table 15: Predicted prenylation products of Orf2 or Orf2 Mutants when using Apigenin as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK47	Apigenin	GPP	C-13/C-15	5.84
UNK48	Apigenin	GPP	C-3	6.77
UNK49	Apigenin	GPP	C-12/C-16	7.36
UNK50	Apigenin	GPP	C-9	7.68
UNK51	Apigenin	GPP	C-5	8.19

[0255] Table 16 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using Apigenin as substrate and GPP as donor. Table 16 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 16: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using Apigenin as substrate and GPP as donor

ID#	**Mutations**	**5.84**	**6.77**	**7.36**	**7.68**	**8.19**
1	Q295C	0.037	0.656	0.079	0.844	0.028
2	Q295E	0.008	0.512	0.01	0.065	0.035
3	Q295F	0.881	8.074	0.332	0.949	0.037
4	Q295G	0.036	0.184	0.032	0.375	0.018
5	Q295H	0.098	1.299	0.007	0.281	0.008
6	Q295I	0.033	0.744	0.118	3.573	0.148
7	Q295L	0.073	1.146	0.221	10.153	0.042
8	Q295M	0.337	3.197	0.213	4.572	0.029
9	Q295N	0.012	0.095	0.024	0.143	0.012
10	Q295D	0.014	0.295	0.024	0.052	0.015
11	Q295K	0.007	0.044	0.021	0.029	0.004
12	Q295P	0.007	0.028	0.003	0.025	0.003
13	Q295R	0.005	0.011	0.001	0.002	0.003
14	Q295S	0.015	0.158	0.023	0.242	0.018
15	Q295T	0.017	0.14	0.016	1.154	0.011
16	Q295V	0.017	0.124	0.039	1.275	0.034
17	Q295Y	0.031	3.792	0.048	3.475	0.053
18	Q295W	0.606	6.037	0.11	0.303	0.014
19	Q295A	0.024	0.17	0.029	0.636	0.032
20	Q295Q	0.051	6.947	0.107	7.634	0.209
21	WT	0.049	5.977	0.104	5.551	0.17
22	S214E	0.008	0.234	0.002	0.221	0.101
23	S214H	0.005	0.216	0.001	0.01	0.013
24	S214Q	0.008	0.107	0.003	0.012	0.038

25	S214R	0.01	0.119	0.003	0.688	0.1
26	Q161A	0.115	40.518	0.579	7.562	0.456
27	Q161C	0.026	19.176	0.487	3.827	0.256
28	Q161D	0.033	0.563	0.016	0.595	0.027
29	Q161E	0.065	0.664	0.019	0.633	0.028
30	Q161F	0.019	5.93	0.096	1.626	0.674
31	Q161G	1.071	36.638	0.561	4.654	0.461
32	Q161H	0.156	10.678	0.221	7.605	0.211
33	Q161I	0.017	32.007	0.281	8.586	0.639
34	Q161K	0.042	27.674	0.412	9.077	0.591
35	Q161L	0.009	3.693	0.115	2.828	0.124
36	Q161M	0.011	2.368	0.145	1.264	0.099
37	Q161N	0.02	3.968	0.078	2.371	0.069
38	Q161P	0.057	31.048	0.831	1.91	0.168
39	Q161Q	0.085	8.857	0.123	7.771	0.229
40	Q161R	0.034	5.103	0.655	33.99	0.143
41	Q161S	0.276	29.936	0.543	6.19	0.204
42	Q161T	0.05	21.028	0.272	8.879	0.163
43	Q161V	0.033	39.061	0.513	7.092	0.539
44	Q161W	0.012	14.605	0.283	19.196	0.013
45	Q161Y	0.018	3.813	0.032	2.387	0.091
46	WT	0.027	3.054	0.066	2.948	0.09
47	V294A_Q161S	0.584	7.832	0.386	6.468	0.235
48	A53T	0.941	11.324	0.131	5.903	0.575
49	Q161S	0.453	11.836	0.18	2.99	0.305
50	Q295A	0.019	0.263	0.019	0.722	0.042
51	Q295W	0.968	8.572	0.161	0.416	0.022
52	V294A	0.144	2.117	0.177	6.328	0.193
53	WT	0.132	7.706	0.103	7.002	0.304

[0256] The amount of each prenylation product was measured by HPLC. FIG. 8 shows a heatmap of the HPLC areas of each prenylation product generated using Apigenin as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 16.

Example 10: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using Naringenin as substrate and GPP as donor

[0257] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to "breakdown" analysis. "Breakdown" analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. "Breakdown" variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position. A subset of Orf2 Mutant enzymes were screened for prenylation when using Naringenin as substrate and GPP as donor.

[0258] The wild type Orf2 prenylation reaction using Naringenin as substrate and GPP as donor produces 2 products as detected by HPLC. The respective retention times of these products are approximately 6.86 and 7.49 minutes.

[0259] Table 17 provides a summary of the prenylation products produced from Naringenin and GPP, their retention times, and the hypothesized prenylation site on Naringenin. FIG. 24 shows the predicted chemical structures of the respective prenylation products.

Table 17: Predicted prenylation products of Orf2 or Orf2 Mutants when using Naringenin as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
RBI-41	Naringenin	GPP	C-3	6.86
RBI-42	Naringenin	GPP	C-5	7.49

[0260] Table 18 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using Naringenin as substrate and GPP as donor. Table 18 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 18: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using Naringenin as substrate and GPP as donor

ID#	**Mutations**	**6.86**	**7.49**
1	WT	8.202	31.829
2	Q295C	2.253	2.131
3	Q295E	0.642	0.105
4	Q295F	6.571	1.125
5	Q295G	0.658	0.37

6	Q295H	3.33	42.881
7	Q295I	0.748	3.277
8	Q295L	1.539	16.474
9	Q295M	3.364	6.71
10	Q295N	0.472	0.522
11	Q295D	0.534	0.051
12	Q295K	0.359	0.04
13	Q295P	0.311	0.039
14	Q295R	0.209	0.006
15	Q295S	0.34	0.2
16	Q295T	0.306	0.199
17	Q295V	0.828	2.854
18	Q295Y	15.157	44.511
19	Q295W	6.094	0.324
20	Q295A	0.703	0.806
21	Q295Q	17.351	24.072
22	WT	16.28	29.481
23	S214E	1.438	0.97
24	S214H	0.85	0.092
25	S214Q	2.065	0.129
26	S214R	0.237	5.428
27	Q161A	9.731	20.938
28	Q161C	22.728	5.655
29	Q161D	3.005	8.28
30	Q161E	2.627	10.858
31	Q161F	11.362	2.239
32	Q161G	4.44	4.066
33	Q161H	5.966	11.015
34	Q161I	34.974	29.071
35	Q161K	18.385	21.875
36	Q161L	22.325	13.502
37	Q161M	14.437	8.335
38	Q161N	4.897	9.208
39	Q161P	4.697	1.86
40	Q161Q	10.32	23.439
41	Q161R	3.622	32.151
42	Q161S	17.823	22.064
43	Q161T	20.046	51.667
44	Q161V	57.983	24.995
45	Q161W	32.888	64.656
46	Q161Y	38.983	19.701
47	WT	8.581	34.506
48	V294A_Q161S	10.737	18.441
49	A53T	19.936	21.86

50	Q161S	15.186	18.466
51	Q295A	2.624	4.295
52	Q295W	9.322	0.573
53	V294A	2.607	15.69
54	WT2	11.047	32.557

[0261] The amount of each prenylation product was measured by HPLC. FIG. 9 shows a heatmap of the HPLC areas of each prenylation product generated using Naringenin as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 18.

Example 11: Generation of ORF2 variants which synthesize an altered amount of prenylated products when using Reservatrol as substrate and GPP as donor

[0262] A rational design approach was used to generate a library of 96 ORF2 triple mutants in which each triple mutant carried amino acid substitutions at 3 of 36 selected residues following the methods described in Example 1. These triple mutants may be interchangeably referred to as tripleton variants or tripleton mutants. Each amino acid substitution was employed 3-5 times in the library. From 66 of the 96 clones each carrying a unique tripleton ORF2 variant, ORF2 mutant proteins were expressed and their activity was analyzed as described in Example 1 . Clones that exhibited improved function relative to the wild type enzyme were subjected to “breakdown” analysis. “Breakdown” analysis involves creating all possible combinations of double mutations and all single combinations from the parental tripleton yielding 6 unique variant enzymes from a single parental tripleton. “Breakdown” variants were used to identify residues for site saturation where all 19 other amino acids were substituted at a single position. A subset of Orf2 Mutant enzymes were screened for prenylation when using Reservatrol as substrate and GPP as donor.

[0263] The wild type Orf2 prenylation reaction using Reservatrol as substrate and GPP as donor produces 4 products as detected by HPLC. The respective retention times of these products are approximately 5.15, 5.87, 7.3, and 8.44 minutes.

[0264] Table 19 provides a summary of the prenylation products produced from Reservatrol and GPP, their retention times, and the hypothesized prenylation site on Reservatrol. FIG. 25 show the predicted chemical structures of the respective prenylation products.

Table 19: Predicted prenylation products of Orf2 or Orf2 Mutants when using Reservatrol as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
RBI-49	Resveratrol	GPP	C-11/C-13	5.15
RBI-48	Resveratrol	GPP	C-3	5.87
UNK52	Resveratrol	GPP	C-10/C-14	7.3
UNK53	Resveratrol	GPP	C-1/5	8.44

[0265] Table 20 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce prenylated products using Reservatrol as substrate and GPP as donor. Table 20 lists the mutations within each of the mutants analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 20: HPLC Area in mAU*min of prenylation products produced by Orf2 and Orf2 Variants when using Reservatrol as substrate and GPP as donor

ID#	**Mutations**	**5.15**	**5.87**	**7.3**	**8.44**
1	WT	0.072	2.459	0.048	0.469
2	Q295C	0.246	18.951	0.212	1.203
3	Q295E	0.014	0.478	0.057	0.109
4	Q295F	0.149	1.98	0.14	0.099
5	Q295G	0.037	3.468	0.09	0.287
6	Q295H	0.489	22.335	0.364	3.931
7	Q295I	0.243	9.527	0.286	1.362
8	Q295L	0.045	5.68	0.13	0.45
9	Q295M	0.136	6.969	0.21	0.819
10	Q295N	0.048	1.249	0.057	0.033
11	Q295D	0.031	1.5	0.076	0.066
12	Q295K	0.032	0.354	0.062	0.001
13	Q295P	0.024	0.604	0.066	0.035
14	Q295R	0.008	0.082	0.07	0.001
15	Q295S	0.05	3.534	0.07	0.126
16	Q295T	0.026	4.023	0.067	0.589
17	Q295V	0.113	11.513	0.156	1.525
18	Q295Y	0.014	2.113	0.084	0.419
19	Q295W	0.308	2.323	0.15	0.24
20	Q295A	0.064	10.437	0.115	0.842
21	Q295Q	0.019	2.981	0.083	0.59
22	WT	0.017	2.104	0.072	0.397
23	S214E	0.032	31.678	0.117	2.491
24	S214H	0.023	33.632	0.018	0.433
25	S214Q	0.033	46.708	0.058	2.431
26	S214R	0.086	0.851	0.02	0.018
27	Q161A	0.254	5.286	0.082	1.987
28	Q161C	0.358	32.321	0.15	2.578

29	Q161D	0.059	13.127	0.173	1.02
30	Q161E	0.073	6.357	0.092	0.347
31	Q161F	0.073	6.956	0.085	0.678
32	Q161G	10.292	2.309	1.037	27.413
33	Q161H	0.048	21.619	0.089	2.828
34	Q161I	0.131	13.601	0.118	2.778
35	Q161K	0.318	3.085	0.09	1.716
36	Q161L	0.023	23.734	0.099	2.929
37	Q161M	0.02	18.21	0.103	2.641
38	Q161N	0.02	1.342	0.041	0.107
39	Q161P	0.054	1.494	0.034	0.481
40	Q161Q	0.031	3.151	0.049	0.894
41	Q161R	0.357	2.428	0.092	2.265
42	Q161S	0.022	9.936	0.101	3.788
43	Q161T	0.019	6.117	0.051	1.709
44	Q161V	0.036	7.982	0.071	1.898
45	Q161W	0.003	1.471	0.045	0.124
46	Q161Y	0.007	2.943	0.049	0.368
47	WT	0.016	1.044	0.047	0.168
48	V294A_Q161S	0.328	17.675	0.288	6.416
49	A53T	0.075	12.785	0.099	3.09223
50	Q161S	0.076	12.144	0.086	4.129
51	Q295A	0.017	3.542	0.031	0.403
52	Q295W	0.588	2.626	0.071	0.288
53	V294A	0.216	11.208	0.131	2.357
54	WT2	0.072	3.864	0.018	0.617

[0266] The amount of each prenylation product was measured by HPLC. FIG. 10 shows a heatmap of the HPLC areas of each prenylation product generated using Reservatrol as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an Orf2 or Orf2 variant. Prenylation products are labeled by retention time. Enzyme variants are labeled by ID# as listed in Table 20.

Example 12: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using ORA as substrate and DMAPP as donor

[0267] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0268] The prenylation reaction using ORA as substrate and DMAPP as donor produces 5 products as detected by HPLC. The respective retention times of these products are approximately 2.5, 2.77, 2.89, 4.78, and 4.96 minutes.

[0269] Table 21 provides a summary of the prenylation products produced from ORA and DMAPP, their retention times, and the hypothesized prenylation site on ORA. FIG. 26 shows the predicted chemical structures of the respective prenylation products.

Table 21: Predicted prenylation products of aromatic prenyltransferase enzymes when using ORA as substrate and DMAPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK25	ORA	DMAPP	CO	2.503
UNK26	ORA	DMAPP	2-O	4.963
UNK27	ORA	DMAPP	4-O	4.797
UNK28	ORA	DMAPP	3-C	2.765
UNK29	ORA	DMAPP	5-C	2.887

[0270] Table 22 provides a summary of the analysis performed on the enzymatic activity of the APT enzymes to produce prenylated products using ORA as substrate and DMAPP as donor. Table 22 lists the APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 22: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using ORA as substrate and DMAPP as donor

ID#	**APT**	**2.503**	**2.765**	**2.887**	**4.797**	**4.963**
1	PB-002	0.806	0.001	1.51	0.022	0.013
2	PB-005	0.209	0.341	0.304	0.01	0.018
3	PB-006	8.57	0.077	15.442	0.001	0.211
4	PB-064	8.833	0.62	1.8872	30.127	2.143
5	PB-065	1.125	0.052	1.3627	0.0227	6.855
6	PBJ	0.021	0.014	0.0031	0.0033	0.002
7	Orf2-A53T	2.384	0.081	0.202	0.008	0.208
8	Orf2-Q295F	0.586	0.004	0.145	0.002	0.186

[0271] The amount of each prenylation product was measured by HPLC. FIG. 11 shows a heatmap of the HPLC areas of each prenylation product generated using ORA as substrate and DMAPP as donor. Each column represents a single prenylation product and each row represents an APT enzyme. Prenylation products are labeled by retention time. APTs are labeled by ID# as listed in Table 22.

Example 13: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using DV as substrate and DMAPP as donor

[0272] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0273] The prenylation reaction using DV as substrate and DMAPP as donor produces 5 products as detected by HPLC. The respective retention times of these products are approximately 4.04, 4.65, 5.26, 6.83, and 7.06 minutes.

[0274] Table 23 provides a summary of the prenylation products produced from DV and DMAPP, their retention times, and the hypothesized prenylation site on DV. FIG. 27 shows the predicted chemical structures of the respective prenylation products.

Table 23: Predicted prenylation products of aromatic prenyltransferase enzymes when using DV as substrate and DMAPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK54	DV	DMAPP	1-C/5-C	4.645
UNK55	DV	DMAPP	2-O/4-O	5.26
UNK56	DV	DMAPP	3-C	4.037
UNK57	DV	DMAPP	5-C + 3-C	6.833
UNK58	DV	DMAPP	5-C + 1-C	7.06

[0275] Table 24 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using DV as substrate and DMAPP as donor. Table 24 lists the APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 24: HPLC Area in mAU*min of prenylation products produced APT enzymes when using DV as substrate and DMAPP as donor

ID#	**Mutations**	**4.037**	**4.645**	**5.26**	**6.833**	**7.06**
1	PB-002	0.249	0.937	0.002	0.178	0.017
2	PB-005	0.646	1.4	2.352	0.321	5.071
3	PB-006	1.814	1.375	0.001	4.782	0.717
4	PB-064	0.144	0.7642	0.001	0.138	0.002
5	PB-065	0.01	0.3027	0.001	0.122	0.116
6	PBJ	0.013	0.3274	0.001	0.052	0.39
7	Orf2-A53T	0.098	0.1293	0.009	0.18	0.001
8	Orf2-Q295F	0.002	0.0213	0.002	0.222	0.001

[0276] The amount of each prenylation product was measured by HPLC. FIG. 12 shows a heatmap of the HPLC areas of each prenylation product generated using DV as substrate and DMAPP as donor. Each column represents a single prenylation product and each row represents APT enzyme. Prenylation products are labeled by retention time. APTs are labeled by ID# as listed in Table 24.

Example 14: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using DV as substrate and GPP as donor

[0277] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0278] The prenylation reaction using DV as substrate and GPP as donor produces 2 products as detected by HPLC. The respective retention times of these products are approximately 6.37 and 6.88 minutes.

[0279] Table 25 provides a summary of the prenylation products produced from DV and GPP, their retention times, and the hypothesized prenylation site on DV. FIG. 28 show the predicted chemical structures of the respective prenylation products.

Table 25: Predicted prenylation products of aromatic prenyltransferase enzymes when using DV as substrate and GPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
RBI-32	DV	GPP	3C	6.368
RBI-33	DV	GPP	1-C/5-C	6.883

[0280] Table 26 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using DV as substrate and GPP as donor. Table 26 lists the APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 26: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using DV as substrate and GPP as donor

ID#	Mutations	6.368	6.883
1	Orf2-A53Q+Y288H	0.185	1.119
2	Orf2-Q161S+V294A	1.959	1.295
3	Orf2-A53T	1.026	2.371
4	Orf2-Q295A	0.409	0.851
5	Orf2-Q295W	0.277	0.711
6	Orf2-V294A	0.692	1.193
7	Orf2-Q295F	0.566	0.758
8	Orf2-Q295H	4.074	1.772
9	Orf2-S214R	0.130	0.377

10	Orf2-WT	0.326	1.077
11	PB-005	0.006	0.086
12	PB-064	0.010	0.059
13	PBJ	0.019	0.430

[0281] The amount of each prenylation product was measured by HPLC. FIG. 13 shows a heatmap of the HPLC areas of each prenylation product generated using DV as substrate and GPP as donor. Each column represents a single prenylation product and each row represents an APT enzyme. Prenylation products are labeled by retention time. APTs are labeled by ID# as listed in Table 26.

Example 15: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using DVA as substrate and DMAPP as donor

[0282] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0283] The prenylation reaction using DVA as substrate and DMAPP as donor produces 4 products as detected by HPLC. The respective retention times of these products are approximately 4.21, 4.29, 4.84, and 5.55 minutes.

[0284] Table 27 provides a summary of the prenylation products produced from DVA and DMAPP, their retention times, and the hypothesized prenylation site on DVA. FIG. 29 shows the predicted chemical structures of the respective prenylation products.

Table 27: Predicted prenylation products of aromatic prenyltransferase enzymes when using DVA as substrate and DMAPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK7	DVA	DMAPP	2-O	5.545
UNK8	DVA	DMAPP	4-O	4.835
UNK9	DVA	DMAPP	3-C	4.213
UNK10	DVA	DMAPP	5-C	4.285

[0285] Table 28 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using DVA as substrate and DMAPP as donor. Table 26 lists the APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 28: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using DVA as substrate and DMAPP as donor

ID#	**Mutations**	**4.213**	**4.285**	**4.835**	**5.545**
1	PB-002	0.001	0.531	0.093	0.2
2	PB-005	0.001	0.312	0.103	0.195
3	PB-006	0.04	39.357	0.189	0.196
4	PB-064	0.76	0.1638	0.134	0.198
5	PB-065	1.304	1.2925	0.126	0.145
6	PBJ	0.003	0.0089	0.005	0.213
7	Orf2-A53T	1.573	0.5925	0.163	0.183
8	Orf2-Q295F	0.114	1.1744	0.069	0.127

[0286] The amount of each prenylation product was measured by HPLC. FIG. 14 shows a heatmap of the HPLC areas of each prenylation product generated using DVA as substrate and DMAPP as donor. Each column represents a single prenylation product and each row represents an APT enzyme. Prenylation products are labeled by retention time. APTs are labeled by ID# as listed in Table 28.

Example 16: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using O as substrate and DMAPP as donor

[0287] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0288] The prenylation reaction using O as substrate and DMAPP as donor produces 5 products as detected by HPLC. The respective retention times of these products are approximately 5.46, 6.04, 6.98, 7.65, and 7.91 minutes.

[0289] Table 29 provides a summary of the prenylation products produced from O and DMAPP, their retention times, and the hypothesized prenylation site on O. FIG. 30 shows the predicted chemical structures of the respective prenylation products.

Table 29: Predicted prenylation products of aromatic prenyltransferase enzymes when using O as substrate and DMAPP as donor

Molecule ID	**Substrate**	**Donor**	**Attachment Site**	**Retention Time**
RBI-09	O	DMAPP	3-C	5.46
RBI-10	O	DMAPP	1-C/5-C	6.04
UNK16	O	DMAPP	2-O/4-O	6.982
RBI-12	O	DMAPP	1-C+5-C	7.91
RBI-11	O	DMAPP	1-C+3-C	7.648

[0290] Table 30-a provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using O as substrate and DMAPP as donor. Table 30-a lists APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 30-a: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using O as substrate and DMAPP as donor

ID#	**Mutations**	**RBI-09**	**6.04**	**6.982**	**7.648**	**7.91**
1	PB-005	1.043	8.722	0.425	0.251	3.148
2	PB-006	4.470	4.243	0.001	2.041	0.667
3	PB-064	0.144	0.280	0.001	0.001	0.001
4	PB-065	0.035	0.719	0.001	0.001	0.326
5	PBJ	0.076	1.003	0.691	0.011	1.239

[0291] The amount of each prenylation product was measured by HPLC. FIG. 14 shows a heatmap of the HPLC areas of each prenylation product generated using O as substrate and DMAPP as donor. Each column represents a single prenylation product and each row represents an APT enzyme. Prenylation products are labeled by retention time with the exception of RBI-09. APTs are labeled by ID# as listed in Table 30-a.

Example 17: Production of Derivative Molecules by refeeding CBGA to Orf2 mutants with DMAPP as a donor

[0292] CBGA produced from an aromatic prenyltransferase reaction with OA and GPP and ORF2 or Orf2 variants as described in Example 3 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction with Orf2 or Orf2 variants and DMAPP as the donor. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride ($MgCl2$), 4 millimolar DMAPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar CBGA, and 40 micrograms Orf2 variant protein. These reactions were incubated for 16 hours at 30°C.

[0293] The prenylation reaction using CBGA as substrate and DMAPP as donor produced a product as detected by HPLC with a retention time of approximately 9.095 minutes.

[0294] Table 30-b provides a summary of the prenylation product produced from CBGA and DMAPP, the retention times, and the hypothesized prenylation site on CBGA. FIG. 31 shows the predicted chemical structure of the prenylation product.

Table 30-b: Predicted prenylation product of Orf2 enzymes when using CBGA as substrate and DMAPP as donor

Molecule	**Prenylation Sites**	**Orf2Clone**	**Mutation**	**mAU*min (9.13)**
RBI-22	5-C (DMAPP) + 3-C (GPP)	33-2	A53T	0.0644
RBI-22	5-C (DMAPP) + 3-C (GPP)	122-2	S214R	0.0644

RBI-22	5-C (DMAPP) + 3-C (GPP)	56-2	Q295F	0.0224

Example 18: Production of Derivative Molecules by refeeding RBI-04 (5-GOA) to Orf2 mutants with DMAPP as a donor

[0295] RBI-04 (5-GOA) produced from an aromatic prenyltransferase reaction with OA and GPP using Orf2 or Orf2 variants as the prenyltransferase as described in Example 3 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 or Orf2 variants as the prenyltransferase. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar DMAPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar CBGA, and 40 micrograms Orf2 variant protein. These reactions were incubated for 16 hours at 30°C.

[0296] The prenylation reaction using RBI-04 (5-GOA) as substrate and DMAPP as donor produced a product as detected by HPLC with a retention time of approximately 9.088 minutes.

[0297] Table 31 provides a summary of the prenylation product produced from RBI-04 (5-GOA) and DMAPP, the retention times and the hypothesized prenylation site on RBI-04 (5-GOA). FIG. 32 shows the predicted chemical structure of the prenylation product.

Table 31: Predicted prenylation product of Orf2 enzymes when using RBI-04 (5-GOA) as substrate and DMAPP as donor

Molecule	Prenylation Sites	Mutation	mAU*min (9.088)
UNK36	5-C (GPP) + 3-C (DMAPP)	Q295F	9.018

Example 19: Production of Derivative Molecules by refeeding RBI-04 (5-GOA) to Orf2 mutants with FPP as a donor

[0298] RBI-04 (5-GOA) produced from an aromatic prenyltransferase reaction with OA and GPP using Orf2 or Orf2 variants as the prenyltransferase as described in Example 3 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 or Orf2 variants as the prenyltransferase. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar FPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-04 (5-GOA), and 40 micrograms Orf2 variant protein. These reactions were incubated for 16 hours at 30°C.

[0299] The prenylation reaction using RBI-04 (5-GOA) as substrate and FPP as donor produced a product as detected by HPLC with a retention time of approximately 16.59 minutes.

[0300] Table 32 provides a summary of the prenylation product produced from RBI-04 (5-GOA) and FPP, the retention times and the hypothesized prenylation site on RBI-04 (5-GOA). FIG. 33 shows the predicted chemical structure of the prenylation product.

Table 32: Predicted prenylation product of Orf2 enzymes when using RBI-04 (5-GOA) as substrate and FPP as donor

Molecule	Prenylation Sites	Mutation	mAU*min (16.59)
UNK42	5-C (GPP) + 3-C (FPP)	Q295F	1.747

Example 20: Production of Derivative Molecules by refeeding RBI-04 (5-GOA) to Orf2 mutants with GPP as a donor

[0301] RBI-04 (5-GOA) produced from an aromatic prenyltransferase reaction with OA and GPP using Orf2 or Orf2 variants as the prenyltransferase as described in Example 3 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 or Orf2 variants as the prenyltransferase. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 2 millimolar GPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-04 (5-GOA), and 20 micrograms Orf2 variant protein. These reactions were incubated for 16 hours at 30°C.

[0302] The prenylation reaction using RBI-04 (5-GOA) as substrate and GPP as donor produced a product as detected by HPLC with a retention time of approximately 11.6 minutes.

[0303] Table 33 provides a summary of the prenylation product produced from RBI-04 (5-GOA) and GPP, the retention times and the hypothesized prenylation site on RBI-04 (5-GOA). FIG. 34 shows the predicted chemical structure of the prenylation product.

Table 33: Predicted prenylation product of Orf2 enzymes when using RBI-04 (5-GOA) as substrate and GPP as donor

Molecule	Prenylation Sites	Mutation	5GOA	mAU*min (11.6)
RBI-07	3-C (GPP) + 5-C (GPP)	Q295A	0.029	2.101
RBI-07	3-C (GPP) + 5-C (GPP)	S214R	0.053	10.7
RBI-07	3-C (GPP) + 5-C (GPP)	A53T	3.516	1.05

Example 21: Production of Derivative Molecules by refeeding RBI-08 to Orf2 mutants with DMAPP as a donor

[0304] RBI-08 produced from an aromatic prenyltransferase reaction with OA and DMAPP using Orf2 or Orf2 variants as the prenyltransferase as described in Example 2 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 or Orf2 variants as the prenyltransferase. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar DMAPP, 100 millimolar HEPES buffer at a pH of 7.5, 1 millimolar RBI-08, and 40 micrograms Orf2 variant protein. These reactions were incubated for 16 hours at 30°C.

[0305] The prenylation reaction using RBI-08 as substrate and DMAPP as donor produced a product as detected by HPLC with a retention time of approximately 7.55 minutes.

[0306] Table 34 provides a summary of the prenylation product produced from RBI-08 and DMAPP, the retention times and the hypothesized prenylation site on RBI-08. FIG. 35 shows the predicted chemical structure of the prenylation product.

Table 34: Predicted prenylation product of Orf2 enzymes when using RBI-08 as substrate and DMAPP as donor

Molecule	Prenylation Sites	Mutation	mAU*min (7.55)
RBI-18	5-C (DMAPP) + 3-C (DMAPP)	S214R	0.1356
RBI-18	5-C (DMAPP) + 3-C (DMAPP)	Q295F	1.3375
RBI-18	5-C (DMAPP) + 3-C (DMAPP)	A53T	7.9273

Example 22: Production of Derivative Molecules by refeeding RBI-08 to Orf2 mutants with GPP as a donor

[0307] RBI-08 produced from an aromatic prenyltransferase reaction with OA and DMAPP using Orf2 or Orf2 variants as the prenyltransferase as described in Example 2 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 or Orf2 variants as the prenyltransferase The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar GPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-08, and 40 micrograms Orf2 variant protein. These reactions were incubated for 16 hours at 30°C.

[0308] The prenylation reaction using RBI-08 as substrate and GPP as donor produced 2 products as detected by HPLC with retention times of approximately 8.22 and 9.1 minutes.

[0309] Table 35 provides a summary of the prenylation products produced from RBI-08 and GPP, the retention times and the hypothesized prenylation sites on RBI-08. FIG. 36 shows the predicted chemical structures of the prenylation products.

Table 35: Predicted prenylation product of Orf2 enzymes when using RBI-09 as substrate and GPP as donor

Molecule	Prenylation Sites	Mutation	mAU*min	Retention Time
UNK38	CO (GPP) + 3-C (DMAPP)	A53T	6.4738	8.22
UNK38	CO (GPP) + 3-C (DMAPP)	S214R	0.0039	8.22
UNK38	CO (GPP) + 3-C (DMAPP)	Q295F	5.9266	8.22

UNK36	5-C (GPP) + 3-C (DMAPP)	A53T	2.5133	9.1
UNK36	5-C (GPP) + 3-C (DMAPP)	S214R	0.0276	9.1
UNK36	5-C (GPP) + 3-C (DMAPP)	Q295F	1.6517	9.1

Example 23: Production of Derivative Molecules by refeeding RBI-09 to Orf2 mutants with GPP as a donor

[0310] RBI-09 produced from an aromatic prenyltransferase reaction with O and DMAPP as described in Example 16 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 or Orf2 variants and GPP as the donor. The first prenyltransferase reaction can include any of the prenyltransferases listed in Example 16. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar GPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-09, and 40 micrograms Orf2 variant protein. These reactions were incubated for 16 hours at 30°C.

[0311] The prenylation reaction using RBI-09 as substrate and GPP as donor produced a product as detected by HPLC with a retention time of approximately 9.26 minutes.

[0312] Table 36 provides a summary of the prenylation product produced from RBI-09 and GPP, the retention times and the hypothesized prenylation sites on RBI-09. FIG. 37 shows the predicted chemical structures of the prenylation products.

Table 36: Predicted prenylation product of Orf2 enzymes when using RBI-09 as substrate and GPP as donor

Molecule	Prenylation Sites	Mutation	mAU*min (9.26)
UNK40	5-C (GPP) + 3-C (DMAPP)	Q295Y	5.6977

Example 24: Production of Derivative Molecules by refeeding RBI-10 to APT enzymes with DMAPP as a donor

[0313] RBI-010 produced from an aromatic prenyltransferase reaction with O and DMAPP as described in Example 16 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using PB-005 or PB-006 as the prenyltransferase and DMAPP as the donor. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 2 millimolar DMAPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-10, and 20 micrograms APT protein. Two APT enzymes were tested. These reactions were incubated for 16 hours at 30°C.

[0314] The prenylation reaction using RBI-10 as substrate and DMAPP as donor produced 2 product as detected by HPLC with a retention times of approximately 7.65 and 7.91 minutes.

[0315] Table 37 provides a summary of the prenylation products produced from RBI-10 and DMAPP, the retention times and the hypothesized prenylation sites on RBI-10. FIG. 38 shows the predicted chemical structures of the prenylation products.

Table 37: Predicted prenylation product of Orf2 enzymes when using RBI-10 as substrate and DMAPP as donor

Molecule	Prenylation Sites	APT	mAU*min	Retention Time
RBI-11	1-C (DMAPP)+3-C (DMAPP)	PB-005	0.5236	7.65
RBI-11	1-C (DMAPP)+3-C (DMAPP)	PB-006	7.401	7.65
RBI-12	1-C (DMAPP)+5-C (DMAPP)	PB-005	4.7233	7.91
RBI-12	1-C (DMAPP)+5-C (DMAPP)	PB-006	1.208	7.91

Example 25: Production of Derivative Molecules by refeeding RBI-10 to APT enzymes with FPP as a donor

[0316] RBI-010 produced from an aromatic prenyltransferase reaction with O and DMAPP as described in Example 16 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using PB-005 or Orf2 variants as the prenyltransferase and FPP as the donor. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar FPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-10, and 40 micrograms APT protein. Two APT enzymes were tested. These reactions were incubated for 16 hours at 30°C.

[0317] The prenylation reaction using RBI-10 as substrate and FPP as donor produced 2 products as detected by HPLC with a retention times of approximately 11.8 and 12.9 minutes.

[0318] Table 38 provides a summary of the prenylation products produced from RBI-10 and FPP, the retention times and the hypothesized prenylation sites on RBI-10. FIG. 39 shows the predicted chemical structures of the prenylation products.

Table 38: Predicted prenylation product of Orf2 enzymes when using RBI-10 as substrate and FPP as donor

Molecule	Prenylation Sites	APT	mAU*Min	Retention Time
UNK44	5-C (DMAPP) + 3-C (FPP)	PB-005	0.5236	11.8
UNK44	5-C (DMAPP) + 3-C (FPP)	Orf2-Q295Y	7.401	11.8
UNK45	5-C (DMAPP) + 1-C(FPP)	PB-005	4.7233	12.9
UNK45	5-C (DMAPP) + 1-C(FPP)	Orf2-Q295Y	1.208	12.9

Example 26: Production of Derivative Molecules by refeeding RBI-10 to Orf2 Variant enzymes with GPP as a donor

[0319] RBI-010 produced from an aromatic prenyltransferase reaction with O and DMAPP as described in Example 16 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 variants as the prenyltransferase and GPP as the donor. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar GPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-10, and 40 micrograms Orf2 Variant protein. These reactions were incubated for 16 hours at 30°C.

[0320] The prenylation reaction using RBI-10 as substrate and GPP as donor produced 2 products as detected by HPLC with a retention times of approximately 9.2 and 9.7 minutes.

[0321] Table 39 provides a summary of the prenylation products produced from RBI-10 and GPP, the retention times and the hypothesized prenylation sites on RBI-10. FIG. 40 shows the predicted chemical structures of the prenylation products.

Table 39: Predicted prenylation product of Orf2 enzymes when using RBI-10 as substrate and GPP as donor

Molecule	Prenylation Sites	Mutation	mAU*min	Retention Time
UNK41	5-C (DMAPP) + 3-C (GPP)	Q295Y	14.558	9.2
UNK41	5-C (DMAPP) + 3-C (GPP)	S214R	8.9769	9.2
UNK66	5-C (DMAPP) + 1-C (GPP)	Q295Y	1.4035	9.7
UNK66	5-C (DMAPP) + 1-C (GPP)	S214R	1.2629	9.7

Example 27: Production of Derivative Molecules by refeeding RBI-12 to Orf2 Variant enzymes with GPP as a donor

[0322] RBI-12 produced from an aromatic prenyltransferase reaction as described in Example 16 (1 reactions) or Example 24 (2 sequential reactions) was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction using Orf2 variants as the prenyltransferase and GPP as the donor. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar GPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-12, and 40 micrograms Orf2 Variant protein. These reactions were incubated for 16 hours at 30°C.

[0323] The prenylation reaction using RBI-12 as substrate and GPP as donor produced a product as detected by HPLC with a retention time of approximately 11.27 minutes.

[0324] Table 40 provides a summary of the prenylation products produced from RBI-12 and GPP, the retention times and the hypothesized prenylation sites on RBI-12. FIG. 41 shows the predicted chemical structures of the prenylation products.

Table 40: Predicted prenylation product of Orf2 enzymes when using RBI-12 as substrate and GPP as donor

Molecule	Prenylation Sites	Mutation	mAU*min (11.27)
UNK67	5-C (DMAPP) + 1-C (DMAPP)+ 3-C (GPP)	Q295Y	9.4062
UNK67	5-C (DMAPP) + 1-C (DMAPP)+ 3-C (GPP)	S214R	2.0624
UNK67	5-C (DMAPP) + 1-C (DMAPP)+ 3-C (GPP)	A53T	2.5475

Example 28: Production of Derivative Molecules by refeeding RBI-03 to APT enzymes with DMAPP as a donor

[0325] RBI-03 produced from an aromatic prenyltransferase reaction with O as substrate and GPP as donor as described in Example 5 was purified and used as a substrate in a subsequent aromatic prenyltransferase reaction with PB-005 as the prenyltransferase and GPP as the donor. The prenylation reaction was performed in a volume of 20 microliters and contained 20 millimolar magnesium chloride (MgCl2), 4 millimolar DMAPP, 100 millimolar HEPES buffer at a pH of 7.5, 2 millimolar RBI-03, and 40 micrograms APT enzyme. These reactions were incubated for 16 hours at 30°C.

[0326] The prenylation reaction using RBI-03 as substrate and DMAPP as donor produced 2 products as detected by HPLC with retention times of approximately 9.3 and 9.7 minutes.

[0327] Table 41 provides a summary of the prenylation products produced from RBI-03 and DMAPP, the retention times and the hypothesized prenylation sites on RBI-03. FIG. 42 shows the predicted chemical structures of the prenylation products.

Table 41: Predicted prenylation product of APT enzymes when using RBI-03 as substrate and DMAPP as donor

Molecule	Prenylation Sites	APT	mAU*min	Retention Time
UNK40	5-C (GPP) + 3-C (DMAPP)	PB005	0.1765	9.26
UNK66	5-C (DMAPP) + 1-C (GPP)	PB005	1.587	9.7

Example 29: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using O as substrate and FPP as donor

[0328] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0329] The prenylation reaction using O as substrate and FPP as donor produces 3 products as detected by HPLC. The respective retention times of these products are approximately 8.52, 9.57, and 10.94 minutes.

[0330] Table 42 provides a summary of the prenylation products produced from O and FPP, their retention times, and the hypothesized prenylation site on O. FIG. 43 shows the predicted chemical structures of the respective prenylation products.

Table 42: Predicted prenylation products of aromatic prenyltransferase enzymes when using O as substrate and FPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
RBI-15	O	FPP	1-C/5-C	9.57
UNK18	O	FPP	4-O/2-O	10.94
UNK19	O	FPP	3-C	8.52

[0331] Table 43 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using O as substrate and FPP as donor. Table 43 lists APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 43: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using O as substrate and FPP as donor

	Mutations	UNK19 (8.52)	RBI-15 (9.57)	UNK18 (10.94)
1	PB-005	0.473	0.393	0.219
2	Q295Y	0.272	0.259	0.177

Example 30: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using ORA as substrate and FPP as donor

[0332] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0333] The prenylation reaction using ORA as substrate and FPP as donor produces 3 products as detected by HPLC. The respective retention times of these products are approximately 7.44, 7.98, and 8.96 minutes.

[0334] Table 44 provides a summary of the prenylation products produced from ORA and FPP, their retention times, and the hypothesized prenylation site on ORA. FIG. 44 shows the predicted chemical structures of the respective prenylation products.

Table 44: Predicted prenylation products of aromatic prenyltransferase enzymes when using ORA as substrate and FPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK33	ORA	FPP	3-C	7.44
UNK34	ORA	FPP	5-C	7.98
UNK31	ORA	FPP	2-O	8.44

[0335] Table 45 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using ORA as substrate and FPP as donor. Table 45 lists APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 45: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using ORA as substrate and FPP as donor

ID#	Mutations	7.44	7.98	8.96
1	Orf2-A53T	4.940	1.264	0.547
2	Orf2-Q295F	0.822	0.162	0.157

Example 31: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using OA as substrate and GGPP as donor

[0336] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0337] The prenylation reaction using OA as substrate and GGPP as donor produces 2 products as detected by HPLC. The respective retention times of these products are approximately 10.29 and 11.18 minutes.

[0338] Table 46 provides a summary of the prenylation products produced from OA and GGPP, their retention times, and the hypothesized prenylation site on OA. FIG. 45 shows the predicted chemical structures of the respective prenylation products.

Table 46: Predicted prenylation products of aromatic prenyltransferase enzymes when using OA as substrate and GGPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK60	OA	GGPP	3C	10.29
UNK61	OA	GGPP	5-C	11.18

[0339] Table 47 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using OA as substrate and GGPP as donor. Table 47 lists APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 47: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using OA as substrate and GGPP as donor

ID#	Mutations	10.29	11.18
1	Orf2-A53T	0.059	0.233
2	Orf2-Q295F	0.607	0.069

Example 32: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using ORA as substrate and GGPP as donor

[0340] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0341] The prenylation reaction using ORA as substrate and GGPP as donor produces 2 products as detected by HPLC. The respective retention times of these products are approximately 8.98 and 9.06 minutes.

[0342] Table 48 provides a summary of the prenylation products produced from ORA and GGPP, their retention times, and the hypothesized prenylation site on ORA. FIG. 46 shows the predicted chemical structures of the respective prenylation products.

Table 48: Predicted prenylation products of aromatic prenyltransferase enzymes when using ORA as substrate and GGPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK62	ORA	GGPP	3C	8.98
UNK63	ORA	GGPP	5-C	9.06

[0343] Table 49 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using ORA as substrate and GGPP as donor. Table 49 lists APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 49: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using OA as substrate and GGPP as donor

ID#	Mutations	8.98	9.06
1	Orf2-A53T	0.094	0.253
2	Orf2-Q295F	0.071	0.069

Example 33: Screening of prenyltransferase enzymes which synthesize an altered amount of prenylated products when using DVA as substrate and GGPP as donor

[0344] Aromatic Prenyltransferase Enzymes were ordered, expressed, purified, and screened for prenylation as described in Example 1.

[0345] The prenylation reaction using DVA as substrate and GGPP as donor produces 2 products as detected by HPLC. The respective retention times of these products are approximately 9.48 and 9.87 minutes.

[0346] Table 50 provides a summary of the prenylation products produced from DVA and GGPP, their retention times, and the hypothesized prenylation site on DVA. FIG. 47 shows the predicted chemical structures of the respective prenylation products.

Table 50: Predicted prenylation products of aromatic prenyltransferase enzymes when using ORA as substrate and GGPP as donor

Molecule ID	Substrate	Donor	Attachment Site	Retention Time
UNK64	DVA	GGPP	3C	9.48
UNK65	DVA	GGPP	5-C	9.87

[0347] Table 51 provides a summary of the analysis performed on the enzymatic activity of the aromatic prenyltransferase enzymes to produce prenylated products using DVA as substrate and GGPP as donor. Table 51 lists APTs analyzed as well mAU*min areas from the HPLC analysis of the reaction products.

