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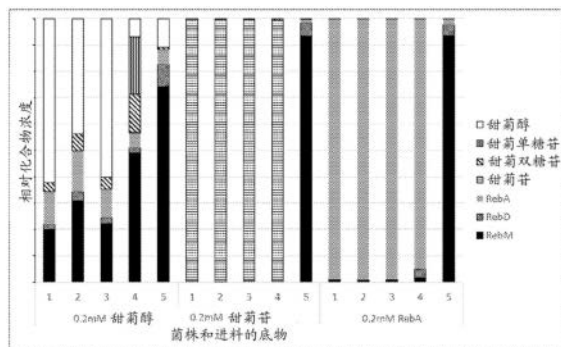
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(54) 发明名称

通过全细胞生物转化产生甜菊醇糖苷

(57) 摘要

在各个方面和实施方案,本发明提供微生物细胞和用于由低级糖基化中间体产生高级糖基化产物的方法。所述微生物细胞在细胞质中表达一种或多种UDP依赖性糖基转移酶,以用于所述中间体的糖基化。当将所述微生物菌株与包含所述中间体的植物提取物或其级分一起孵育时,这些糖基化的中间体可用于由所述细胞进行进一步糖基化,并且所述高级糖基化产物能够从培养基和/或微生物细胞中回收。



1. 一种用于从糖苷中间体产生糖基化产物的方法,所述方法包括:

提供在细胞内表达一种或多种重组UDP依赖性糖基转移酶(UGT酶)的微生物细胞,其中所述UGT酶使甜菊醇糖苷中间体糖基化,其中所述微生物细胞是大肠杆菌(*Escherichia coli*),其中所述UGT酶选自1-3'糖基化UGT酶、1-2'糖基化UGT酶、C19 0-糖基化UGT酶和C13 0-糖基化UGT酶中的一种或多种,并且其中所述微生物细胞具有:

磷酸葡萄糖变位酶(pgm)和UTP-葡萄糖-1-磷酸尿苷酰转移酶(galU)的过表达,以及ushA(UDP糖水解酶)、pgi(葡萄糖-6磷酸酯异构酶)和galETKM的缺失,

将所述微生物菌株与所述糖苷中间体一起孵育,其中所述糖苷中间体是来自甜叶菊叶提取物的甜菊醇糖苷,以及

回收所述糖基化产物。

2. 如权利要求1所述的方法,其中所述糖基化产物包含RebM。

3. 如权利要求1所述的方法,其中所述糖基化产物包含RebD和/或RebI。

4. 如权利要求1所述的方法,其中所述微生物细胞表达一种或多种UGT酶,所述一种或多种UGT酶包含SEQ ID NO:1至45中任一个的氨基酸序列。

5. 如权利要求1所述的方法,其中所述微生物细胞表达1-3'糖基化UGT酶。

6. 如权利要求5所述的方法,其中所述1-3'糖基化UGT酶选自SrUGT76G1、MbUGT1-3、MbUGT1-3\_1、MbUGT1-3\_1.5和MbUGT1-3\_2。

7. 如权利要求6所述的方法,其中所述1-3'糖基化UGT酶是经L200A取代的SrUGT76G1。

8. 如权利要求6所述的方法,其中所述1-3'糖基化UGT酶包含与SEQ ID NO:15、SEQ ID NO:16或SEQ ID NO:17的氨基酸序列相同的氨基酸序列。

9. 如权利要求1所述的方法,其中所述微生物细胞表达1-2'糖基化UGT酶。

10. 如权利要求9所述的方法,其中所述1-2'糖基化UGT酶选自SrUGT91D2、SrUGT91D1、SrUGT91D2e、OsUGT1-2、MbUGT1,2和MbUGT1,2.2。

11. 如权利要求1所述的方法,其中所述微生物细胞表达C13 0-糖基化UGT酶。

12. 如权利要求11所述的方法,其中所述C13 0-糖基化UGT酶是SrUGT85C2,任选地所述C13 0-糖基化UGT酶经P215T取代。

13. 如权利要求1所述的方法,其中所述微生物细胞表达C19 0-糖基化UGT酶。

14. 如权利要求13所述的方法,其中所述C19 0-糖基化UGT酶选自SrUGT74G1和MbUGTc19。

15. 如权利要求4所述的方法,其中所述微生物细胞表达1-3'糖基化UGT酶和1-2'糖基化UGT酶,以及任选的C13 0-糖基化UGT酶和C19 0-糖基化酶。

16. 如权利要求15所述的方法,其中所述微生物细胞表达1-3'糖基化UGT酶、1-2'糖基化UGT酶、C19 0-糖基化UGT酶和C13 0-糖基化UGT酶。

17. 如权利要求16所述的方法,其中所述微生物细胞表达: SrUGT85C2; MbUGT1,2; SrUGT74G1; 以及选自SrUGT76G1、MbUGT1-3\_1、MbUGT1-3\_1.5和MbUGT1-3\_2的酶。

18. 如权利要求1所述的方法,其中所述UGT酶被整合到所述微生物细胞的染色体中。

19. 如权利要求1所述的方法,其中所述UGT酶在染色体外表达。

20. 如权利要求1所述的方法,其中所述方法使得糖苷中间体按重量计至少40%转化为糖基化产物。

21. 如权利要求20所述的方法,其中所述方法使得糖苷中间体按重量计至少50%转化为糖基化产物。

22. 如权利要求21所述的方法,其中所述方法使得糖苷中间体按重量计至少75%转化为糖基化产物。

23. 如权利要求1所述的方法,其中至少5:1比例的RebM与RebD被回收为所述糖基化产物。

24. 如权利要求1至23中任一项所述的方法,其中所述方法通过分批发酵、连续发酵或半连续发酵进行。

25. 如权利要求24所述的方法,其中所述方法通过补料分批发酵进行。

26. 如权利要求25所述的方法,其中使所述糖苷中间体与所述细胞一起孵育约72小时或更少。

## 通过全细胞生物转化产生甜菊醇糖苷

[0001] 优先权

[0002] 本申请要求2018年7月16日提交的美国临时申请号62/698,617的优先权和权益,所述临时申请特此以引用的方式整体并入。

### 背景技术

[0003] 小分子的糖基转移酶由植物界中的大的多基因家族编码。这些酶将糖从核苷酸糖转移至广泛范围的次级代谢物,从而改变受体分子的理化性质。例如,甜菊醇糖苷是在甜叶菊(*Stevia rebaudiana* Bertoni)的叶中发现的一类化合物,甜叶菊是原产于南美洲的某些地区的一种菊科(Asteraceae (Compositae))多年生灌木。它们的结构特征是单碱基甜菊醇,不同之处在于位置C13和C19处存在碳水化合物残基。它们积聚在甜叶菊叶中,占总干重的大约10%至20%。按干重计,在甜叶菊叶中发现的四种主要糖苷通常包括甜菊苷(9.1%)、莱鲍迪苷A(3.8%)、莱鲍迪苷C(0.6%-1.0%)和杜克苷A(0.3%)。其他甜菊醇糖苷以少量或痕量存在,包括莱鲍迪苷B、D、E、F、G、H、I、J、K、L、M和O、杜克苷B、甜菊双糖苷和甜茶苷。