Table 51: HPLC Area in mAU*min of prenylation products produced by APT enzymes when using DVA as substrate and GGPP as donor

ID#	Mutations	9.48	9.87
1	Orf2-A53T	0.063	0.440
2	Orf2-Q295F	0.350	0.064

[0348] Example 34 - Generation of ORF2 variants which synthesize an altered amount of CBFA and/or 5-FOA, compared to WT ORF2

[0349] Table 52 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce CBFA and 5-FOA using Olivetolic Acid (OA) as substrate and FPP as donor. Table 52 lists the mutations within each of the tripleton mutants as well the nMol of CBFA produced, nMol of 5-FOA produced, total prenylated products produced (nMol of CBFA + 5-FOA), %CBFA within total prenylated products (nMol of CBFA / [nMol of CBFA+5-FOA]), % enzymatic activity (total prenylated products produced by a mutant/ total prenylated products produced by wild-type ORF2), CBFA production (%CBFA among total prenylated products * % enzymatic activity), and %5-FOA within prenylated products (nMol of 5-FOA / [nMol of CBFA + 5-FOA]) for each of the ORF2 variants.

[0350] Table 52: Analysis of ORF2 mutants and WT ORF2 based on production of CBFA from OA and FPP

CLONE	**Mutations**	nMol CBFA	nMol 5-FOA	Total Products	%CBFA	%Activity	CBFA Production Potential	%5-FOA
WT	WT	0.055962156	0.364360073	0.42032223	13.31%	100.00%	0.133141082	86.7%
H03	V24_A17T_F213M_S214R	0.297527669	0.012775302	0.310302971	95.88%	58.75%	0.563316386	4.1%
A4	V25_L219F_V294N_Q295A	0.213539807	0.066199295	0.279739102	76.34%	66.55%	0.508038338	23.7%
C6	V43_Q161A_M162F_Q295A	0.120001785	0.009713453	0.129715238	92.51%	30.86%	0.285499497	7.5%
C5	V35_A53Q_S177Y_Y288H	0.111656551	0.089215955	0.200872507	55.59%	47.79%	0.265645125	44.4%
A9	V65_V49A_Q161S_V294A	0.083050696	0.040754271	0.123804967	67.08%	29.45%	0.19758816	32.9%
H9	V72_E112G_G205M_L298W	0.120715816	3.345756699	3.466472515	3.48%	338.13%	0.117748184	96.5%
C11	V83_E112D_L219F_V294F	0.049223492	1.057816162	1.107039654	4.45%	263.38%	0.117108942	95.6%
H2	V16_A53Q_S177W_L219F	0.118930739	0.129125578	0.248056317	47.95%	24.20%	0.116006991	52.1%
D12	V92_A53T_E112D_G205M	0.112995359	2.775408071	2.888403429	3.91%	281.74%	0.110217524	96.1%
D4	V28_A53T_D166E_Q295W	0.045073188	0.208522499	0.253595687	17.77%	60.33%	0.107234843	82.2%
A2	V9_Q38G_E112D_F123H	0.043779007	1.308359905	1.352138912	3.24%	321.69%	0.104155822	96.8%
G12	V95_A17T_Q161W_A232S	0.090994288	0.022488756	0.113483043	80.18%	11.07%	0.088757319	19.8%
F9	V70_Q38G_D166E_Q295A	0.083853981	0.261946492	0.345800472	24.25%	33.73%	0.081792546	75.8%
A5	V33_A17T_C25V_E112G	0.030569439	0.172308212	0.202877652	15.07%	48.27%	0.072728581	84.9%
D11	V84_F123H_L174V_S177E	0.05315066	0.163122664	0.216273324	24.58%	21.10%	0.051844025	75.4%
E9	V69_A53T_M106E_Q161S	0.051544091	0.182338408	0.2338825	22.04%	22.81%	0.050276951	78.0%
G3	V23_L219F_Y283L_L298W	0.048777222	1.532825137	1.581602359	3.08%	154.27%	0.047578102	96.9%
B12	V90_A17T_F123W_L298A	0.018966441	0.074645776	0.093612216	20.26%	22.27%	0.045123572	79.7%
G08	V63_F123W_M162F_C209G	0.012540164	0.00316743	0.015707595	79.84%	5.16%	0.041205063	20.2%
G11	V87_S177W_Y288H_V294N	0.025660478	0.00422324	0.029883719	85.87%	2.91%	0.025029651	14.1%
G9	V71_M106E_G205L_C209G	0.025526598	0.004117659	0.029644257	86.11%	2.89%	0.024899061	13.9%
H5	V40_S177E_S214R_R228E	0.02418779	0.000211162	0.024398952	99.13%	2.38%	0.023593167	0.9%
A3	V17_V49L_F123A_Y283L	0.00941628	0.011825073	0.021241353	44.33%	5.05%	0.022402527	55.7%
A7	V49_G205L_R228E_C230N	0.009059265	0.004856727	0.013915991	65.10%	3.31%	0.021553142	34.9%
A8	V57_C25V_A232S_V271E	0.009059265	0.004856727	0.013915991	65.10%	3.31%	0.021553142	34.9%
A10	V73_V49S_K118Q_S177E	0.008389861	0.039381718	0.047771578	17.56%	11.37%	0.019960545	82.4%
B8	V58_K118Q_L174V_R228Q	0.008389861	0.003589754	0.011979615	70.03%	2.85%	0.019960545	30.0%
B10	V74_M106E_Y121W_D166E	0.007854338	0.003589754	0.011444092	68.63%	2.72%	0.018686468	31.4%

C8	V59_V49S_S214G_V294A	0.007765084	0.053529573	0.061294657	12.67%	14.58%	0.018474121	87.3%
H4	V32_M162A_C209G_Y288H	0.018163156	0.00517347	0.023336626	77.83%	2.28%	0.01771664	22.2%
H7	V56_F123A_M162F_S214G	0.01767226	0.453048124	0.470720384	3.75%	45.91%	0.017237812	96.2%
D6	V44_A53E_Q161A_V294N	0.00709568	0.030090589	0.037186269	19.08%	8.85%	0.016881525	80.9%
B4	V26_A53E_A108G_K118N	0.00709568	0.004012078	0.011107759	63.88%	2.64%	0.016881525	36.1%
G5	V39_A53T_K118N_S214F	0.016467333	0.004434403	0.020901736	78.78%	2.04%	0.016062506	21.2%
D8	V60_E112D_K119A_N173D	0.016065691	0.002745106	0.018810797	85.41%	1.83%	0.015670738	14.6%
F10	V78_K119D_Q161W_L298Q	0.014905391	0.008129738	0.023035129	64.71%	2.25%	0.014538962	35.3%
B2	V10_V49A_S177Y_C209G	0.006024634	0.005279051	0.011303685	53.30%	2.69%	0.01433337	46.7%
H6	V48_V49L_E112D_G286E	0.014548376	0.002428363	0.016976739	85.70%	1.66%	0.014190724	14.3%
C10	V75_A53Q_L274V_Q295A	0.005890753	0.004962308	0.010853061	54.28%	2.58%	0.014014851	45.7%
B6	V42_D166E_S177Y_S214F	0.005489111	0.003061849	0.00855096	64.19%	2.03%	0.013059293	35.8%
D9	V68_K118N_C209G_R228Q	0.013209568	0.003273011	0.016482579	80.14%	1.61%	0.012884829	19.9%
A12	V89_Y121W_S177Y_G286E	0.00535523	0.000844648	0.006199878	86.38%	1.48%	0.012740773	13.6%
F8	V62_A53T_N173D_S214R	0.012540164	0.000422324	0.012962488	96.74%	1.26%	0.012231882	3.3%
A11	V81_V49L_D166E_L274V	0.005132096	0.001372553	0.006504649	78.90%	1.55%	0.012209908	21.1%
D3	V20_D227E_C230N_Q295W	0.005132096	0.007390671	0.012522767	40.98%	2.98%	0.012209908	59.0%
C1	V3_V49S_M162A_Y283L	0.005087469	0.18360538	0.188692849	2.70%	44.89%	0.012103735	97.3%
D8	V60_E112D_K119A_N173D	0.012406283	0.003589754	0.015996038	77.56%	1.56%	0.012101292	22.4%
H8	V64_M106E_M162A_Y216A	0.01187076	0.007073928	0.018944688	62.66%	1.85%	0.011578934	37.3%
C3	V19_V49L_S214R_V271E	0.004685826	0.00211162	0.006797447	68.94%	1.62%	0.011148177	31.1%
D05	V36_F123H_L274V_L298A	0.005622992	0.034313829	0.039936821	14.08%	7.56%	0.010646147	85.9%
B5	V34_A53Q_Y121W_A232S	0.004462692	0.002217201	0.006679893	66.81%	1.59%	0.010617311	33.2%
B11	V82_V49S_K119D_F213M	0.004328811	0.001689296	0.006018107	71.93%	1.43%	0.010298792	28.1%
G2	V15_A53E_F213M_R228Q	0.010487326	0.016153895	0.026641221	39.37%	2.60%	0.010229509	60.6%
H1	V8_K119A_Q161A_R228Q	0.010308818	0.001266972	0.01157579	89.05%	1.13%	0.01005539	10.9%
F12	V94_A17T_V49A_C230N	0.010264191	0.001900458	0.01216465	84.38%	1.19%	0.01001186	15.6%
D7	V52_K119A_S214G_L298A	0.010130311	0.016365057	0.026495368	38.23%	2.58%	0.009881271	61.8%
C7	V51_V49L_K119D_G205M	0.004150303	0.001900458	0.006050762	68.59%	1.44%	0.009874099	31.4%
D10	V76_V49A_F123A_Y288H	0.010041057	0.001266972	0.011308029	88.80%	1.10%	0.009794211	11.2%
C2	V11_K118N_K119A_V271E	0.003971796	0.000844648	0.004816444	82.46%	1.15%	0.009449407	17.5%
H10	V80_M162A_N173D_S214F	0.009505534	0.102624744	0.112130278	8.48%	10.94%	0.009271853	91.5%
G10	V79_V49A_Y121W_C230S	0.009460907	0.00316743	0.012628337	74.92%	1.23%	0.009228323	25.1%
G7	V55_V49S_Y216A_V294N	0.009371653	0.00422324	0.013594893	68.94%	1.33%	0.009141264	31.1%
H11	V88_A108G_Q161S_G205M	0.009282399	0.017632029	0.026914428	34.49%	2.63%	0.009054204	65.5%
D1	V4_K118Q_Q161W_S214F	0.00361478	0.001478134	0.005092915	70.98%	1.21%	0.008600022	29.0%

C9	V67_A108G_K119D_L298A	0.002632988	0.001478134	0.004111122	64.05%	0.98%	0.006264214	36.0%
B9	V66_C25V_F213M_Y216A	0.002499107	0.001583715	0.004082823	61.21%	0.97%	0.005945694	38.8%
C12	V91_N173D_F213M_V294F	0.002454481	0.010558101	0.013012582	18.86%	3.10%	0.005839521	81.1%
G4	V31_D227E_R228E_L298Q	0.004462692	0.004645565	0.009108256	49.00%	0.89%	0.004352983	51.0%
G6	V47_K118Q_F123A_R228E	0.003570154	0.002639525	0.006209679	57.49%	0.61%	0.003482386	42.5%

[0351] The amount of CBFA or 5-FOA (in nMols) generated by each of the ORF2 triple mutant clones was measured using HPLC. FIG. 53 shows the total nMols of prenylated products generated using OA as substrate and FPP as donor by each of the ORF2 triple mutants, and the proportion of CBFA and 5-FOA within the total amount of prenylated products. An exemplary Wild Type ORF2 replicate is included in the graph for comparison purposes.

[0352] FIG. 54 shows the %CBFA within the total prenylated products produced by each of the ORF2 triple mutant clones using OA as substrate and FPP as donor. In this graph, the mutant clones are ordered based on decreasing %CBFA (from left to right) they produce, with the %5-FOA depicted in red. The black threshold line on the graph indicates the %CBFA that is produced by the wild type enzyme.

[0353] FIG. 55 shows the ORF2 enzymatic activity (using OA as substrate and FPP as donor) of each of the triple mutant ORF2 clones relative to the wild type enzyme. %activity was calculated by dividing the nMols of total prenylated products produced by a mutant by the nMols of total prenylated products produced by the wild type control, and expressed as a percentage. The red threshold line is the wild type Orf2 %activity.

[0354] FIG. 56 shows the CBFA production potential of each of the ORF2 triple mutant clones when using OA as substrate and FPP as donor. CBFA production potential (interchangeably referred to herein as CBFA production quotient) represents the improvement in CBFA production vs. the wild type enzyme. CBFA production potential was calculated by multiplying the % CBFA by the % activity of each mutant. For instance, a wild type ORF2, which makes ~20% CBFA, and has an activity of 100%, would have a CBFA Production Potential of 0.2. The red threshold line on the graph represents this wild type value of 0.2.

[0355] While the CBFA production potential analysis shown in FIG. 56 is useful to rank ORF2 mutant clones based on the amount of CBFA produced, such an analysis would not differentiate between a mutant that made 100% CBFA but was 20% as active as wild-type ORF2; or a mutant that made 10% CBFA and was 200% as active as wild type ORF2. Therefore, we employed a cluster analysis by plotting the CBFA Production Potential vs. %5-FOA (FIG. 57). %5-FOA was calculated in a similar manner as %CBFA. We used the top 16 mutants ranked based on their CBFA production potential for this analysis. High 5-FOA producing mutants cluster together towards the right of the graph and high CBFA producing mutants cluster towards the left of the graph.

[0356] Based on the analysis performed in FIG. 57, 12 mutants which cluster to the left of the graph were selected (Table 53). These clones were targeted for "breakdown" analysis. Breakdown analysis involves breaking a parent triple mutant into all pair wise doubleton combinations of mutations as well as all singleton mutations that make up the parental clone. For each parental clone targeted six unique mutants are generated (3 doubles and 3 singles).

[0357] Table 53 - Clones targeted for breakdown analysis based on CBFA production potential and %5-FOA produced, using OA as substrate and FPP as donor

CBFA Production Rank	Clone ID	Mutations
1	H03	V24_A17T_F213M_S214R
2	A04	V25_L219F_V294N_Q295A
3	C06	V43_Q161A_M162F_Q295A
4	C05	V35_A53Q_S177Y_Y288H
5	A09	V65_V49A_Q161S_V294A
8	H02	V16_A53Q_S177W_L219F
10	D04	V28_A53T_D166E_Q295W
12	G12	V95_A17T_Q161W_A232S
13	F09	V70_Q38G_D166E_Q295A
14	A05	V33_A17T_C25V_E112G
15	D11	V84_F123H_L174V_S177E
16	E09	V69_A53T_M106E_Q161S

[0358] For the singleton and doubleton mutants resulting from the breakdown of triple mutants - H03, A04, C06, C05, A09, H02, D04, G12, F09, A05, D11 and E09- the total amount of prenylated products (and the respective proportion of CBFA and 5-FOA); and %CBFA within the prenylated products was calculated. FIGs 58-65 depict the total amount of prenylated products and %CBFA produced using OA as substrate and FPP as donor for the mutants derived from A04 (FIG. 58); C05 (FIG. 59); A09 (FIG. 60); H02 (FIG. 61); D04 (FIG. 62); F09 (FIG. 63); D11 (FIG. 64); and E09 (FIG. 65). The %CBFA for these clones, along with the mutations they carry, are listed in Table 54.

[0359] In a similar manner, the triple mutants, H03, C06, A05 and G12, will also be subjected to "breakdown" analysis. Further, the singleton and double mutants resulting from the breakdown of H03, C06, A05 and G12, will be analyzed to determine the total amount of prenylated products (and the respective proportion of CBFA and 5-FOA); and %CBFA within the prenylated products produced by these mutants, as described above.

[0360] Table 54 - Breakdown CBFA Shift Summary Table using OA as substrate and FPP as donor

RBP CLONE ID	Mutations	%CBFA
A04	V25_L219F_V294N_Q295A	76.34%
004	L219F_V294N	26.34%
005.1	L219F_Q295A	80.15%
006	V294N_Q295A	25.26%
039.2	L219F	22.55%
042	Q295A	82.32%
050	V294N	29.66%
C05	V35_A53Q_S177Y_Y288H	55.59%
019	A53Q_S177Y	6.48%
020	A53Q_Y288H	79.03%
021	S177Y_Y288H	69.79%
032	A53Q	12.50%
047.2	S177Y	11.08%
052	Y288H	89.32%
A09	V65_V49A_Q161S_V294A	67.08%
022	V49A_Q161S	59.70%
023	V49A_V294A	33.33%
024	Q161S_V294A	61.84%
041	Q161S	63.19%
049	V294A	26.57%
051	V49A	29.48%
H02	V16_A53Q_S177W_L219F	47.95%
007.1	A53Q_S177W	55.80%
008	A53Q_L219F	10.06%
009	S177W_L219F	61.76%
032	A53Q	12.50%
039.2	L219F	22.55%
046	S177W	73.48%
D04	V28_A53T_D166E_Q295W	17.77%
016	A53T_D166E	4.36%
017	A53T_Q295W	22.07%
018	D166E_Q295W	36.56%
033	A53T	8.62%
034	D166E	14.98%
043	Q295W	47.86%
F09	V70_Q38G_D166E_Q295A	24.25%
001	Q38G_D166E	12.60%
002	Q38G_Q295A	14.58%
003	D166E_Q295A	66.80%
034	D166E	14.98%
042	Q295A	82.32%
044	Q38G	20.42%
D11	V84_F123H_L174V_S177E	24.58%

013	F123H_L174V	6.11%
014	F123H_S177E	21.97%
015	L174V_S177E	10.43%
035	F123H	6.34%
045	S177E	18.97%
038	L174V	19.23%
E09	V69_A53T_M106E_Q161S	22.04%
025	A53T_M106E	5.13%
026	A53T_Q161S	26.79%
027	M106E_Q161S	47.19%
033	A53T	8.62%
040	M106E	19.05%
041	Q161S	63.19%

[0361] This analysis provided important insights into which positions on ORF2, when mutated, are likely to give rise to significant effects on the enzymatic activity of ORF2 in the reaction using Olivetolic Acid (OA) as substrate and FPP as donor. Based on this analysis, the amino acid sites listed in Table 55 were selected for targeted amino acid site saturation mutagenesis.

[0362] Table 55 - Site Saturation Target Table for CBFA shift using OA as substrate and FPP as donor

Parental Clone	Mutations	Apparent CBFA Shift Controlling Residue	Target for Site Saturation
A4	V25_L219F_V294N_Q295A	Q295A	Q295
C5	V35_A53Q_S177Y_Y288H	Y288H	Y288
A9	V65_V49A_Q161S_V294A	Q161S	Q161
		V49A	V49
H2	V16_A53Q_S177W_L219F	S177W	S177
D4	V28_A53T_D166E_Q295W	Q295W	Q295
F9	V70_Q38G_D166E_Q295A	Q295A	Q295
E9	V69_A53T_M106E_Q161S	Q161S	Q161
G5	V39_A53T_K118N_S214F	S214F	S214
H11	V88_A108G_Q161S_G205M	Q161S	Q161

[0363] Site saturated mutagenesis was done for Q295, Q161, and S214 by replacing the wild type residue with each of the other 19 standard amino acids. The amount of total prenylated products, the CBFA production potential and GOA production potential was measured for each of the site saturated mutants. These results are depicted in FIGs. 66, 67 and 68; and Tables 56, 57 and 58.

[0364] Table 56. Q295 site saturated mutants OA+FPP

Mutations	nMol CBFA	nMol 5-FOA	Total Products	%CBFA	%Activity	CBFA Production	%5-FOA	5-FOA Production
Q295F	4.27418779	0.16998543	4.44417322	96.18%	437.21%	4.20	3.82%	0.17
Q295L	2.10848804	0.170724497	2.279212537	92.51%	224.22%	2.07	7.49%	0.17
Q295V	1.427258122	0.13556602	1.562824142	91.33%	153.75%	1.40	8.67%	0.13
Q295I	0.724473402	0.086893173	0.811366575	89.29%	79.82%	0.71	10.71%	0.09
Q295M	2.435469475	0.376924214	2.812393689	86.60%	276.68%	2.40	13.40%	0.37
Q295A	0.57894502	0.144223663	0.723168682	80.06%	71.14%	0.57	19.94%	0.14
Q295C	1.090324884	0.27324366	1.363568544	79.96%	134.14%	1.07	20.04%	0.27
Q295E	0.077740093	0.030724075	0.108464167	71.67%	10.67%	0.08	28.33%	0.03
Q295T	0.082916815	0.038537069	0.121453885	68.27%	11.95%	0.08	31.73%	0.04
Q295G	0.266601214	0.162594759	0.429195973	62.12%	42.22%	0.26	37.88%	0.16
Q295P	0.157086755	0.101357772	0.258444527	60.78%	25.43%	0.15	39.22%	0.10
Q295S	0.159942878	0.144012501	0.303955378	52.62%	29.90%	0.16	47.38%	0.14
Q295W	1.019903606	1.181451528	2.201355134	46.33%	216.56%	1.00	53.67%	1.16
Q295N	0.18814709	0.287919421	0.476066511	39.52%	46.83%	0.19	60.48%	0.28
Q295R	0.025481971	0.049834238	0.075316209	33.83%	7.41%	0.03	66.17%	0.05
Q295K	0.019189575	0.039804042	0.058993617	32.53%	11.17%	0.04	67.47%	0.08
Q295H	0.403471974	0.870937771	1.274409745	31.66%	125.37%	0.40	68.34%	0.86
Q295D	0.264905391	0.69250586	0.957411251	27.67%	181.27%	0.50	72.33%	1.31
Q295Y	0.130667619	0.700635598	0.831303216	15.72%	157.39%	0.25	84.28%	1.33

[0365] Table 57. Q161 site saturated mutants OA+FPP

Mutations	CBFA (8.362)	5-FOA (8.805)	nMol CBFA	nMol 5-FOA	Total Products	%CBFA	%Activity	CBFA Production	%5-FOA	5-FOA Production Potential
Q161E										
Q161V										
Q161L	0.16	0.1715	0.07140307	0.181071436	0.252474506	28.28%	78.08%	0.22	71.72%	0.56
Q161A	0.1471	0.346	0.065646198	0.365310303	0.4309565	15.23%	63.83%	0.10	84.77%	0.54
Q161I	0.0683	0.1596	0.030480186	0.168507296	0.198987481	15.32%	61.54%	0.09	84.68%	0.52
Q161N	0.1186	0.232	0.052927526	0.244947949	0.297875474	17.77%	56.40%	0.10	82.23%	0.46
Q161T	0.0924	0.1156	0.041235273	0.12205165	0.163286923	25.25%	50.50%	0.13	74.75%	0.38
Q161C	0.0424	0.0787	0.018921814	0.083092257	0.10201407	18.55%	31.55%	0.06	81.45%	0.26
Q161Y	0.5214	0.0721	0.232684755	0.07612391	0.308808665	75.35%	95.50%	0.72	24.65%	0.24
Q161K	0.3091	0.1306	0.137941806	0.137888802	0.275830609	50.01%	40.85%	0.20	49.99%	0.20
Q161R	0.5209	0.0589	0.232461621	0.062187216	0.294648837	78.89%	91.12%	0.72	21.11%	0.19
Q161H	11.4099	0.1017	5.091886826	0.10737589	5.199262716	97.93%	770.04%	7.54	2.07%	0.16
Q161M	0.1041	0.0444	0.046456623	0.046877969	0.093334592	49.77%	28.86%	0.14	50.23%	0.14
Q161F	0.3662	0.0404	0.163423777	0.042654729	0.206078506	79.30%	63.73%	0.51	20.70%	0.13
Q161S	0.0787	0.0319	0.035121385	0.033680343	0.068801728	51.05%	21.28%	0.11	48.95%	0.10

Q161P	0.0752	0.0658	0.033559443	0.069472306	0.103031749	32.57%	15.43%	0.05	67.43%	0.10
Q161G	0.0685	0.0403	0.030569439	0.042549148	0.073118587	41.81%	13.84%	0.06	58.19%	0.08
Q161W	0.0553	0.0372	0.024678686	0.039276137	0.063954823	38.59%	9.58%	0.04	61.41%	0.06
Q161D	0.0711	0.0036	0.031729739	0.003800916	0.035530656	89.30%	5.32%	0.05	10.70%	0.01

[0366] Table 58. S214 site saturated mutants OA+FPP

Mutations	nMol CBFA	nMol 5-FOA	Total Products	%CBFA	%Activity	CBFA Production	%5-FOA	5-FOA Production
S214A								
S214G								
S214Q								
S214T	0.13803106	0.678041261	0.816072321	16.91%	154.51%	0.26	83.09%	1.28375
S214V	0.110942521	0.534451084	0.645393605	17.19%	122.19%	0.21	82.81%	1.01189
S214D	0.076535166	0.353379648	0.429914814	17.80%	81.40%	0.14	82.20%	0.66906
S214N	0.053507676	0.241569356	0.295077032	18.13%	55.87%	0.10	81.87%	0.45737
S214C	0.016199572	0.126697215	0.142896786	11.34%	0.439674	0.05	88.66%	0.38983
S214I	0.113620136	0.123635365	0.237255501	47.89%	44.92%	0.22	52.11%	0.23408
S214W	0.009014638	0.016153895	0.025168533	35.82%	4.77%	0.02	64.18%	0.03058
S214H	0.536058551	0.014886923	0.550945473	97.30%	104.31%	1.01	2.70%	0.02819
S214E	0.047616923	0.014464599	0.062081521	76.70%	11.75%	0.09	23.30%	0.02739
S214K	0.027713317	0.017315286	0.045028603	61.55%	6.67%	0.04	38.45%	0.02565
S214F	0.063816494	0.01351437	0.077330864	82.52%	14.64%	0.12	17.48%	0.02559
S214M	0.034139593	0.009713453	0.043853046	77.85%	8.30%	0.06	22.15%	0.01839
S214R	1.079926812	0.008974386	1.088901198	99.18%	206.16%	2.04	0.82%	0.01699
S214P	0.00303463	0.005384632	0.00841926 2	36.04%	0.025905	0.01	63.96%	0.01657
S214Y	0.013254195	0.006123699	0.019377894	68.40%	3.67%	0.03	31.60%	0.01159
S214L	0.02128704	0.004117659	0.0254047	83.79%	4.81%	0.04	16.21%	0.0078

[0367] Similarly, site saturated mutagenesis will also be completed for the other amino acid residues targeted for site saturation listed in Table 55; and the amount of total prenylated products and the CBFA production potential will be measured for each of these site saturated mutants.

[0368] From the results described above, multiple mutations of Q295, Q161 and S214 that have significantly higher CBFA production potential and/or the total amount of prenylated products, as compared to WT ORF2, were identified. Thus, the ORF2 mutants disclosed herein have unexpectedly superior enzymatic functions, in a reaction using OA as a substrate and FPP as donor, as compared to WT ORF2.

[0369] Finally, ORF2 stacking mutants, that carry different novel combinations of the mutations identified by our analysis as being important for ORF2's enzymatic activity, were analyzed to determine the total amount of prenylated products they produce; % enzymatic activity, %CBFA, and CBFA production potential. The analysis of the stacking mutants shows that multiple stacking mutants have significantly higher % enzymatic activity, %CBFA, and CBFA production potential, compared to the WT ORF2 or either singleton substitution variant on its own, thereby indicating that the ORF2 stacking mutants disclosed herein have synergistically enhanced effects compared to the individual single mutants. Thus, the ORF2 stacking mutants disclosed herein have unexpectedly superior enzymatic functions, in a reaction using OA and FPP, as compared to WT ORF2.

[0370] For instance, ORF2 double mutants - S214R-Q295F; S177W-Q295A; A53T-Q295F; and Q161S-Q295L have synergistically enhanced CBFA production potential and % activity as compared to either of the single mutants. See FIGs. 69-72; and Table 59.

[0371] More stacking mutants will be generated as described above, based on the breakdown analysis of additional triple mutants and planned site saturation mutagenesis experiments described above. These stacking mutants will further be analyzed to determine their % enzymatic activity, %CBFA, %5-FOA and CBFA production potential.

[0372] Table 59 - Stacking Representative Results (using OA as substrate and FPP as donor) by ORF2 stacking mutants

RBP CLONE ID	Mutations	CBFA (8.362)	5-FOA (8.805)	nMol CBFA	nMol 5-FOA	Total Products	%CBFA	%Activity	CBFA Production	%5-FOA
BB05	S214R	2.4199	0.0085	1.079926812	0.008974386	1.088901198	99.18%	206.16%	2.04	0.82%
056.2	Q295F	9.5776	0.161	4.27418779	0.16998543	4.44417322	96.18%	437.21%	4.20	3.82%
ST13	S214R_Q295F	10.6601	0.0249	4.757274188	0.026289672	4.78356386	99.45%	708.48%	7.05	0.55%
046	S177W	0.413	0.063	0.184309175	0.066516038	0.250825213	73.48%	37.57%	0.28	26.52%
042.3	Q295A	1.2973	0.1366	0.57894502	0.144223663	0.723168682	80.06%	71.14%	0.57	19.94%
ST01	S177W_Q295A	10.3347	0.0119	4.612058194	0.01256414	4.624622334	99.73%	684.94%	6.83	0.27%
033	A53T	0.3639	1.6305	0.162397358	1.721498406	1.883895764	8.62%	282.15%	0.24322	91.38%
056.2	Q295F	9.5776	0.161	4.27418779	0.16998543	4.44417322	96.18%	437.21%	4.20	3.82%
ST08	A53T_Q295F	6.8272	0.4389	3.046769011	0.463395063	3.510164074	86.80%	519.88%	4.51	13.20%
EE06	Q161S	0.0787	0.0319	0.035121385	0.033680343	0.068801728	51.05%	21.28%	0.11	48.95%
061.2	Q295L	4.7247	0.1617	2.10848804	0.170724497	2.279212537	92.51%	224.22%	2.07	7.49%
ST11L	Q161S_Q295L	5.2287	0.0436	2.333407712	0.046033321	2.379441033	98.07%	352.41%	3.46	1.93%

[0373] Example 35 - Generation of ORF2 variants which synthesize an altered amount of 5-DOA and/or 3-DOA, compared to WT ORF2

[0374] Table 60 provides a summary of the analysis performed on the enzymatic activity of the ORF2 variants to produce CBGA and 5-DOA using Olivetolic Acid (OA) as substrate and DMAPP as donor. Table 60 lists the mutations within each of the tripleton mutants as well the nMol of 3-DOA produced, nMol of 5-DOA produced, total prenylated products produced (nMol of 3-DOA + 5-DOA), %3-DOA within total prenylated products (nMol of 3-DOA / [nMol of 3-DOA+5-DOA]), % enzymatic activity (total prenylated products produced by a mutant/ total prenylated products produced by wild-type ORF2), 3-DOA production (%3-DOA among total prenylated products * % enzymatic activity), and %5-DOA within prenylated products (nMol of 5-DOA / [nMol of 3-DOA + 5-DOA]) for each of the ORF2 variants.

[0375] Table 60: Analysis of ORF2 mutants and WT ORF2 based on production of 3-DOA from OA and DMAPP

CLONE	Mutations	nMol 3-DOA	nMol 5-DOA	nMol Total Products	%3-DOA	%5-DOA	%Activity	3-DOA Production	5-DOA Production
WT	WT	0.070427374	0.032532794	0.102960168	68.40%	31.60%	100.00%	0.68	0.32
C6	V43_Q161A_M162F_Q295A	0.655232239	0.005112296	0.660344535	99.23%	0.77%	640.01%	6.35	0.05
A9	V65_V49A_Q161S_V294A	0.290469974	0.058210464	0.348680438	83.31%	16.69%	337.94%	2.82	0.56
A4	V25_L219F_V294N_Q295A	0.260581283	0.02649099	0.287072273	90.77%	9.23%	278.23%	2.53	0.26
G12	V95_A17T_Q161W_A232S	0.16662086	0.038923165	0.205544025	81.06%	18.94%	164.32%	1.33	0.31
H03	V24_A17T_F213M_S214R	0.095334616	0.002904714	0.09823933	97.04%	2.96%	122.88%	1.19	0.04

D6	V44_A53E_Q161A_V294N	0.11757936	0.036250828	0.153830187	76.43%	23.57%	149.09%	1.14	0.35
F9	V70_Q38G_D166E_Q295A	0.120241858	0.04647542	0.166717278	72.12%	27.88%	133.28%	0.96	0.37
D12	V92_A53T_E112D_G205M	0.10478219	0.081912928	0.186695119	56.12%	43.88%	149.25%	0.84	0.65
C5	V35_A53Q_S177Y_Y288H	0.085285832	0.055073373	0.140359205	60.76%	39.24%	136.04%	0.83	0.53
D4	V28_A53T_D166E_Q295W	0.081592689	0.054550525	0.136143214	59.93%	40.07%	131.95%	0.79	0.53
A2	V9_Q38G_E112D_F123H	0.077384224	0.098063137	0.175447361	44.11%	55.89%	170.04%	0.75	0.95
E9	V69_A53T_M106E_Q161S	0.091040264	0.032532794	0.123573058	73.67%	26.33%	98.79%	0.73	0.26
D11	V84_F123H_L174V_S177E	0.089322523	0.033113737	0.12243626	72.95%	27.05%	97.88%	0.71	0.26
H2	V16_A53Q_S177W_L219F	0.079874948	0.04008505	0.119959998	66.58%	33.42%	95.90%	0.64	0.32
C11	V83_E112D_L219F_V294F	0.065445926	0.038167939	0.103613864	63.16%	36.84%	100.42%	0.63	0.37
H9	V72_E112G_G205M_L298W	0.052391095	0.094693669	0.147084764	35.62%	64.38%	117.58%	0.42	0.76
A5	V33_A17T_C25V_E112G	0.040452796	0.029105232	0.069558028	58.16%	41.84%	67.42%	0.39	0.28
A3	V17_V49L_F123A_Y283L	0.03134877	0.009469367	0.040818137	76.80%	23.20%	39.56%	0.30	0.09
B12	V90_A17T_F123W_L298A	0.031005222	0.010979818	0.04198504	73.85%	26.15%	40.69%	0.30	0.11
C1	V3_V49S_M162A_Y283L	0.030404013	0.043861178	0.074265191	40.94%	59.06%	71.98%	0.29	0.43
H11	V88_A108G_Q161S_G205M	0.034354817	0.05054202	0.084896836	40.47%	59.53%	67.87%	0.27	0.40
C8	V59_V49S_S214G_V294A	0.027741514	0.020391091	0.048132605	57.64%	42.36%	46.65%	0.27	0.20
H7	V56_F123A_M162F_S214G	0.032637076	0.026723367	0.059360442	54.98%	45.02%	47.45%	0.26	0.21
A12	V89_Y121W_S177Y_G286E	0.026453209	0.012083609	0.038536818	68.64%	31.36%	37.35%	0.26	0.12
H4	V32_M162A_C209G_Y288H	0.030060464	0.004066599	0.034127064	88.08%	11.92%	27.28%	0.24	0.03
A11	V81_V49L_D166E_L274V	0.024649581	0.012838835	0.037488416	65.75%	34.25%	36.33%	0.24	0.12
D3	V20_D227E_C230N_Q295W	0.024134259	0.013768343	0.037902602	63.67%	36.33%	36.74%	0.23	0.13
A10	V73_V49S_K118Q_S177E	0.024048372	0.012374081	0.036422452	66.03%	33.97%	35.30%	0.23	0.12
C10	V75_A53Q_L274V_Q295A	0.023017727	0.005518956	0.028536683	80.66%	19.34%	27.66%	0.22	0.05
C7	V51_V49L_K119D_G205M	0.022588292	0.006041805	0.028630097	78.90%	21.10%	27.75%	0.22	0.06
H5	V40_S177E_S214R_R228E	0.026624983	0.004066599	0.030691582	86.75%	13.25%	24.54%	0.21	0.03
A7	V49_G205L_R228E_C230N	0.021042325	0.010747441	0.031789766	66.19%	33.81%	30.81%	0.20	0.10
G3	V23_L219F_Y283L_L298W	0.024907242	0.024980538	0.04988778	49.93%	50.07%	39.88%	0.20	0.20
H1	V8_K119A_Q161A_R228Q	0.024907242	0.002904714	0.027811956	89.56%	10.44%	22.23%	0.20	0.02
C9	V67_A108G_K119D_L298A	0.020527003	0.004821825	0.025348828	80.98%	19.02%	24.57%	0.20	0.05
B9	V66_C25V_F213M_Y216A	0.020269342	0.006216087	0.026485429	76.53%	23.47%	25.67%	0.20	0.06
B6	V42_D166E_S177Y_S214F	0.020183455	0.00639037	0.026573825	75.95%	24.05%	25.76%	0.20	0.06
C3	V19_V49L_S214R_V271E	0.020011681	0.005344673	0.025356354	78.92%	21.08%	24.58%	0.19	0.05
H10	V80_M162A_N173D_S214F	0.024048372	0.006971313	0.031019685	77.53%	22.47%	24.80%	0.19	0.06
D1	V4_K118Q_Q161W_S214F	0.01975402	0.011212195	0.030966215	63.79%	36.21%	30.01%	0.19	0.11
B4	V26_A53E_A108G_K118N	0.019238697	0.01603402	0.035272717	54.54%	45.46%	34.19%	0.19	0.16

G11	V87_S177W_Y288H_V294N	0.023189501	0.002904714	0.026094215	88.87%	11.13%	20.86%	0.19	0.02
B11	V82_V49S_K119D_F213M	0.018465714	0.004531353	0.022997067	80.30%	19.70%	22.29%	0.18	0.04
G5	V39_A53T_K118N_S214F	0.022330631	0.05054202	0.07287265	30.64%	69.36%	58.26%	0.18	0.40
B8	V58_K118Q_L174V_R228Q	0.01829394	0.006680842	0.024974781	73.25%	26.75%	24.21%	0.18	0.06
C12	V91_N173D_F213M_V294F	0.017692731	0.011909326	0.029602057	59.77%	40.23%	28.69%	0.17	0.12
B2	V10_V49A_S177Y_C209G	0.017435069	0.006506559	0.023941628	72.82%	27.18%	23.20%	0.17	0.06
B10	V74_M106E_Y121W_D166E	0.017177408	0.004357071	0.021534479	79.77%	20.23%	20.87%	0.17	0.04
H6	V48_V49L_E112D_G286E	0.02061289	0.003485657	0.024098546	85.54%	14.46%	19.27%	0.16	0.03
F8	V62_A53T_N173D_S214R	0.02061289	0.002323771	0.022936661	89.87%	10.13%	18.34%	0.16	0.02
B5	V34_A53Q_Y121W_A232S	0.016662086	0.009411273	0.026073359	63.90%	36.10%	25.27%	0.16	0.09
A8	V57_C25V_A232S_V271E	0.016490312	0.009469367	0.025959679	63.52%	36.48%	25.16%	0.16	0.09
G10	V79_V49A_Y121W_C230S	0.01975402	0.002904714	0.022658733	87.18%	12.82%	18.11%	0.16	0.02
D05	V36_F123H_L274V_L298A	0.012883056	0.009876027	0.022759083	56.61%	43.39%	25.94%	0.15	0.11
D10	V76_V49A_F123A_Y288H	0.018036279	0.004647542	0.022683821	79.51%	20.49%	18.13%	0.14	0.04
D7	V52_K119A_S214G_L298A	0.018036279	0.003485657	0.021521935	83.80%	16.20%	17.21%	0.14	0.03
F10	V78_K119D_Q161W_L298Q	0.018036279	0.003485657	0.021521935	83.80%	16.20%	17.21%	0.14	0.03
G08	V63_F123W_M162F_C209G	0.018036279	0.002904714	0.020940992	86.13%	13.87%	16.74%	0.14	0.02
H8	V64_M106E_M162A_Y216A	0.014600797	0.004647542	0.019248339	75.85%	24.15%	18.69%	0.14	0.05
C2	V11_K118N_K119A_V271E	0.014429023	0.004415165	0.018844188	76.57%	23.43%	18.26%	0.14	0.04
D9	V68_K118N_C209G_R228Q	0.017177408	0.004066599	0.021244008	80.86%	19.14%	16.98%	0.14	0.03
G2	V15_A53E_F213M_R228Q	0.017177408	0.002904714	0.020082122	85.54%	14.46%	16.05%	0.14	0.02
D8	V60_E112D_K119A_N173D	0.016318538	0.004066599	0.020385137	80.05%	19.95%	16.30%	0.13	0.03
D8	V60_E112D_K119A_N173D	0.016318538	0.001742828	0.018061366	90.35%	9.65%	14.44%	0.13	0.01
G7	V55_V49S_Y216A_V294N	0.014600797	0.002904714	0.017505511	83.41%	16.59%	13.99%	0.12	0.02
F12	V94_A17T_V49A_C230N	0.014600797	0.002323771	0.016924568	86.27%	13.73%	13.53%	0.12	0.02
G6	V47_K118Q_F123A_R228E	0.013741927	0.002323771	0.016065698	85.54%	14.46%	12.84%	0.11	0.02
G4	V31_D227E_R228E_L298Q	0.012883056	0.001742828	0.014625884	88.08%	11.92%	11.69%	0.10	0.01

[0376] The amount of 3-DOA or 5-DOA (in nMols) generated by each of the ORF2 triple mutant clones was measured using HPLC. FIG. 73 shows the total nMols of prenylated products generated using OA as substrate and DMAPP as donor by each of the ORF2 triple mutants, and the proportion of 3-DOA and 5-DOA within the total amount of prenylated products. An exemplary Wild Type ORF2 replicate is included in the graph for comparison purposes.

[0377] FIG. 74 shows the %3-DOA within the total prenylated products produced by each of the ORF2 triple mutant clones using OA as substrate and DMAPP as donor. In this graph, the mutant clones are ordered based on decreasing %3-DOA (from left to right) they produce, with the %5-DOA depicted in red. The black threshold line on the graph indicates the %3-DOA that is produced by the wild type enzyme.

[0378] FIG. 75 shows the ORF2 enzymatic activity (using OA as substrate and DMAPP as donor) of each of the triple mutant ORF2 clones relative to the wild type enzyme. %activity was calculated by dividing the nMols of total prenylated products produced by a mutant by the nMols of total prenylated products produced by the wild type control, and expressed as a percentage. The red threshold line is the wild type Orf2 %activity.

[0379] FIG. 76 shows the 3-DOA production potential of each of the ORF2 triple mutant clones when using OA as substrate and DMAPP as donor. 3-DOA production potential (interchangeably referred to herein as 3-DOA production quotient) represents the improvement in 3-DOA production vs. the wild type enzyme. 3-DOA production potential was calculated by multiplying the % 3-DOA by the % activity of each mutant. For instance, a wild type ORF2, which makes ~20% 3-DOA, and has an activity of 100%, would have a 3-DOA Production Potential of 0.2. The red threshold line on the graph represents this wild type value of 0.2.

[0380] While the 3-DOA production potential analysis shown in FIG. 76 is useful to rank ORF2 mutant clones based on the amount of 3-DOA produced, such an analysis would not differentiate between a mutant that made 100% 3-DOA but was 20% as active as wild-type ORF2; or a mutant that made 10% 3-DOA and was 200% as active as wild type ORF2. Therefore, we employed a cluster analysis by plotting the 3-DOA Production Potential vs. %5-DOA (FIG. 77). %5-DOA was calculated in a similar manner as %3-DOA. We used the top 16 mutants ranked based on their 3-DOA production potential for this analysis. High 5-DOA producing mutants cluster together towards the right of the graph and high 3-DOA producing mutants cluster towards the left of the graph.

[0381] Based on the analysis performed in FIG. 77, 10 mutants which cluster to the left of the graph were selected (Table 61). These clones were targeted for “breakdown” analysis. Breakdown analysis involves breaking a parent triple mutant into all pair wise doubleton combinations of mutations as well as all singleton mutations that make up the parental clone. For each parental clone targeted six unique mutants are generated (3 doubles and 3 singles).