[0004] 较小的糖基化产物莱鲍迪苷M(RebM)的效力估计比蔗糖高约200-350倍,并且被描述为具有纯净的甜味,略带苦味或甘草余味。Prakash I.等人,Development of Next Generation Stevia Sweetener:Rebaudioside M,*Foods* 3(1),162-175(2014)。尽管RebM在全球食品工业中引起极大的兴趣,但其在甜叶菊提取物中的低普及率需要创新的合成方法。

[0005] 仍然需要经济的方法来产生作为天然植物提取物的次要产物的高价值糖苷。

### 发明内容

[0006] 在各个方面和实施方案,本发明提供微生物细胞和用于由低级糖基化中间体产生高级糖基化产物的方法。所述微生物细胞表达一种或多种用于所述中间体的糖基化的UDP依赖性糖基转移酶。当将微生物菌株与含糖苷中间体的植物提取物或其级分一起孵育时,这些中间体可用于通过微生物细胞进一步糖基化。在各种实施方案中,从培养基和/或微生物细胞回收所述高级糖基化产物。

[0007] 糖苷中间体可以是任何糖基化的次级代谢物,如在植物提取物(包括糖基化萜类化合物、类黄酮、大麻素、聚酮化合物、芪类化合物和多酚等)中天然发现那些。在一些实施方案中,所述糖基化的中间体是糖基化的萜类化合物(如甜菊醇糖苷或罗汉果苷),并且可用作甜味剂。在一些实施方案中,所述产物的生物合成涉及微生物细胞中糖苷中间体的至少两种糖基化反应。

[0008] 在一些实施方案中,所述糖苷中间体来自甜叶菊叶提取物。例如,RebM和其他高级糖基化产物可由甜菊醇糖苷中间体如甜菊苷、甜菊双糖苷、莱鲍迪苷A(RebA)和莱鲍迪苷C(RebC)等生物合成。所述微生物细胞在细胞质中表达一种或多种UDP依赖性糖基转移酶,以用于较低价值中间体的糖基化。当将微生物菌株与含糖菊醇糖苷中间体的甜叶菊叶提取

物或其级分一起孵育时,这些中间体可用于细胞进行进一步糖基化,并且可从培养基和/或微生物细胞中回收这些产物。因此,这种方法在甜叶菊叶提取物中使用高级中间体,即具有一至五种糖基化的甜菊醇糖苷(以及在一些实施方案中,糖苷配基核心,甜菊醇)。来自甜叶菊叶提取物的高级中间体可从甜菊醇糖苷的现有工业提取物中容易地获得。

[0009] 在各种实施方案中,微生物细胞表达使糖苷中间体糖基化的至少一种或至少两种或至少三种或至少四种UGT酶,并且可选自表1中列出的那些和本文提供为SEQ ID NO:1-45的那些及其衍生物。在各种实施方案中,所述UGT酶独立地选自1-2'糖基化UGT酶、1-3'糖基化UGT酶和0-糖基化UGT酶。在各种实施方案中,这些酶在细胞内表达,因为它们不含膜易位或分泌信号。

[0010] 在一些实施方案中,特别是关于甜叶菊叶提取物中的甜菊醇糖苷中间体的糖基化,所述微生物细胞表达四种UGT酶,如13-0 UGT糖基化酶、19-0 UGT糖基化酶、1-2'UGT糖基化酶和1-3'UGT糖基化酶。

[0011] 在各种实施方案中,所述微生物细胞表达1-3'糖基化UGT酶。例如,所述1-3'糖基化UGT酶可选自SrUGT76G1、MbUGT1-3及其衍生物(例如,UGT76G1\_L200A或MbUGT1-3\_1、MbUGT1-3\_1.5或MbUGT1-3\_2)。

[0012] 在这些和其他实施方案中,所述微生物细胞表达1-2'糖基化UGT酶。例如,所述1-2'糖基化UGT酶可选自SrUGT91D2、SrUGT91D1、SrUGT91D2e、OsUGT1-2、MbUGT1,2、MbUGT1,2.2及其衍生物。

[0013] 在这些或其它实施方案中,所述微生物细胞表达C13 0-糖基化UGT酶。例如,所述C13 0-糖基化UGT酶可选自SrUGT85C2及其衍生物(例如,MbUGTC13)。

[0014] 在这些或其它实施方案中,所述微生物细胞表达C19 0-糖基化UGT酶。例如,所述C19 0-糖基化酶可选自SrUGT74G1、MbUGTc19及其衍生物(例如,MbUGTc19-2)。

[0015] 全细胞转化需要在细胞内表达的酶作用于外部进料的底物(例如,糖基化中间体),并且细胞提供UDP-葡萄糖辅因子再生。这与依赖于在细胞外纯化或分泌的酶的过程相反,所述过程需要外源性UDP-葡萄糖供应或UDP-葡萄糖前体或UDP-葡萄糖再生机制或UDP-葡萄糖再生酶系统。在本发明的实施方案中,用活的微生物细胞进行催化(糖基化)。UDP-葡萄糖辅因子再循环使用天然细胞代谢发生,而无需其他外部提供的酶或底物进料。

[0016] 根据本公开,对微生物细胞的遗传修饰使得糖苷中间体可用于通过细胞内表达的酶进行进一步糖基化。在一些实施方案中,与从裂解细胞中提取相反,可从培养基中回收高级糖基化产物。在一些实施方案中,所述微生物细胞具有一种或多种遗传修饰,所述遗传修饰增加UDP-葡萄糖可用性。在一些实施方案中,不希望受理论束缚,这些修饰也可使细胞对葡萄糖可用性产生应力,从而导致内源性转运蛋白的表达增加,以将糖苷中间体输入细胞。在一些实施方案中,不希望受理论束缚,通过遗传修饰或培养基组分使细胞具有渗透性,从而允许产物和底物的被动扩散。

[0017] 在各种实施方案中,所述微生物细胞具有一种或多种内源性转运蛋白的过表达(例如,与亲本微生物菌株相比),或在某些实施方案中,经修饰以表达重组和/或工程化的转运蛋白。在一些实施方案中,所述微生物细胞表达内源性转运蛋白或其衍生物的一个或多个其他拷贝。例如,转运蛋白的表达或活性可进行修饰以增加甜菊醇糖苷底物(例如,甜菊苷、甜菊双糖苷、RebA和/或RebC中的一种或多种)转运至细胞中,同时输出产物,如RebM

和/或RebD以及其他高级糖基化产物。可过表达或工程化以改变微生物细胞中的活性或底物特异性的示例性转运蛋白包括大肠杆菌、acrAD、xylE、ascF、bglF、chbA、ptsG/crr、wzxE、rfbX以及其直向同源物或衍生物。其他转运蛋白包括选自转运所需糖苷中间体的细菌或内源性转运蛋白的那些。例如,转运蛋白可来自宿主物种,或另一种细菌或酵母物种,并且可被工程化以调节其对特定糖苷中间体或产物的亲和力。

[0018] 在各种实施方案中,所述方法使得糖苷中间体至少40%转化、或至少50%转化或至少75%转化为所需产物(例如,甜菊苷、甜菊双糖苷和RebA转化为RebD、RebM和/或其他高度糖基化的莱鲍迪苷)。在RebM的产生中,与RebD相比,产品特征可能更偏向RebM。

[0019] 所述方法可通过分批发酵、补料-分批发酵、连续发酵或半连续发酵进行。例如,在一些实施方案中,所述方法通过分批发酵进行,其中孵育时间小于约72小时,或者在一些实施方案中小于约48小时或小于约24小时。

[0020] 在一些方面,本发明提供了用于制备包含高级甜菊醇糖苷如RebM的产品的的方法。所述方法包括将目标甜菊醇糖苷掺入产品,如食品、饮料、口腔护理产品、甜味剂、调味剂或其他产品中。在一些方面,本发明提供了用于制备包含多种高甜度甜味剂的甜味剂组合物的方法,所述高甜度甜味剂如甜菊醇糖苷、罗汉果苷、三氯蔗糖、阿斯巴甜、纽甜、爱德万甜(advantame)、乙酰磺胺酸钾、糖精、环己基氨基磺酸盐、新橙皮苷二氢查尔酮、买麻藤素E和/或白皮杉醇4'-O-β-D-吡喃葡萄糖苷中的两种或更多种。

[0021] 本发明的其他方面和实施方案将根据以下具体实施方式而是显而易见的。

## 附图说明

[0022] 图1.RebM的结构。甜菊醇支架(二萜类化合物)显示为加框。RebM含有六种糖基化:(1)C13 0-糖基化,(2)C13 1-2'糖基化,(3)C131-3'糖基化,(4)C19 0-糖基化,(5)C19 1-2'糖基化,和(6)C19 1-3'糖基化。

[0023] 图2.通过UGT酶对甜菊醇和甜菊醇糖苷中间体的作用产生的糖基化产物。

[0024] 图3.通过UGT酶对含有鼠李糖的甜菊醇糖苷中间体的作用产生的糖基化产物。

[0025] 图4.甜菊双糖苷转化为RebM。对于RebM途径中的五种主要化合物,化合物浓度是相对于缺少UGT酶的对照菌株中这些化合物的总浓度示出。向菌株进料0.2mM甜菊双糖苷,并在提取之前与底物一起孵育48小时。菌株3和4显示没有甜菊双糖苷的转化,而菌株5显示实质性转化为RebD和RebM。

[0026] 图5.甜菊苷和RebA转化为RebM。使用菌株1至5示出叶提取物中发现的两种市售中间体甜菊苷和RebA生物转化为RebM。还示出甜菊醇核心的生物转化。所述值报告为总甜菊醇糖苷组合物的百分比。所有菌株将甜菊醇转化为RebM表明,所有菌株都能够形成RebM。与甜菊双糖苷一样,甜菊苷和RebA两者均被转化为RebM,但仅在菌株5中。对于从甜菊苷和RebA转化,RebD:RebM的比例强烈有利于RebM(1:20)。

[0027] 图6.96孔板中甜菊醇糖苷的生物转化。对于RebM途径中的五种主要化合物,在有或没有表达的UGT酶情况下示出化合物浓度。向菌株进料0.5g/L的甜菊苷/RebA叶提取物,并在取样前与底物一起孵育48小时。

[0028] 图7.在生物反应器规模上甜菊苷/RebA叶提取物的生物转化。对于RebM途径中的五种主要化合物,在整个发酵过程中的不同时间点对培养基中的化合物浓度进行采样。向

菌株进料2g/L的甜菊苷/RebA叶提取物,并与底物一起孵育总共30小时。

[0029] 图8.遗传修饰以增加至UDP-葡萄糖的天然大肠杆菌通量。代谢物:G6P,葡萄糖-6-磷酸;G1P,葡萄糖-1-磷酸;6PGL,6-磷酸葡萄糖酸-1,5-内酯;6PG,6-磷酸葡萄糖酸酯;R5P,核糖-5-磷酸;PRPP,5-磷酸核糖基-1-焦磷酸酯;UMP,尿苷一磷酸;UDP,尿苷二磷酸;UTP,尿苷三磷酸;UDP-Glu,UDP-葡萄糖;UDP-Gal,UDP-半乳糖;UDP-GlcNAc,UDP-N-乙酰氨基葡萄糖;CTP,胞苷三磷酸;dUDP,脱氧尿苷-二磷酸;RebA,莱鲍迪苷A;RebM,莱鲍迪苷M;酶:1.glk,葡萄糖激酶;2.pgi,葡萄糖-6-磷酸异构酶;3.zwf,葡萄糖-6-磷酸-1-脱氢酶;4.pgl,6-磷酸葡萄糖酸内酯酶;5.edd,磷酸葡萄糖酸脱水酶;6.gnd,6-磷酸葡萄糖酸脱氢酶;7.rpiA,核糖-5-磷酸异构酶或rpe,核糖-磷酸3-差向异构酶;8.prs,核糖-磷酸二磷酸激酶;9.pyrEF,乳清酸磷酸核糖基转移酶和乳清苷-5'磷酸-脱羧酶;10.pyrK,尿苷酸激酶;11.ndk,UDP激酶;12.murG,N-乙酰葡萄糖胺基转移酶;13.nrdABEF,核糖核苷-二磷酸还原酶;14.pyrG,CTP合酶;15.pgm,磷酸葡萄糖变位酶;16.galU,UDP-葡萄糖焦磷酸化酶;17.galETKM,UDP-葡萄糖-4-差向异构酶/半乳糖-1-磷酸尿苷酰转移酶/半乳糖激酶/半乳糖-1-差向异构酶;18.ushA,5'-核苷酸酶/UDP-糖水解酶;19.UGT,UDP-糖基转移酶。所述示意图说明从菌株1至菌株5的顺序基因组修饰。菌株1是基础大肠杆菌菌株。菌株2缺失17,并且菌株3具有添加缺失18。通过缺失2从菌株3构建菌株4。通过过表达15和16从菌株4构建菌株5。

### 具体实施方式

[0030] 在各个方面和实施方案,本发明提供微生物细胞和用于由低级糖基化中间体产生高级糖基化产物的方法。在各种实施方案中,所述微生物细胞表达一种或多种UDP依赖性糖基转移酶(例如,细胞内),以用于中间体的糖基化。在各种实施方案中,所述微生物细胞具有一种或多种遗传修饰,所述遗传修饰增加UDP-葡萄糖的可用性。当将微生物菌株与糖苷中间体(例如,来自植物提取物或其级分)一起孵育时,这些糖苷中间体可用于细胞,以通过细胞内表达的糖基转移酶进一步糖基化。在一些实施方案中,所述高级糖基化产物可从培养基中回收,或者在一些实施方案中,可从培养基和微生物细胞中回收。

[0031] 糖苷中间体可以是任何糖基化的次级代谢物,如在植物提取物(包括糖基化萜类化合物、糖基化类黄酮、糖基化大麻素、糖基化聚酮化合物、糖基化芪类化合物和糖基化多酚等)中天然发现那些。在一些实施方案中,所述糖苷中间体是糖基化的萜类化合物,如甜菊醇糖苷或罗汉果苷。在一些实施方案中,所述糖苷中间体具有一个、两个、三个、四个或五个糖基,包括选自葡萄糖基、半乳糖基、甘露糖基、木糖基和鼠李糖基等的糖基。在各种实施方案中,所述糖基化产物具有至少四个糖基、或至少五个糖基、或至少六个糖基。在其他实施方案中,所述产物具有七个、八个、九个或更多个糖基。在一些实施方案中,所述产物的生物合成涉及微生物细胞对中间体的至少两次糖基化。