[0382] Table 61 - Clones targeted for breakdown analysis based on 3-DOA production potential and %5-DOA produced, using OA as substrate and DMAPP as donor

3-DOA Production Rank	Clone ID	Mutations	Targeted for Breakdown
1	C6	V43_Q161A_M162F_Q295A	YES
2	A9	V65_V49A_Q161S_V294A	YES
3	A4	V25_L219F_V294N_Q295A	YES
4	A2	V9_Q38G_E112D_F123H	NO-HIGH 5-DOA CLUSTER
5	G12	V95_A17T_Q161W_A232S	YES
6	D12	V92_A53T_E112D_G205M	NO-MIDDLE 5-DOA CLUSTER
7	D6	V44_A53E_Q161A_V294N	YES
8	C5	V35_A53Q_S177Y_Y288H	NO-MIDDLE 5-DOA CLUSTER
9	F9	V70_Q38G_D166E_Q295A	YES
10	D4	V28_A53T_D166E_Q295W	NO-MIDDLE 5-DOA CLUSTER
11	H03	V24_A17T_F213M_S214R	YES
12	H9	V72_E112G_G205M_L298W	NO-HIGH 5-DOA CLUSTER
13	C11	V83_E112D_L219F_V294F	NO-HIGH 5-DOA CLUSTER
14	E9	V69_A53T_M106E_Q161S	YES
15	D11	V84_F123H_L174V_S177E	YES
16	H2	V16_A53Q_S177W_L219F	NO- WT CLUSTER
17	C1	V3_V49S_M162A_Y283L	NO-HIGH 5-DOA CLUSTER
18	H11	V88_A108G_Q161S_G205M	NO-HIGH 5-DOA CLUSTER
19	A5	V33_A17T_C25V_E112G	NO-MIDDLE 5-DOA CLUSTER
20	G5	V39_A53T_K118N_S214F	YES-HIGH 5-DOA CLUSTER REPRESENTATIVE

[0383] Breakdown analysis for these triple mutants will be performed as described above in Example 34. The singleton and double mutants resulting from the breakdown of these mutants will be analyzed to determine the total amount of prenylated products (and the respective proportion of 5-DOA and 3-DOA); and %3-DOA within the prenylated products produced by these mutants.

[0384] Further, based on the analysis of the breakdown mutants, amino acid sites will be selected for targeted amino acid site saturation mutagenesis, as described above in Example 34; and mutants that have significantly higher 3-DOA production potential and/or the total amount of prenylated products, as compared to WT ORF2, will be identified. Finally, ORF2 stacking mutants that carry different novel combinations of the mutations identified by the analysis as being important for ORF2's enzymatic activity will be generated. These stacking mutants will further be analyzed to determine their % enzymatic activity, %3-DOA, %5-DOA and 3-DOA production potential.

[0385] Example 36 - Proton NMR signals of selected compounds

[0386] The Proton NMR signals of selected compound were obtained in DMSO at 600MHz and the proton NMR assignments of these compounds were shown in FIGs. 84A-84K, including RBI-01 (FIG. 84A); RBI-02 (FIG. 84B); RBI-03 (FIG. 84C); RBI-04 (FIG. 84D);

RBI-05 (FIG. 84E); RBI-07 (FIG. 84F); RBI-08 (FIG. 84G); RBI-09 (FIG. 84H); RBI-10 (FIG. 84I); RBI-11 (FIG. 84J); and RBI-12 (FIG. 84K).

CLAIMS

1. A recombinant polypeptide comprising an amino acid sequence with at least 80% identity to the amino acid sequence of a prenyltransferase, wherein the recombinant polypeptide comprises at least one amino acid substitution compared to the amino acid sequence of the prenyltransferase, wherein said recombinant polypeptide converts a substrate and a prenyl donor to at least one prenylated product, and wherein the recombinant polypeptide produces a ratio of an amount of the at least one prenylated product to an amount of total prenylated products that is higher than the prenyltransferase under the same condition.

2. The recombinant polypeptide of claim 1, wherein the recombinant polypeptide comprises an amino acid sequence with at least 95% identity to the amino acid sequence of the prenyltransferase.

3. The recombinant polypeptide of claim 1 or 2, wherein said amino acid sequence has at least 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of the prenyltransferase.

4. The recombinant polypeptide of claim 1, wherein said at least one amino acid substitution comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions to the amino acid sequence of the prenyltransferase.

5. The recombinant polypeptide of any of claims 1-4, wherein the prenyltransferase is selected from the group consisting of ORF2, HypSc, PB002, PB005, PB064, PB065, and Atapt.

6. The recombinant polypeptide of any of claims 1-5, wherein the prenyl donor is selected from the group consisting of DMAPP, GPP, FPP, GGPP, and any combination thereof.

7. The recombinant polypeptide of any of claims 1-6, wherein the prenyl donor is not a naturally occurring donor of the prenyltransferase.

8. The recombinant polypeptide of any of claims 1-7, wherein the substrate is selected from the group consisting of olivetolic acid (OA), divarinolic acid (DVA), olivetol (O), divarinol (DV), orsellinic acid (ORA), dihydroxybenzoic acid (DHBA), apigenin, naringenin and resveratrol.

9. The recombinant polypeptide of any of claims 1-8, wherein the substrate is not a naturally occurring substrate of the prenyltransferase.

10. The recombinant polypeptide of any of claims 1-9, wherein the at least one prenylated product comprises a prenyl group attached to any position on an aromatic ring of the substrate.

11. The recombinant polypeptide of any of claims 1-10, wherein the at least one prenylated product is selected from the group consisting of UNK1, UNK2, UNK3, RBI-08, RBI-17 (5-DOA), RBI-05, RBI-06, 4-O-GOA, RBI-02 (CBGA), RBI-04 (5-GOA), UNK4, RBI-56, UNK5, RBI-14 (CBFA), RBI-16 (5-FOA), RBI-24, RBI-28, RBI-26 (CBGVA), RBI-27, RBI-38, RBI-39, RBI-09, RBI-10, RBI-03 (5-GO), RBI-20, RBI-01 (CBG), RBI-15, RBI-34, RBI-32, RBI-33, RBI-07, RBI-29, RBI-30, RBI-12, and RBI-11.

12. The recombinant polypeptide of any of claims 1-10, wherein the prenyltransferase is ORF2.

13. The recombinant polypeptide of claim 12, wherein the substrate is OA and the prenyl donor is DMAPP.

14. The recombinant polypeptide of claim 13, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; 5-C; or 5-C and 3-C on the aromatic ring of OA.

15. The recombinant polypeptide of claim 13, wherein the at least one prenylated product comprises UNK1, UNK2, UNK3, RBI-08, RBI-17, or RBI-18.

16. The recombinant polypeptide of claim 12, wherein the substrate is OA and the prenyl donor is GPP.

17. The recombinant polypeptide of claim 16, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; 5-C; or 3-C and 5-C on the aromatic ring of OA.

18. The recombinant polypeptide of claim 17, wherein the at least one prenylated product comprises RBI-05, RBI-06, UNK-4, RBI-02 (CBGA), RBI-04 (5-GOA) or RBI-07.

19. The recombinant polypeptide of claim 12, wherein the substrate is OA and the prenyl donor is FPP.

20. The recombinant polypeptide of claim 19, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 2-O; 4-O; 3-C; and 5-C on the aromatic ring of OA.

21. The recombinant polypeptide of claim 20, wherein the at least one prenylated product comprises RBI-56, UNK5, RBI-14 (CBFA), or RBI-16 (5-FOA).

22. The recombinant polypeptide of claim 12, wherein the substrate is DVA and the prenyl donor is DMAPP.

23. The recombinant polypeptide of claim 22, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; and 5-C on the aromatic ring of DVA.

24. The recombinant polypeptide of claim 12, wherein the substrate is DVA and the prenyl donor is GPP.

25. The recombinant polypeptide of claim 24, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; 5-C; 3-C and 5-C; or 5-C and 2-O on the aromatic ring of DVA.

26. The recombinant polypeptide of claim 24, wherein the at least one prenylated product comprises RBI-24, RBI-28, UNK11, RBI-26, RBI-27, RBI-29, or RBI-30.

27. The recombinant polypeptide of claim 12, wherein the substrate is DVA and the prenyl donor is FPP.

28. The recombinant polypeptide of claim 27, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO; 2-O; 4-O; 3-C; and 5-C on the aromatic ring of DVA.

29. The recombinant polypeptide of claim 28, wherein the at least one prenylated product comprises UNK12, UNK13, UNK14, RBI-38, or RBI-39.

30. The recombinant polypeptide of claim 12, wherein the substrate is O and the prenyl donor is DMAPP.

31. The recombinant polypeptide of claim 30, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C; 5-C; 2-O; 4-O; or 3-C on the aromatic ring of O.

32. The recombinant polypeptide of claim 31, wherein the at least one prenylated product comprises RBI-10, UNK16, or RBI-09.

33. The recombinant polypeptide of any of claims 1-10, wherein the prenyltransferase is HypSc.

34. The recombinant polypeptide of claim 33, wherein the substrate is O and the prenyl donor is DMAPP.

35. The recombinant polypeptide of claim 34, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C; 5-C; 2-O; 4-O; or 3-C on the aromatic ring of O.

36. The recombinant polypeptide of claim 35, wherein the at least one prenylated product comprises RBI-10, UNK16 or RBI-09.

37. The recombinant polypeptide of any of claims 1-10, wherein the prenyltransferase is PB005.

38. The recombinant polypeptide of claim 37, wherein the substrate is O and the prenyl donor is DMAPP.

39. The recombinant polypeptide of claim 38, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C, 5-C; 2-O, 4-O; 3-C; 1-C and 5-C; or 1-C and 3-C on the aromatic ring of O.

40. The recombinant polypeptide of claim 38, wherein the at least one prenylated product comprises RBI-10, UNK16, RBI-09, RBI-11 or RBI-12.

41. The recombinant polypeptide of claim 12, wherein the substrate is O and the prenyl donor is GPP.

42. The recombinant polypeptide of claim 41, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C, 5-C; 2-O, 4-O; or 3-C on the aromatic ring of O.

43. The recombinant polypeptide of claim 42, wherein the at least one prenylated product comprises RBI-20, RBI-01 (CBG), or RBI-03 (5-GO).

44. The recombinant polypeptide of claim 12, wherein the substrate is O and the prenyl donor is FPP.

45. The recombinant polypeptide of claim 44, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C, 5-C, 2-O, 4-O, or 3-C on the aromatic ring of O.

46. The recombinant polypeptide of claim 45, wherein the at least one prenylated product comprises RBI-15, UNK18 or UNK19.

47. The recombinant polypeptide of claim 37, wherein the substrate is DV and the prenyl donor is DMAPP.

48. The recombinant polypeptide of claim 47, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 1-C; 5-C; 2-O; 4-O; or 3-C on the aromatic ring of DV.

49. The recombinant polypeptide of claim 48, wherein the at least one prenylated product comprises UNK54, UNK55 or UNK56.

50. The recombinant polypeptide of claim 12, wherein the substrate is ORA and the prenyl donor is GPP.

51. The recombinant polypeptide of claim 50, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, 4-O, 3-C, 5-C, or 5-C and 3-C on the aromatic ring of ORA.

52. The recombinant polypeptide of claim 12, wherein the substrate is ORA and the prenyl donor is DMAPP.

53. The recombinant polypeptide of claim 52, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, or 5-C on the aromatic ring of ORA.

54. The recombinant polypeptide of claim 33, wherein the substrate is ORA and the prenyl donor is DMAPP.

55. The recombinant polypeptide of claim 54, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, or 4-O on the aromatic ring of ORA.

56. The recombinant polypeptide of claim 37, wherein the substrate is ORA and the prenyl donor is DMAPP.

57. The recombinant polypeptide of claim 56, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, or 3-C on the aromatic ring of ORA.

58. The recombinant polypeptide of any of claims 1-10, wherein the prenyltransferase is PB064.

59. The recombinant polypeptide of claim 58, wherein the substrate is ORA and the prenyl donor is DMAPP.

60. The recombinant polypeptide of claim 59, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O or 3-C on the aromatic ring of ORA.

61. The recombinant polypeptide of any of claims 1-10, wherein the prenyltransferase is PB065.

62. The recombinant polypeptide of claim 61, wherein the substrate is ORA and the prenyl donor is DMAPP.

63. The recombinant polypeptide of claim 62, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, or 2-O on the aromatic ring of ORA.

64. The recombinant polypeptide of any of claims 1-10, wherein the prenyltransferase is PB002.

65. The recombinant polypeptide of claim 64, wherein the substrate is ORA and the prenyl donor is DMAPP.

66. The recombinant polypeptide of claim 65, wherein the at least one prenylated product comprises a prenyl group attached to a position CO on the aromatic ring of ORA.

67. The recombinant polypeptide of any of claims 1-10, wherein the prenyltransferase is Atapt.

68. The recombinant polypeptide of claim 67, wherein the substrate is ORA and the prenyl donor is DMAPP.

69. The recombinant polypeptide of claim 68, wherein the at least one prenylated product comprises a prenyl group attached to a position 4-O on the aromatic ring of ORA.

70. The recombinant polypeptide of claim 12, wherein the substrate is ORA and the prenyl donor is FPP.

71. The recombinant polypeptide of claim 70, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, 4-O, 3-C, or 5-C on the aromatic ring of ORA.

72. The recombinant polypeptide of claim 12 or claim 37, wherein the substrate is DHBA and the prenyl donor is DMAPP.

73. The recombinant polypeptide of claim 72, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from CO, 2-O, 4-O, 3-C, or 5-C on the aromatic ring of DHBA.

74. The recombinant polypeptide of claim 12, wherein the substrate is DV and the prenyl donor is GPP.

75. The recombinant polypeptide of claim 74, wherein the at least one prenylated product comprises a prenyl group attached to positions 5-C and 1-C; or 3-C and 5-C on the aromatic ring of DV.

76. The recombinant polypeptide of claim 75, wherein the at least one prenylated product comprises RBI-36, or UNK35.

77. The recombinant polypeptide of claim 12, wherein the substrate is OA and the prenyl donor is GPP, DMAPP or both.

78. The recombinant polypeptide of claim 77, wherein the at least one prenylated product comprises a prenyl group attached to positions 5-C and 3-C; or CO and 3-C on the aromatic ring of OA.

79. The recombinant polypeptide of claim 12, wherein the substrate is OA and the prenyl donor is GPP, FPP or both.

80. The recombinant polypeptide of claim 79, wherein the at least one prenylated product comprises a prenyl group attached to positions 5-C and 3-C on the aromatic ring of OA.

81. The recombinant polypeptide of claim 12, wherein the substrate is O and the prenyl donor is GPP, FPP or both.

82. The recombinant polypeptide of claim 81, wherein the at least one prenylated product comprises a prenyl group attached to positions 5-C and 3-C on the aromatic ring of O.

83. The recombinant polypeptide of any of claims 1-82, wherein the substrate is a prenylated molecule.

84. The recombinant polypeptide of claim 83, wherein the prenylated molecule is selected from the group consisting of UNK1, UNK2, UNK3, RBI-08, 5-DOA, RBI-05, RBI-06, 4-O-GOA, RBI-02 (CBGA), RBI-04 (5-GOA), UNK4, RBI-56, UNK5, RBI-14 (CBFA), RBI-16 (5-FOA), RBI-24, RBI-28, RBI-26 (CBGVA), RBI-27, RBI-38, RBI-39, RBI-09, RBI-10, RBI-03 (5-GO), RBI-20, RBI-01 (CBG), RBI-15, RBI-34, RBI-32, RBI-33, RBI-07, RBI-29, RBI-30, RBI-12, and RBI-11.

85. The recombinant polypeptide of claim 12, wherein the amino acid sequence of ORF2 comprises SEQ ID NO: 1, and the at least one amino acid substitution comprises at least one amino acid substitution in SEQ ID NO: 1 on a position chosen from the group consisting of amino acid positions 17, 25, 38, 49, 53, 106, 108, 112, 118, 119, 121, 123, 161, 162, 166, 173, 174, 177, 205, 209, 213, 214, 216, 219, 227, 228, 230, 232, 271, 274, 283, 286, 288, 294, 295, and 298.

86. The recombinant polypeptide of claim 85, wherein the at least one amino acid substitution is located on a position chosen from the group consisting of amino acid positions 17, 25, 38, 49, 53, 106, 108, 112, 118, 119, 162, 166, 173, 174, 205, 209, 213, 219, 227, 228, 230, 232, 271, 274, 283, 286, 288, and 298.

87. The recombinant polypeptide of claim 12, wherein the amino acid sequence of ORF2 comprises SEQ ID NO: 1, and the at least one amino acid substitution is chosen from the group consisting of A17T, C25V, Q38G, V49A, V49L, V49S, A53C, A53D, A53E, A53F, A53G, A53H, A53I, A53K, A53L, A53M, A53N, A53P, A53Q, A53R, A53S, A53T, A53V, A53W, A53Y, M106E, A108G, E112D, E112G, K118N, K118Q, K119A, K119D, Y121W, F123A, F123H, F123W, Q161A, Q161C, Q161D, Q161E, Q161F, Q161G, Q161H, Q161I, Q161K, Q161L, Q161M, Q161N, Q161P, Q161R, Q161S, Q161T, Q161V, Q161W, Q161Y, M162A, M162F, D166E, N173D, L174V, S177E, S177W, S177Y, G205L, G205M, C209G, F213M, S214A, S214C, S214D, S214E, S214F, S214G, S214H, S214I, S214K, S214L, S214M, S214N, S214P, S214Q, S214R, S214T, S214V, S214W, S214Y, Y216A, L219F, D227E, R228E, R228Q, C230N, C230S, A232S, V271E, L274V, Y283L, G286E, Y288A, Y288C, Y288D, Y288E, Y288F, Y288G, Y288H, Y288I, Y288K, Y288L, Y288M, Y288N, Y288P, Y288Q, Y288R, Y288S, Y288T, Y288V, Y288W, V294A, V294F, V294N, Q295A,

Q295C, Q295D, Q295E, Q295F, Q295G, Q295H, Q295I, Q295K, Q295L, Q295M, Q295N, Q295P, Q295R, Q295S, Q295T, Q295V, Q295W, Q295Y, L298A, L298Q, and L298W.

88. The recombinant polypeptide of claim 12, wherein the amino acid sequence of ORF2 comprises SEQ ID NO: 1, and the at least one amino acid substitution to SEQ ID NO: 1 comprises two or more amino acid substitutions to SEQ ID NO: 1 selected from the group consisting of:

(a) A17T, C25V, Q38G, V49A, V49L, V49S, A53C, A53D, A53E, A53F, A53G, A53H, A53I, A53K, A53L, A53M, A53N, A53P, A53Q, A53R, A53S, A53T, A53V, A53W, A53Y, M106E, A108G, E112D, E112G, K118N, K118Q, K119A, K119D, Y121W, F123A, F123H, F123W, Q161A, Q161C, Q161D, Q161E, Q161F, Q161G, Q161H, Q161I, Q161K, Q161L, Q161M, Q161N, Q161P, Q161R, Q161S, Q161T, Q161V, Q161W, Q161Y, M162A, M162F, D166E, N173D, L174V, S177E, S177W, S177Y, G205L, G205M, C209G, F213M, S214A, S214C, S214D, S214E, S214F, S214G, S214H, S214I, S214K, S214L, S214M, S214N, S214P, S214Q, S214R, S214T, S214V, S214W, S214Y, Y216A, L219F, D227E, R228E, R228Q, C230N, C230S, A232S, V271E, L274V, Y283L, G286E, Y288A, Y288C, Y288D, Y288E, Y288F, Y288G, Y288H, Y288I, Y288K, Y288L, Y288M, Y288N, Y288P, Y288Q, Y288R, Y288S, Y288T, Y288V, Y288W, V294A, V294F, V294N, Q295A, Q295C, Q295D, Q295E, Q295F, Q295G, Q295H, Q295I, Q295K, Q295L, Q295M, Q295N, Q295P, Q295R, Q295S, Q295T, Q295V, Q295W, Q295Y, L298A, L298Q, and L298W;

OR

(b) A53T and S214R; S177W and Q295A; S214R and Q295F; Q161S and S214R; S177W and S214R; Q161S and Q295L; Q161S and Q295F; V49A and S214R; A53T and Q295F; Q161S and S177W; Q161S, V294A and Q295W; A53T, Q161S and Q295W; A53T and S177W; A53T, Q161S, V294A and Q295W; A53T, V294A and Q295A; V49A and Q295L;

A53T, Q161S, V294N and Q295W; A53T and Q295A; Q161S, V294A and Q295A; A53T and Q295W; A53T, V294A and Q295W; A53T, Q161S and Q295A; A53T, Q161S, V294A and Q295A; and A53T, Q161S, V294N and Q295A.

89. A nucleic acid molecule, comprising a nucleotide sequence encoding the recombinant polypeptide of any of claims 1-88, or a codon degenerate nucleotide sequence thereof.

90. The nucleic acid molecule of claim 89, wherein said nucleotide sequence comprises at least 500, 600, 700, 800, or 900 nucleotides.

91. The nucleic acid molecule of claim 89 or 90, wherein said nucleic acid molecule is isolated and purified.

92. A cell vector, construct or expression system comprising said nucleic acid molecule of any one of claims 89-91.

93. A cell, comprising said cell vector, construct or expression system of claim 92.

94. The cell of claim 93, wherein said cell is a bacteria, yeast, insect, mammalian, fungi, vascular plant, or non-vascular plant cell.

95. The cell of claim 93, wherein said cell is a microalgae cell.

96. The cell of claim 93, wherein said cell is an E. coli cell.

97. A plant, comprising said cell of claim 93 or 94.

98. The plant of claim 97, wherein said plant is a terrestrial plant.

99. A method of producing at least one prenylated product, comprising, contacting the recombinant polypeptide of any one of claims 1-88 with a substrate and a prenyl donor, thereby producing at least one prenylated product.

100. The method of claim 99, wherein the recombinant polypeptide is the recombinant polypeptide of any one of claims 13, 16, 19, 22, 24, 27, 30, 34, 38, 41, 44, 47, 50, 52, 54, 56, 59, 62, 65, 68, 70, 72, 74, 77, 79, and 81.

101. A method of producing at least one prenylated product, comprising, a) contacting a first recombinant polypeptide with a substrate and a first prenyl donor, wherein the first recombinant polypeptide is the recombinant polypeptide of any one of claims 1-88, thereby producing a first prenylated product; and b) contacting the first prenylated product and a second prenyl donor with a second recombinant polypeptide, thereby producing a second prenylated product.

102. The method of claim 101, wherein the first recombinant polypeptide and the second recombinant polypeptide are selected from the recombinant polypeptide of any one of claims 13, 16, 19, 22, 24, 27, 30, 34, 38, 41, 44, 47, 50, 52, 54, 56, 59, 62, 65, 68, 70, 72, 74, 77, 79, and 81.

103. The method of claim 101 or 102, wherein the first recombinant polypeptide is the same as the second recombinant polypeptide.

104. The method of claim 101 or 102, wherein the first recombinant polypeptide is different from the second recombinant polypeptide.

105. The method of any one of claims 101-104, wherein the first prenyl donor is the same as the second prenyl donor.

106. The method of any one of claims 101-104, wherein the first prenyl donor is different from the second prenyl donor.

107. The method of any one of claims 101-106, wherein the first prenylated product is the same as the second prenylated product.

108. The method of any one of claims 101-106, wherein the first prenylated product is different from the second prenylated product.

109. The method of any one of claims 101-108, wherein (a) the first recombinant polypeptide is the recombinant polypeptide of claim 12, and the second recombinant polypeptide is the recombinant polypeptide of claim 37; or the first recombinant polypeptide is the recombinant polypeptide of claim 37 and the second recombinant polypeptide is the recombinant polypeptide of claim 12; (b) the first prenyl donor is GPP and the second prenyl donor is DMAPP; or the first prenyl donor is DMAPP, and the second prenyl donor is GPP; and (c) the substrate is O.

110. The method of claim 109, wherein the first prenylated product or the second prenylated product comprises a prenyl group attached to positions of 5-C and 3-C; 5-C and 1-C; and 5-C, 1-C and 3-C on the aromatic ring of O.

111. The method of any one of claims 101-108, wherein (a) the first recombinant polypeptide is the recombinant polypeptide of claim 12, and the second recombinant polypeptide is the recombinant polypeptide of claim 37; or the first recombinant polypeptide is the recombinant polypeptide of claim 37 and the second recombinant polypeptide is the recombinant polypeptide of claim 12; (b) the first prenyl donor is

FPP and the second prenyl donor is DMAPP; or the first prenyl donor is DMAPP, and the second prenyl donor is FPP; and (c) the substrate is O.

112. The method of claim 111, wherein the first prenylated product or the second prenylated product comprises a prenyl group attached to positions 5-C and 3-C; or 5-C and 1-C on the aromatic ring of O.

113. The method of any one of claims 101-112, wherein the second recombinant polypeptide is a cyclase.

114. The recombinant polypeptide of claim 12, wherein the substrate is apigenin and the prenyl donor is GPP.

115. The recombinant polypeptide of claim 114, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from C-13; C-15; C-3; C-12; C-16; C-9; or C-5 on the aromatic ring of apigenin.

116. The recombinant polypeptide of claim 12, wherein the substrate is naringenin and the prenyl donor is GPP.

117. The recombinant polypeptide of claim 116, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from C-3; or C-5 on the aromatic ring of naringenin.

118. The recombinant polypeptide of claim 116, wherein the at least one prenylated product comprises RBI-41 or RBI-42.

119. The recombinant polypeptide of claim 12, wherein the substrate is resveratrol and the prenyl donor is GPP.

120. The recombinant polypeptide of claim 119, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from C-11; C-13; C-3; C-10; C-14; C-1; or C-5 on the aromatic ring of resveratrol.

121. The recombinant polypeptide of claim 119, wherein the at least one prenylated product comprises UNC52, UNC53, RBI-48 or RBI-49.

122. The recombinant polypeptide of any of claims 1-7, wherein the substrate comprises olivetolic acid (OA), divarinolic acid (DVA), olivetol (O), resveratrol, piceattanol and related stilbenes, naringenin, apigenin and related flavanones and flavones, respectively, Isoliquiritigenin, 2'-O-methylisoliquiritigenin and related chalcones, catechins and epi-catechins of all possible stereoisomers, biphenyl compounds such as 3,5-dihydroxy-biphenyl, benzophenones such as phlorobenzophenone, isoflavones such as biochanin A, genistein, daidzein, 2,4-dihydroxybenzoic acid, 1,3-benzenediol, 2,4-dihydroxy-6-methylbenzoic acid; 1,3-Dihydroxy-5-methylbenzene; 2,4-Dihydroxy-6-aethyl-benzoesaeure; 5-ethylbenzene-1,3-diol 2,4-dihydroxy-6-propylbenzoic acid; 5-propylbenzene-1,3-diol; 2-butyl-4,6-dihydroxybenzoic acid; 5-butylbenzene-1,3-diol; 2,4-dihydroxy-6-pentyl-benzoic acid; 5-pentylbenzene-1,3-diol; 5-hexylbenzene-1,3-diol; 2-heptyl-4,6-dihydroxy-benzoic acid; 5-heptylbenzene-1,3-diol; 5-Dodecylbenzene-1,3-diol; 5-nonadecylbenzene-1,3-diol; 1,3-Benzenediol; 3,4',5-Trihydroxystilbene; 4'5-Tetrahydroxystilbene; 1,2-Diphenylethylene; 2-Phenylbenzopyran-4-one; 2-Phenylchroman-4-one; 1,3-benzenediol; 5,7,4'-Trihydroxyflavone; (E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one; 4,4'-dihydroxy-2'-methoxychalcone; 1,3-Diphenylpropenone; (2R,3S)-2-(3,4-Dihydroxyphenyl)chroman-3,5,7-triol; (2R,3R)-2-(3,4-Dihydroxyphenyl)-3,5,7-chromanetriol; Phenylbenzene; 5-Phenylresorcinol; diphenylmethanone; 3-phenyl-4H-

chromen-4-one; 5,7-Dihydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one; 4',5,7-Trihydroxyisoflavone; 4',7-Dihydroxyisoflavone; 4-Hydroxy-6-methyl-2H-pyran-2-one; 1,6-DHN; or any combination thereof.

123. The method of claim 113, wherein the cyclase comprises cannabidiolic acid synthase (CBDAS) or tetrahydrocannabinolic acid synthase (THCAS).

124. The method of claim 113, wherein the cyclase is derived from a plant belonging to the *Rhododendron* genus and wherein the cyclase cyclizes an FPP moiety.

125. The recombinant polypeptide of claim 23, wherein the at least one prenylated product comprises UNK6, UNK7, UNK8, UNK9, or UNK10.

126. The recombinant polypeptide of claim 51, wherein the at least one prenylated product comprises UNK20, UNK21, UNK22, UNK23, UNK24, or UNK59.

127. The recombinant polypeptide of claim 53, wherein the at least one prenylated product comprises UNK25, UNK26, or UNK29.

128. The recombinant polypeptide of claim 55, wherein the at least one prenylated product comprises UNK25, UNK26 or UNK27.

129. The recombinant polypeptide of claim 57, wherein the at least one prenylated product comprises UNK25 or UNK28.

130. The recombinant polypeptide of claim 60, wherein the at least one prenylated product comprises UNK25, UNK26 or UNK28.

131. The recombinant polypeptide of claim 63, wherein the at least one prenylated product comprises UNK25 or UNK26.

132. The recombinant polypeptide of claim 66, wherein the at least one prenylated product comprises UNK25.

133. The recombinant polypeptide of claim 69, wherein the at least one prenylated product comprises UNK27.

134. The recombinant polypeptide of claim 71, wherein the at least one prenylated product comprises UNK30, UNK31, UNK32, UNK33, or UNK34.

135. The recombinant polypeptide of claim 78, wherein the at least one prenylated product comprises UNK36, UNK38, or RBI-22.

136. The recombinant polypeptide of claim 80, wherein at least one prenylated product comprises UNK42.

137. The recombinant polypeptide of claim 82, wherein at least one prenylated product comprises UNK46.

138. The recombinant polypeptide of claim 37, wherein the substrate is DV and the prenyl donor is GPP.

139. The recombinant polypeptide of claim 138, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, 1-C, or 5-C on the aromatic ring of DV.

140. The recombinant polypeptide of claim 139, wherein the at least one prenylated product comprises RBI-32 or RBI-33.

141. The recombinant polypeptide of claim 12, wherein the substrate is OA and the prenyl donor is GGPP.

142. The recombinant polypeptide of claim 141, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, or 5-C on the aromatic ring of OA.

143. The recombinant polypeptide of claim 139, wherein the at least one prenylated product comprises UNK60 or UNK61.

144. The recombinant polypeptide of claim 12, wherein the substrate is ORA and the prenyl donor is GGPP.

145. The recombinant polypeptide of claim 144, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, or 5-C on the aromatic ring of ORA.

146. The recombinant polypeptide of claim 145, wherein the at least one prenylated product comprises UNK62 or UNK63.

147. The recombinant polypeptide of claim 12, wherein the substrate is DVA and the prenyl donor is GGPP.

148. The recombinant polypeptide of claim 147, wherein the at least one prenylated product comprises a prenyl group attached to a position selected from 3-C, or 5-C on the aromatic ring of DVA.

149. The recombinant polypeptide of claim 148, wherein the at least one prenylated product comprises UNK64 or UNK65.

150. The method of claim 110, wherein the at least one prenylated product comprises UNK40, UNK41, UNK66 or UNK67.

151. The method of claim 112, wherein the at least one prenylated product comprises UNK44 or UNK45.

152. The method of claim 101, wherein the first recombinant polypeptide is PB005, and the second recombinant polypeptide is HypSc; or the first recombinant polypeptide is HypSc, and the second recombinant polypeptide is PB005.

153. The method of claim 152, wherein the substrate is DV; and the first prenyl donor and the second prenyl donor is DMAPP.

154. The method of claim 153, wherein the at least one prenylated product comprises a prenyl group attached to positions of 5C and 3C; or 5C and 1C on the aromatic ring of DV.

155. The method of claim 154, wherein the at least one prenylated product comprises UNK57 or UNK58.

156. A composition comprising the at least one prenylated product produced by the method of claim 99 or 100.

157. A composition comprising the first prenylated product and/or the second prenylated product produced by the method of any one of claims 101-113, 123 and 124.

158. The recombinant polypeptide of claim 115, wherein the at least one prenylated product comprises UNK47, UNK48, UNK49, UNK50, or UNK51.

159. A composition comprising a prenylated product, wherein the prenylated product comprises a substitution by a prenyl donor on an aromatic ring of a substrate, wherein the substrate is selected from the group consisting of olivetolic acid (OA), divarinolic

acid (DVA), olivetol (O), divarinol (DV), orsellinic acid (ORA), dihydroxybenzoic acid (DHBA), apigenin, naringenin and resveratrol.

160. The composition of claim 159, wherein the prenyl donor is selected from the group consisting of DMAPP, GPP, FPP, GGPP, and any combination thereof.

161. The composition of claim 159, wherein the prenylated product is selected from any of the prenylated products in Table C.

162. The composition of claim 159, wherein the prenylated product is selected from the group consisting of UNK1, UNK2, UNK3, RBI-08, RBI-17, RBI-05, RBI-06, UNK4, RBI-02 (CBGA), RBI-04 (5-GOA), RBI-56, UNK5, RBI-14 (CBFA), RBI-16 (5-FOA), UNK6, UNK7, UNK8, UNK9, UNK10, RBI-24, RBI-28, UNK11, RBI-26 (CBGVA), RBI-27, UNK12, UNK13, UNK14, RBI-38, RBI-39, RBI-10, UNK16, RBI-09, RBI-10, UNK16, RBI-09, RBI-10, UNK16, RBI-09, RBI-10, RBI-03 (5-GO), RBI-20, RBI-01 (CBG), RBI-03 (5-GO), RBI-15, UNK18, UNK19, RBI-15, UNK54, UNK55, UNK56, UNK54, UNK20, UNK21, UNK22, UNK23, UNK24, UNK25, UNK26, UNK27, UNK28, UNK29, RBI-32, RBI-33, UNK30, UNK31, UNK32, UNK33, UNK34, UNK60, UNK61, UNK62, UNK63, UNK64, UNK65, RBI-07, RBI-29, RBI-30, RBI-36, UNK35, UNK36, RBI-22, UNK38, RBI-18, UNK40, UNK41, UNK42, RBI-12, RBI-11, UNK44, UNK45, UNK46, UNK57, UNK58, UNK59, UNK66, and UNK67.

163. The composition of claim 162, wherein the prenylated product is selected from the group consisting of RBI-01, RBI-02, RBI-03, RBI-04, RBI-05, RBI-07, RBI-08, RBI-09, RBI-10, RBI-11, and RBI-12.

164. The composition of claim 162, wherein the prenylated product is RBI-29 or UNK59.

FIG. 1: Heatmap of Prenylated Products produced from Orf2 Variants using OA as substrate and DMAPP as donor

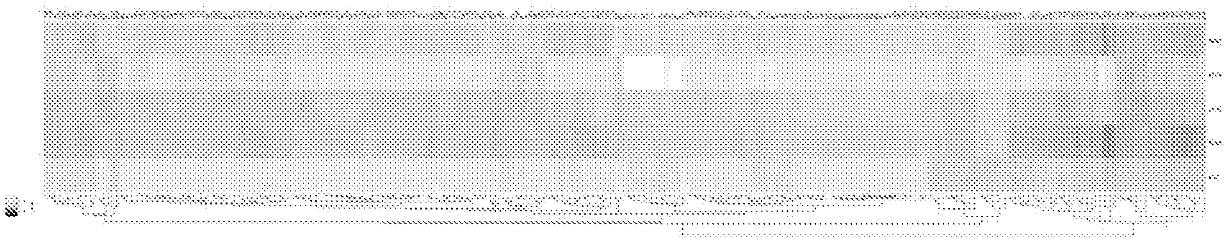
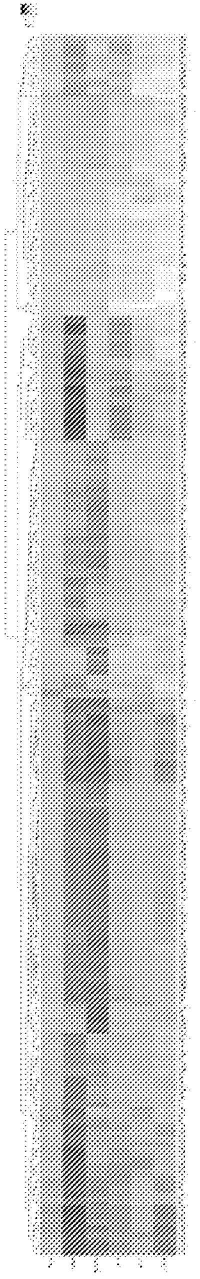


FIG. 2: Heatmap of Prenylated Products produced from Orf2 Variants using OA as substrate and GPP as donor



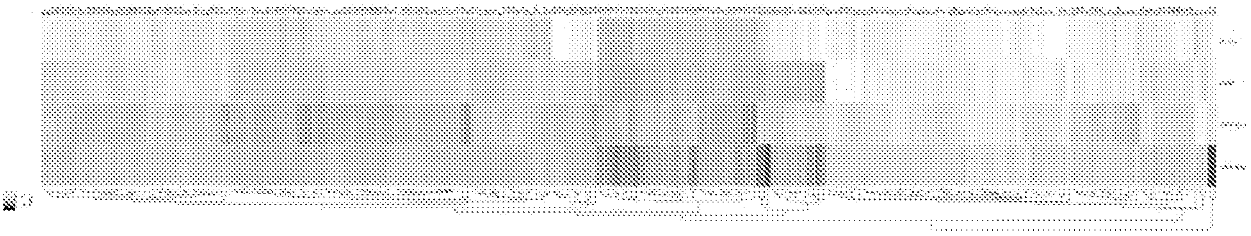


FIG. 3: Heatmap of Prenylated Products produced from Orf2 Variants using OA as substrate and FPP as donor

FIG. 4: Heatmap of Prenylated Products produced from Orf2 Variants using O as substrate and GPP as donor

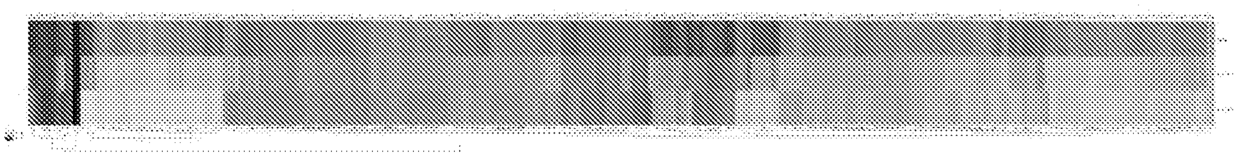
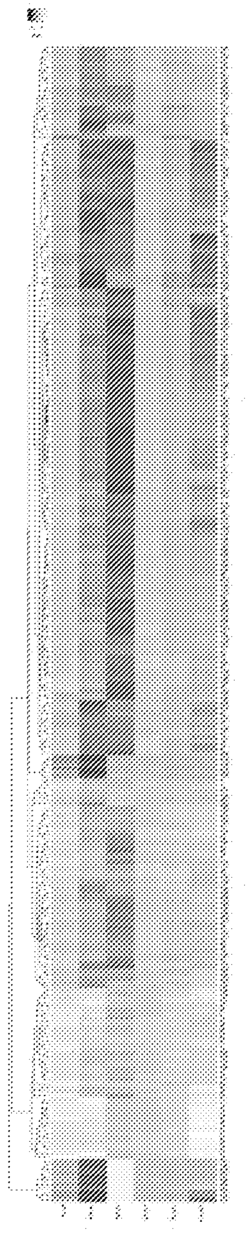


FIG. 5: Heatmap of Prenylated Products produced from Orf2 Variants using DVA as substrate and GPP as donor



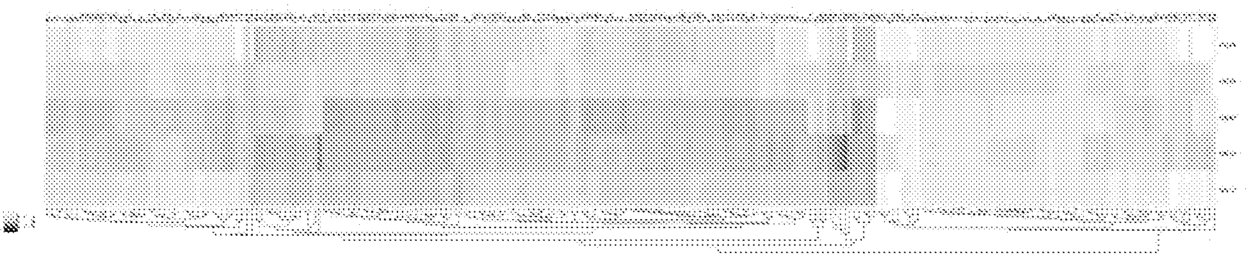


FIG. 6: Heatmap of Prenylated Products produced from Orf2 Variants using DVA as substrate and FPP as donor

FIG. 7: Heatmap of Prenylated Products produced from select Orf2 Variants using ORA as substrate and GPP as donor

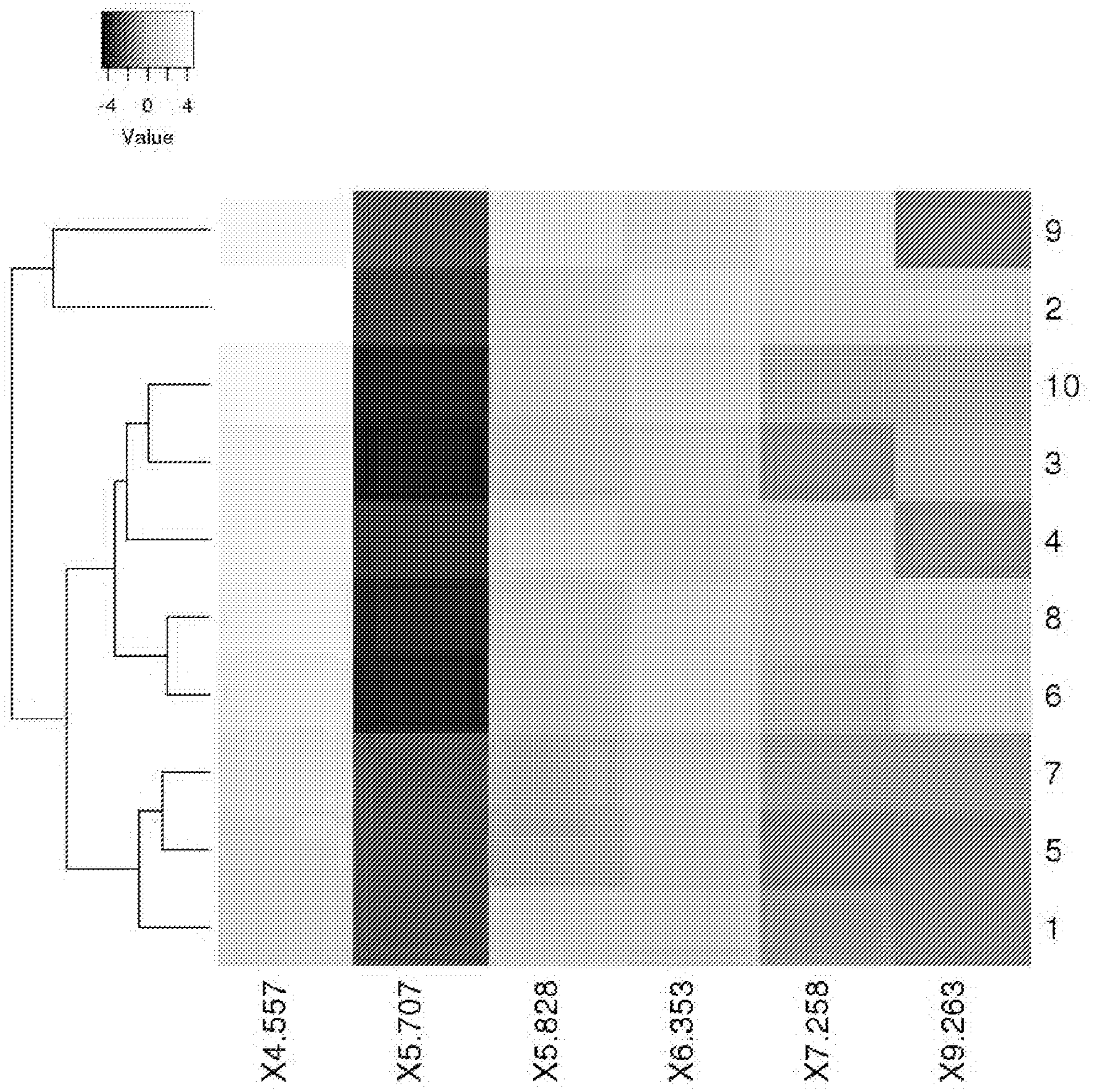


FIG. 8: Heatmap of Prenylated Products produced from select Orf2 Variants using Apigenin as substrate and GPP as donor