[0032] 在一些实施方案中,所述糖苷中间体来自甜叶菊叶提取物。例如,可从甜菊醇糖苷中间体如甜菊苷、甜菊双糖苷、莱鲍迪苷A、杜克苷A、杜克苷B、莱鲍迪苷C和莱鲍迪苷F等生物合成具有五种、六种或更多种糖基化的甜菊醇糖苷(如RebD或RebM)。所述微生物细胞表达一种或多种UDP依赖性糖基转移酶,以用于这些较低价值前体的糖基化。当将微生物菌株与包含甜菊醇糖苷中间体的甜叶菊叶提取物或其级分一起孵育时,这些中间体可用于进一步糖基化为RebD或RebM或其他高级糖基化产物(例如,RebI),其可从培养基和/或微生物细

胞中回收。

[0033] 在各种实施方案中,所述UDP依赖性糖基转移酶“在细胞内”表达,因为所述酶不具有膜易位或分泌肽或结构域。因此,UGT酶的表达在细胞质中发生,并且这些酶不經由分泌或转运信号定向到细胞外。在各种实施方案中,所述UGT酶不含膜锚定结构域。即,在各种实施方案中,所述UGT酶不包含跨膜结构域。

[0034] 在一些实施方案中,所述方法使用甜叶菊叶提取物中的高级中间体,即具有一至五种糖基化的甜菊醇(糖苷配基中间体)和甜菊醇糖苷,其可用于微生物细胞进行进一步糖基化。来自甜叶菊叶提取物的高级中间体可从甜菊醇糖苷的现有工业提取物中容易地获得。如表2所示,叶提取物可主要含有途径中间体甜菊苷和莱鲍迪苷A(RebA)。在各种实施方案中,甜叶菊叶提取物是甜菊醇糖苷的提取。在一些实施方案中,所述提取物包含甜菊苷、甜菊双糖苷和莱鲍迪苷A中的一种或多种作为主要组分。在一些实施方案中,所述提取物包含杜克苷A、杜克苷B、RebC和/或RebF中的一种或多种作为主要组分。主要组分通常占提取物或其级分中的甜菊醇糖苷的至少约10%,但在一些实施方案中,可占提取物或其级分中的甜菊醇糖苷的至少约20%、或至少约25%、或至少约30%。

[0035] RebM在图1中示出。RebM包含甜菊醇核心的六种糖基化:(1)C13 0-糖基化,(2)C13 1-2'糖基化,(3)C13 1-3'糖基化,(4)C19 0-糖基化,(5)C19 1-2'糖基化,和(6)C19 1-3'糖基化。尽管各种糖基化产物是可能的(图2),但是RebM可通过四种UGT酶的作用由甜菊醇合成,并且UGT糖基化酶各自能够作用于许多底物。例如,UGT91D2和OsUGT1-2两者均是1-2'糖基化酶,它们可由甜菊单糖苷(通过C13作用)产生甜菊双糖苷,也可以由RebA(通过C19处的作用)产生RebD。此外,UGT76G1是1-3'糖基化酶,可由甜菊苷(通过C13的作用)产生RebA,也可由RebD(通过C19的作用)产生RebM。

[0036] 用于甜菊醇和甜菊醇糖苷的糖基化的UGT酶(包括用于RebM的生物合成)在US 2017/0332673中公开,其特此以引用的方式整体并入。示例性UGT酶列于以下表1中(参考专利申请特此以引用的方式整体并入):

[0037] 表1:示例性UGT酶

糖基化的类型	酶	基因 ID	蛋白质 ID	描述
C13	SrUGT85C2	AY345978.1	AAR06916.1	
	MbUGTC13			US 2017/0332673
C19	SrUGT74G1	AY345982.1	AAR06920.1	
	MbUGTc19	-	-	US 2017/0332673
	MbUGTc19-2			US 2017/0332673
1-2'	SrUGT91D1	AY345980.1	AAR06918.1	
	SrUGT91D2	ACE87855.1	ACE87855.1	
	SrUGT91D2e	-	-	US 2011/038967
	OsUGT1-2	NM_00105754 2.1	NP_001051007. 2	WO 2013/022989
	MbUGT1,2	-	-	US 2017/0332673
	MbUGT1,2.2	-	-	US 2017/0332673
1-3'	SrUGT76G1	FB917645.1	CAX02464.1	
	MbUGT1-3			US 2017/0332673
	76G1_L200A			US 2017/0332673
	MbUGT1-3_1			US 62/866,148
[0039]	MbUGT1-3_1 .5			本公开
	MbUGT1-3_2			US 62/866,148

[0040] 示例性UGT酶的氨基酸序列由本公开提供为SEQ ID NO:1-17。SEQ ID NO:1是甜叶菊UGT85C2。SEQ ID NO:13是具有P215T取代的SrUGT85C2,并且在第2位置插入A1a以增加稳定性。SEQ ID NO:2是甜叶菊UGT74G1(在第2位置插入A1a)。SEQ ID NO:8和12是基于SrUGT74G1的环状变换体(MbUGTC19和MbUGTC19-2)。SEQ ID NO:3是甜叶菊UGT76G1。具有1-3'糖基化活性的环状变换体公开为SEQ ID NO:10(MbUGT1-3)和SEQ ID NO:15、16和17(分别MbUGT1-3\_1、MbUGT1-3\_1.5和MbUGT1-3\_2)。具有L200A取代和位置2处A1a的UGT76G1公开为SEQ ID NO:14。甜叶菊UGT91D1、UGT91D2和UGT91D2e公开为SEQ ID NO:4、5和6。水稻UGT1-2公开为SEQ ID NO:7。MbUGT1,2和MbUGT1,2.2是具有1-2'糖基化活性的环状变换体酶(SEQ ID NO:9和11)。

[0041] 另外的UGT酶提供为SEQ ID NO:18至46,来自物种罗汉果(*Siraitia grosvenorii*/monk fruit)、苦瓜(*Momordica charantia*/bitter melon)、黄瓜(*Cucumis sativa*/Cucumber)、笋瓜(*Cucurbita maxima*/squash)、南瓜(*Cucurbita moschata*/squash/pumpkin)、碧桃(桃)、可可(*Theobroma cacao*/cacao)、圆果种黄麻(白黄麻)、枣(红枣)、葡萄(葡萄藤)、核桃(*Juglans regia*/walnut)、橡胶树(*Hevea brasiliensis*/rubber tree)、木薯(*Manihot esculenta*/cassava)、土瓶草(猪笼草)和小粒咖啡(咖啡)。可基于所需的产物和可用的中间体选择UGT酶并任选地进行工程化。

[0042] 在各种实施方案中,所述微生物细胞表达至少一种、或至少两种、或至少三种、或至少四种UGT酶。在一些实施方案中,所述UGT酶使甜菊醇糖苷底物糖基化,包括表2中列出的那些。在一些实施方案中,所述微生物细胞表达使甜菊醇糖苷中间体糖基化的四种UGT酶,如13-0 UGT糖基化酶、19-0 UGT糖基化酶、1-2'UGT糖基化酶和1-3'UGT糖基化酶。这些

通用类别的UGT酶对甜叶菊叶的糖基化中间体的作用描绘于图2和3中。

[0043] 在各种实施方案中,所述微生物细胞表达1-3'糖基化UGT酶。例如,所述1-3'糖基化UGT酶可选自SrUGT76G1、MbUGT1-3\_1、MbUGT1-3\_1.5和MbUGT1-3\_2及其衍生物。

[0044] 在这些和其他实施方案中,所述微生物细胞表达1-2'糖基化UGT酶。例如,所述1-2'糖基化UGT酶可选自SrUGT91D2、SrUGT91D1、SrUGT91D2e、OsUGT1-2、MbUGT1,2、MbUGT1,2.2及其衍生物。

[0045] 在这些或其它实施方案中,所述微生物细胞表达C13 0-糖基化UGT酶。例如,所述C13 0-糖基化UGT酶可选自SrUGT85C2及其衍生物(例如,MbUGTC13)。

[0046] 在这些或其它实施方案中,所述微生物细胞表达C19 0-糖基化UGT酶。例如,所述C19 0-糖基化酶可选自SrUGT74G1、MbUGTc19及其衍生物(例如,MbUGTc19-2)。

[0047] 在这些或其它实施方案中,所述微生物细胞表达1-3'糖基化UGT酶和1-2'糖基化UGT酶。在一些实施方案中,所述微生物细胞表达1-3'糖基化UGT酶、1-2'糖基化UGT酶和C13 0-糖基化UGT酶。在一些实施方案中,所述微生物细胞表达1-3'糖基化UGT酶、1-2'糖基化UGT酶、C19 0-糖基化UGT酶和C19 0-糖基化UGT酶。在一些实施方案中,所述微生物细胞表达1-3'糖基化UGT酶、1-2'糖基化UGT酶、C19 0-糖基化UGT酶和C13 0-糖基化UGT酶。在一些实施方案中,所述微生物细胞表达SrUGT85C2或其衍生物(例如,MbUGTC13)、MbUGT1,2.2或其衍生物、SrUGT74G1或其衍生物(例如,MbUGTc19或MbUGTc19-2)以及SrUGT76G1或MbUGT1-3\_1或其衍生物(例如,76G1\_L200A、MbUGT1-3\_1.5或MbUGT1-3\_2)。不受理论束缚,工程化的UGT酶可提供增加的至RebM的碳通量(以及较高的糖基化产物),并且尤其是由于底物结合袋能够更好地容纳更多的底物,而对较低糖基化中间体的活性没有实质性损失。在这些实施方案中,UGT酶(如1-3'和1-2'糖基化UGT酶)可具有增加的活性速率(例如,底物结合和周转速率)与更高度糖基化的甜菊醇底物(如RebA或RebD)。

[0048] UGT酶的衍生物通常包含与SEQ ID NO:1至46中的一个或多个具有至少约50%同一性、至少约60%同一性、至少约70%同一性、至少约80%同一性、至少约85%同一性或至少约90%同一性、或至少约95%同一性、或至少96%、97%、98%或99%同一性的氨基酸序列。在一些实施方案中,相对于SEQ ID NO:1至46中的一个,所述衍生物具有1至20或1至10或1至5个氨基酸修饰(独立地选自氨基酸取代、插入和缺失)。在一些实施方案中,例如关于由甜菊醇糖苷中间体产生RebM和其他高级甜菊醇糖苷(例如,具有至少5个糖基),这些UGT酶的衍生物包含与SEQ ID NO:1至17中的一个或多个具有至少约50%同一性、至少约60%同一性、至少约70%同一性、至少约80%同一性、至少约85%同一性或至少约90%同一性、或至少约95%同一性、或至少96%、97%、98%或99%同一性的氨基酸序列。在一些实施方案中,相对于SEQ ID NO:1至17中的一个,所述衍生物具有1至20或1至10或1至5个氨基酸修饰(独立地选自氨基酸取代、插入和缺失)。

[0049] 在一些实施方案中,所述微生物细胞表达1-3'UGT酶,所述1-3'UGT酶包含与SEQ ID NO:15、SEQ ID NO:16或SEQ ID NO:17的氨基酸序列至少约75%相同的氨基酸序列。在一些实施方案中,所述1-3'UGT包含与SEQ ID NO:15、16或17至少约80%相同的氨基酸序列。在一些实施方案中,所述1-3'UGT酶的氨基酸序列与SEQ ID NO:15、16或17至少约85%相同,或与SEQ ID NO:15、16或17至少约90%相同,或与SEQ ID NO:15、16或17至少约95%相同,或与SEQ ID NO:15、16或17至少约98%相同。在一些实施方案中,所述1-3'UGT酶的氨

氨基酸序列包含SEQ ID NO:15、16或17的氨基酸。

[0050] 例如,相对于氨基酸序列SEQ ID NO:15、16或17,所述氨基酸序列可具有1至20个独立地选自取代、缺失和插入的氨基酸修饰。在一些实施方案中,相对于SEQ ID NO:15、16或17的氨基酸序列,所述氨基酸序列具有1至10个(例如,1至5个)独立地选自取代、缺失和插入的氨基酸修饰。对SEQ ID NO:15、16或17的氨基酸序列的氨基酸修饰可通过可用的酶结构和同源性模型的构建来指导。示例性结构描述于例如Li等人,Crystal Structure of Medicago truncatula UGT85H2-insights into the Structural Basis of a Multifunctional (iso)Flavonoid Glycosyltransferase, J.of Mol.Biol.370.5(2007): 951-963中。公开可用的晶体结构(例如,PDB条目:2PQ6)可用于告知氨基酸修饰。例如,可对活性位点或在活性位点附近进行一种或多种氨基酸修饰,以改进底物的结合,和/或改进这些底物与催化侧链的反应几何形状。

[0051] 在一些实施方案中,1-3'UGT酶在对应于SEQ ID NO:3(甜叶菊UGT76G1)的位置29、200、357和414的位置处包含氨基酸取代。包含在SEQ ID NO:15和17的酶中的这些位置的取代(分别在SEQ ID NO:15中的位置183、354、54和111)可提供活性的显著改进。在一些实施方案中,在对应于SEQ ID NO:15的位置183、354、54和111的位置处的氨基酸的同一性允许在其他位置处的进一步修饰。例如,在一些实施方案中,1-3'UGT酶包含与SEQ ID NO:15或17的氨基酸序列至少约60%相同的氨基酸序列,其中所述UGT酶包含:在对应于SEQ ID NO:15的位置54的位置处的甘氨酸(G)或苏氨酸(T);在对应于SEQ ID NO:15的位置111的位置处的亮氨酸(L)或异亮氨酸(I);在对应于SEQ ID NO:15的位置183的位置处的甲硫氨酸(M)或亮氨酸(L);以及在对应于SEQ ID NO:15的位置354的位置处的丙氨酸(A)、甘氨酸(G)或丝氨酸(S)。在一些实施方案中,1-3'UGT酶在对应于SEQ ID NO:15的位置183的位置处包含甲硫氨酸(M)。在一些实施方案中,1-3'UGT酶在对应于SEQ ID NO:15的位置54的位置处包含甘氨酸(G)。在一些实施方案中,1-3'UGT酶在对应于SEQ ID NO:15的位置111的位置处包含亮氨酸(L)。在一些实施方案中,1-3'UGT具有以下中的两个或三个:在对应于SEQ ID NO:15的位置183的位置处的蛋氨酸(M)、在对应于SEQ ID NO:15的位置54的位置处的甘氨酸(G)以及在对应于SEQ ID NO:15的位置111的位置处的亮氨酸(L)。这些修饰可提供对酶的活性的显著改进。