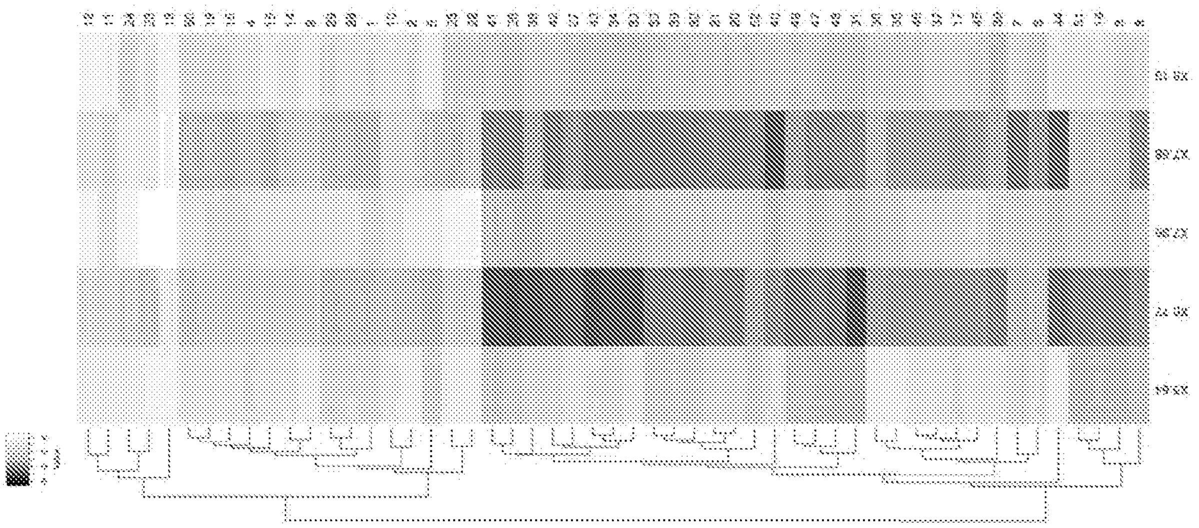


FIG. 9: Heatmap of Prenylated Products produced from select Orf2 Variants using Naringenin as substrate and GPP as donor

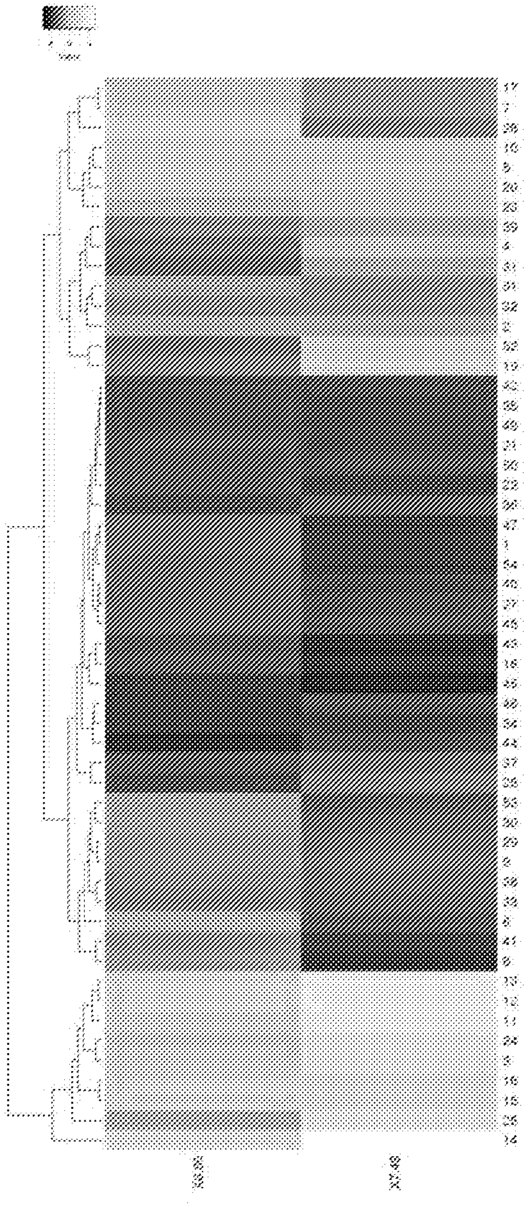


FIG. 10: Heatmap of Prenylated Products produced from select Orf2 Variants using Resveratrol as substrate and GPP as donor

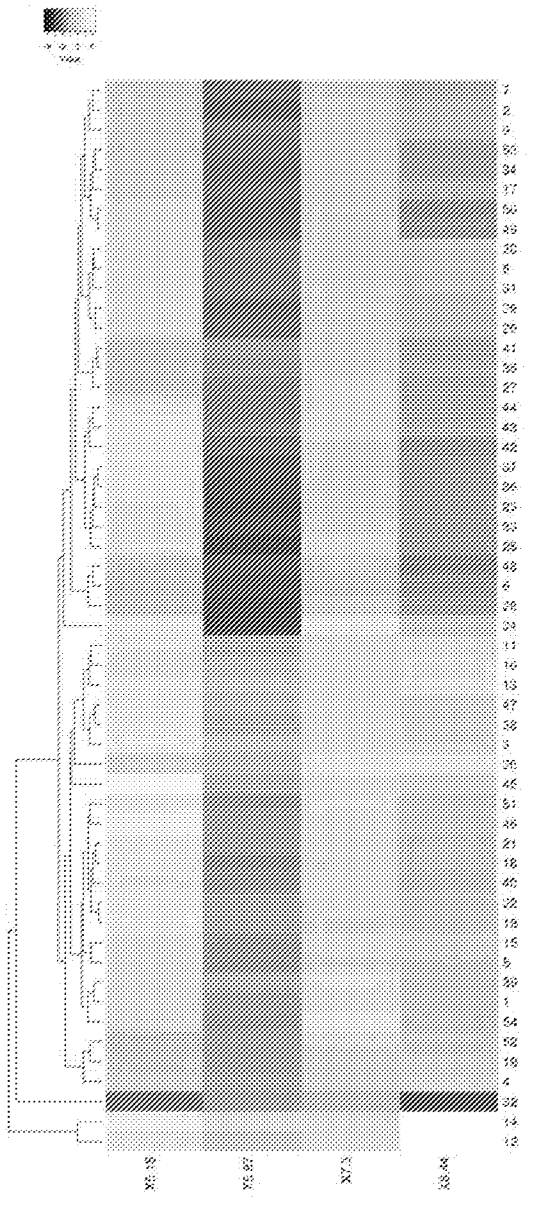


FIG. 11: Heatmap of Prenylated Products produced from prenyltransferase enzymes using ORA as substrate and DMAPP as donor

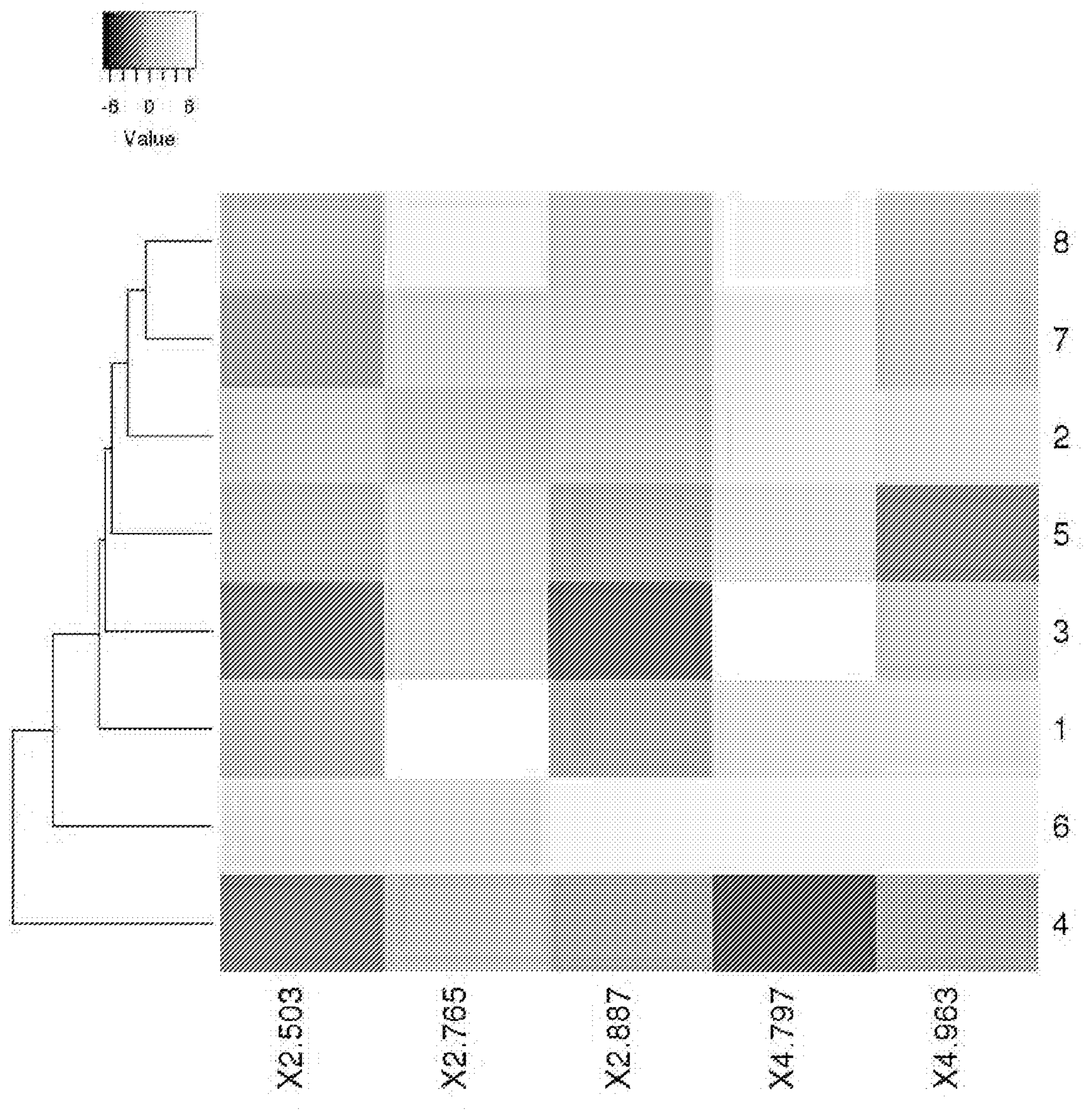


FIG. 12: Heatmap of Prenylated Products produced from prenyltransferase enzymes using DV as substrate and DMAPP as donor

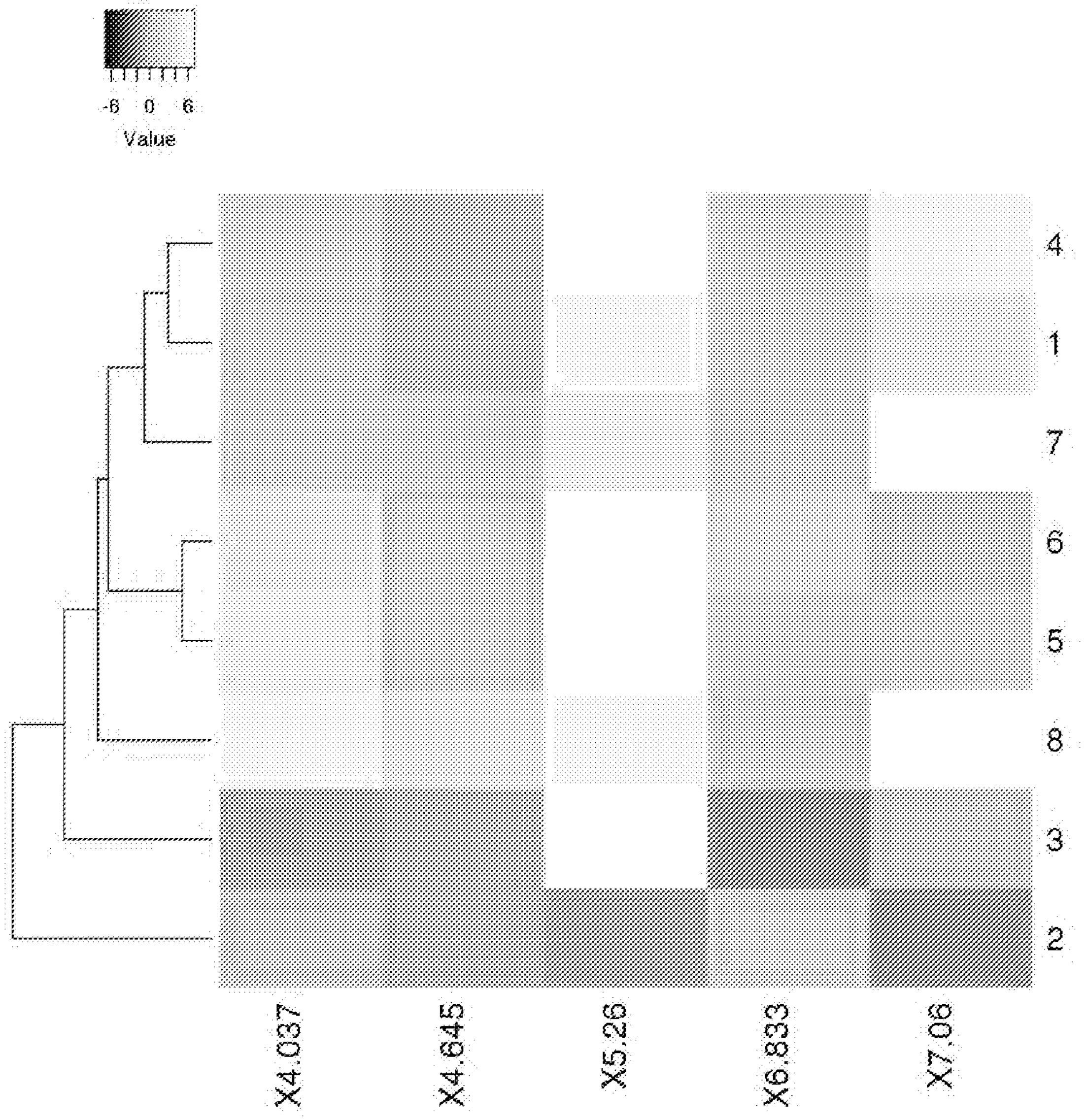


FIG. 13: Heatmap of Prenylated Products produced from prenyltransferase enzymes using DV as substrate and GPP as donor

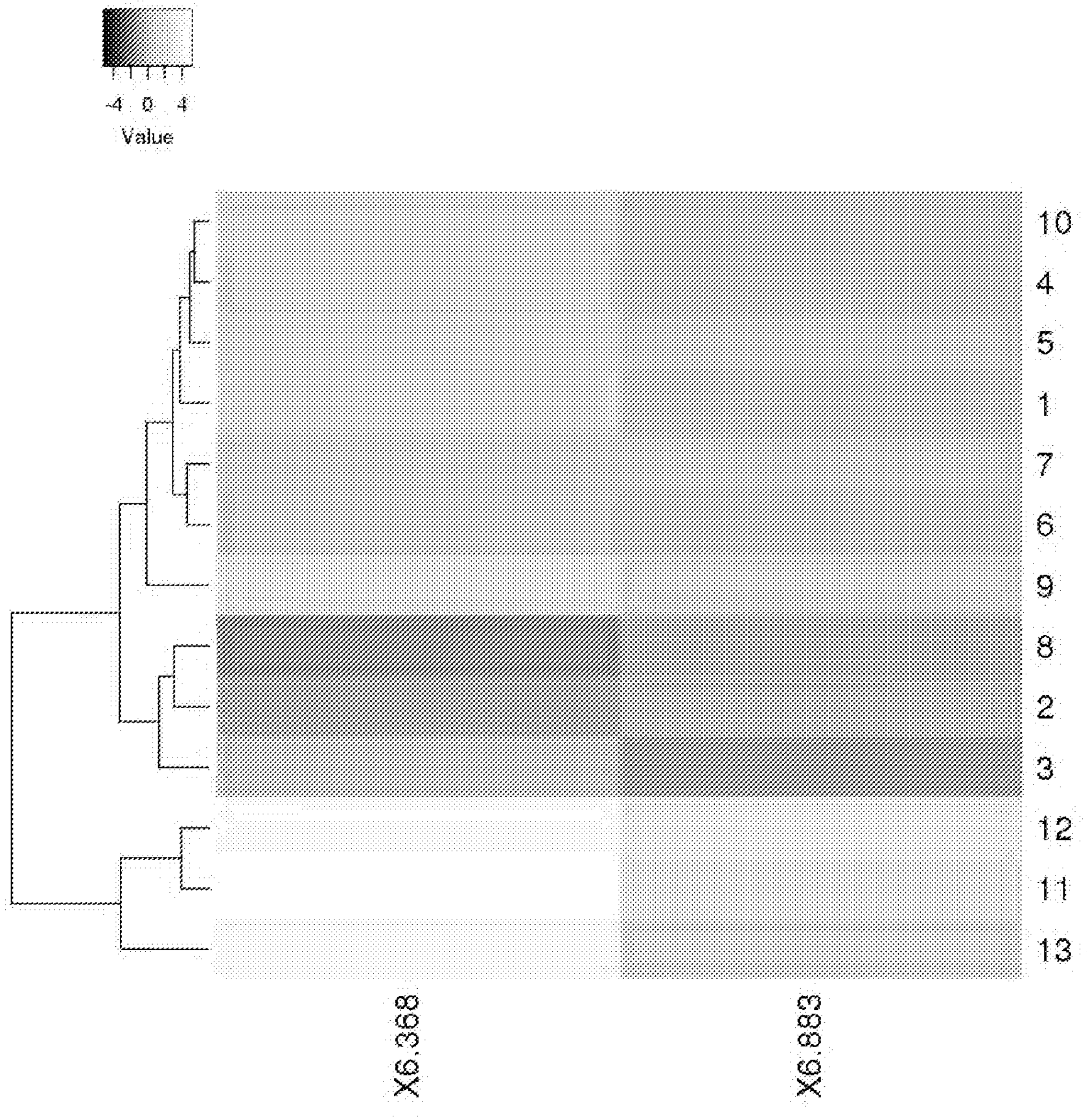


FIG. 14: Heatmap of Prenylated Products produced from prenyltransferase enzymes using DVA as substrate and DMAPP as donor

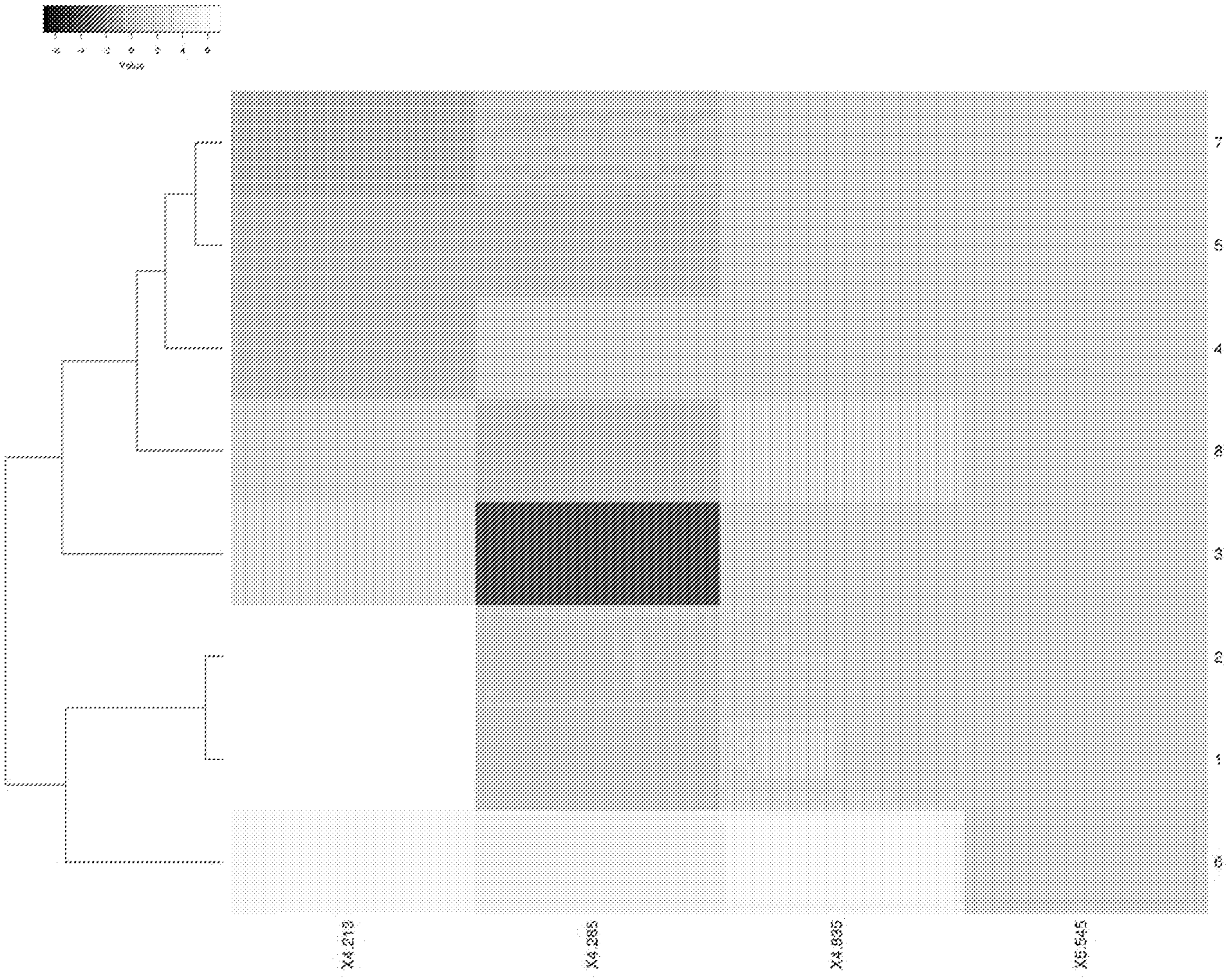


FIG. 15: Heatmap of Prenylated Products produced from prenyltransferase enzymes using O as substrate and DMAPP as donor

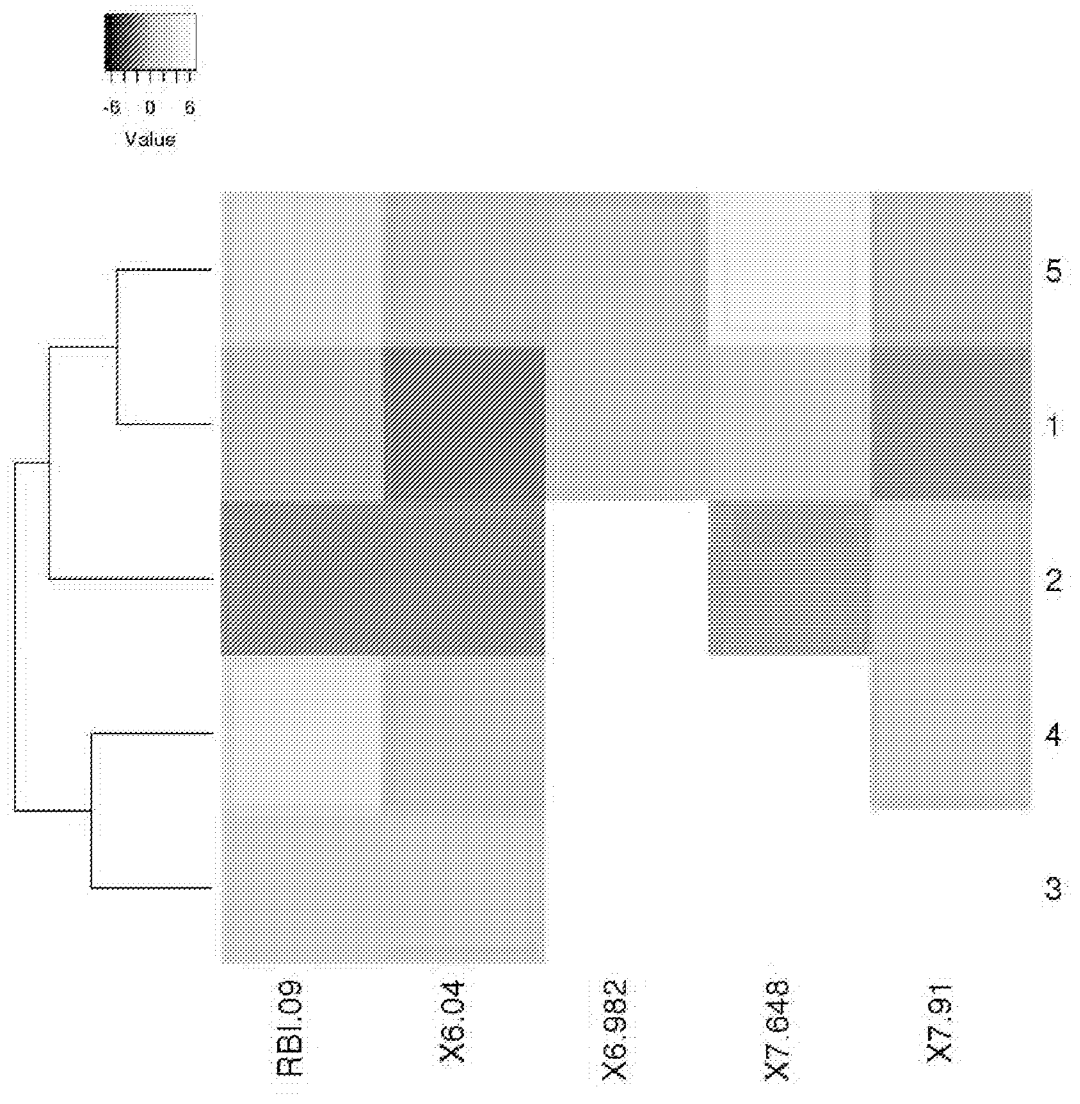
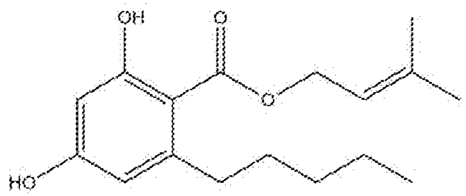


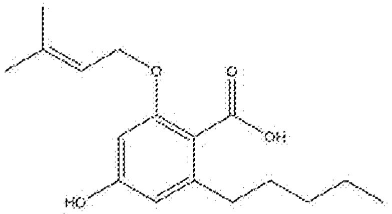
FIG. 16: Predicted Prenylation Products using OA as substrate and DMAPP as Donor

UNK1



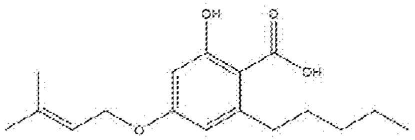
3-methylbut-2-en-1-yl 2,4-dihydroxy-6-pentylbenzoate

UNK2



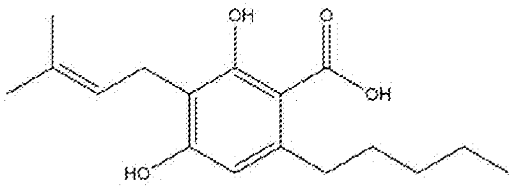
4-hydroxy-2-((3-methylbut-2-en-1-yl)oxy)-6-pentylbenzoic acid

UNK3



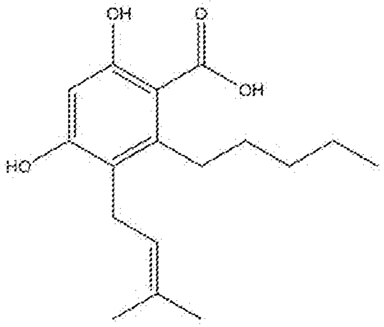
2-hydroxy-4-((3-methylbut-2-en-1-yl)oxy)-6-pentylbenzoic acid

RBI-08



2,4-dihydroxy-3-(3-methylbut-2-en-1-yl)-6-pentylbenzoic acid

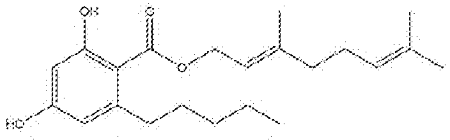
RBI-17



4,6-dihydroxy-3-(3-methylbut-2-en-1-yl)-2-pentylbenzoic acid

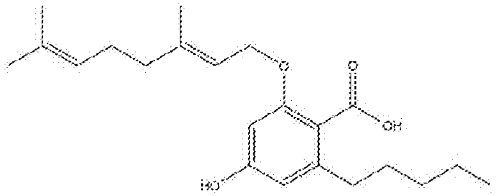
FIG. 17: Predicted Prenylation Products using OA as substrate and GPP as Donor

RBI-05



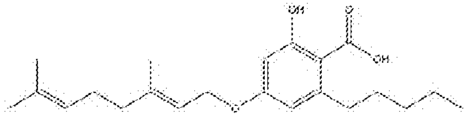
(E)-3,7-dimethylocta-2,6-dien-1-yl 2,4-dihydroxy-6-pentylbenzoate

RBI-06



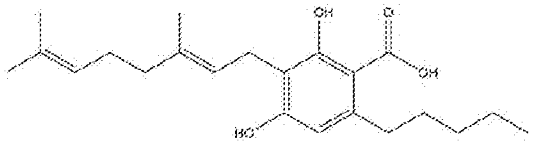
(E)-2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-hydroxy-6-pentylbenzoic acid

UNS4



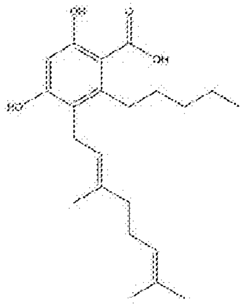
(E)-4-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-2-hydroxy-6-pentylbenzoic acid

RBI-02 (CBGA)



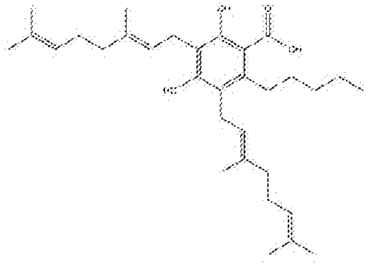
(E)-3-(3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-pentylbenzoic acid

RBI-04



(E)-3-(3,7-dimethylocta-2,6-dien-1-yl)-4,6-dihydroxy-2-pentylbenzoic acid

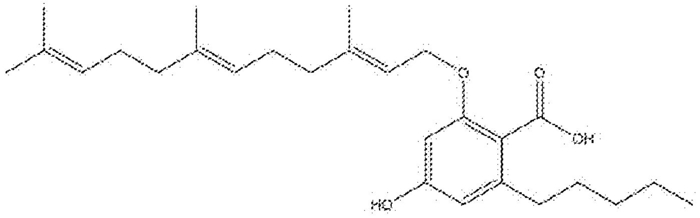
RBI-07



3,5-bis((E)-3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-pentylbenzoic acid

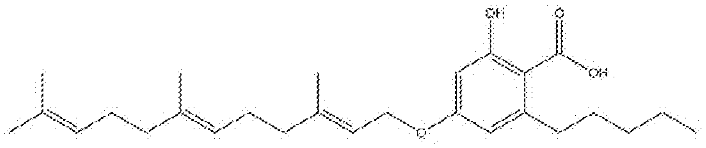
FIG. 18: Predicted Prenylation Products using OA as substrate and FPP as Donor

RBI-56



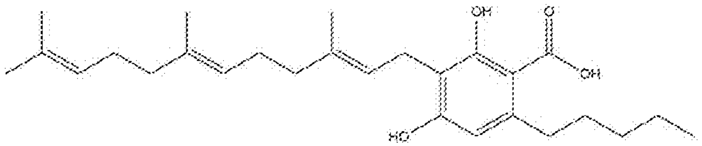
4-hydroxy-2-pentyl-6-(((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)benzoic acid

UNK5



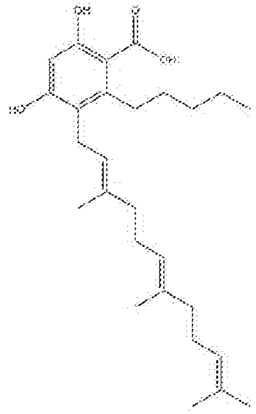
2-hydroxy-6-pentyl-4-(((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)benzoic acid

RBI-14



2,4-dihydroxy-6-pentyl-3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic acid

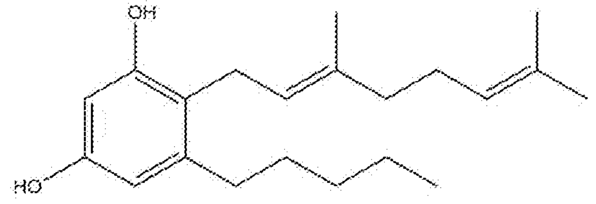
RBI-16



4,6-dihydroxy-2-pentyl-3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic acid

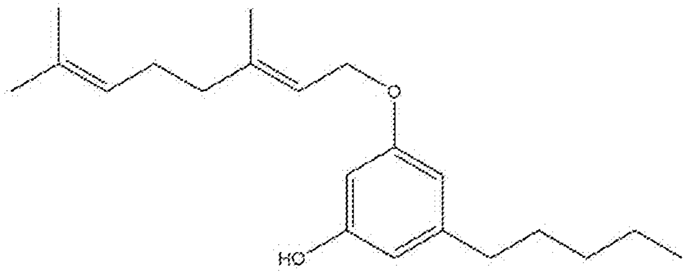
FIG. 19: Predicted Prenylation Products using O as substrate and GPP as Donor

RBI-03



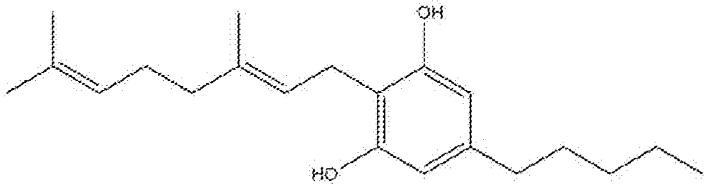
(*E*)-4-(3,7-dimethylocta-2,6-dien-1-yl)-5-pentylbenzene-1,3-diol

RBI-20



(*E*)-3-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-5-pentylphenol

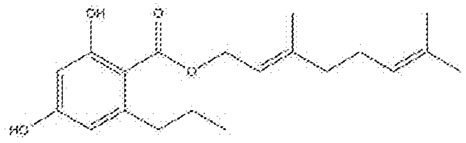
RBI-01 (CBG)



(*E*)-2-(3,7-dimethylocta-2,6-dien-1-yl)-5-pentylbenzene-1,3-diol

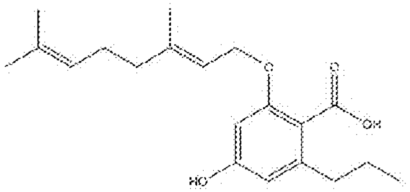
FIG. 20: Predicted Prenylation Products using DVA as substrate and GPP as Donor

RBI-24



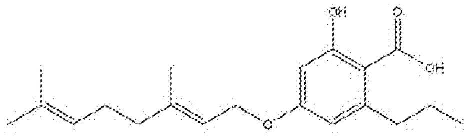
(*E*)-3,7-dimethylocta-2,6-dien-1-yl 2,4-dihydroxy-6-propylbenzoate

RBI-28



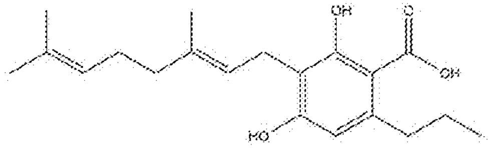
(*E*)-2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-hydroxy-6-propylbenzoic acid

CNK11



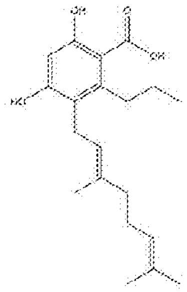
(*E*)-4-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-2-hydroxy-6-propylbenzoic acid

RBI-26



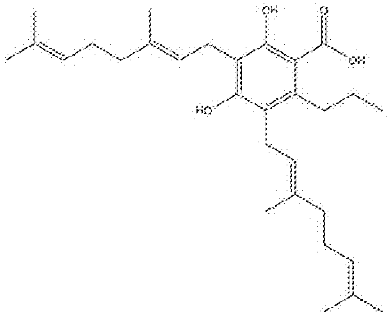
(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-propylbenzoic acid

RBI-27



(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-4,6-dihydroxy-2-propylbenzoic acid

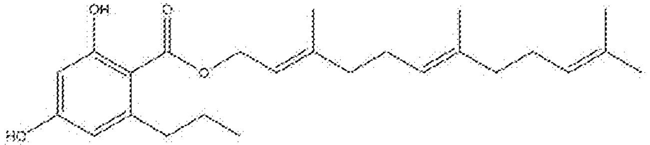
RBI-29



3,5-bis((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-propylbenzoic acid

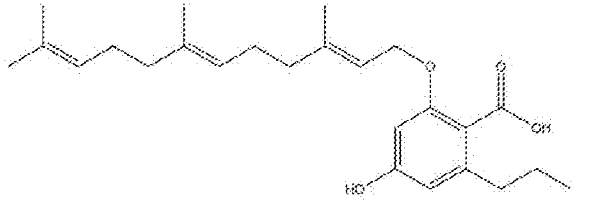
FIG. 21: Predicted Prenylation Products using DVA as substrate and FPP as Donor

UNK12



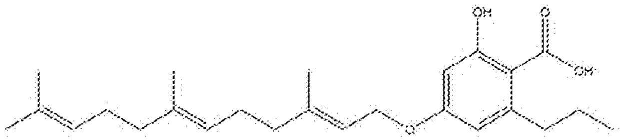
(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl 2,4-dihydroxy-6-propylbenzoate

UNK13



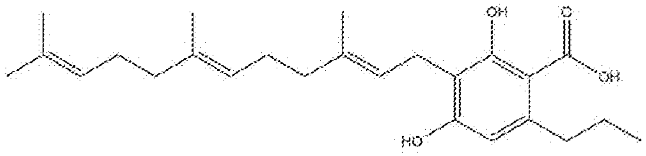
4-hydroxy-2-propyl-6-(((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)benzoic acid

UNK14



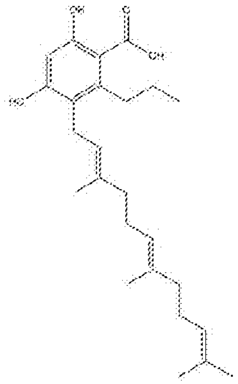
2-hydroxy-6-propyl-4-(((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)benzoic acid

RBI-38



2,4-dihydroxy-6-propyl-3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic acid

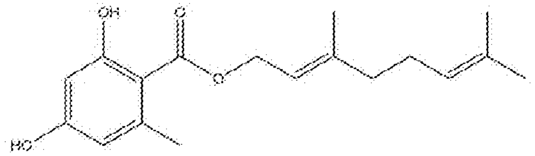
RBI-39



4,6-dihydroxy-2-propyl-3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic acid

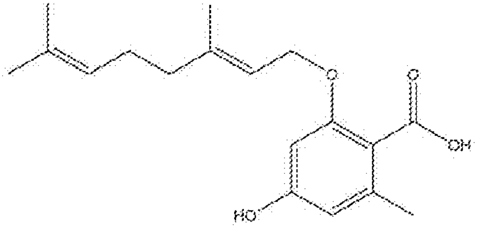
FIG. 22: Predicted Prenylation Products using ORA as substrate and GPP as Donor

UNK20



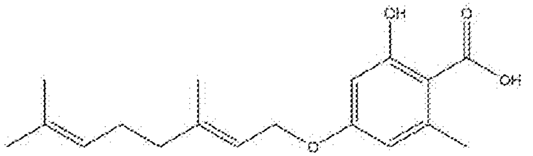
(*E*)-3,7-dimethylocta-2,6-dien-1-yl 2,4-dihydroxy-6-methylbenzoate

UNK21



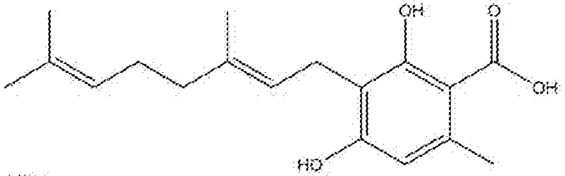
(*E*)-2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-hydroxy-6-methylbenzoic acid

UNK22



(*E*)-4-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-2-hydroxy-6-methylbenzoic acid

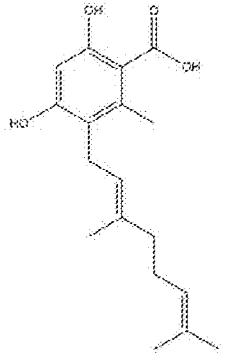
UNK23



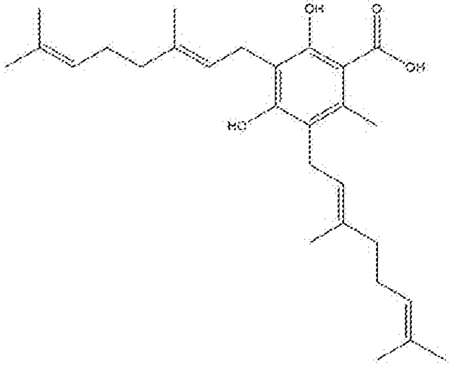
UNK59

(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-methylbenzoic acid

UNK24



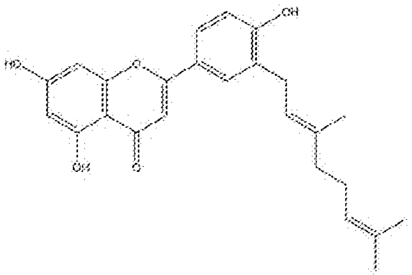
(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-4,6-dihydroxy-2-methylbenzoic acid



3,5-bis((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-methylbenzoic acid

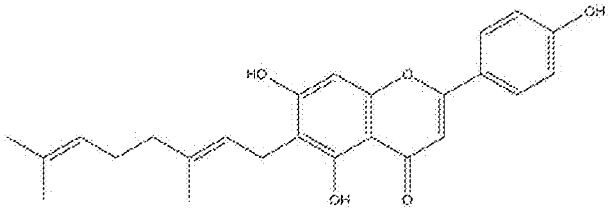
FIG. 23: Predicted Prenylation Products using Apigenin as substrate and GPP as Donor

UNK47



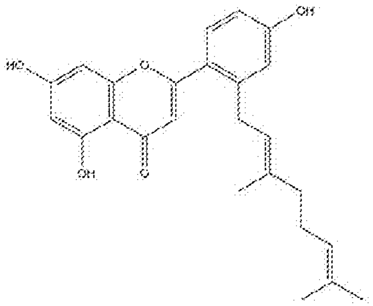
(*E*)-2-(3-(3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one

UNK48



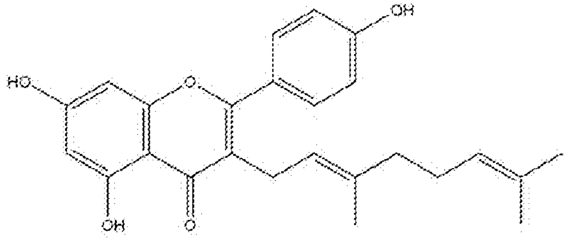
(*E*)-6-(3,7-dimethylocta-2,6-dien-1-yl)-5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one

UNK49



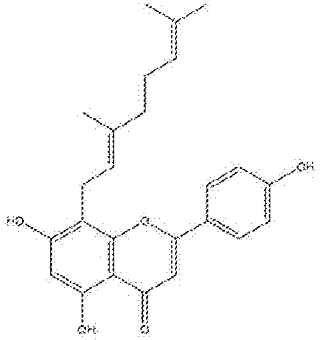
(*E*)-2-(2-(3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one

UNK50



(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one

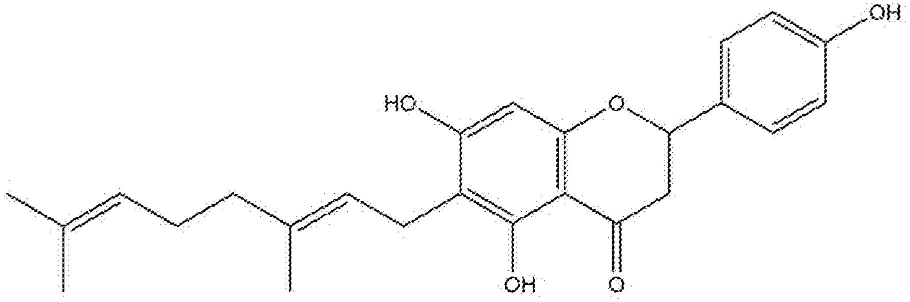
UNK51



(*E*)-8-(3,7-dimethylocta-2,6-dien-1-yl)-5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one

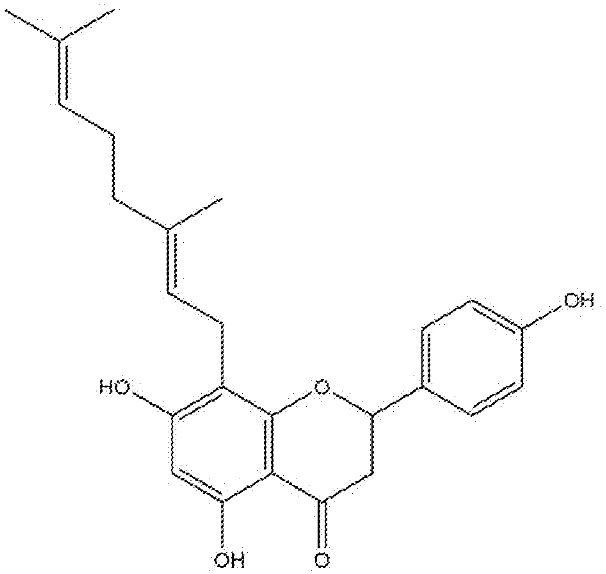
FIG. 24: Predicted Prenylation Products using Naringenin as substrate and GPP as Donor

RBI-41



(*E*)-6-(3,7-dimethylocta-2,6-dien-1-yl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one

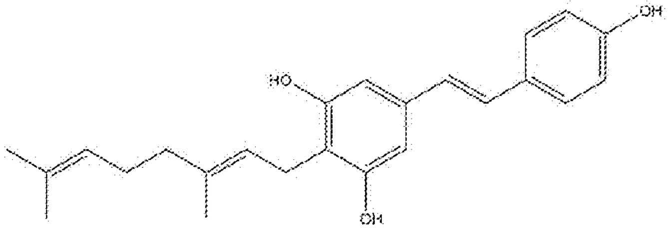
RBI-42



(*E*)-8-(3,7-dimethylocta-2,6-dien-1-yl)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one

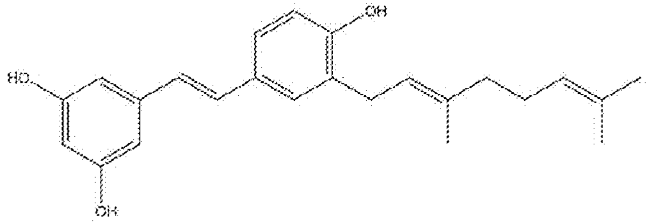
FIG. 25: Predicted Prenylation Products using Reservatrol as substrate and GPP as Donor

RBI-48



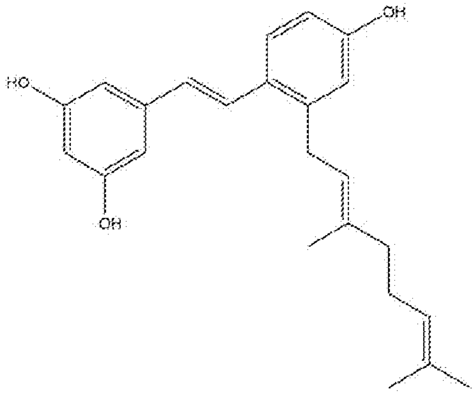
2-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-5-((*E*)-4-hydroxystyryl)benzene-1,3-diol

RBI-49



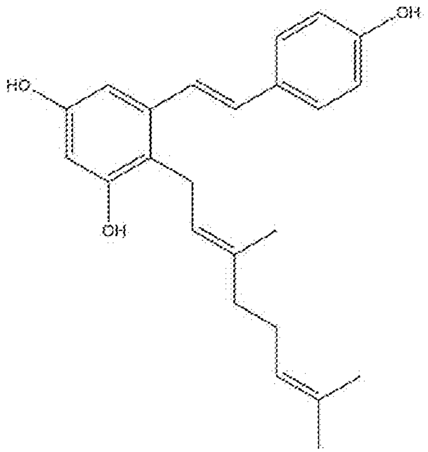
5-((*E*)-3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxystyryl)benzene-1,3-diol

UNK52



5-((*E*)-2-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxystyryl)benzene-1,3-diol

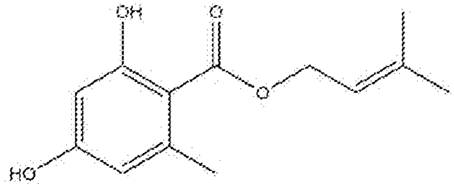
UNK53



4-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-5-((*E*)-4-hydroxystyryl)benzene-1,3-diol

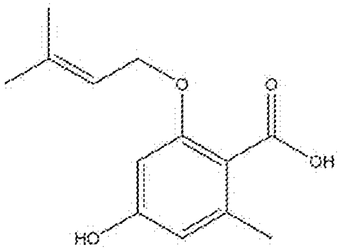
FIG. 26: Predicted Prenylation Products using ORA as substrate and DMAPP as Donor

UNK25



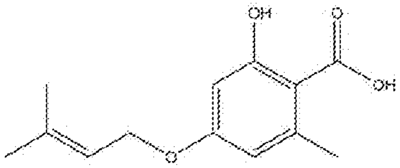
3-methylbut-2-en-1-yl 2,4-dihydroxy-6-methylbenzoate

UNK26



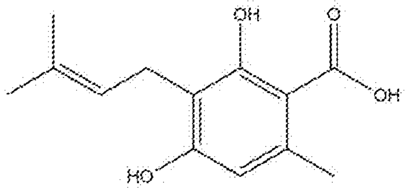
4-hydroxy-2-methyl-6-((3-methylbut-2-en-1-yl)oxy)benzoic acid

UNK27



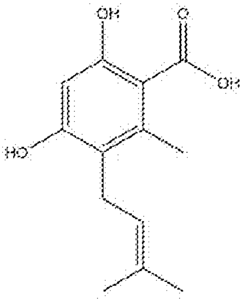
2-hydroxy-6-methyl-4-((3-methylbut-2-en-1-yl)oxy)benzoic acid

UNK28



2,4-dihydroxy-6-methyl-3-(3-methylbut-2-en-1-yl)benzoic acid

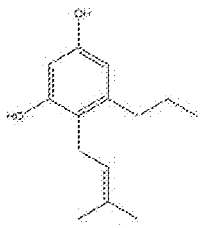
UNK29



4,6-dihydroxy-2-methyl-3-(3-methylbut-2-en-1-yl)benzoic acid

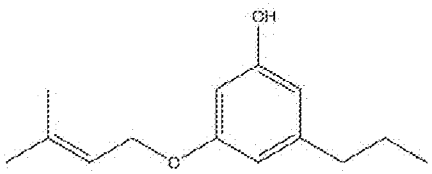
FIG. 27: Predicted Prenylation Products using DV as substrate and DMAPP as Donor

UNK54



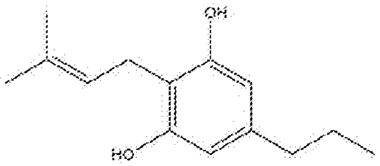
4-(3-methylbut-2-en-1-yl)-5-propylbenzene-1,3-diol

UNK55



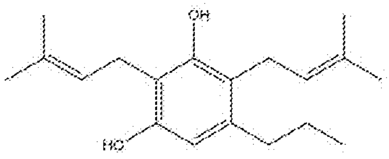
3-((3-methylbut-2-en-1-yl)oxy)-5-propylphenol

UNK56



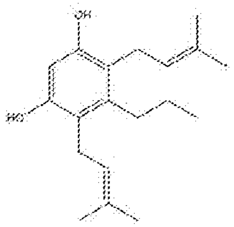
2-(3-methylbut-2-en-1-yl)-5-propylbenzene-1,3-diol

UNK57



2,4-bis(3-methylbut-2-en-1-yl)-5-propylbenzene-1,3-diol

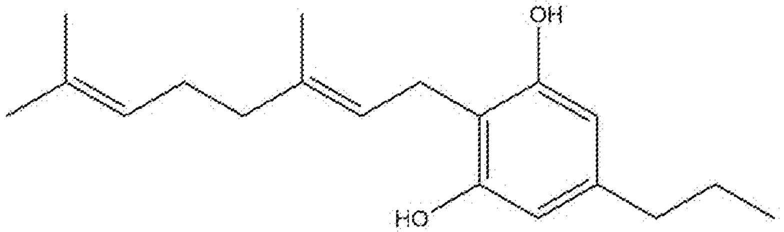
UNK58



4,6-bis(3-methylbut-2-en-1-yl)-5-propylbenzene-1,3-diol

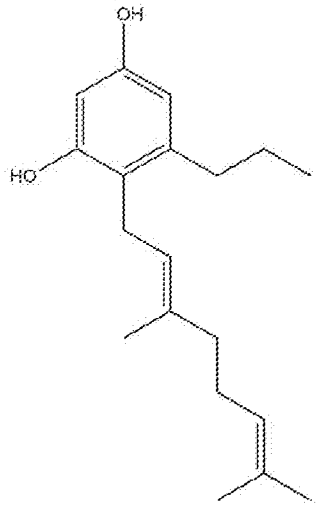
FIG. 28: Predicted Prenylation Products using DV as substrate and GPP as Donor

RBI-32



(*E*)-2-(3,7-dimethylocta-2,6-dien-1-yl)-5-propylbenzene-1,3-diol

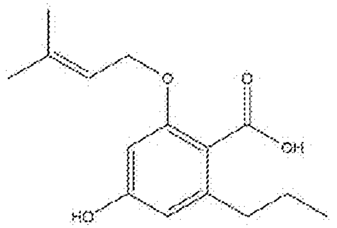
RBI-33



(*E*)-4-(3,7-dimethylocta-2,6-dien-1-yl)-5-propylbenzene-1,3-diol

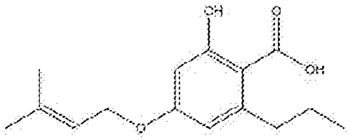
FIG. 29: Predicted Prenylation Products using DVA as substrate and DMAPP as Donor

UNK7



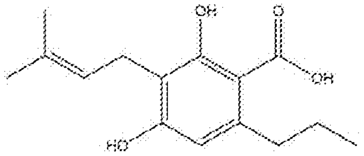
4-hydroxy-2-((3-methylbut-2-en-1-yl)oxy)-6-propylbenzoic acid

UNK8



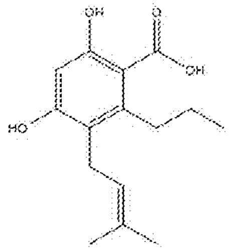
2-hydroxy-4-((3-methylbut-2-en-1-yl)oxy)-6-propylbenzoic acid

UNK9



2,4-dihydroxy-3-(3-methylbut-2-en-1-yl)-6-propylbenzoic acid

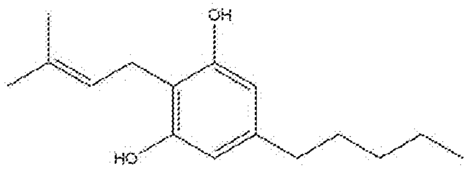
UNK10



4,6-dihydroxy-3-(3-methylbut-2-en-1-yl)-2-propylbenzoic acid

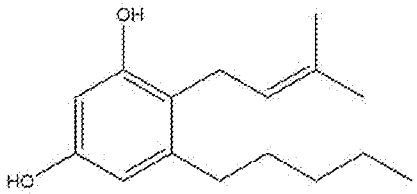
FIG. 30: Predicted Prenylation Products using O as substrate and DMAPP as Donor

RBI-09



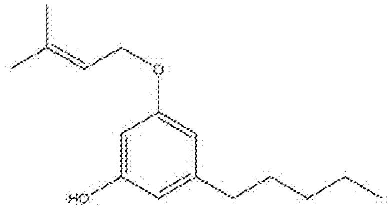
2-(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

RBI-10



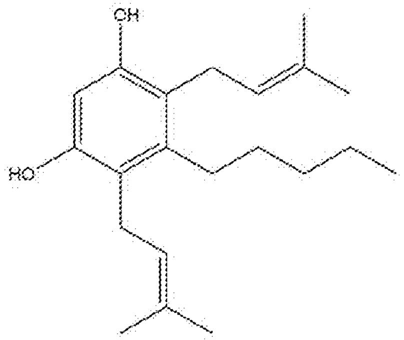
4-(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

UNK16



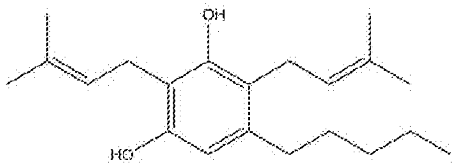
3-((3-methylbut-2-en-1-yl)oxy)-5-pentylphenol

RBI-12



4,6-bis(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

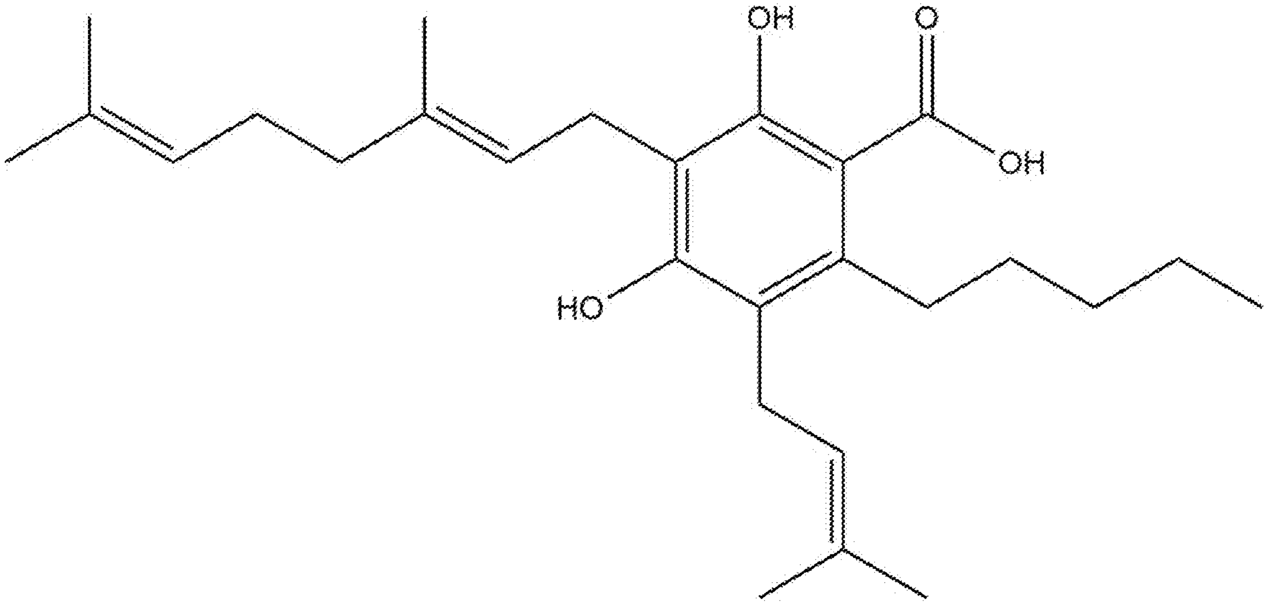
RBI-11



2,4-bis(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

FIG. 31: Predicted Prenylation Products using CBGA as substrate and DMAPP as Donor

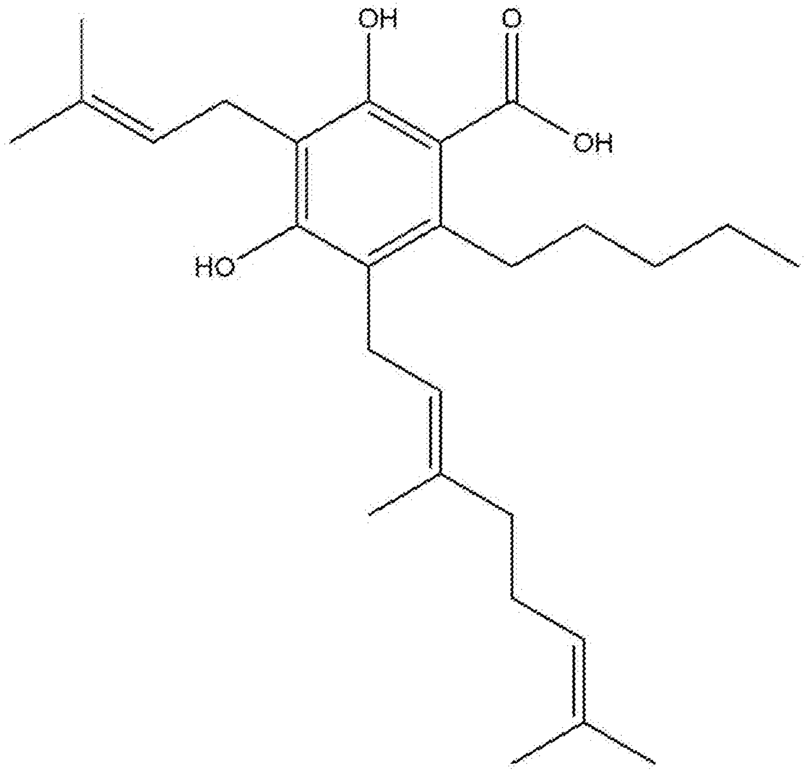
RBI-22



(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-5-(3-methylbut-2-en-1-yl)-6-pentylbenzoic acid

FIG. 32: Predicted Prenylation Products using RBI-04 as substrate and DMAPP as Donor

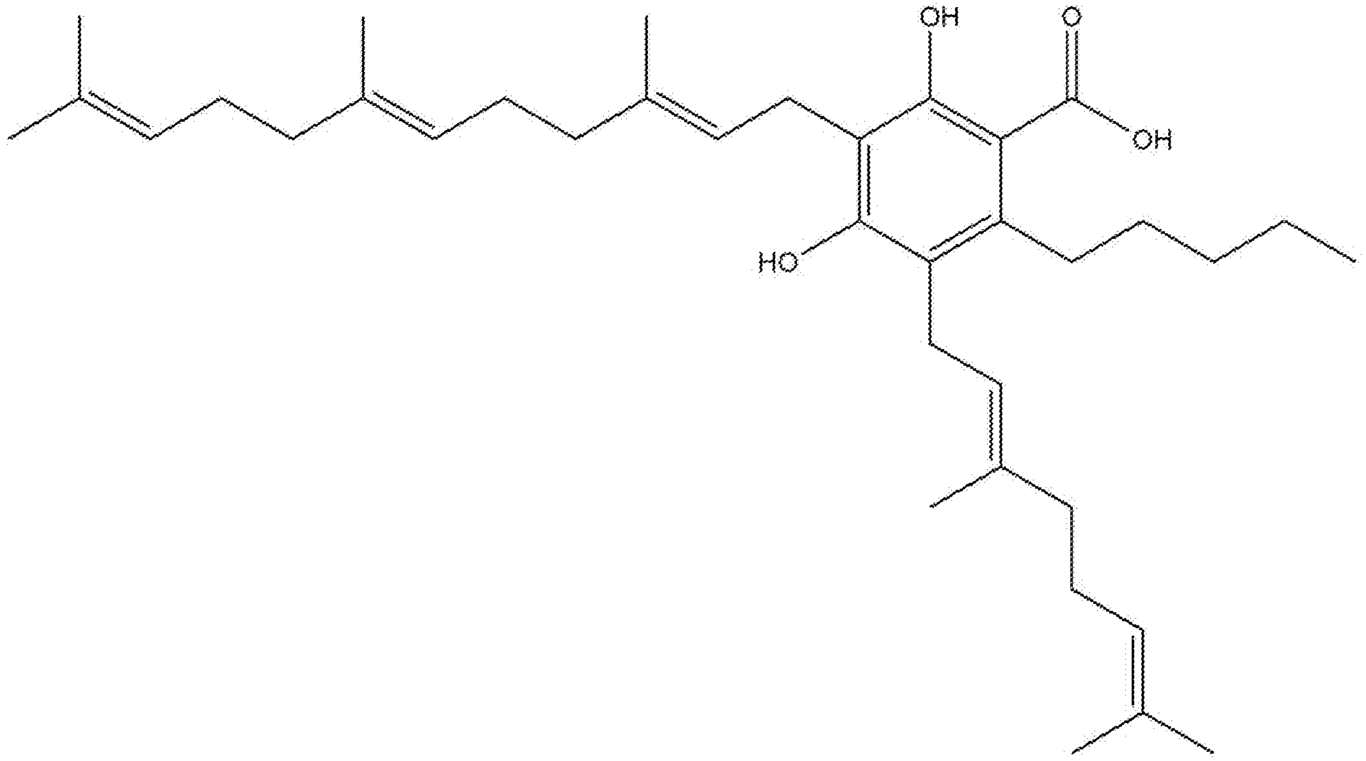
UNK36



(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-4,6-dihydroxy-5-(3-methylbut-2-en-1-yl)-2-pentylbenzoic acid

FIG. 33: Predicted Prenylation Products using RBI-04 as substrate and FPP as Donor

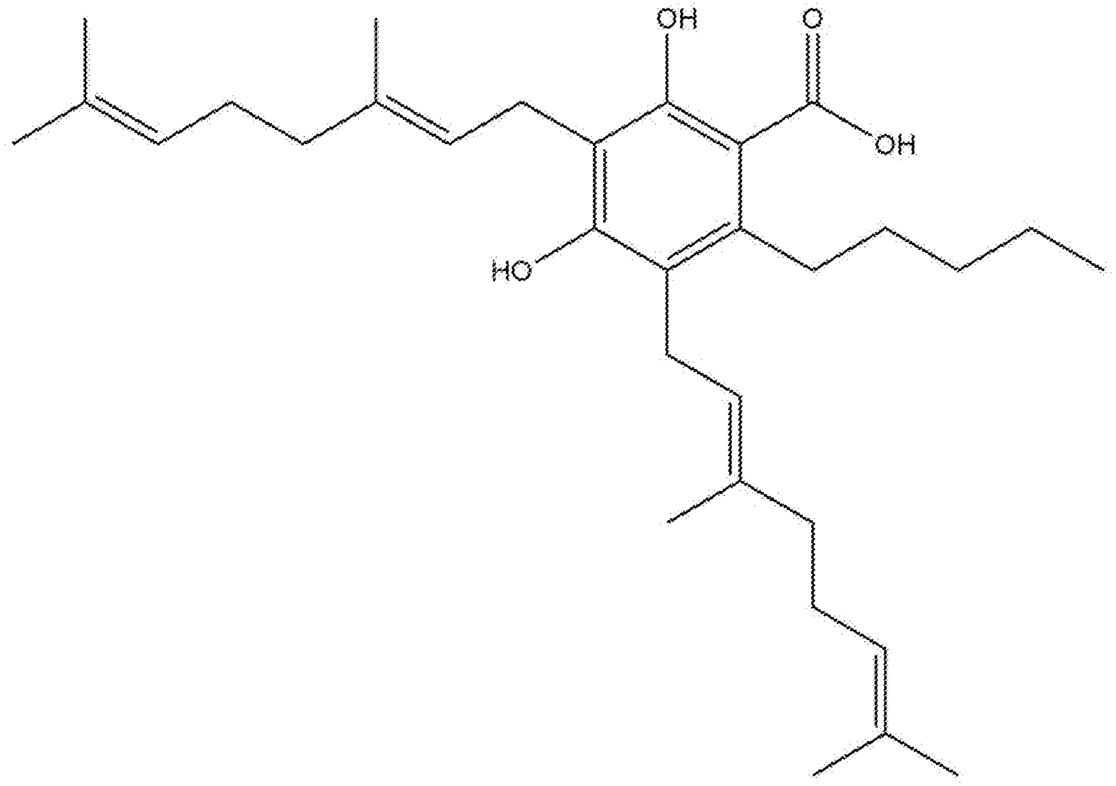
UNK42



3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-4,6-dihydroxy-2-pentyl-5-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic acid

FIG. 34: Predicted Prenylation Products using RBI-04 as substrate and GPP as Donor

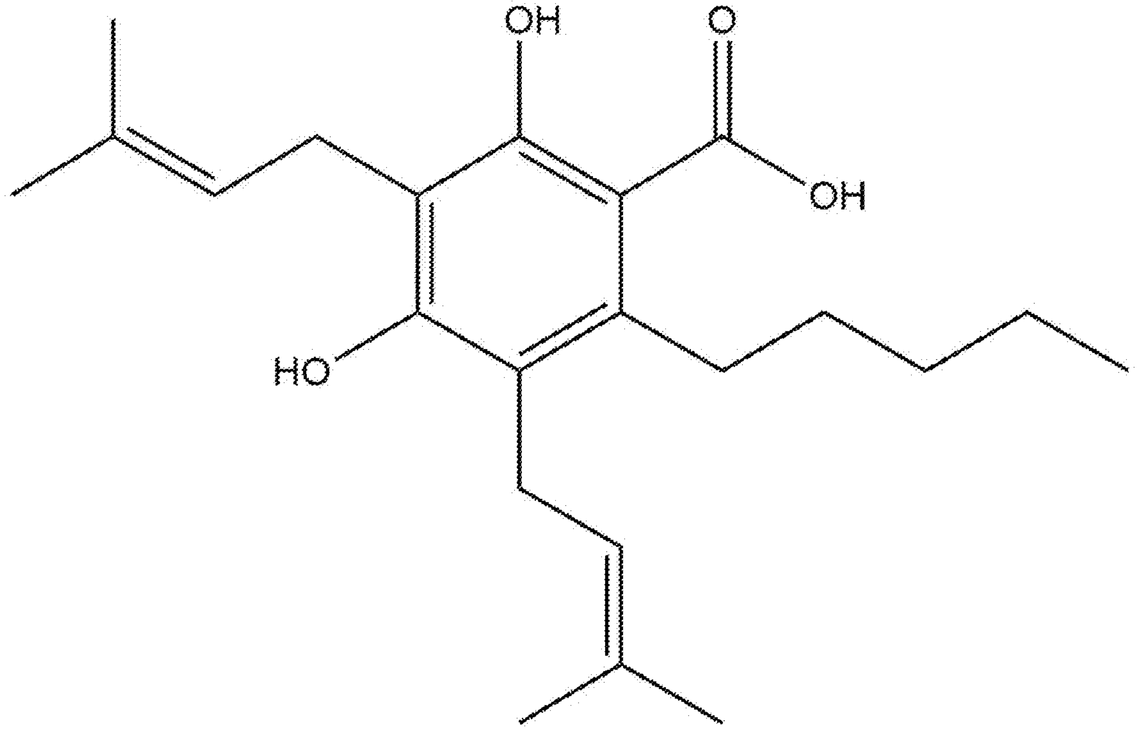
RBI-07



3,5-bis((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-pentylbenzoic acid

FIG. 35: Predicted Prenylation Products using RBI-08 as substrate and DMAPP as Donor

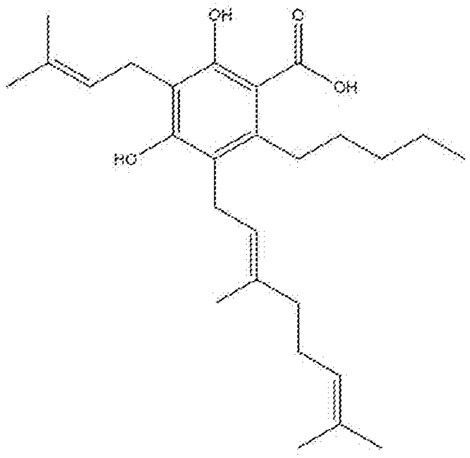
RBI-18



2,4-dihydroxy-3,5-bis(3-methylbut-2-en-1-yl)-6-pentylbenzoic acid

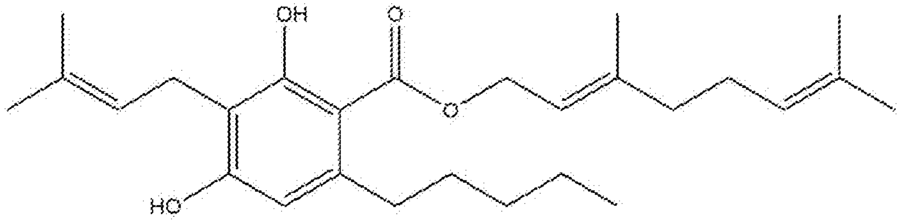
FIG. 36: Predicted Prenylation Products using RBI-08 as substrate and GPP as Donor

UNK36



(*E*)-3-(3,7-dimethylocta-2,6-dien-1-yl)-4,6-dihydroxy-5-(3-methylbut-2-en-1-yl)-2-pentylbenzoic acid

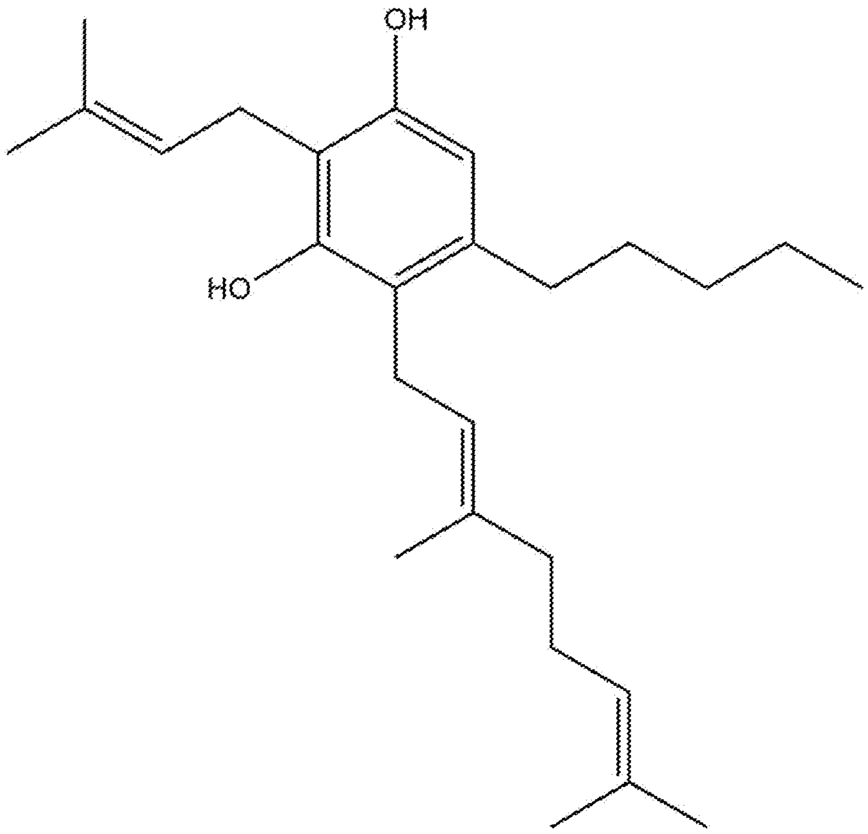
UNK38



(*E*)-3,7-dimethylocta-2,6-dien-1-yl 2,4-dihydroxy-3-(3-methylbut-2-en-1-yl)-6-pentylbenzoate

FIG. 37: Predicted Prenylation Products using RBI-09 as substrate and GPP as Donor

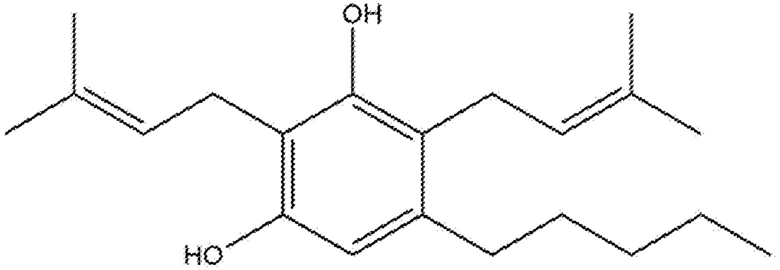
UNK40



(*E*)-4-(3,7-dimethylocta-2,6-dien-1-yl)-2-(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

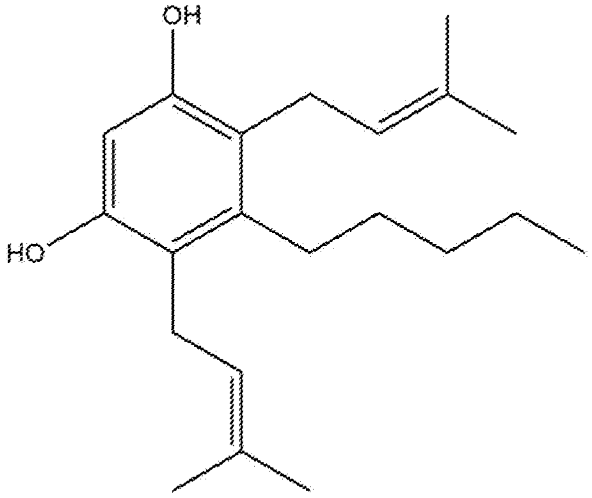
FIG. 38: Predicted Prenylation Products using RBI-10 as substrate and DMAPP as Donor

RBI-11



2,4-bis(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

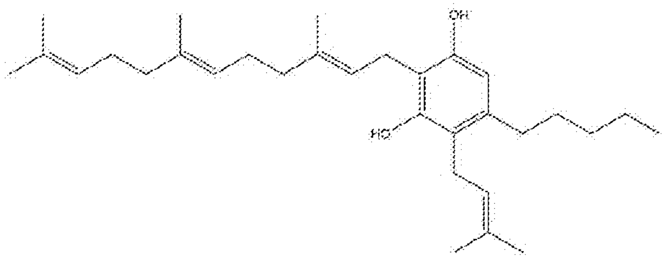
RBI-12



4,6-bis(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

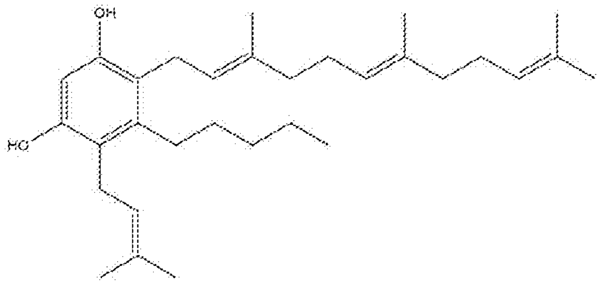
FIG. 39: Predicted Prenylation Products using RBI-10 as substrate and FPP as Donor

UNK44



4-(3-methylbut-2-en-1-yl)-5-pentyl-2-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzene-1,3-diol

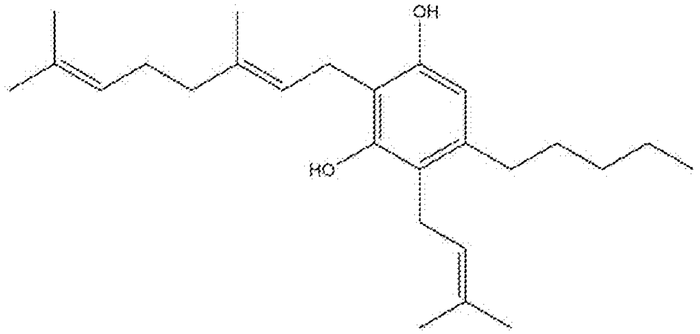
UNK45



4-(3-methylbut-2-en-1-yl)-5-pentyl-6-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzene-1,3-diol

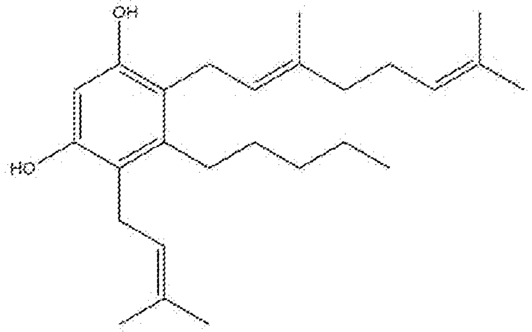
FIG. 40: Predicted Prenylation Products using RBI-10 as substrate and GPP as Donor

UNK41



(*E*)-2-(3,7-dimethylocta-2,6-dien-1-yl)-4-(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

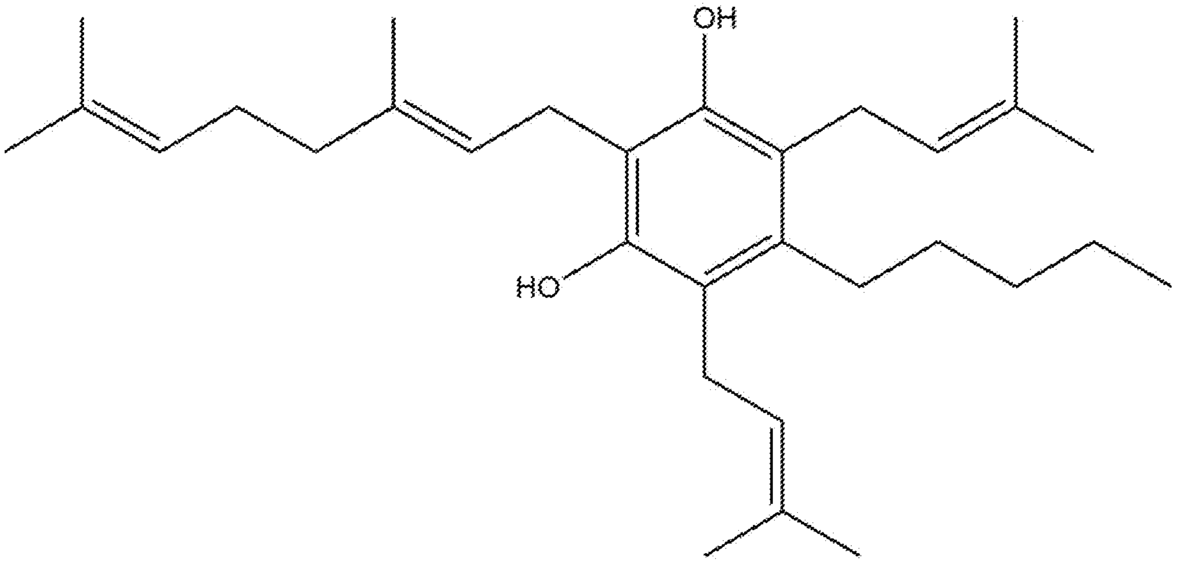
UNK66



(*E*)-4-(3,7-dimethylocta-2,6-dien-1-yl)-6-(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

FIG. 41: Predicted Prenylation Products using RBI-12 as substrate and GPP as Donor

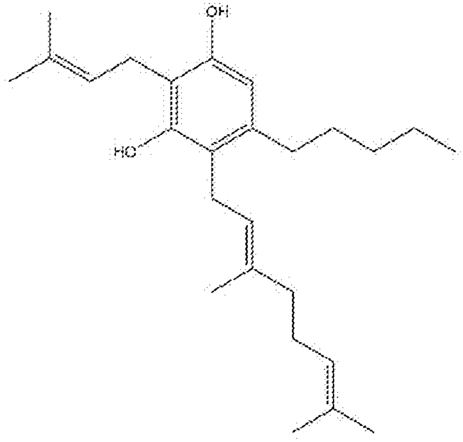
UNK67



(*E*)-2-(3,7-dimethylocta-2,6-dien-1-yl)-4,6-bis(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

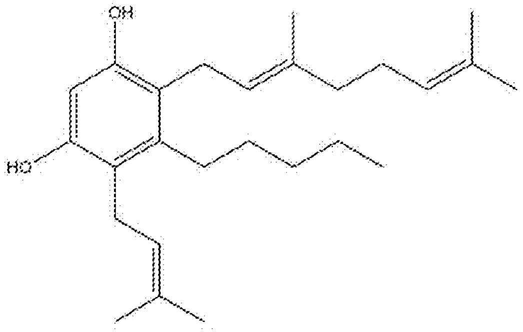
FIG. 42: Predicted Prenylation Products using RBI-03 as substrate and DMAPP as Donor

UNK40



(*E*)-4-(3,7-dimethylocta-2,6-dien-1-yl)-2-(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

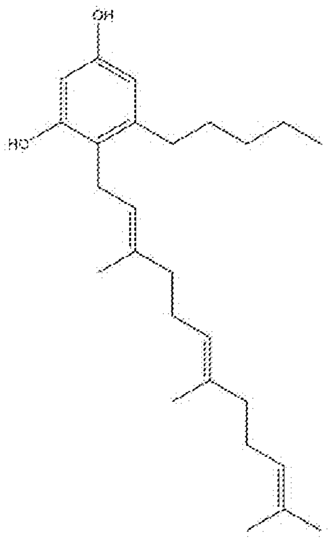
UNK66



(*E*)-4-(3,7-dimethylocta-2,6-dien-1-yl)-6-(3-methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol

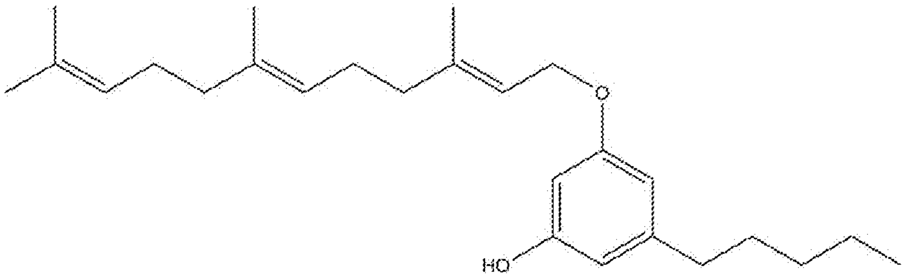
FIG. 43: Predicted Prenylation Products using O as substrate and FPP as Donor

RBI-15



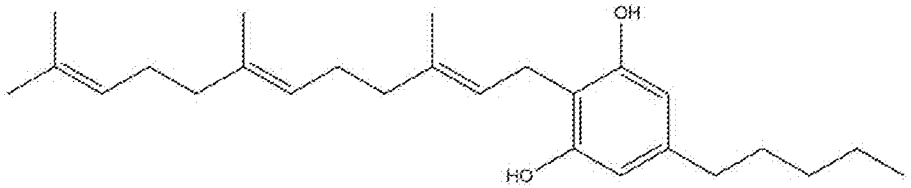
5-pentyl-4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzene-1,3-diol