[0052] 在一些实施方案中,1-3'UGT酶在对应于SEQ ID NO:15的位置155的位置之后包含5至约15个氨基酸,如6至12个氨基酸或约6或约11个氨基酸的插入。在一些实施方案中,插入是柔性和亲水性序列,所述序列主要是甘氨酸和丝氨酸残基。在一些实施方案中,所述序列是GSGGSG(SEQ ID NO:47)或GSGGSGGSG(SEQ ID NO:48)。

[0053] 在各种实施方案中,相较于UGT76G1-L200A(SEQ ID NO:14),1-3'UGT酶显示出改进的甜菊苷转化为Reb A,和RebD至RebM的改进的转化。这种改进的转化在生物转化测定中得到了体现,其中将甜菊苷或RebD底物进料至表达1-3'UGT酶的微生物细胞。在与含有重组表达的1-3'UGT的细胞裂解物反应,或与纯化或部分纯化的1-3'UGT的体外反应中可证明改进的转化。

[0054] 对酶的氨基酸序列的变化可改变其活性或没有可测量的影响。没有可测量的影响的沉默变化通常是保守性取代以及远离活性位点和底物结合位点的溶剂暴露表面上的小插入或缺失。相比之下,酶活性更可能受到非保守取代、大的插入或缺失以及活性位点、底

物结合位点以及对于蛋白质折叠或构象重要的掩埋位置处的变化的影响。改变酶活性的变化可增加或降低反应速率或增加或降低对特定底物的亲和力或特异性。例如,增加底物结合位点大小的变化可允许酶作用于更大的底物,而使催化氨基酸侧链更靠近底物上的靶位点定位的变化可增加酶促速率。

[0055] 酶的三维结构和相关活性位点、底物结合位点和其他相互作用位点的位置的知识可有助于衍生物的合理设计,并提供对特定变化的表型的机械洞察力。植物UGT具有高度保守的二级和三级结构,同时具有相对低的氨基酸序列同一性。Osmani等人,Substrate specificity of plant UDP-dependent glycosyltransferases predicted from crystal structures and homology modeling, *Phytochemistry* 70(2009) 325-347。UGT的糖受体和糖供体底物容纳在N与C末端结构域之间形成的槽中。一级序列的若干区域有助于底物结合袋的形成,包括结构保守的结构域以及在氨基酸序列和序列长度方面均不同的环区域。

[0056] 与已知结构相比,UGT衍生物的构建可基于同源性建模进行指导。例如,根据SrUGT76G1\_L200A的晶体结构分析和SrUGT76G1至MbUGT3-1\_1的氨基酸序列比对,可预测甜菊苷的甜菊醇核心与MbUGT3-1\_1的以下残基接近(在4Å之内):I244、L280、W351、A354、I357、M362和T438。此外,C19 1-2糖基化预计与T438接近(在4Å之内)。RebD的甜菊醇核心预计与MbUGT3-1\_1的以下疏水性侧链接近(在4Å之内):L239、M242、I244、L280、I353、A354和I357。C13 1-2'糖基化预计与MbUGT3-1\_1的以下氢键合侧链接近(4Å之内):S301和D77。MbUGT3-1\_1的V341-Q352和K355-A367螺旋的位置和氨基酸含量可能对催化很重要,因为对应于L200A的突变位于这些螺旋之间的环中。MbUGT3-1\_1的位置L76和/或D77可与甜菊苷的C13糖基化相互作用。

[0057] 所述UGT酶中的一者或多者的氨基酸序列可任选地包含在2位插入或取代的丙氨酸以降低细胞中的周转。在各种实施方案中,一种或多种UGT酶包含在位置2插入或取代的丙氨酸氨基酸残基,以在体内提供额外的稳定性。

[0058] 氨基酸序列的同一性,即序列同一性百分比,可经由序列比对来测定。此类比对可用几种本领域已知的算法,诸如用Karlin和Altschul (Karlin&Altschul (1993) *Proc.Natl.Acad.Sci.USA* 90:5873-5877)的数学算法、hmmalign(HMMER软件包)或CLUSTAL算法(Thompson,J.D.,Higgins,D.G.和Gibson,T.J.(1994) *Nucleic Acids Res.* 22,4673-80)进行。可使用例如BLAST、BLAT或BlastZ(或BlastX)来计算序列同一性(序列匹配)的等级。将类似算法并入到Altschul等人(1990) *J.Mol.Biol.* 215:403-410的BLASTN和BLASTP程序中。BLAST蛋白质比对可使用BLASTP程序进行,得分=50,字长=3。为了获得空位比对用于比较目的,如Altschul等人(1997) *Nucleic Acids Res.* 25:3389-3402中所述利用空位BLAST。利用BLAST和空位BLAST程序时,使用各个程序的默认参数。

[0059] UGT酶可整合到微生物细胞的染色体中,或者可替代地在染色体外表达。例如,UGT酶可从细菌人工染色体(BAC)或酵母人工染色体(YAC)表达。

[0060] 可使用例如基因模块(例如,操纵子)或UGT酶的独立表达调节UGT酶的表达以获得最佳酶活性。例如,基因或操纵子的表达可以通过选择具有不同强度(例如强、中或弱)的启动子诸如诱导型启动子或组成型启动子来调节。具有不同强度的启动子的几个非限制性实

例包括Trc、T5和T7。另外,可以通过操纵细胞中基因或操纵子的拷贝数来调节基因或操纵子的表达。在一些实施方案中,所述细胞表达每种UGT酶的单个拷贝。在一些实施方案中,可以通过操纵模块内基因的顺序来调节基因或操纵子的表达,其中先转录的基因通常以更高的水平表达。在一些实施方案中,通过将一个或多个基因或操纵子整合到染色体中来调节基因或操纵子的表达。

[0061] 对UGT表达的优化也可通过选择适当的启动子和核糖体结合位点来实现。在一些实施方案中,这可以包括选择高拷贝数的质粒,或单拷贝数、低拷贝数或中拷贝数的质粒。通过引入或消除诸如茎环之类的结构,也可以靶向转录终止的步骤进行基因表达的调控。

[0062] 全细胞转化需要底物(例如,糖苷中间体)可用于细胞通过所表达的酶(例如,细胞内表达的酶)进行糖基化,并且优选可从细胞外培养基中提取产物。全细胞系统具有优点,因为所述细胞提供UDP-葡萄糖辅因子再生。这与依赖于使用来自细胞外溶解或分泌的酶的过程相反,所述过程需要外源性UDP-葡萄糖供应或UDP-葡萄糖前体或UDP-葡萄糖再生机制或UDP-葡萄糖再生酶系统。在本发明的实施方案中,用活的微生物细胞进行催化(糖基化),并且使用细胞代谢来进行UDP-葡萄糖辅因子再循环,而无需酶补料或底物补料来进行UDP-葡萄糖再生。