UNK18



3-pentyl-5-(((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)phenol

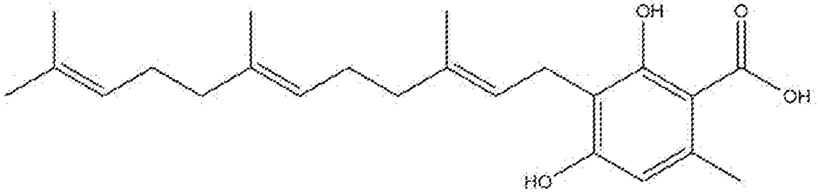
UNK19



5-pentyl-2-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzene-1,3-diol

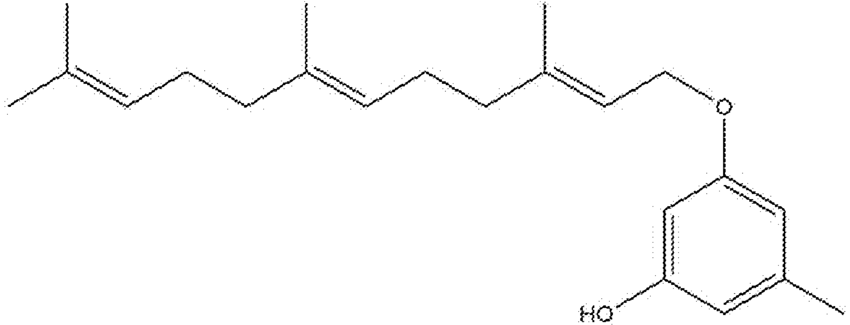
FIG. 44: Predicted Prenylation Products using ORA as substrate and FPP as Donor

UNK33



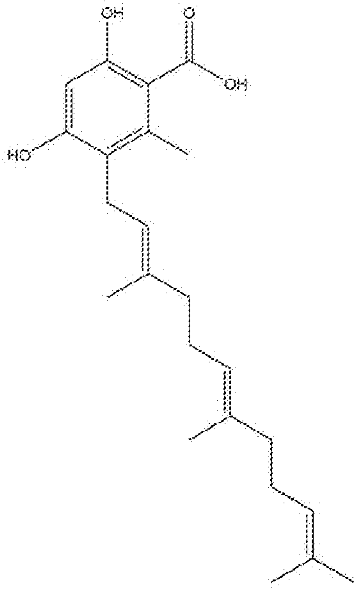
2,4-dihydroxy-6-methyl-3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic acid

UNK31



3-methyl-5-(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)phenol

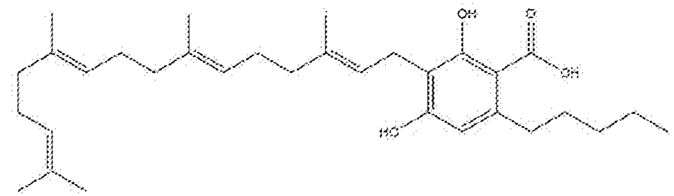
UNK34



4,6-dihydroxy-2-methyl-3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic acid

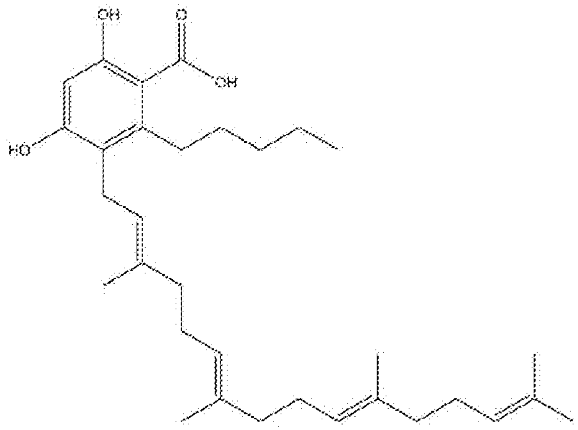
FIG. 45: Predicted Prenylation Products using OA as substrate and GGPP as Donor

UNK60



2,4-dihydroxy-6-pentyl-3-((2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl)benzoic acid

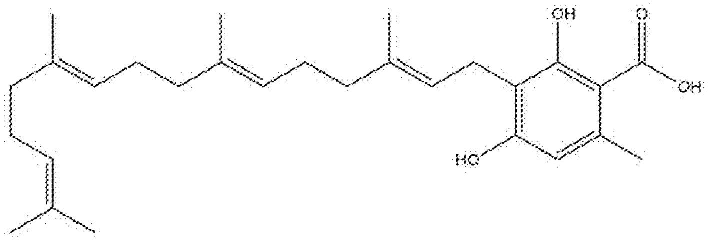
UNK61



4,6-dihydroxy-2-pentyl-3-((2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl)benzoic acid

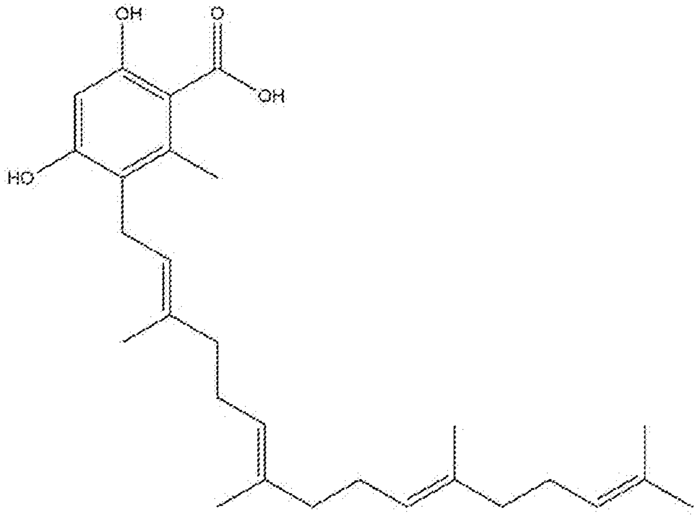
FIG. 46: Predicted Prenylation Products using ORA as substrate and GGPP as Donor

UNK62



2,4-dihydroxy-6-methyl-3-((2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl)benzoic acid

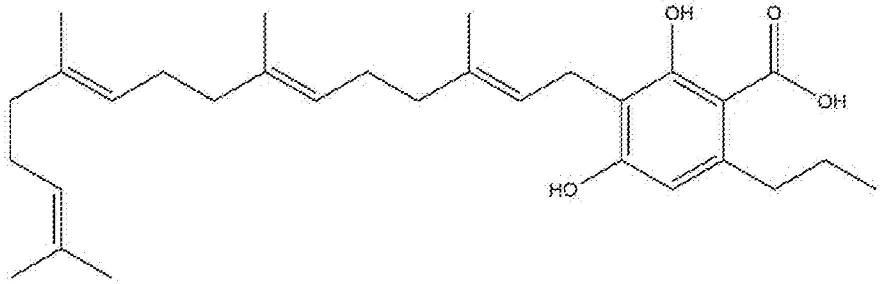
UNK63



4,6-dihydroxy-2-methyl-3-((2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl)benzoic acid

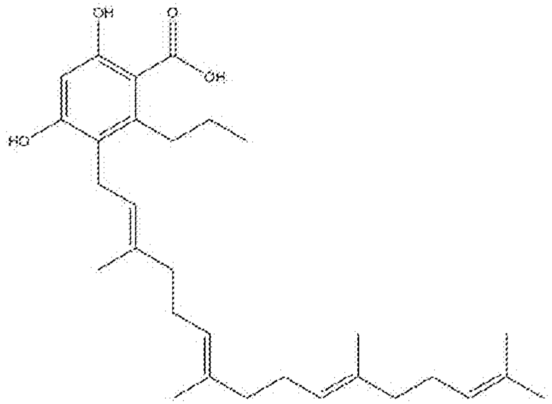
FIG. 47: Predicted Prenylation Products using DVA as substrate and GGPP as Donor

UNK64



2,4-dihydroxy-6-propyl-3-((2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl)benzoic acid

UNK65



4,6-dihydroxy-2-propyl-3-((2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl)benzoic acid

FIG. 48: Alkylresorcinol (i.e. DV, O, etc) prenylation site numbering

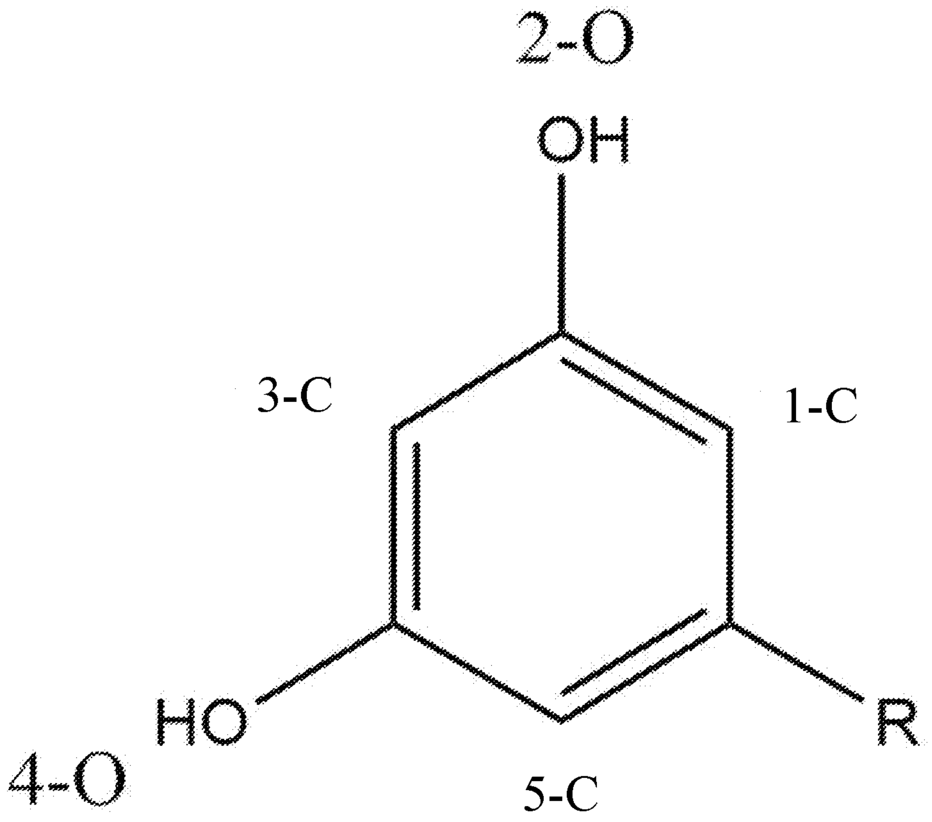


FIG. 49: Alkylresorcyclic Acid (i.e. ORA, DVA, OA, etc) prenylation site numbering

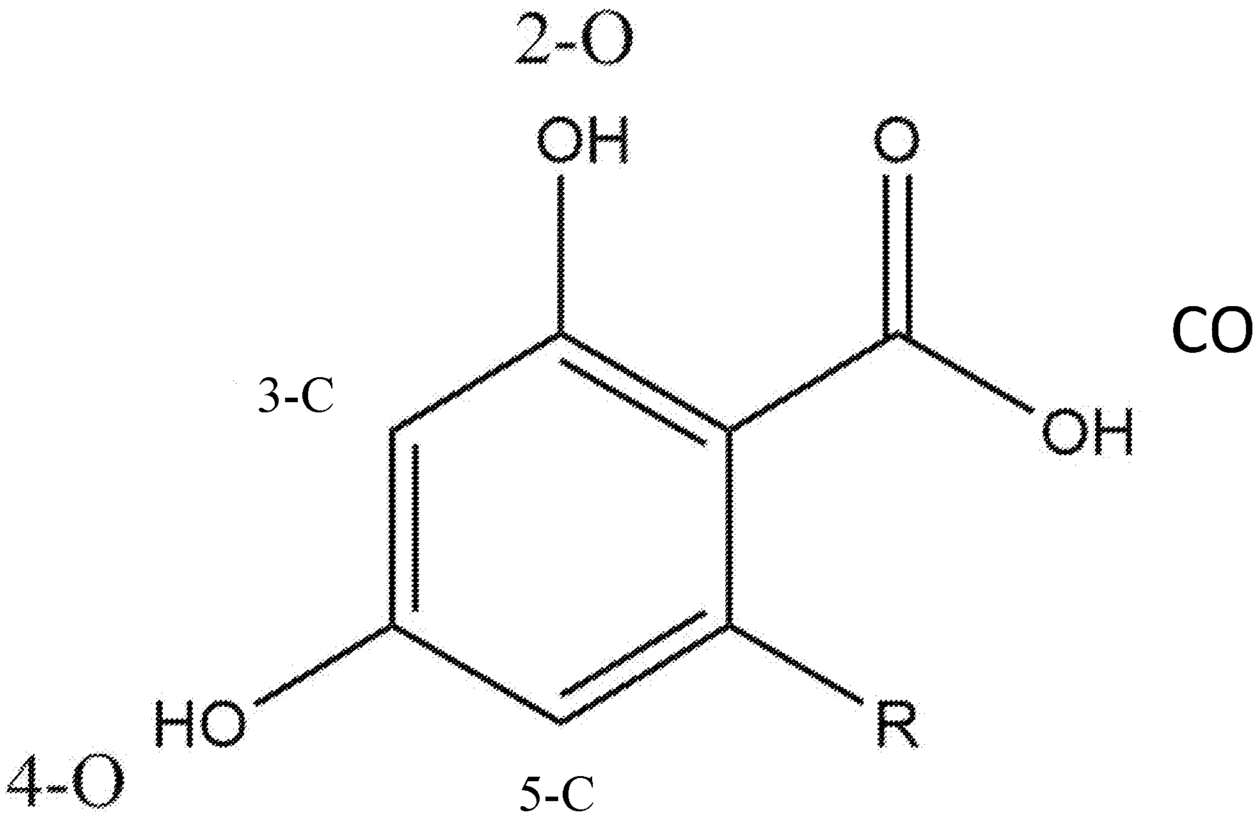


FIG. 50: Apigenin prenylation site numbering

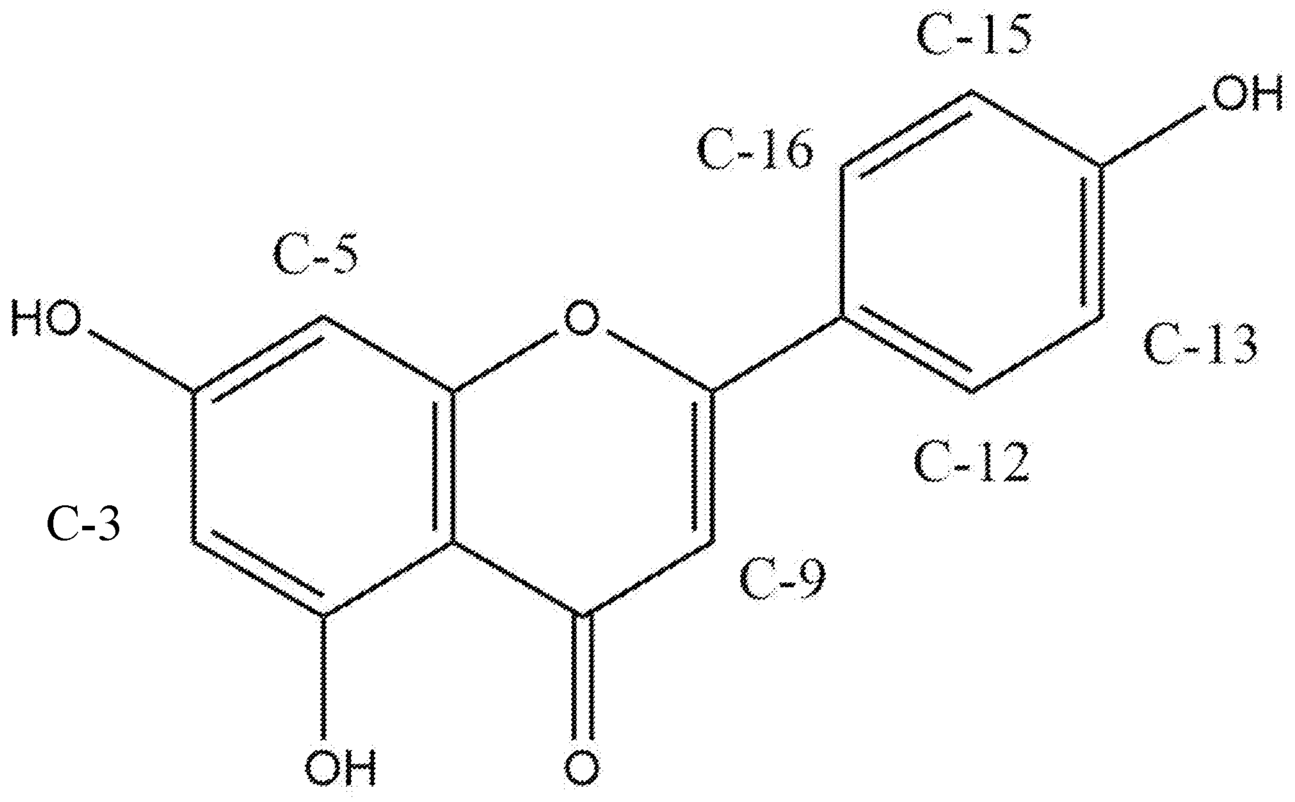


FIG. 51: Naringenin prenylation site numbering

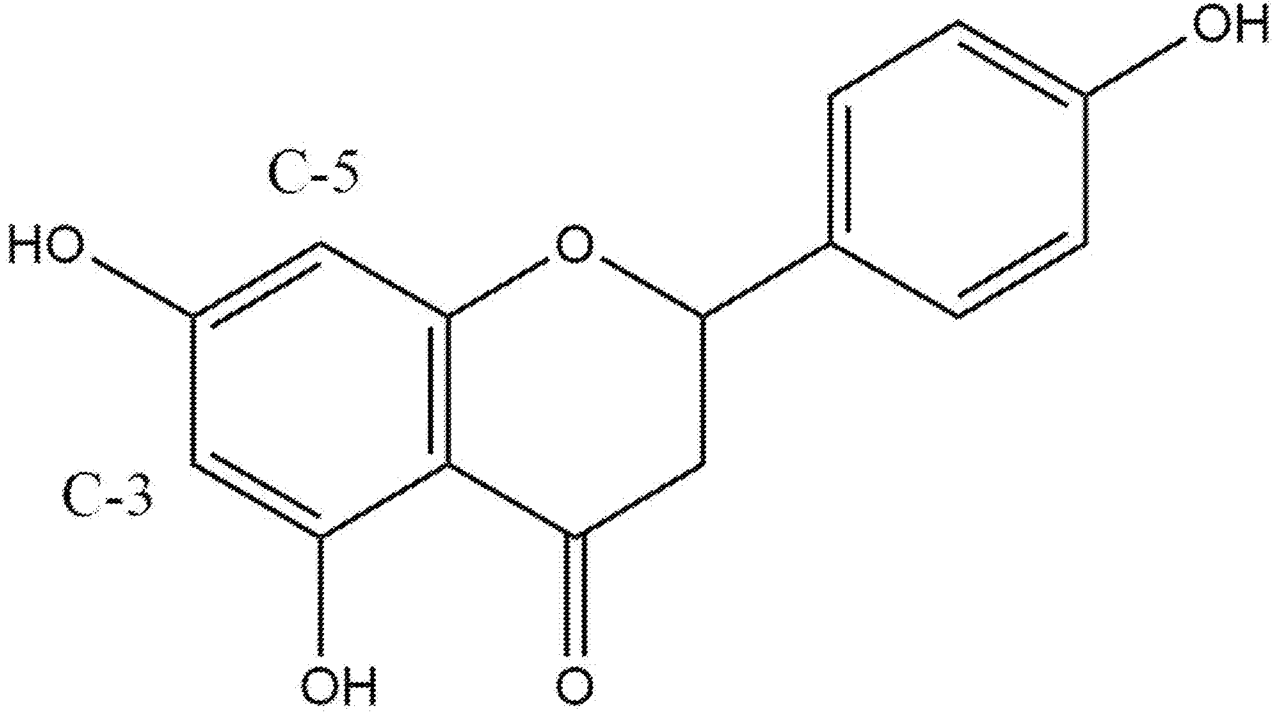


FIG. 52: Reservatrol prenylation site numbering

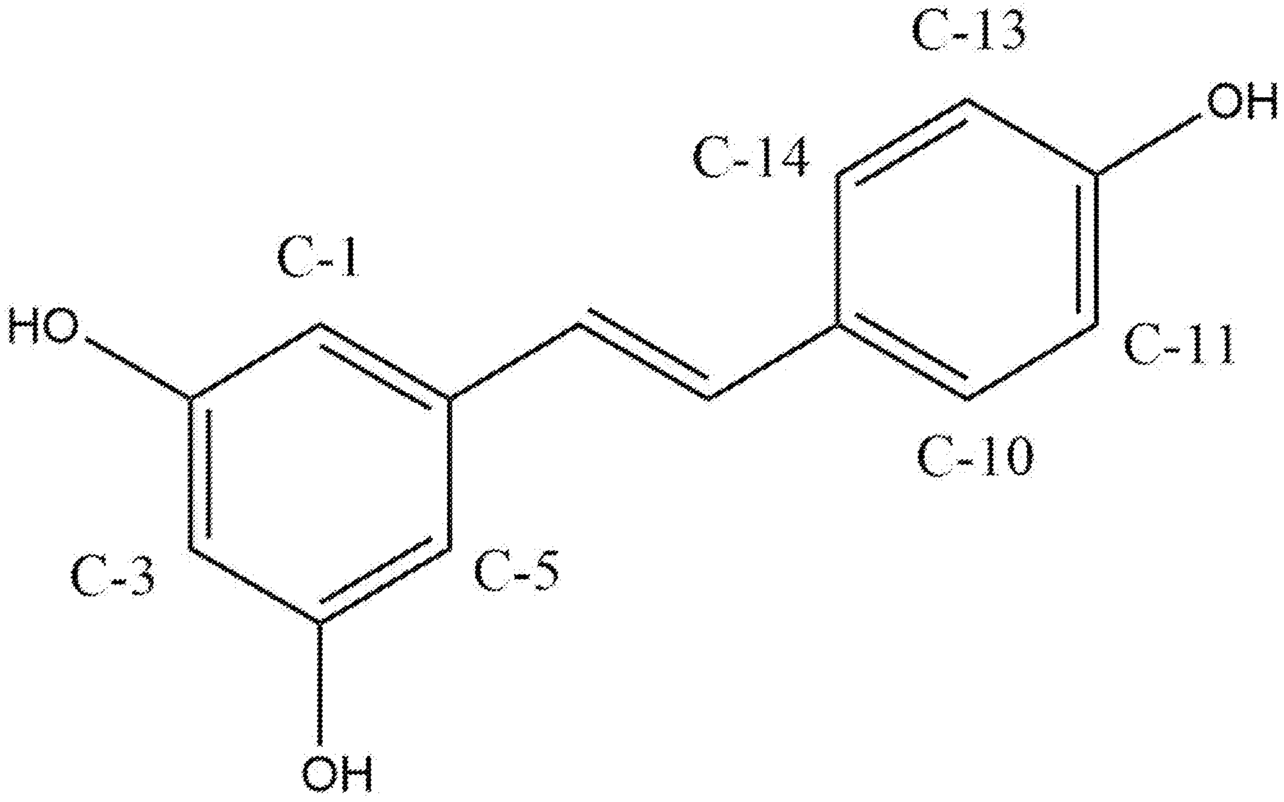


FIG. 53: Total nMol of prenylated products produced by ORF2 triple mutants using OA as substrate and FPP as donor

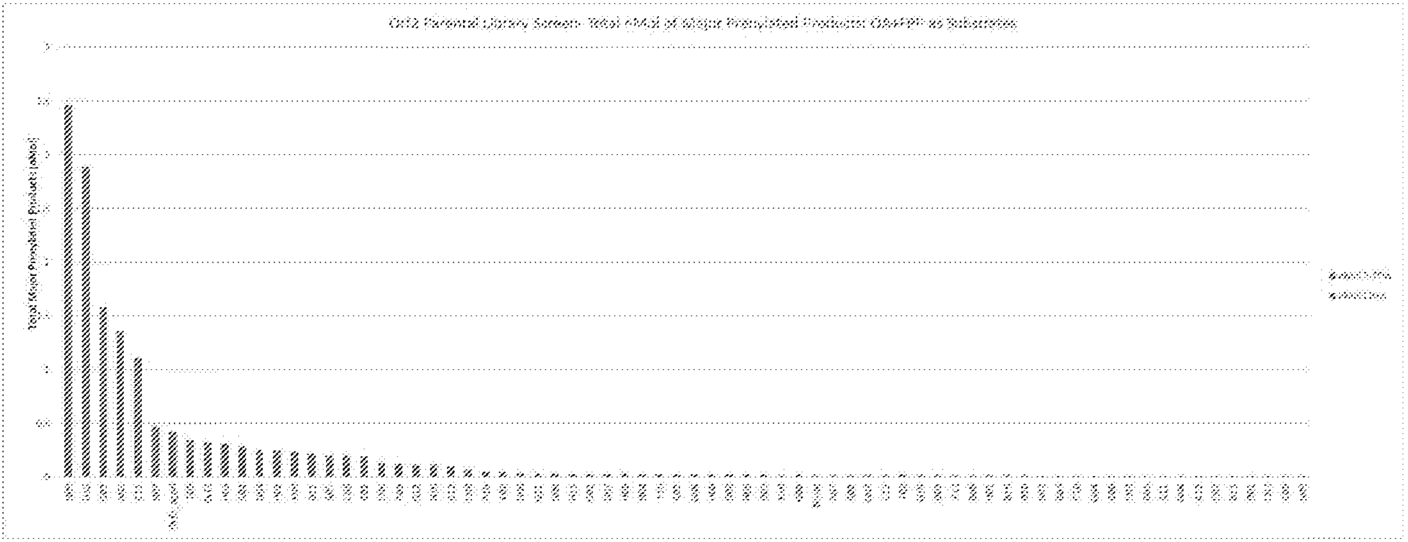


FIG. 54: % CBFA produced by ORF2 triple mutants using OA as substrate and FPP as donor

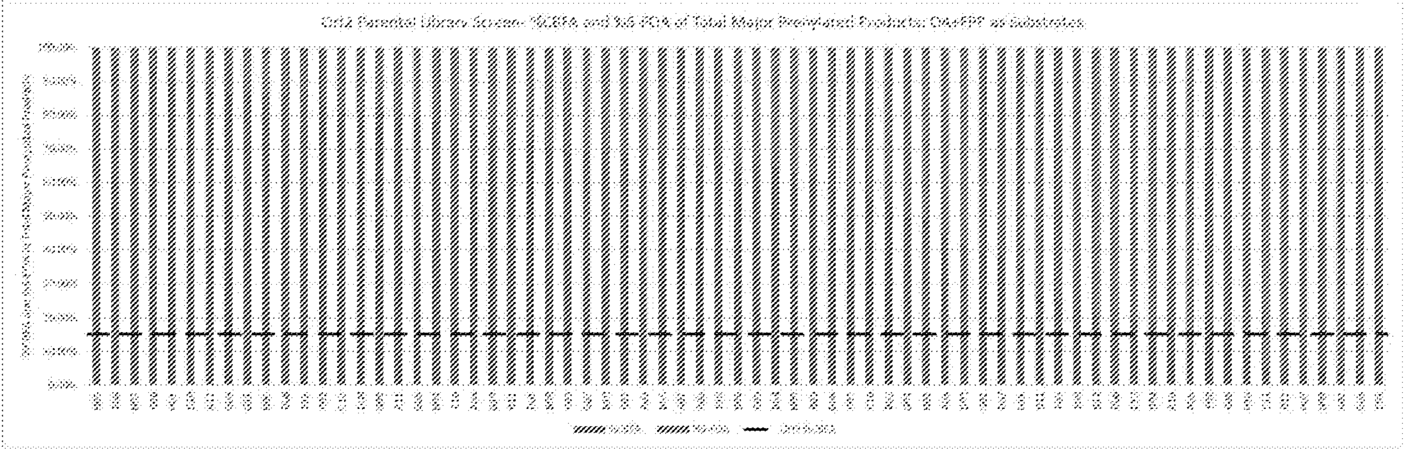


FIG. 55: % enzymatic activity of ORF2 triple mutants using OA as substrate and FPP as donor

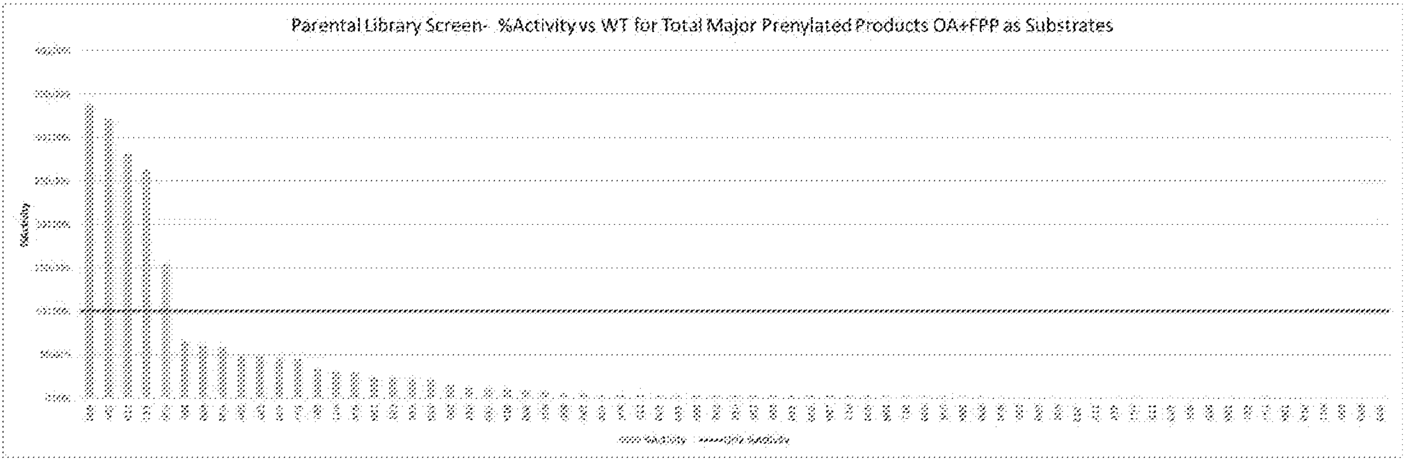


FIG. 56: CBFA production potential of ORF2 triple mutants using OA as substrate and FPP as donor

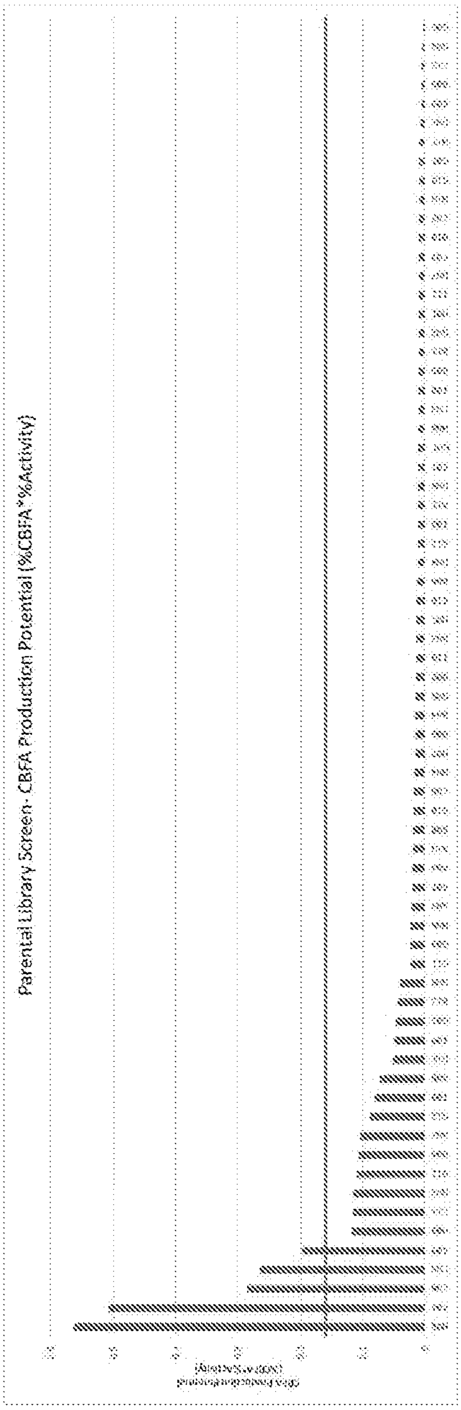


FIG. 57: Cluster map of ORF2 triple mutants clustered based on CBFA production potential and %5-FOA produced, using OA as substrate and FPP as donor

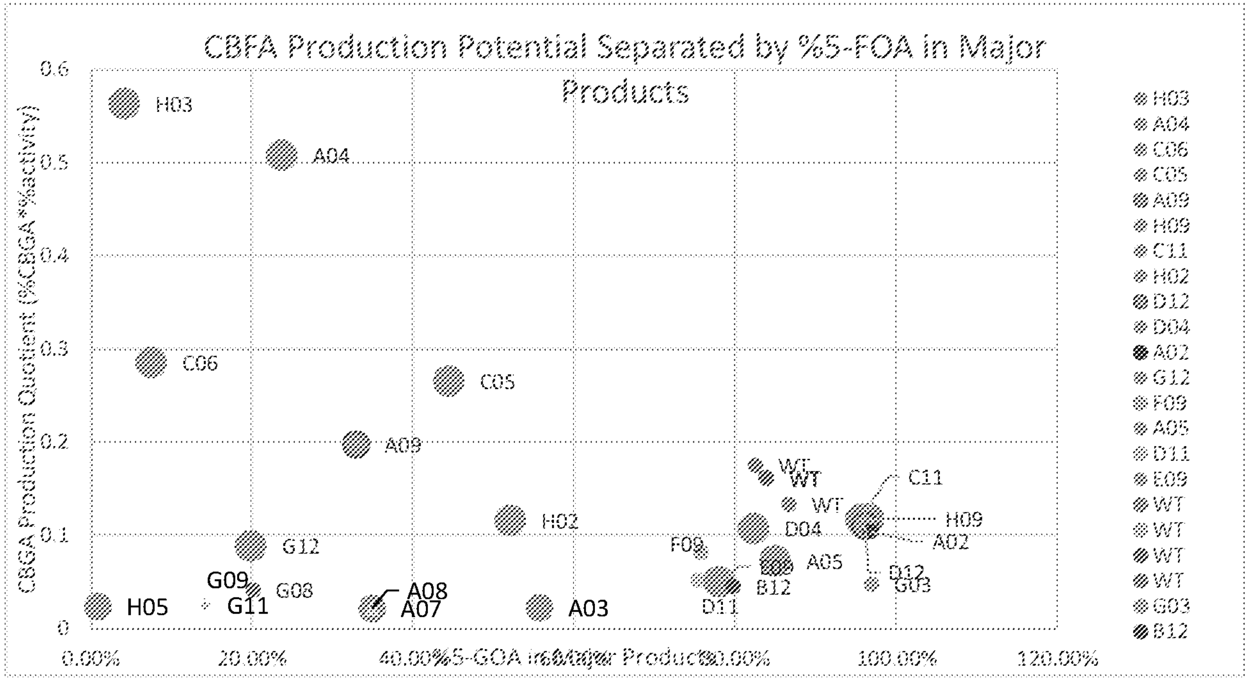


FIG. 58: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone A04

FIG. 58A

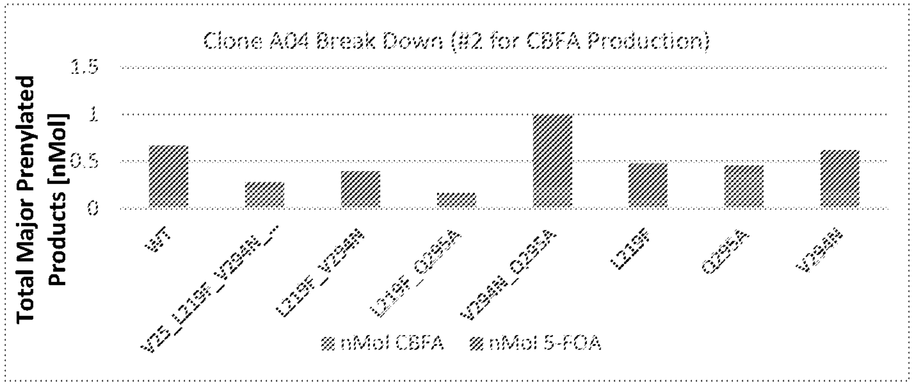


FIG. 58B

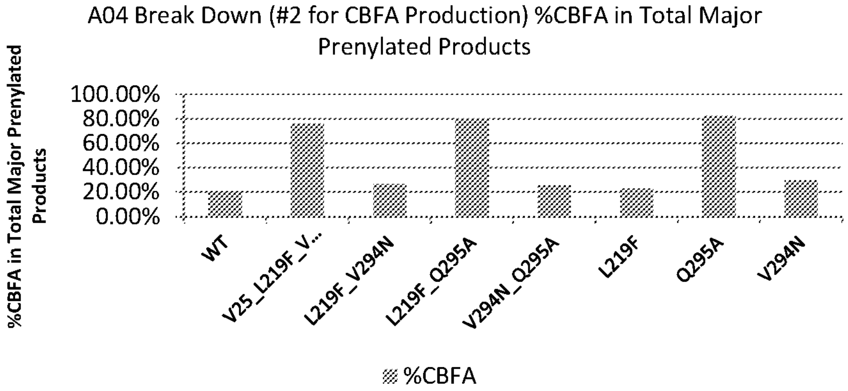


FIG. 59: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone C05

FIG. 59A

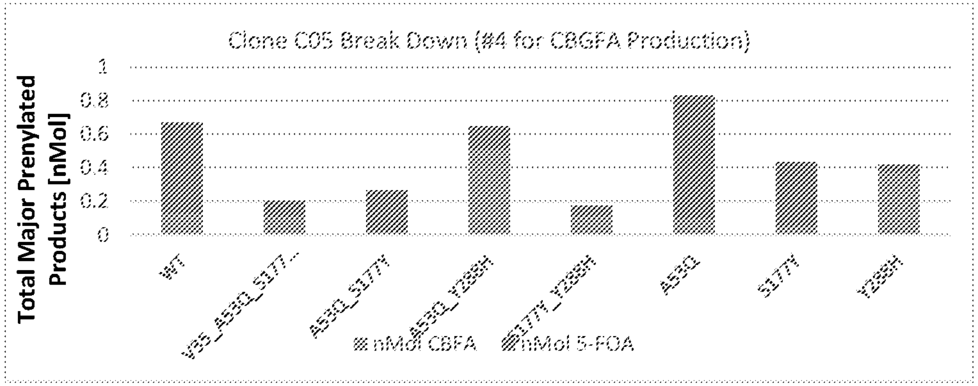


FIG. 59B

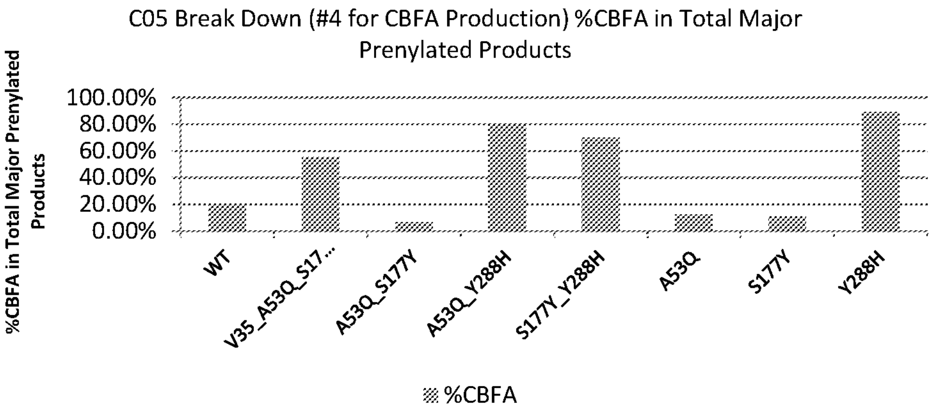


FIG. 60: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone A09

FIG. 60A

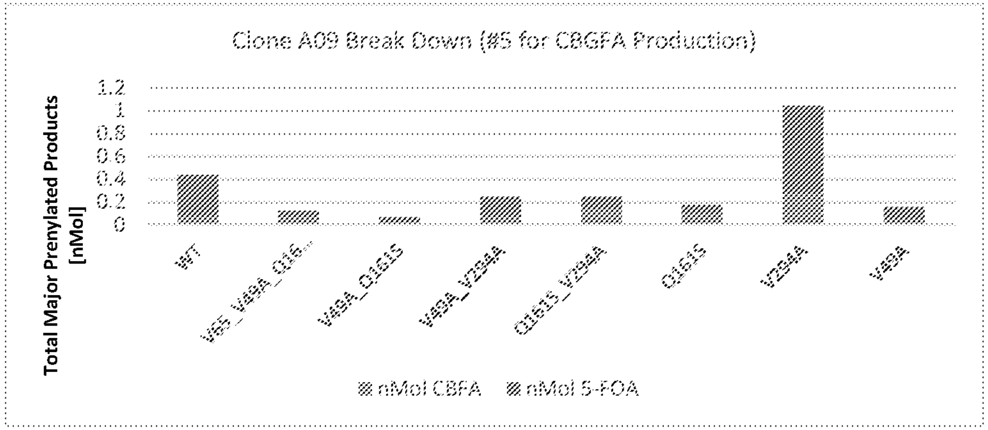


FIG. 60B

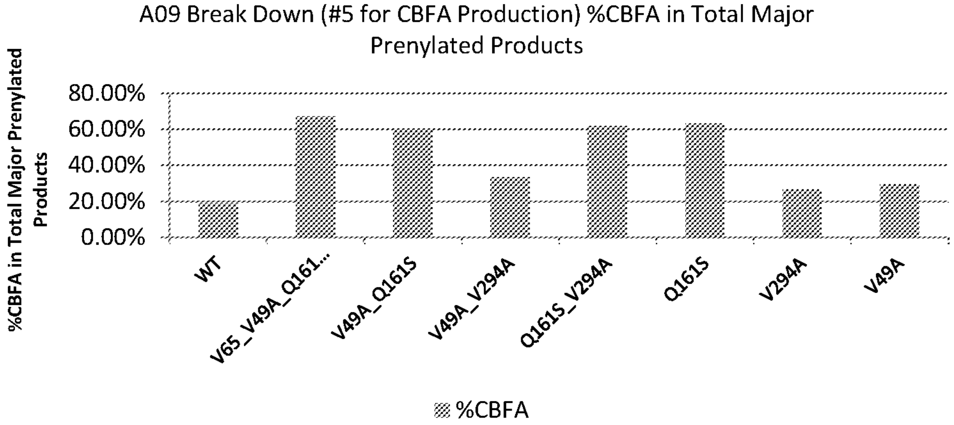


FIG. 61: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant H02

FIG. 61A

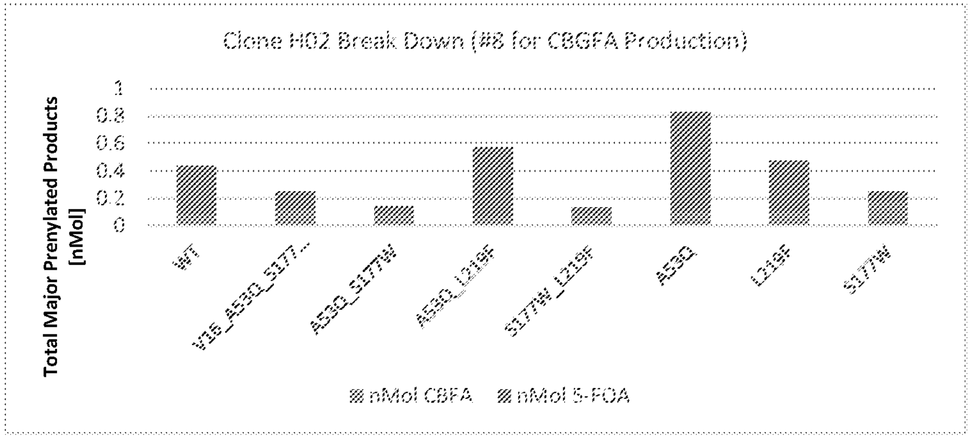


FIG. 61B

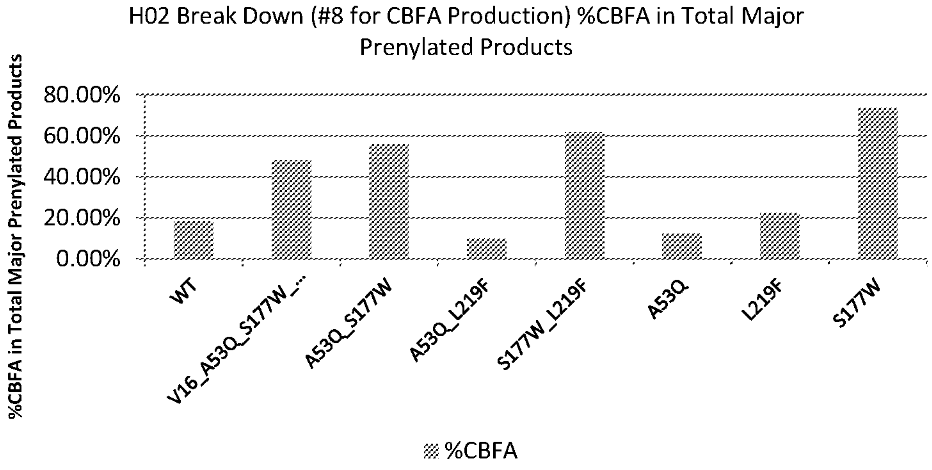


FIG. 62: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone D04

FIG. 62A

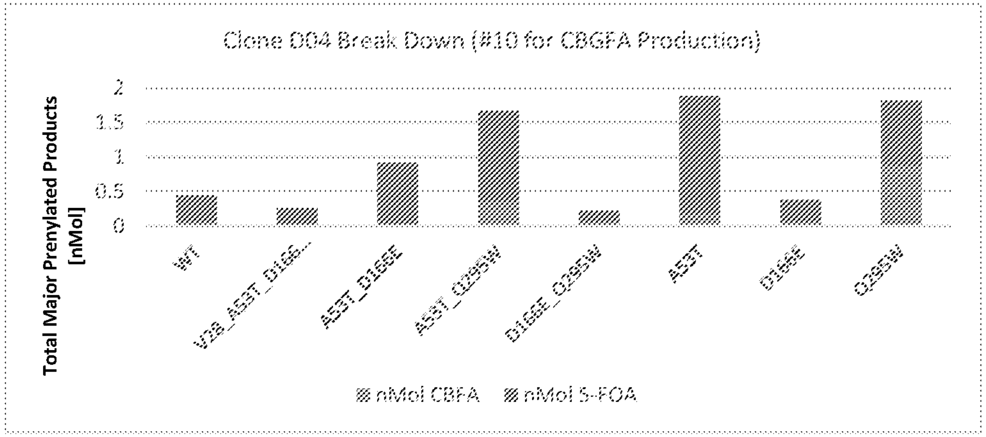


FIG. 62B

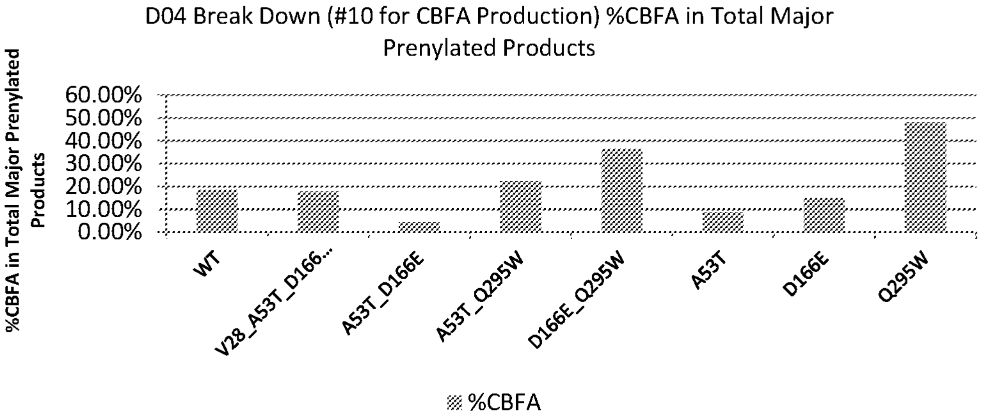


FIG. 63: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone F09

FIG. 63A

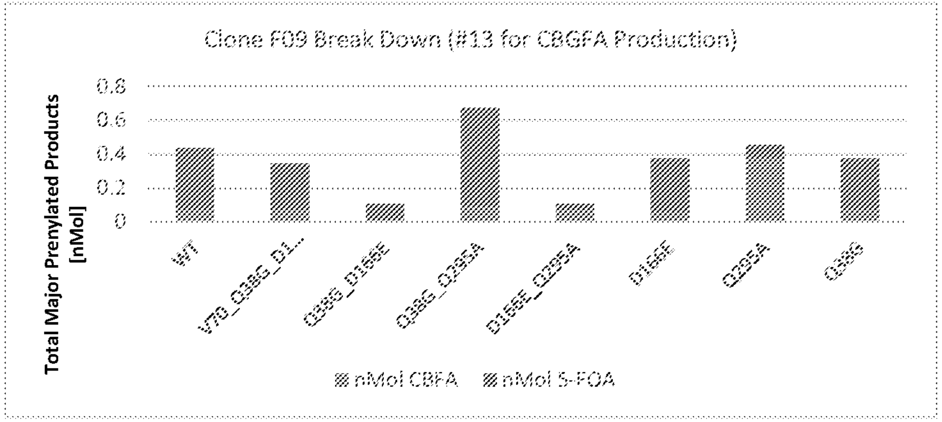


FIG. 63B

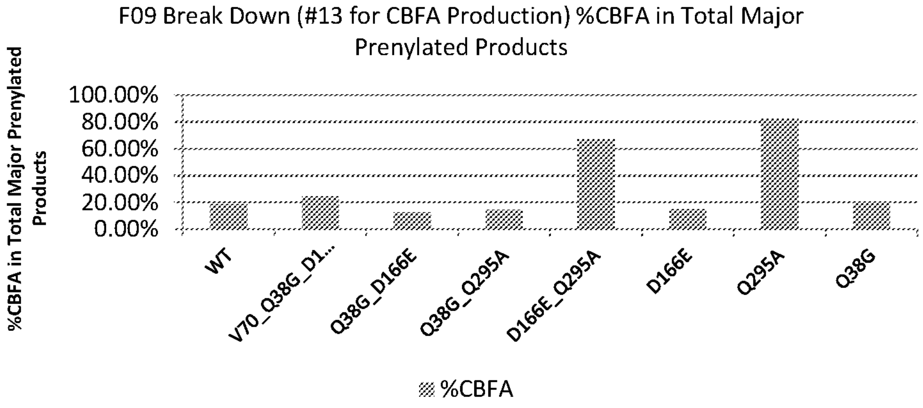


FIG. 64: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone D11

FIG. 64A

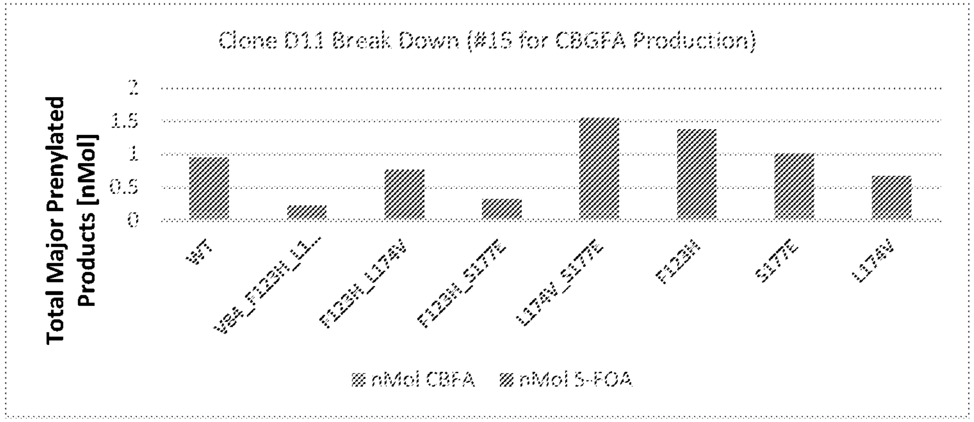


FIG. 64B

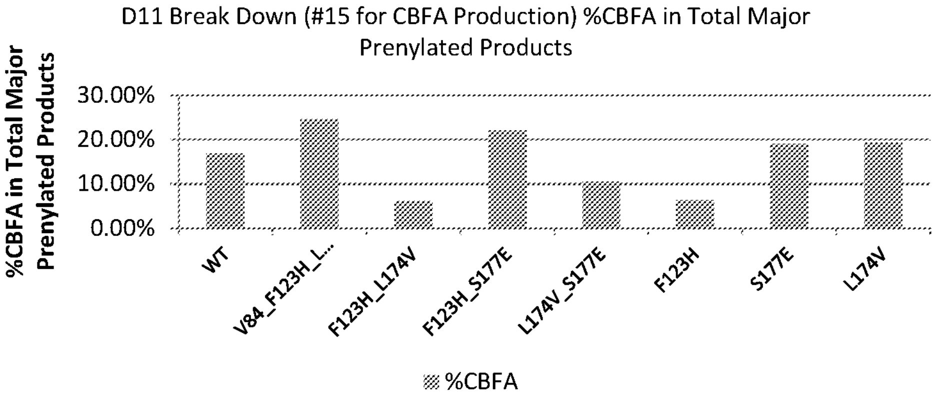


FIG. 65: Analysis of ORF-2 enzymatic function of mutants derived from the breakdown of ORF-2 triple mutant clone E09

FIG. 65A

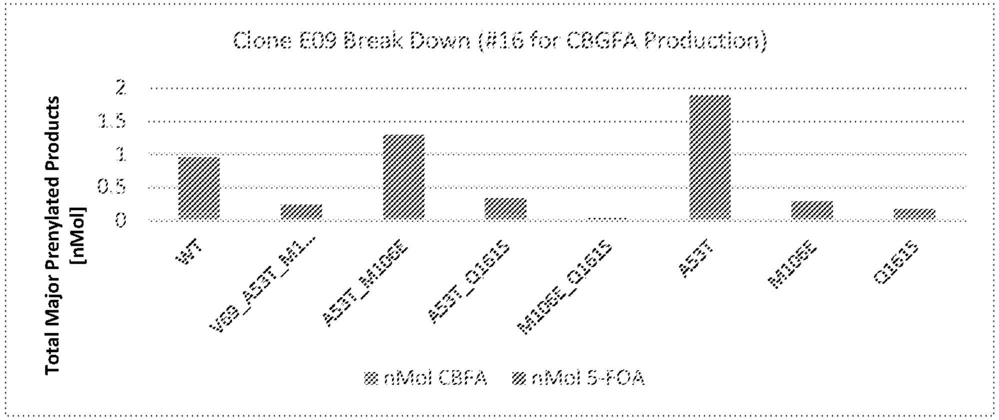


FIG. 65B

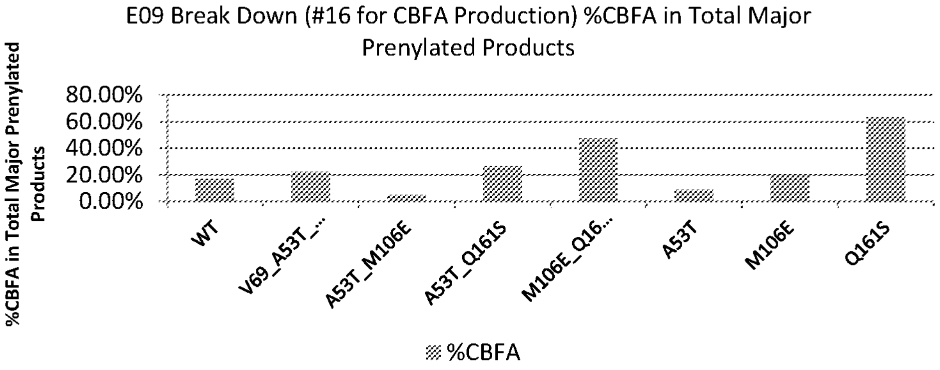


FIG. 66: Analysis of enzymatic activity of site-saturated ORF2 mutants of Q295 using OA as substrate and FPP as donor

FIG. 66A

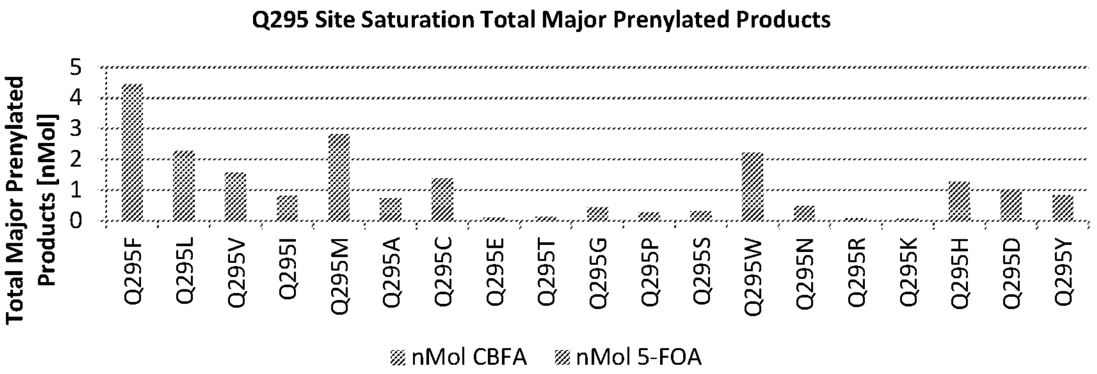


FIG. 66B

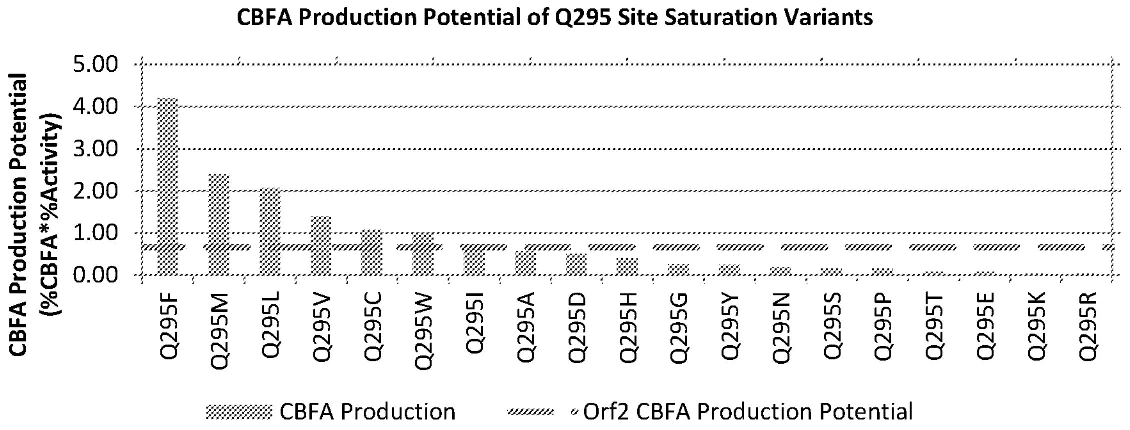


FIG. 66C: 5-FOA production (using OA as substrate and FPP as donor) by ORF2 mutants carrying site saturation Q295 mutations

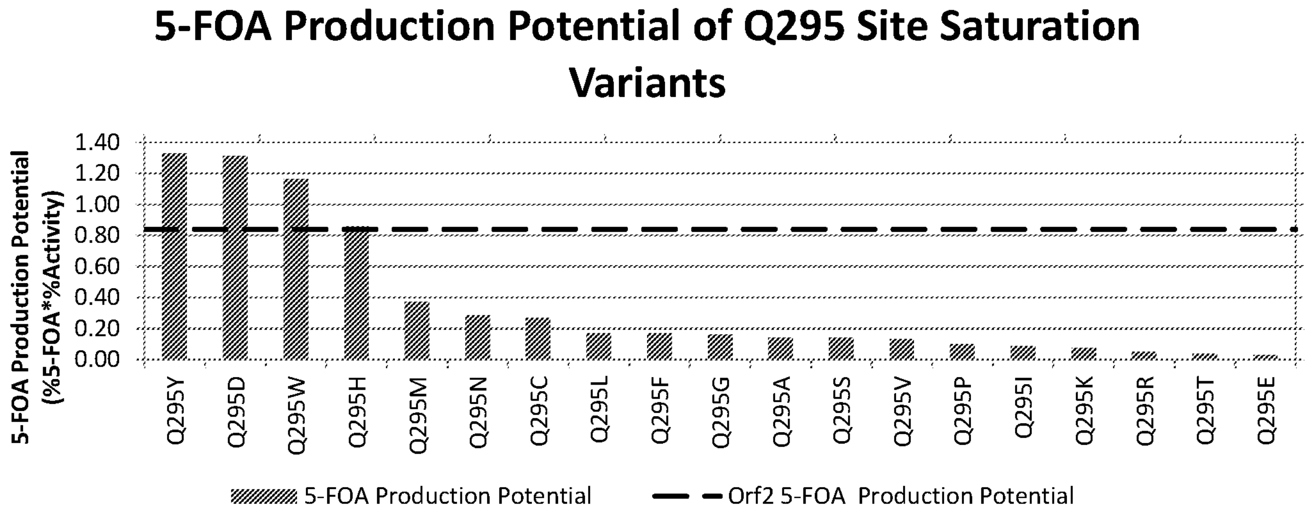


FIG. 67: Analysis of enzymatic activity of site-saturated ORF2 mutants of Q161 using OA as substrate and FPP as donor

FIG. 67A

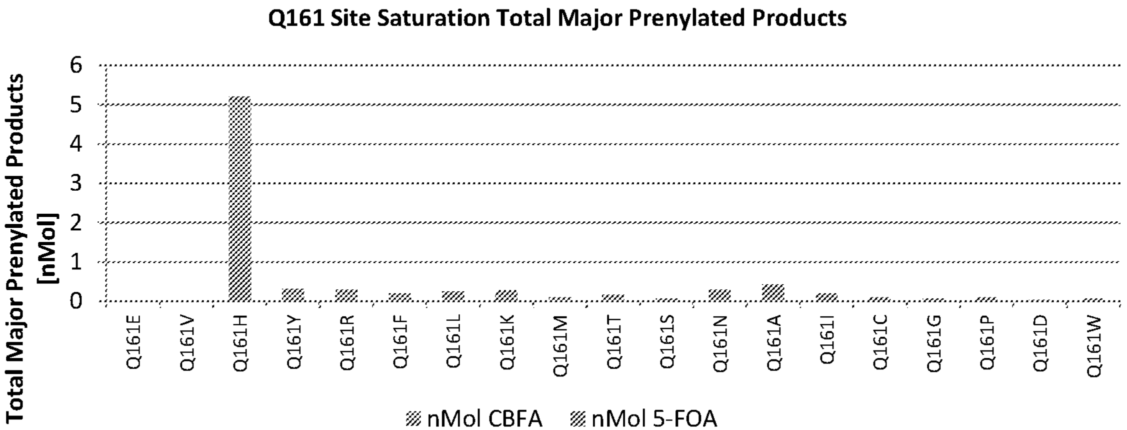


FIG. 67B

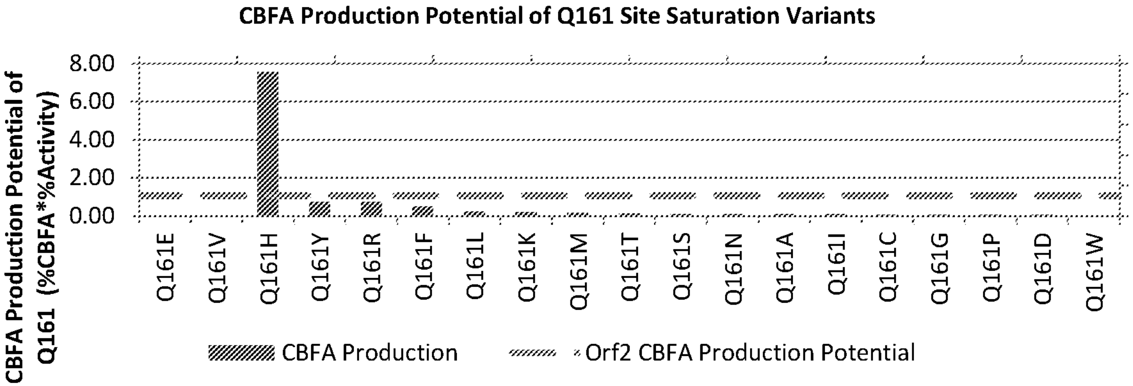


FIG. 67C: 5-GOA production (using OA as substrate and FPP as donor) by ORF2 mutants carrying site saturation Q161 mutations

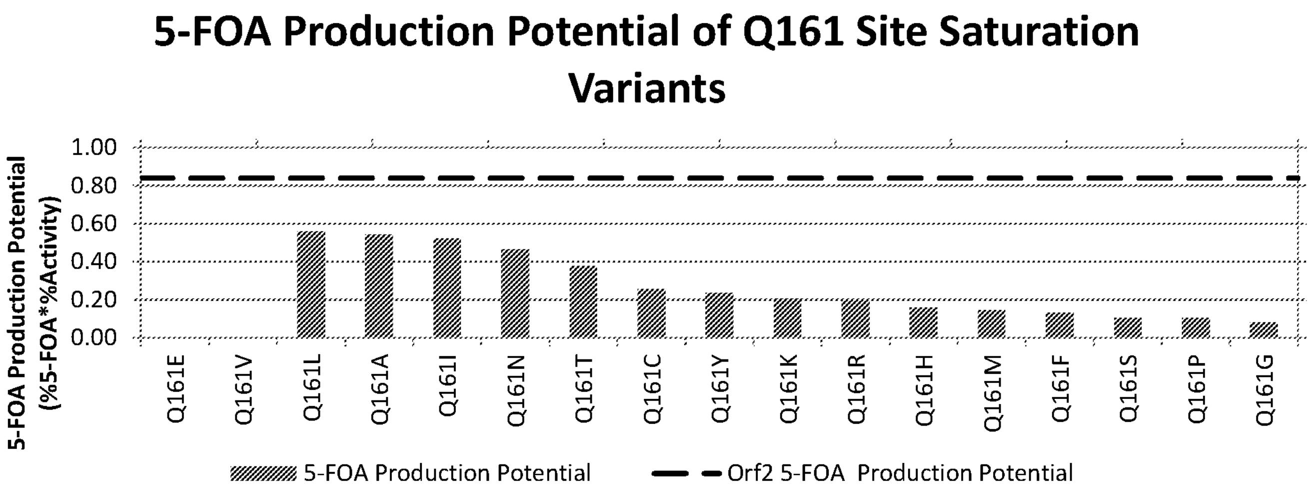


FIG. 68: Analysis of enzymatic activity of site-saturated ORF2 mutants of S214 using OA as substrate and FPP as donor

FIG. 68A

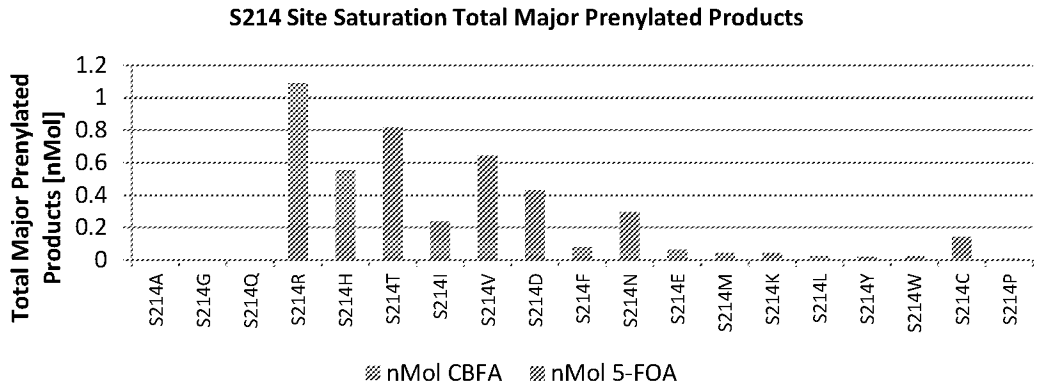


FIG. 68B

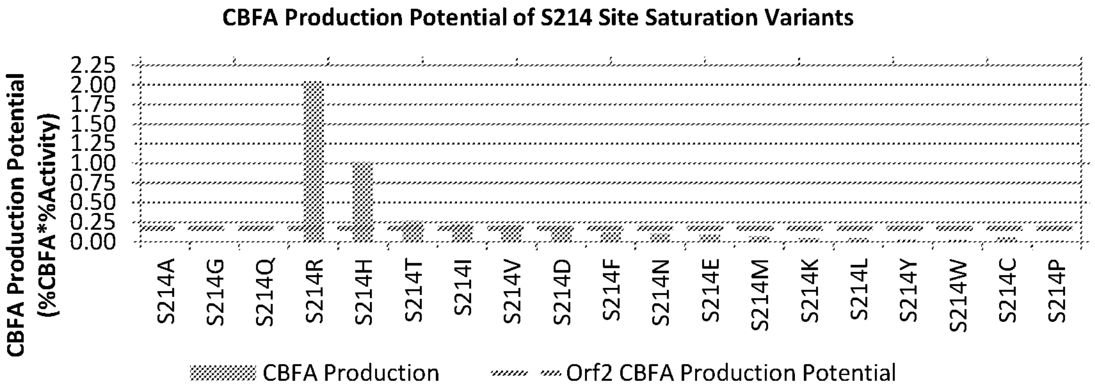


FIG. 68C: 5-GOA production (using OA as substrate and FPP as donor) by ORF2 mutants carrying site saturation S214 mutations

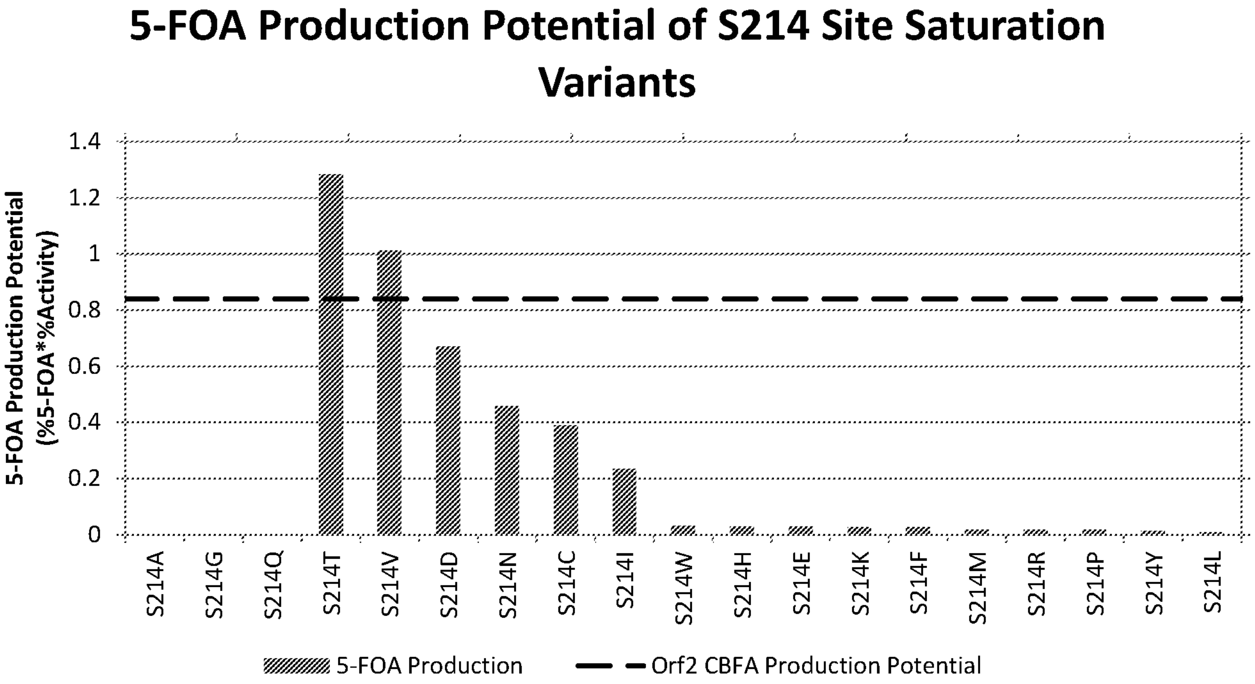


FIG. 69: ORF-2 activity (using OA as substrate and FPP as donor) of S214R-Q295F Stacking variant

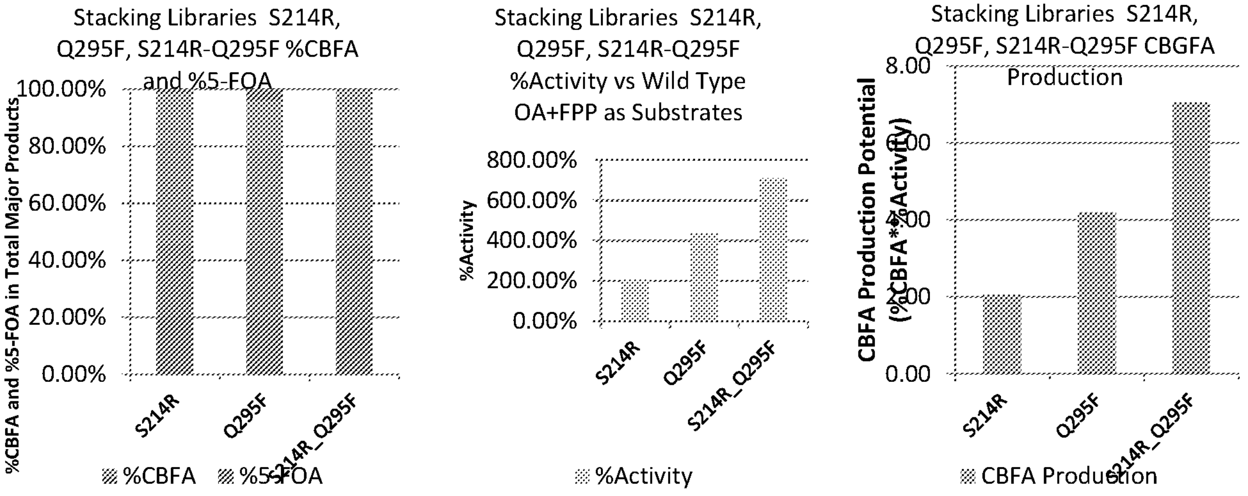
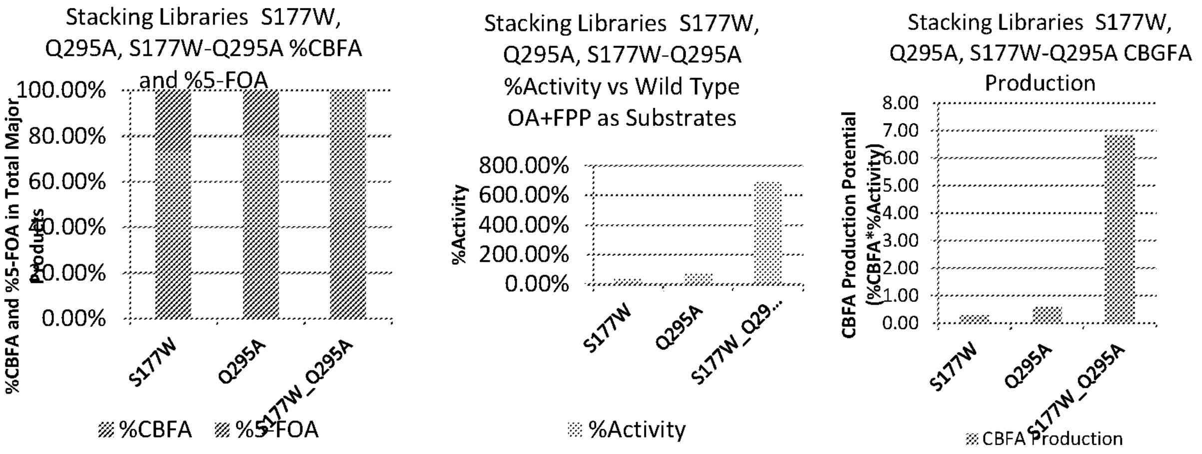


FIG. 70: ORF-2 activity (using OA as substrate and FPP as donor) of S177W-Q295A Stacking variant


Stacking Libraries S177W, Q295A, S177W-Q295A %CBFA and %5-FOA

Stacking Libraries S177W, Q295A, S177W-Q295A %Activity vs Wild Type OA+FPP as Substrates

Stacking Libraries S177W, Q295A, S177W-Q295A CBGFA Production

FIG. 71: ORF-2 activity (using OA as substrate and FPP as donor) of A53T-Q295F Stacking variant

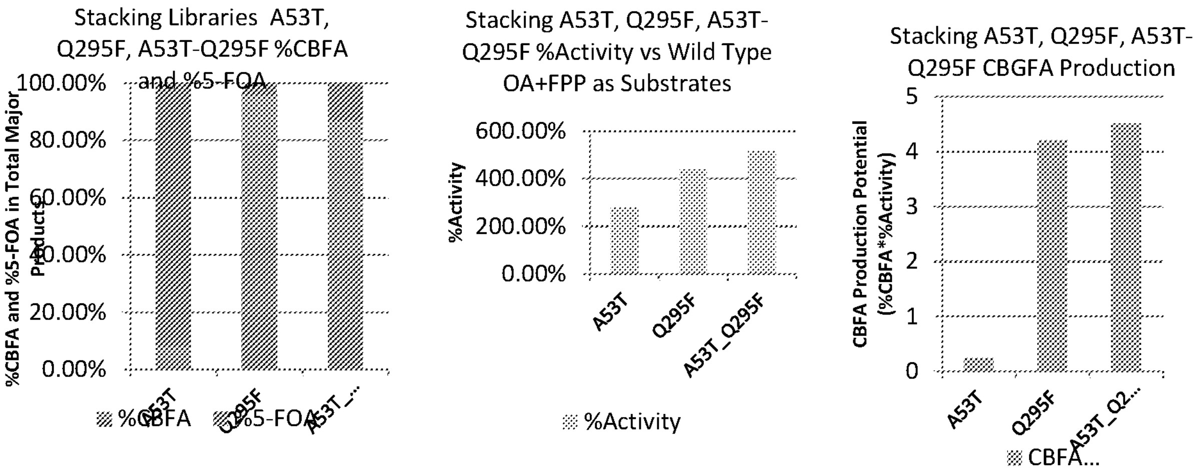
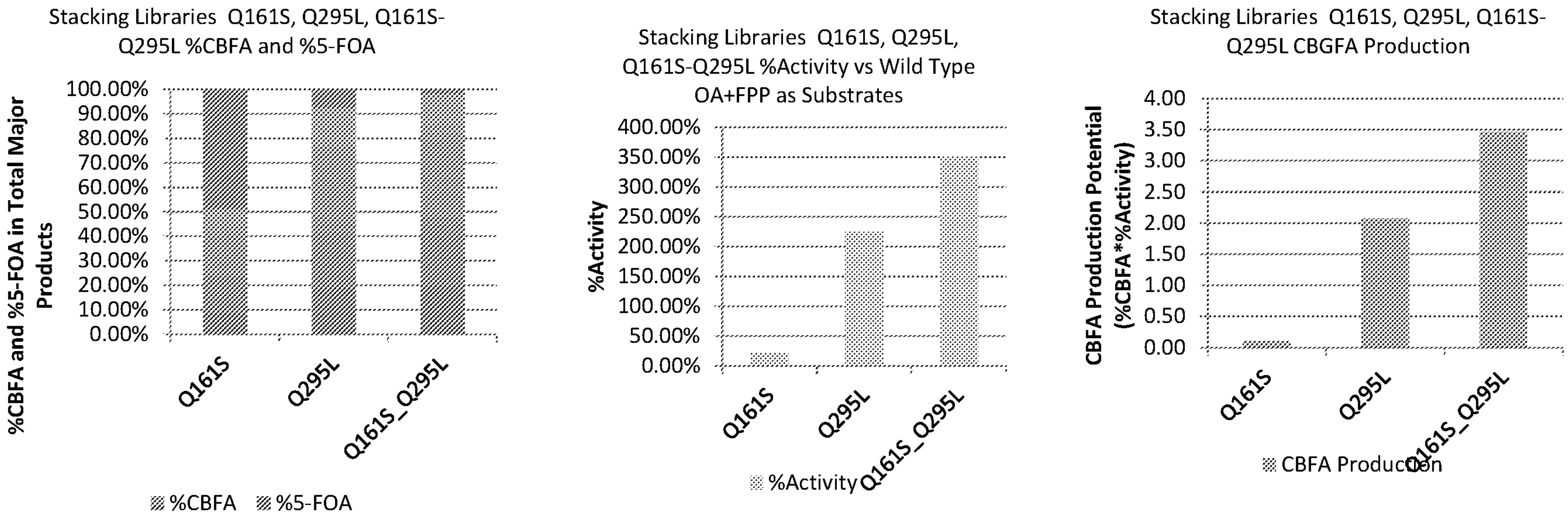


FIG. 72: ORF-2 activity (using OA as substrate and FPP as donor) of Q161S-Q295L Stacking variant



Stacking Libraries Q161S, Q295L, Q161S-Q295L %CBFA and %5-FOA

Stacking Libraries Q161S, Q295L, Q161S-Q295L %Activity vs Wild Type OA+FPP as Substrates

Stacking Libraries Q161S, Q295L, Q161S-Q295L CBGFA Production

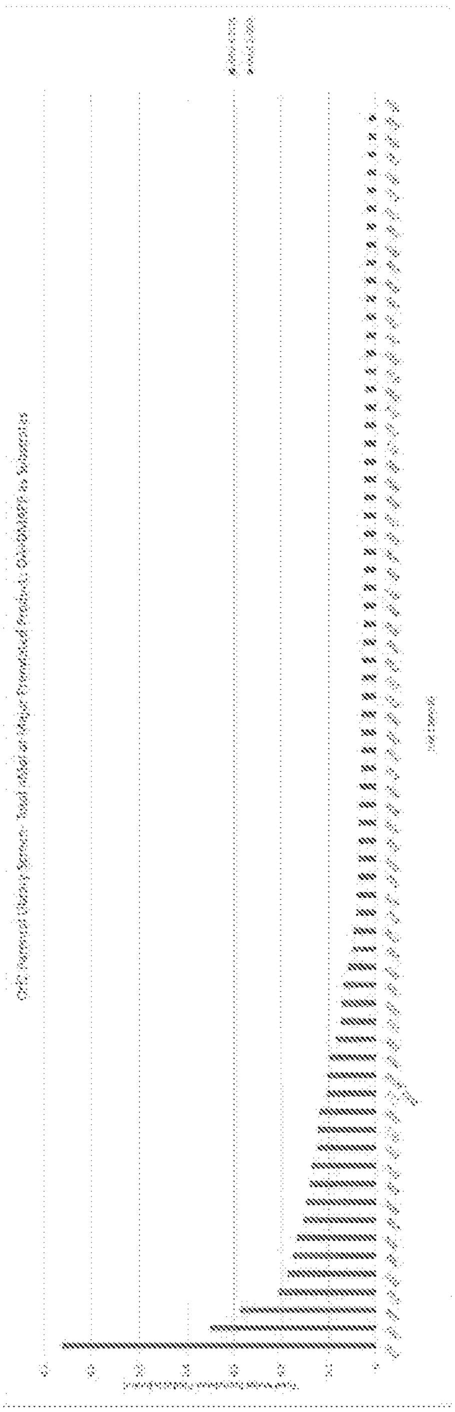


FIG. 73: Total nMol of prenylated products produced by ORF2 triple mutants using OA as substrate and DMAPP as donor

FIG. 74: % 3-DOA and %5-DOA produced by ORF2 triple mutants using OA as substrate and DMAPP as donor

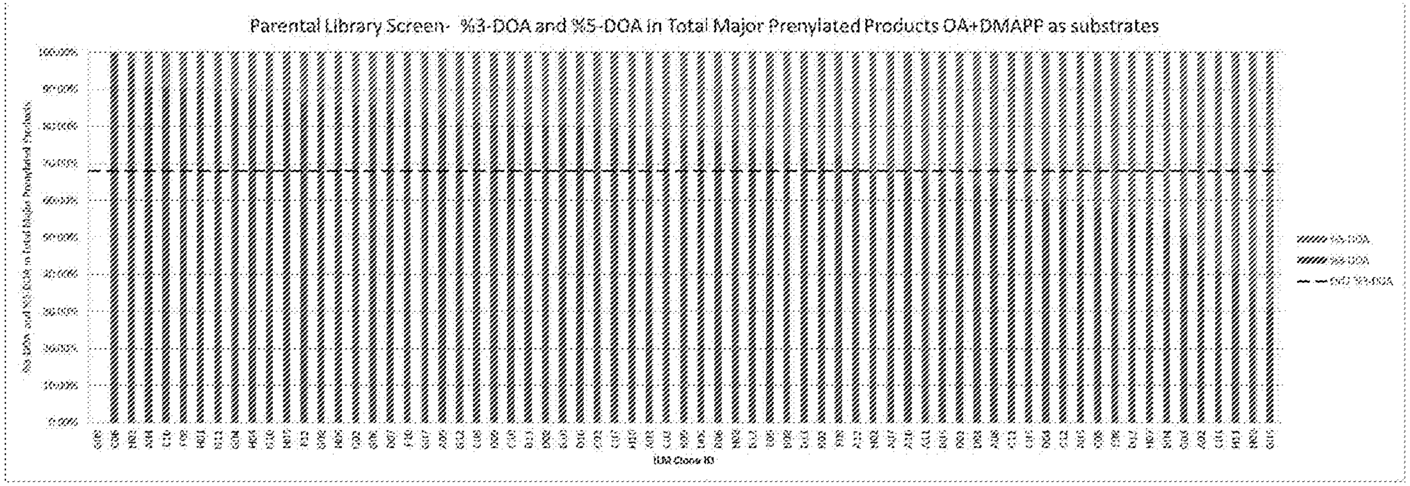


FIG. 75: % enzymatic activity of ORF2 triple mutants using OA as substrate and DMAPP as donor

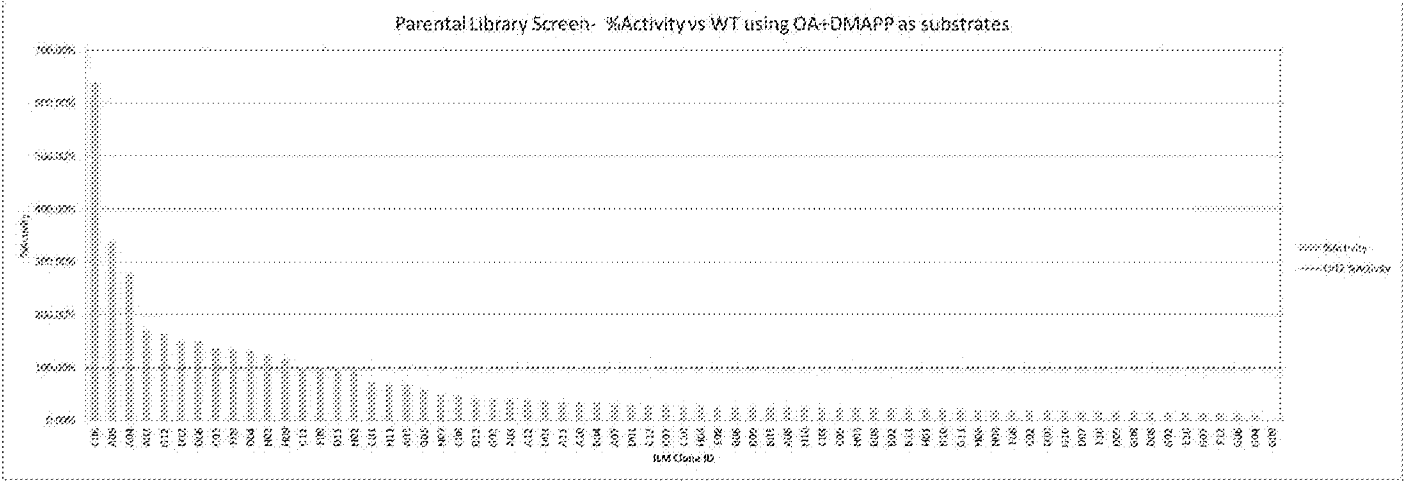


FIG. 76: 3-DOA production potential of ORF2 triple mutants using OA as substrate and DMAPP as donor