[0063] US 2017/0332673描述过表达MEP途径酶连同下游的甜菊醇生物合成途径的以及UGT酶以驱动由葡萄糖产生RebM的大肠杆菌菌株。然而,这些菌株不进行进料的甜菊醇糖苷中间体至RebM的生物催化,这可能部分是由于宿主细胞无法接近甜菊醇糖苷底物。根据本公开,对微生物细胞的遗传修饰使得糖基化中间体可用于全细胞系统中的进一步糖基化。此外,可从细胞外培养基中回收产物,这有助于产物的下游纯化和加工。

[0064] 在一些实施方案中,所述微生物细胞具有一种或多种遗传修饰,所述遗传修饰增加UDP-葡萄糖可用性。在一些实施方案中,不希望受理论束缚,这些修饰也可使细胞对葡萄糖可用性产生应力,从而导致内源性转运蛋白的表达增加,以将甜菊醇糖苷输入细胞。指数生长的大肠杆菌中的野生型UDP葡萄糖水平是约2.5mM (Bennett BD, Kimball EH, Gao M, Osterhout R, Van dien SJ, Rabinowitz JD. Absolute metabolite concentrations and implied enzyme active site occupancy in Escherichia coli. Nat Chem Biol. 2009; 5(8):593-9.)。在一些实施方案中,对宿主细胞的遗传修饰进行工程化以在指数生长细胞(例如,不具有UGT酶的重组表达)中使UDP-葡萄糖例如增加到至少5mM或至少10mM。

[0065] 在一些实施方案中,微生物细胞具有编码消耗UDP-葡萄糖的酶的基因的缺失、失活或活性或表达降低。例如,微生物细胞可具有微生物物种中ushA (UDP糖水解酶) 和/或galE、galT、galK和galM(其负责来自UDP-葡萄糖的UDP-半乳糖生物合成) 或其直向同源物中的一者或多者的缺失、失活或活性降低。在一些实施方案中,galETKM基因失活、缺失或表达显著降低。或者或另外地,微生物细胞具有微生物物种中大肠杆菌otsA(海藻糖-6-磷酸合酶) 或其直向同源物的缺失、失活或活性或表达降低。或者或另外,微生物细胞具有微生物物种中大肠杆菌ugd (UDP-葡萄糖6-脱氢酶) 或其直向同源物的缺失、失活或活性或表达降低。降低或消除otsA和ugd的活性可分别将UDP-葡萄糖槽除去或还原为海藻糖或UDP-葡萄糖醛酸。

[0066] 其他可减少或消除的UDP-葡萄糖槽包括消除或降低负责脂质糖基化和LPS生物合成的基因以及负责使十一异戊烯二磷酸(UPP)糖基化的基因的活性或表达。参与使脂质糖

基化或LPS生物合成的基因包括大肠杆菌waaG(脂多糖葡萄糖基转移酶1) 大肠杆菌waaO(UDP-D-葡萄糖:(葡萄糖基)LPS $\alpha$ -1,3-葡萄糖基转移酶)) 和大肠杆菌waaJ(UDP-葡萄糖:(葡萄糖基)LPS $\alpha$ -1,2-葡萄糖基转移酶))。负责使十一异戊烯二磷酸(UPP)糖基化的基因包括大肠杆菌yfdG(推定的细菌萜醇连接的葡萄糖转位酶)、大肠杆菌yfdH(细菌萜醇葡萄糖基转移酶)、大肠杆菌yfdI(血清型特异性葡萄糖基转移酶) 和大肠杆菌wcaJ(十一异戊烯-磷酸葡萄糖磷酸转移酶)。这些基因产物中的一种或多种(或微生物细胞中的相应直向同源物)的缺失、失活或活性或表达的降低可增加UDP-葡萄糖可用性。

[0067] 在这些或其它实施方案中,微生物细胞具有编码消耗UDP-葡萄糖的前体的酶的基因的缺失、失活或活性或表达降低。例如,在一些实施方案中,微生物细胞具有宿主细胞的微生物物种中pgi(葡萄糖-6磷酸酯异构酶)或其直向同源物的缺失、失活或活性或表达降低。

[0068] 在这些或其它实施方案中,所述细胞具有一种或多种编码参与将葡萄糖-6-磷酸转化为UDP-葡萄糖的酶的基因的过表达或活性增加。例如,可过表达或修饰pgm(磷酸葡萄糖变位酶)和/或galU(UTP-葡萄糖-1-磷酸尿苷酰转移酶)(或其直向同源物或衍生物)以提高酶生产率。或者或此外,可过表达或修饰大肠杆菌ycjU( $\beta$ -磷酸葡萄糖变位酶)(其将葡萄糖-6-磷酸转化为葡萄糖-1-磷酸)和两歧双歧杆菌ugpA(其将葡萄糖-1-磷酸转化为UDP)或这些酶的直向同源物或衍生物以提高酶生产率。

[0069] 或者或此外,所述微生物细胞具有一种或多种遗传修饰,所述遗传修饰增加至戊糖磷酸途径(PPP)的通量,如大肠杆菌zwf(或其同源物或工程化衍生物)的过表达或活性增加,其是NADP<sup>+</sup>依赖性葡萄糖-6-磷酸脱氢酶。

[0070] 或者或此外,所述微生物细胞具有增加葡萄糖转运的一种或多种遗传修饰。此类修饰包括大肠杆菌galP(半乳糖:H<sup>+</sup>同向转运体) 和大肠杆菌g1k(葡萄糖激酶) 或可替代地运动发酵单胞菌g1f和大肠杆菌g1k或这些基因的同源物、直向同源物或工程化的衍生物的表达或活性增加。

[0071] 或者或此外,所述微生物细胞具有增加UTP产生和再循环的一种或多种遗传修饰。此类修饰包括大肠杆菌pyrH(UMP激酶)、大肠杆菌cmk(胞苷酸激酶)、大肠杆菌adk(腺苷酸激酶) 或大肠杆菌ndk(核苷二磷酸激酶) 或这些酶的同源物、直向同源物或工程化的衍生物的表达或活性增加。

[0072] 或者或此外,所述微生物细胞具有增加UDP产生的一种或多种遗传修饰。此类修饰包括大肠杆菌upp(尿嘧啶磷酸核糖基转移酶)、大肠杆菌dctA(C4二羧酸/乳清酸:H<sup>+</sup>同向转运体)、大肠杆菌pyrE(乳清酸磷酸核糖基转移酶) 和大肠杆菌pyrF(乳清苷-5'-磷酸脱羧酶),包括其同源物、直向同源物或工程化的衍生物中的一者或多者的过表达或活性增加。例如,在一些实施方案中,所述微生物细胞过表达upp、pyrH和cmk或其同源物或工程化的衍生物,或具有增加的upp、pyrH和cmk或其同源物或工程化的衍生物活性。或者,所述微生物细胞过表达dctA、pyrE、pyrH和cmk或其同源物或工程化的衍生物,或具有增加的dctA、pyrE、pyrH和cmk或其同源物或工程化的衍生物活性。

[0073] 或者或此外,所述微生物细胞可具有一种或多种遗传修饰以除去或减少对葡萄糖摄取的调控。例如,所述微生物细胞可具有sgrS的缺失、失活或减少的表达,sgrS是大肠杆菌中的小调控RNA。