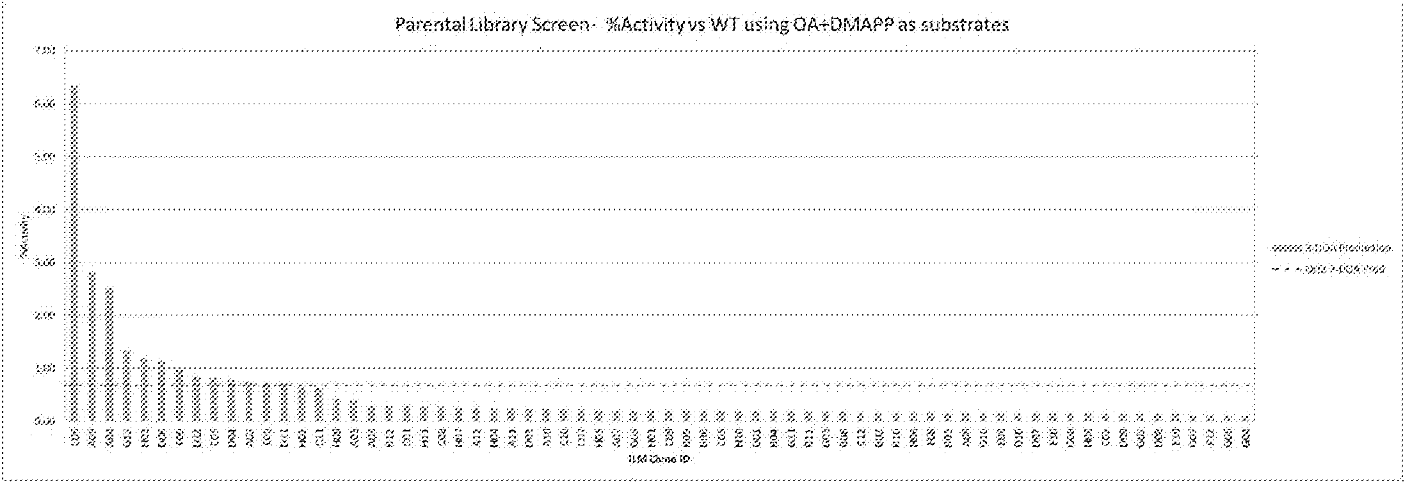


FIG. 77: Cluster map of ORF2 triple mutants clustered based on 3-DOA production potential and %5-DOA produced, using OA as substrate and DMAPP as donor

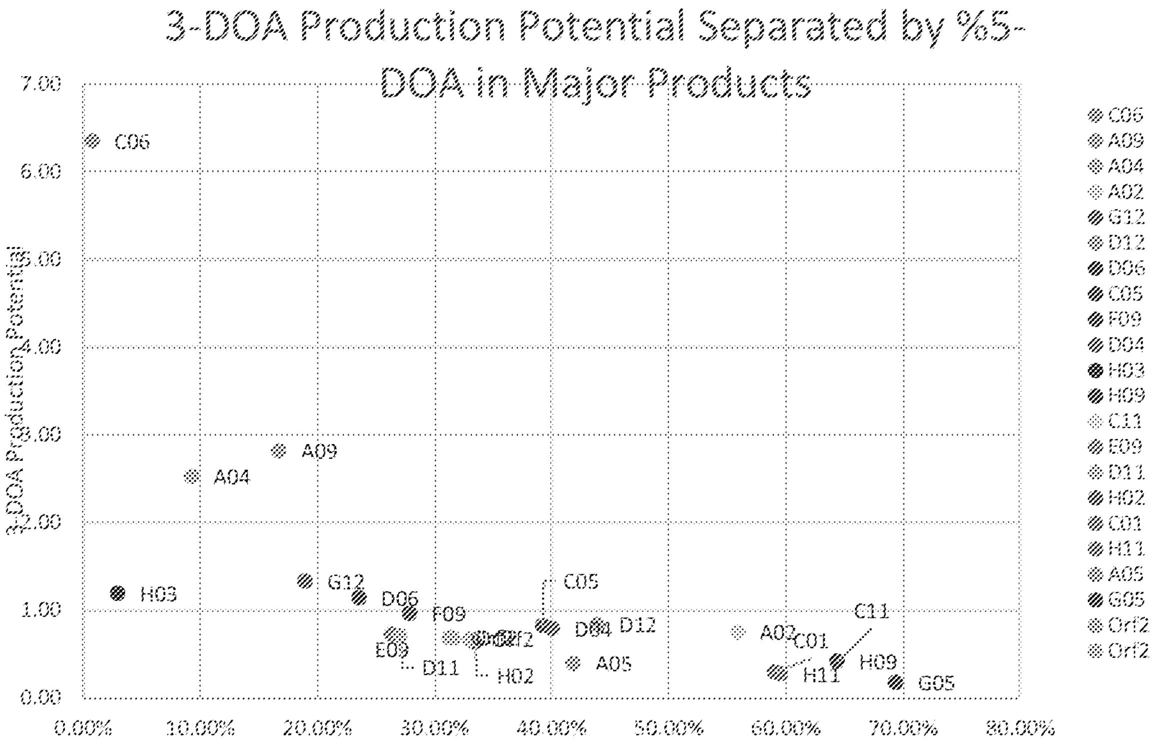


FIG. 78: Complete amino acid replacement at position Q161 and S214 in Orf2 allows a structure function mechanism for CBGA production and regiospecific prenylation

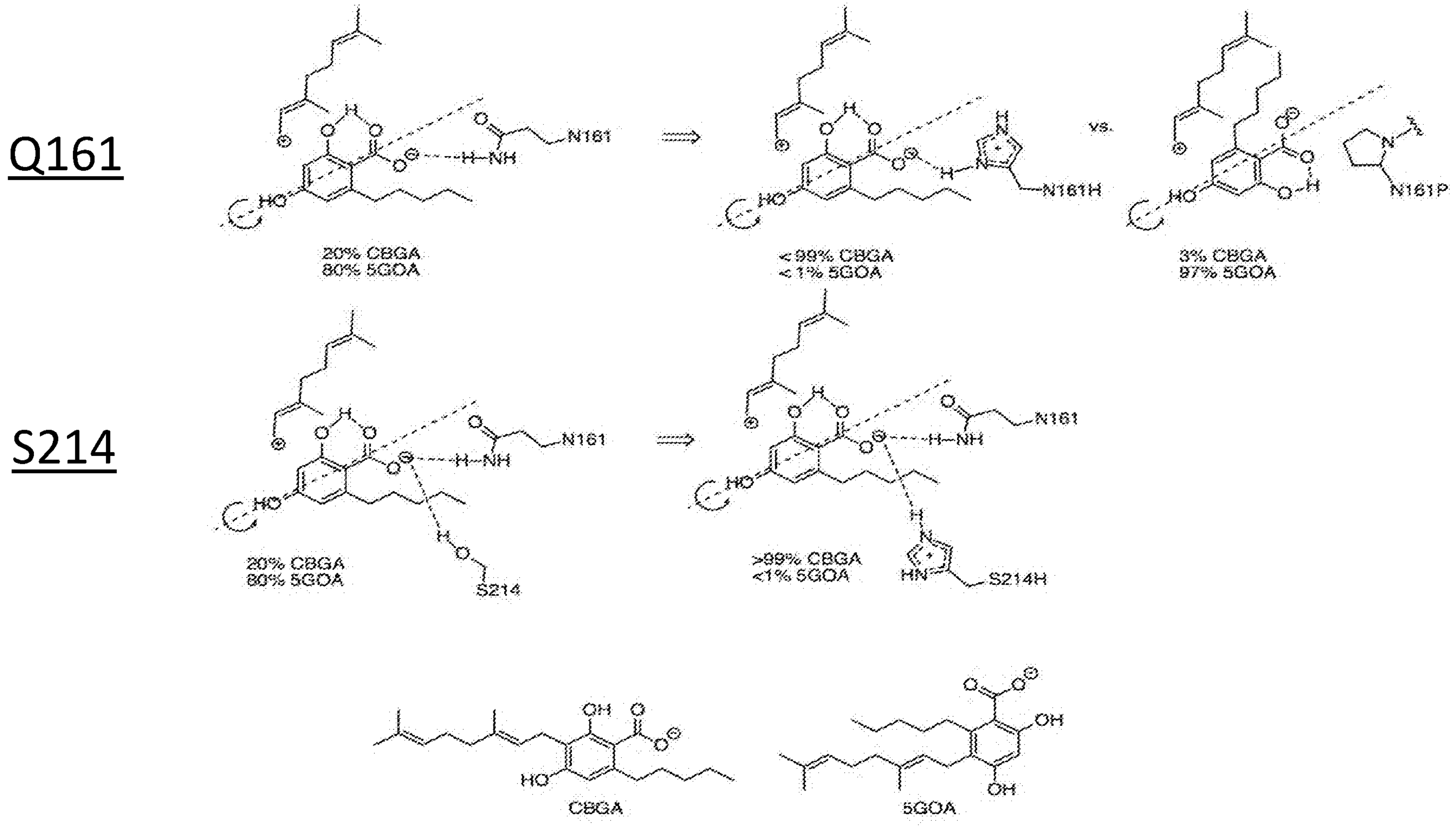


FIG. 79: Complete amino acid replacement at position Q295 in Orf2 allows a structure function mechanism for CBGA production and regiospecific prenylation

Q295

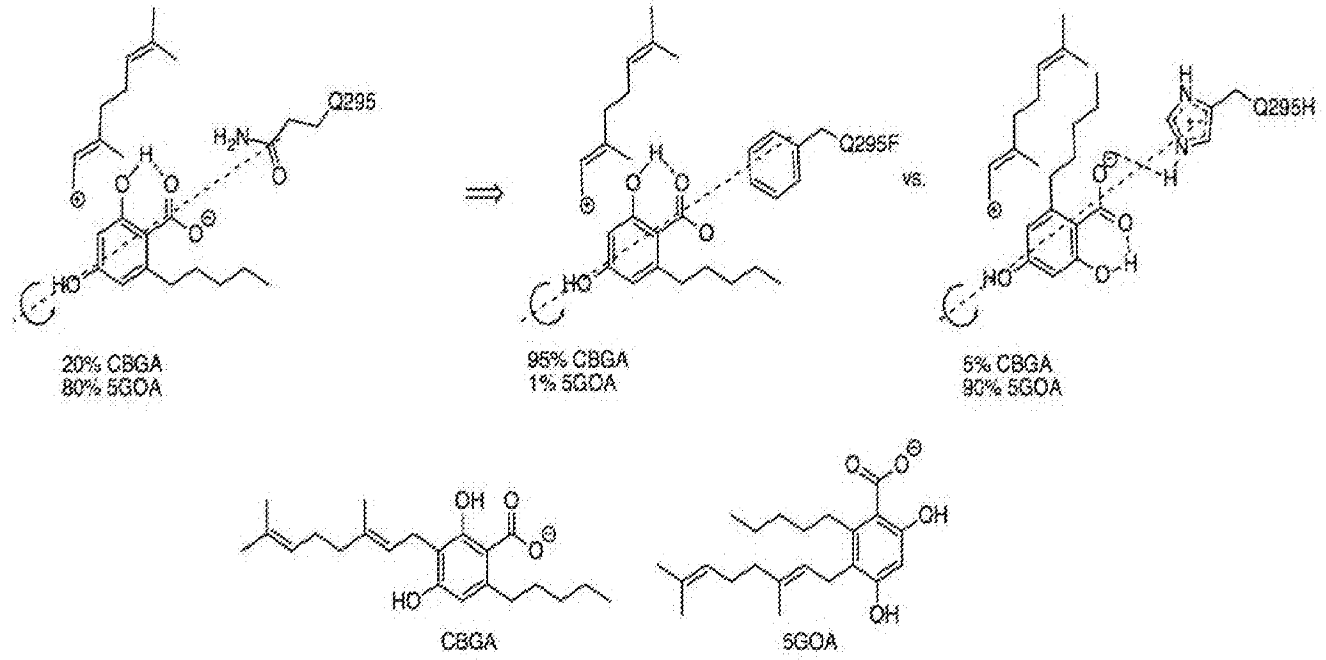
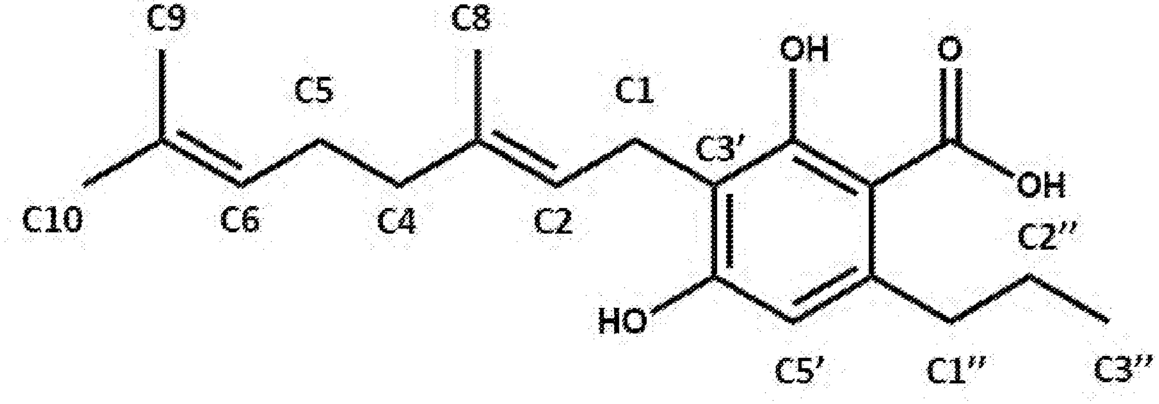


FIG. 80

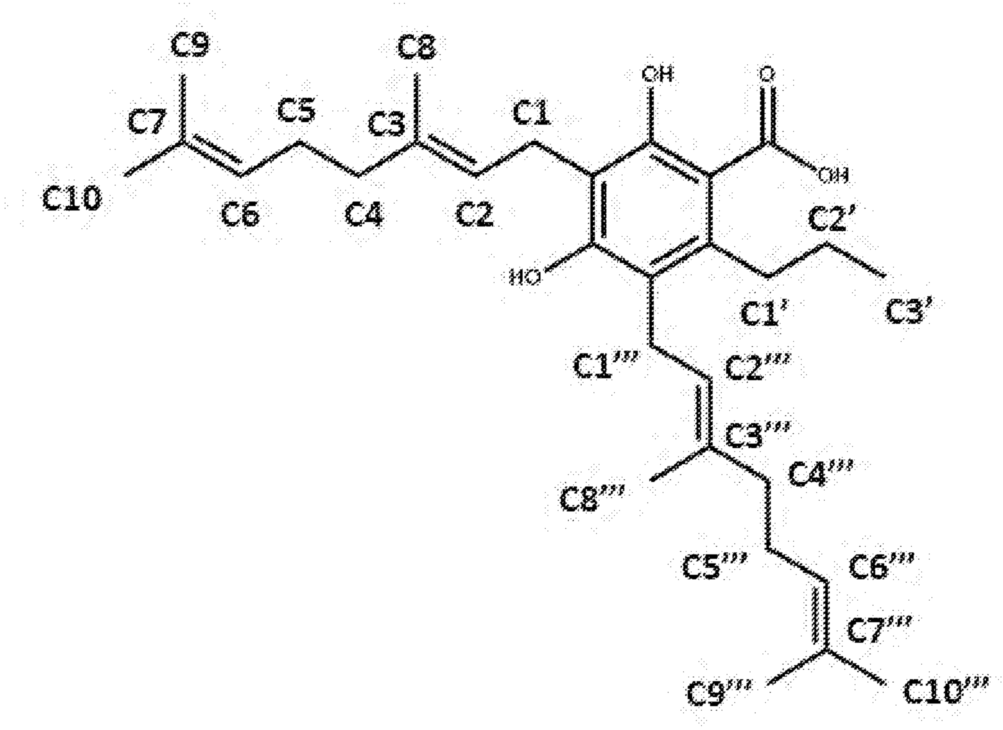
Carbon and Proton NMR Assignments for CBGVA



Protons: 28
Carbons: 20

FIG. 81

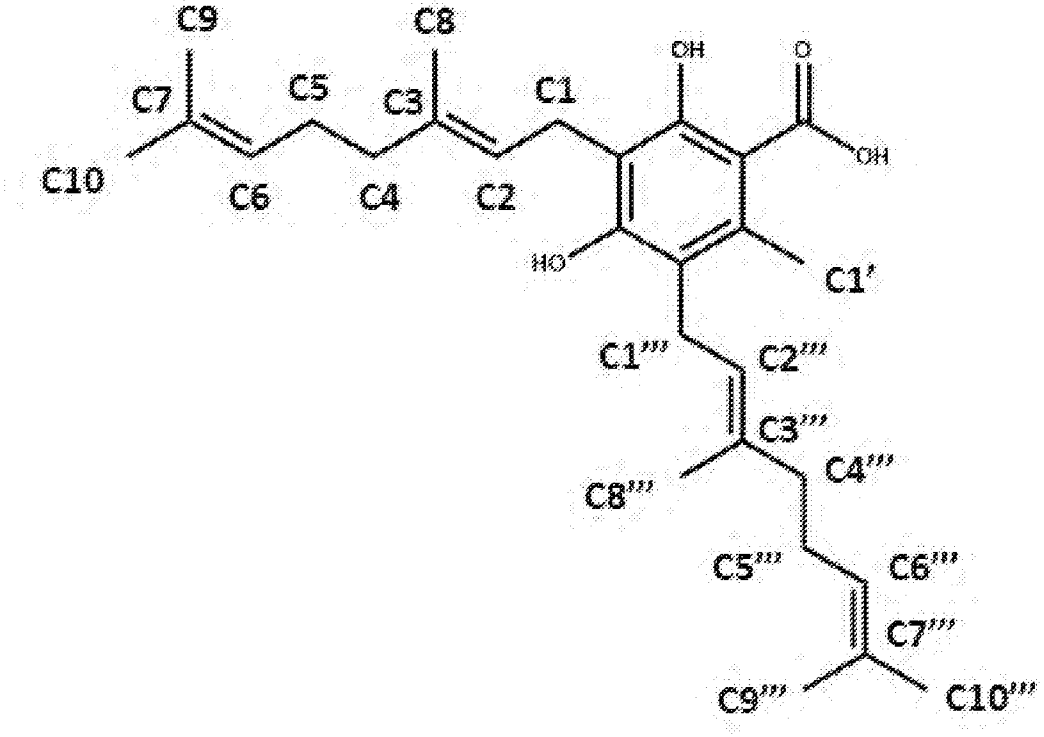
Carbon and Proton NMR Assignments for RBI-29



Protons: 44
Carbons: 30

FIG. 82

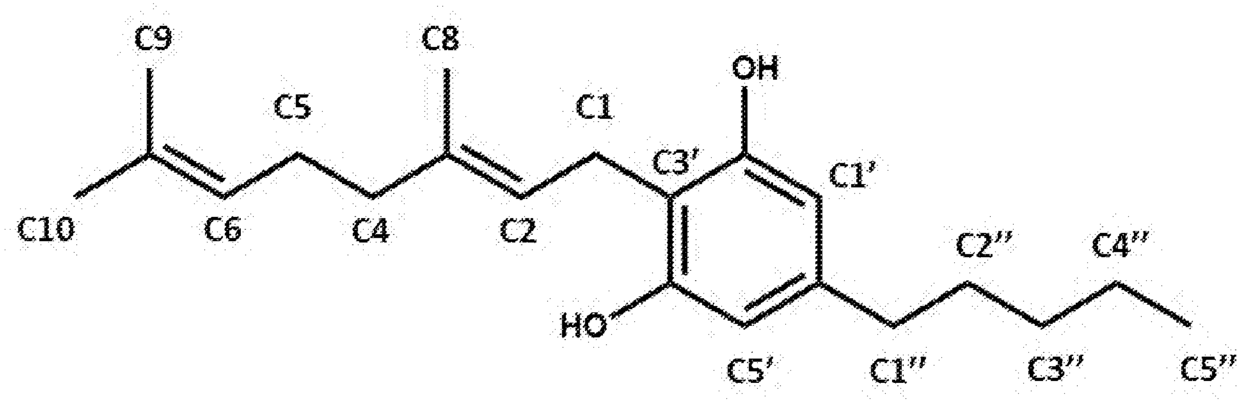
Carbon and Proton NMR Assignments for UNK59



Protons: 40
Carbons: 28

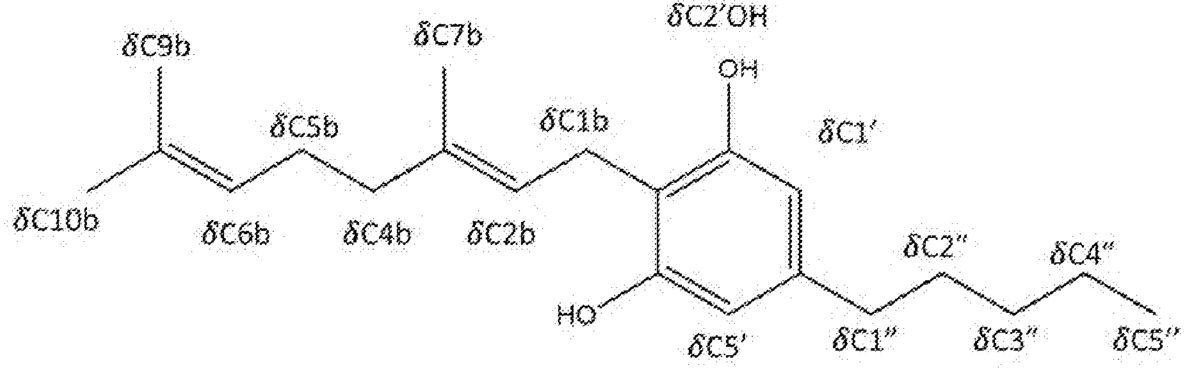
FIG. 83

Carbon and Proton NMR Assignments for CBG



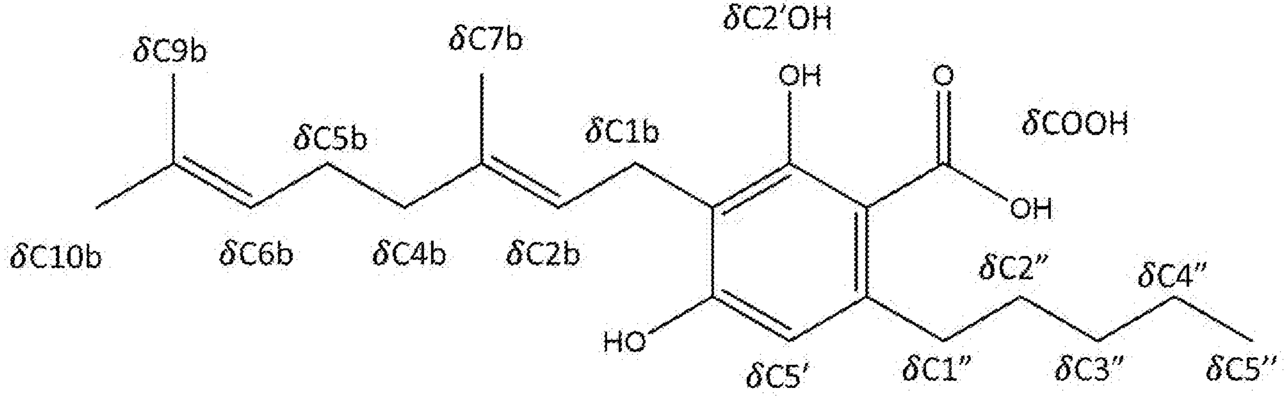
Protons: 32
Carbons: 21

FIG. 84A



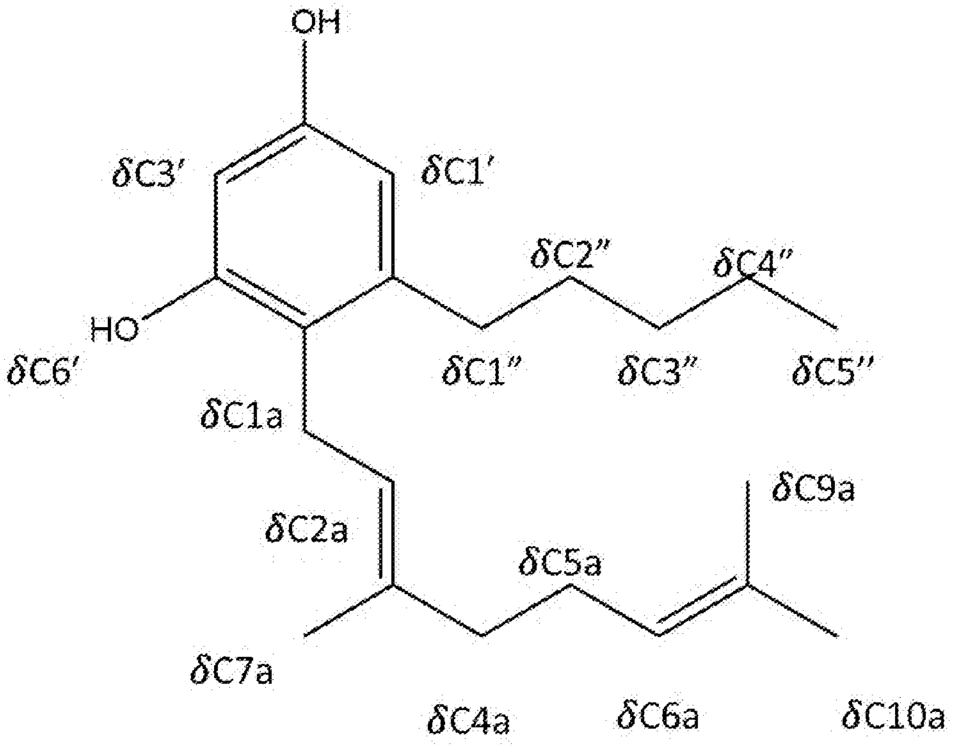
RBI-01	
Proton	Shift
C1b	3.344
C2b	5.28
C7b	1.823
C4b	2.11
C5b	2.083
C6b	5.062
C9b	1.688
C10b	1.601
2' OH	7.274
C5'+C1'	6.261
C1''	2.78
C2''	1.575
C3''+C4''	1.322
C5''	0.895

FIG. 84B



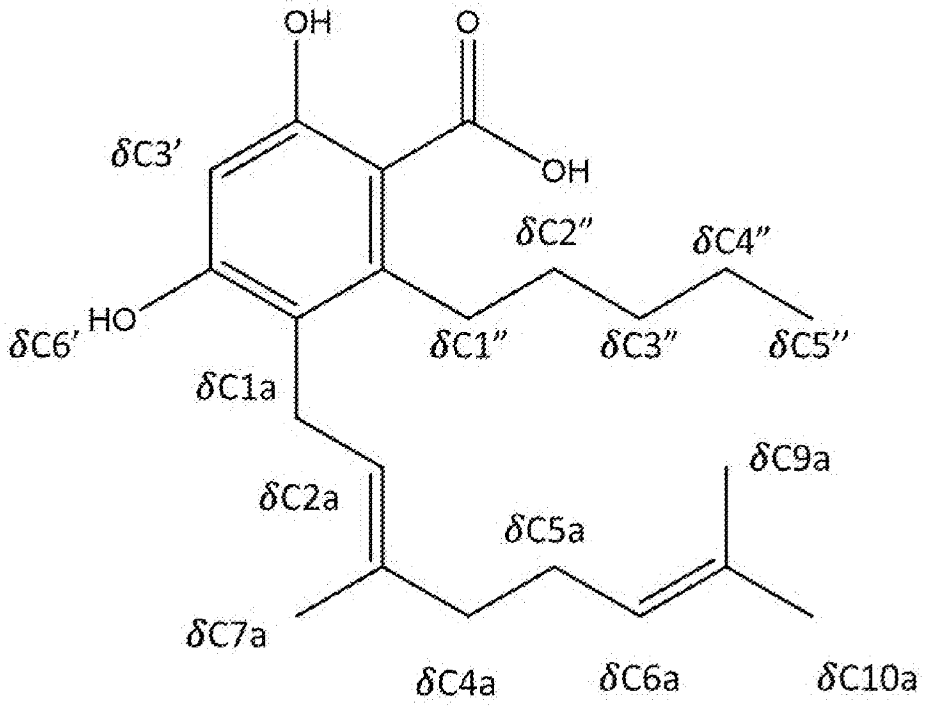
RBI-02	
Proton	Shift
C1b	3.443
C2b	5.292
C7b	1.827
C4b	2.117
C5b	2.092
C6b	5.066
C9b	1.688
C10b	1.605
2' OH	7.272
C5'	6.261
C1''	2.885
C2''	1.585
C3''+C4''	1.353
C5''	0.909

FIG. 84C



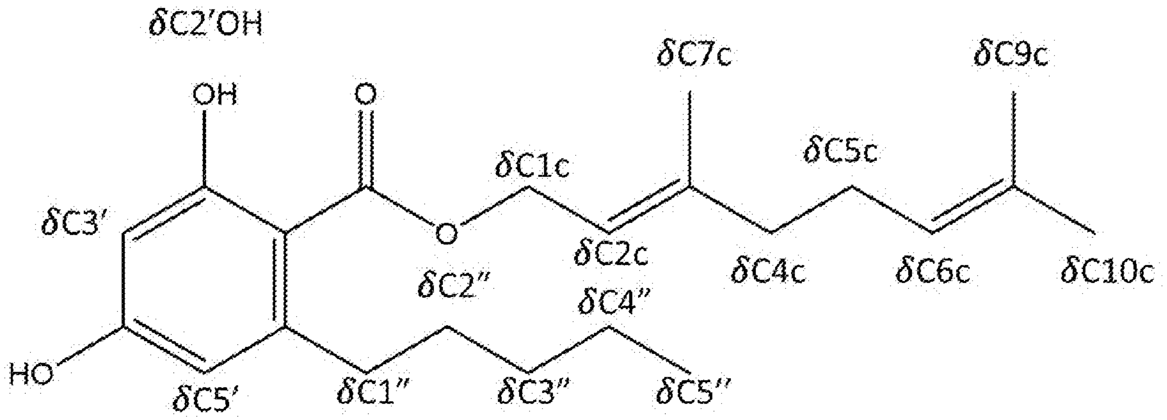
RBI-03	
Proton	Shift
C1a	3.3
C2a	5.152
C7a	1.816
C4a	2.099
C5a	2.068
C6a	5.055
C9a	1.684
C10a	1.6
C3'	6.266
C1'	6.234
C1''	2.541
C2''	1.533
C3''+C4''	1.343
C5''	0.909

FIG. 84D



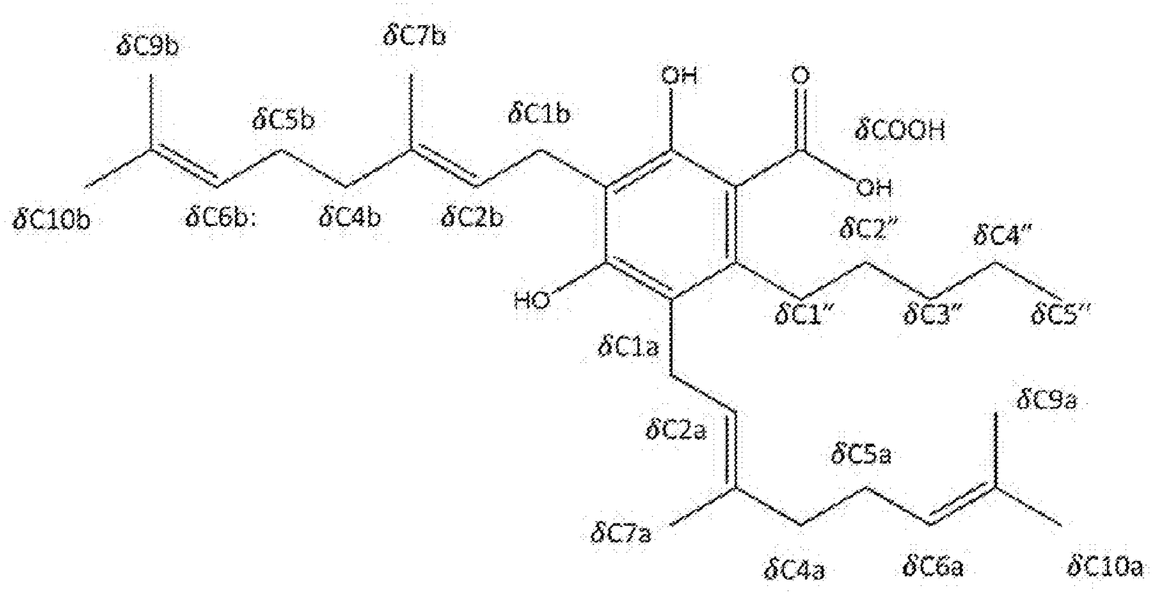
RBI-04	
Proton	Shift
C1a	3.348
C2a	5.152
C7a	1.814
C4a	2.099
C5a	2.064
C6a	5.055
C9a	1.679
C10a	1.601
C3'	6.343
C1''	2.959
C2''	1.555
C3''+C4''	1.391
C5''	0.927

FIG. 84E



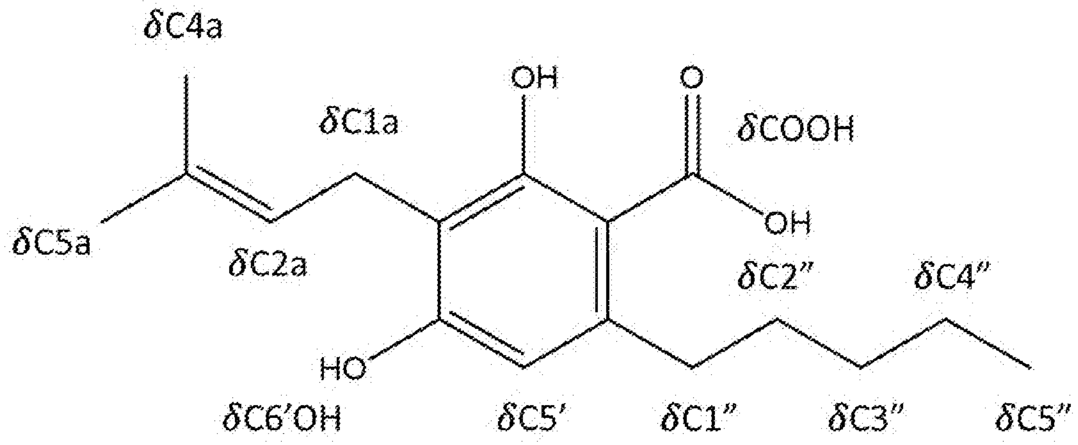
RBI-05	
Proton	Shift
C5''	0.891
C3''+C4''	1.348
C10c	1.611
C9c	1.687
C7c	1.747
C5c+C4c	2.108
C1''	2.929
C1c	4.651
C6c	5.076
C2c	5.499
C5'+C3'	6.387
C2''	1.57

FIG. 84F



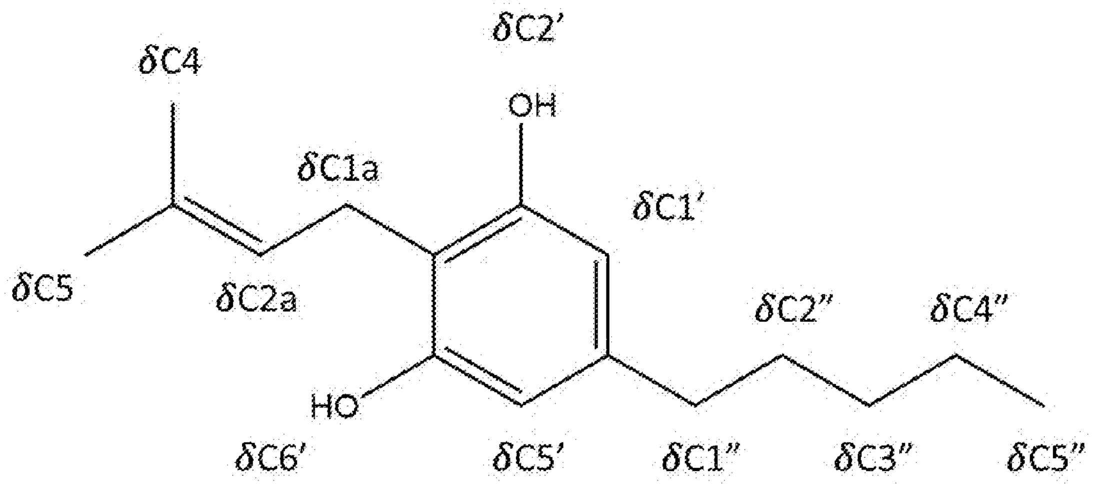
RBI-07	
Proton	Shift
C5''	0.919
C3''+C4''	1.268
C10a+C10b	1.601
C9a+C9b	1.681
C7a	1.796
C7b	1.828
C5ab+C4ab	2.02-2.1
C1a	3.353
C1b	3.454
C2a+C6b+C6a	5.069
C2b	5.263
COOH	11.801
C1''	2.911
C2''	1.571

FIG. 84G



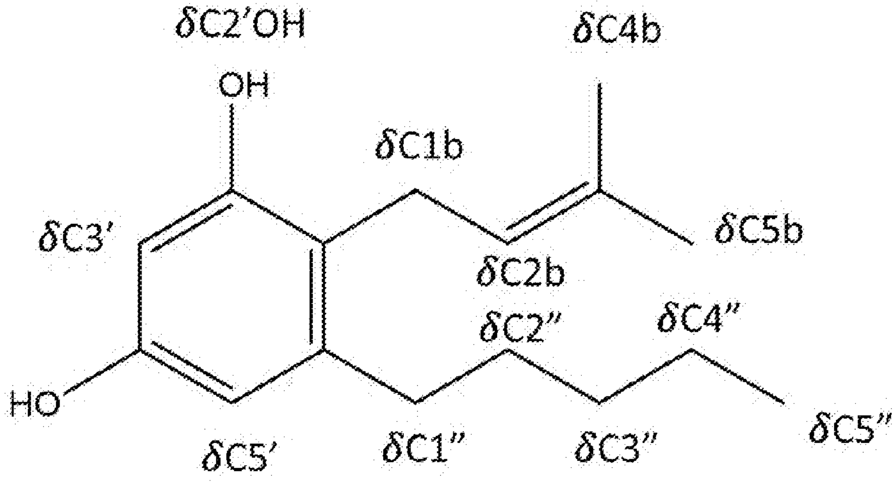
RBI-08	
Proton	Shift
C1a	3.424
C2a	5.283
C4a	1.836
C5a	1.772
C1''	2.874
C2''	1.57
C3''+C4''	1.345
C5''	0.904
C5'	6.274
COOH	12.022
C6'OH	5.845

FIG. 84H



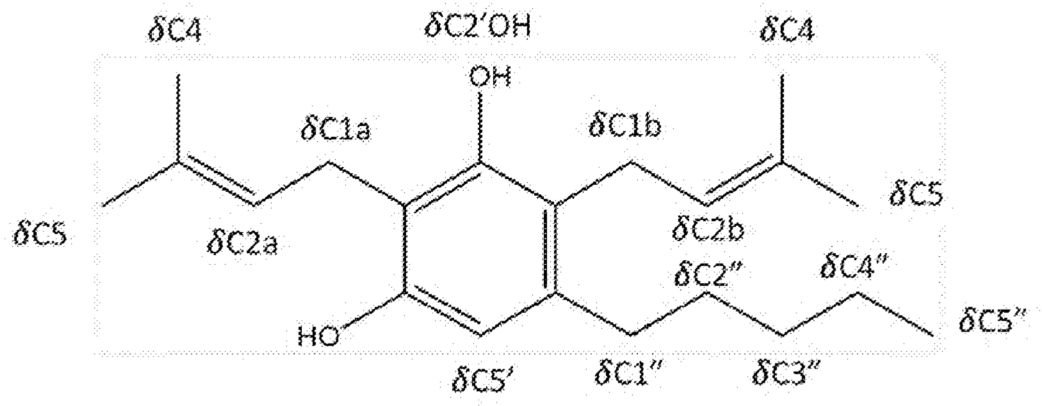
RBI-09	
Proton	Shift
C1a	3.386
C1"	2.463
C2a	5.276
C2"	1.573
C3"+C4"	1.32
C1'+C5'	6.257
C4a	1.834
C5"	0.896
C5a	1.767

FIG. 84I



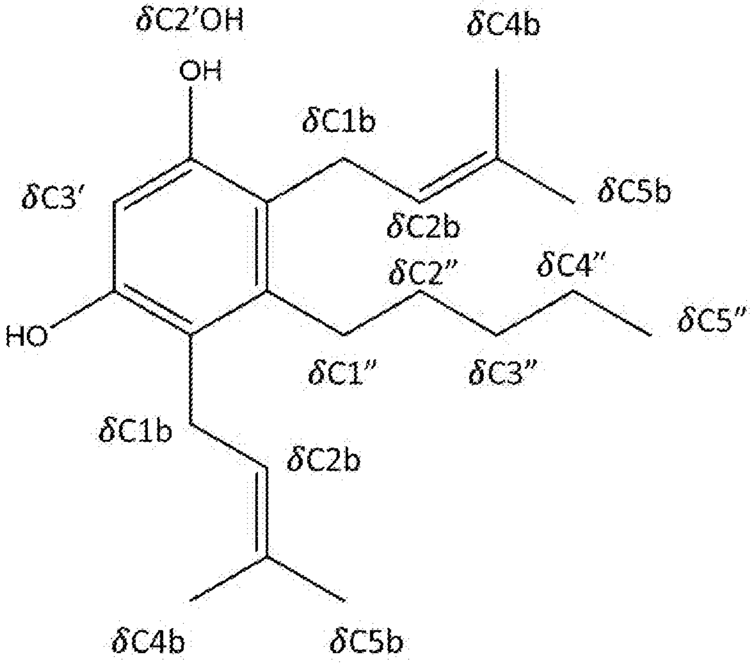
RBI-10	
Proton	Shift
C1b	3.282
C3'	6.219
C1"	2.524
C2b	5.139
C2"	1.52
C3"+C4"	1.33
C4b	1.813
C5b	1.74
C5'	6.256
C5"	0.896

FIG. 84J



RBI-11	
Proton	Shift
C1a	3.382
C1b	3.293
C1"	2.504
C2b	5.125
C2"	1.506
C3"+C4"	1.342
C4a+C4b	1.819
C5a+C5b	1.744
C5'	6.257
C5"	0.896

FIG. 84K



RBI-12	
Proton	Shift
C1b+C1b	3.285
C1"	2.553
C2b+C2b	5.119
C2"	1.551
C3"+C4"	1.355
C4b+C4b	1.798
C5b+C5b	1.715
C3'	6.235
C5"	0.896

FIG. 49: Alkylresorcyclic Acid (i.e. ORA, DVA, OA, etc) prenylation site numbering