[0074] 或者或此外,所述微生物细胞可具有减少葡萄糖-1-磷酸的去磷酸化的一种或多种遗传修饰。示例性修饰包括微生物细胞中大肠杆菌agp(葡萄糖-1-磷酸酶)、大肠杆菌yihX( $\alpha$ -D-葡萄糖-1-磷酸磷酸酶)、大肠杆菌ybiV(糖磷酸酶)、大肠杆菌yidA(糖磷酸酶)、大肠杆菌yigL(磷酸糖磷酸酶)和大肠杆菌phoA(碱性磷酸酶)或其直向同源物中的一者或多者的缺失、失活或表达或活性降低。

[0075] 或者或此外,所述微生物细胞可具有减少葡萄糖-1-磷酸至TDP-葡萄糖的转化的一种或多种遗传修饰。示例性修饰包括微生物细胞中大肠杆菌rffH(dTDP-葡萄糖焦磷酸化酶)和大肠杆菌rfbA(DTDP葡萄糖焦磷酸)或其直向同源物中的一者或多者的缺失、失活或表达或活性降低。

[0076] 或者或此外,所述微生物细胞可具有减少葡萄糖-1-磷酸至ADP-葡萄糖的转化的一种或多种遗传修饰。示例性修饰包括微生物细胞中大肠杆菌gigC(葡萄糖-1-磷酸腺苷酰转移酶)或其直向同源物的缺失、失活或表达或活性降低。

[0077] 在一些实施方案中,所述微生物细胞是包含遗传修饰的细菌细胞:ushA和galETKM缺失、失活或表达降低;pgi缺失、失活或表达降低;并且pgm和galU过表达或互补。

[0078] 在一些实施方案中,对内源性基因进行编辑以通过改变所编码蛋白质的氨基酸序列而失活或降低酶活性,或通过表达控制序列的编辑而降低表达。编辑可修饰内源启动子、核糖体结合序列或其它表达控制序列,和/或在一些实施方案中,修饰基因调控中的反式作用因子和/或顺式作用因子。基因组编辑可以使用CRISPR/Cas基因组编辑技术,或采用锌指核酸酶和TALEN的类似技术进行。在一些实施方案中,通过同源重组置换内源性基因。

[0079] 各种实施方案中的微生物细胞不表达用于产生前体(例如,包含一种或多种植物酶)的重组生物合成途径。例如,对于根据本发明的实施方案的产生RebM(和其他高级糖基化产物)的微生物细胞,不表达甜菊醇生物合成途径,如椰油酰基合酶、月桂烯合酶、月桂烯氧化酶和/或月桂烯酸羟化酶,以使得RebM和其他高级糖基化产物的产生取决于将甜菊醇糖苷中间体进料至细胞。

[0080] 在各种实施方案中,微生物细胞是来自埃希氏菌属、芽孢杆菌属、红杆菌属、发酵单胞菌属或假单胞菌属的细菌。在一些实施方案中,细菌物种选自大肠杆菌、枯草芽孢杆菌、荚膜红细菌、球形红细菌、运动发酵单胞菌或恶臭假单胞菌。在一些实施方案中,细菌细胞是大肠杆菌。

[0081] 在其他实施方案中,细胞是真菌细胞,如酵母细胞,例如酵母属、裂殖酵母属、毕赤酵母属、法夫酵母属、克鲁维酵母属、假丝酵母属、篮状菌属、酒香酵母属、管囊酵母属、德巴利氏酵母属、耶氏酵母属和工业多倍体酵母菌株。在一个实施方案中,酵母可以是酵母属、毕赤酵母属或耶氏酵母属的物种,包括酿酒酵母、巴斯德毕赤酵母和解脂耶氏酵母。在一些实施方案中,酵母细胞表达一种或多种细菌转运蛋白或其衍生物,其将糖苷中间体输入细胞中以进行进一步糖基化。

[0082] 在各种实施方案中,所述微生物细胞具有一种或多种内源性转运蛋白的过表达(例如,与亲本微生物菌株相比),或在某些实施方案中,经修饰以表达重组和/或工程化的转运蛋白。在一些实施方案中,所述微生物细胞表达内源性转运蛋白或其衍生物的一个或多个其他拷贝。例如,转运蛋白的表达或活性可进行修饰以增加甜菊醇糖苷中间体(例如,甜菊苷、甜菊双糖苷和RebA等中的一种或多种)转运到细胞中,同时输出产物,如RebM和/或

RebD和/或其他高级糖基化甜菊醇糖苷。

[0083] 可过表达或工程化以改变微生物细胞中的活性或底物特异性的示例性转运蛋白包括大肠杆菌、acrAD、xylE、ascF、bglF、chbA、ptsG/crr、wzxE、rfbX以及其直向同源物或衍生物。这些蛋白质的衍生物和直向同源物通常包含与大肠杆菌转运蛋白具有至少约30%、或至少约40%、或至少约50%同一性、至少约60%同一性、至少约70%同一性、至少约80%同一性、至少约85%同一性、或至少约90%同一性、或至少约95%同一性、或至少96%、97%、98%或99%同一性的氨基酸序列。

[0084] acrAD是在Aires JR and Nikaido H., Aminoglycosides are captured from both periplasm and cytoplasm by the AcrD multidrug efflux transporter of Escherichia coli, *J Bacteriol.* 2005;187 (6) :1923-9中描述的大肠杆菌多药外排泵。此外,同源物结构描述于Yu, EW等人, Structural basis of multiple drug-binding capacity of the AcrB multidrug efflux pump, *Science* 2003;300 (5621) :976-80中。

[0085] xylE是与葡萄糖转运蛋白具有同源性的木糖转运蛋白。xylE描述于Sumiya M, 等人, Molecular genetics of a receptor protein for D-xylose, encoded by the gene xylF, in Escherichia coli, *Recept Channels* 1995;3 (2) :117-28中,其中结构由Sun L, 等人, Crystal structure of a bacterial homologue of glucose transporters GLUT-1-4, *Nature* 2012;490 (7420) :361-6描述。

[0086] ascF由Hall BG和Xu L, Nucleotide sequence, function, activation, and evolution of the cryptic asc operon of Escherichia coli K12, *Mol. Biol. Evol.* 1992; 9 (4) :688-706描述。所述asc操纵子被认为在大肠杆菌中的纤维二糖代谢中起作用。

[0087] bglF由Schnetz K, 等人, Identification of catalytic residues in the beta-glucoside permease of Escherichia coli by site-specific mutagenesis and demonstration of interdomain cross-reactivity between the beta-glucoside and glucose systems, *J. Biol. Chem.* 1990;265 (23) :13464-71描述。同源结构描述于Herzberg O., An atomic model for protein-protein phosphoryl group transfer, *J. Biol. Chem.* 1992;276 (34) :24819-23中。bglF催化 $\beta$ -葡萄糖苷的转运和磷酸化。

[0088] chbA描述于Keyhani NO, 等人, The transport/phosphorylation of N,N'-diacetylchitobiose in Escherichia coli. Characterization of phosphor-IIB (Chb) and of a potential transition state analogue in the phosphotransfer reaction between the proteins IIA (Chb) and IIB (Chb). *J. Biol. Chem.* 2000;275 (42) :33102-9中;其中结构描述于Tang C, 等人, Solution structure of enzyme IIA (Chitobiose) from the N,N'-diacetylchitobiose branch of the Escherichia coli phosphotransferase system. *J. Biol. Chem.* 2005;280 (12) :11770-80中。磷酸烯醇丙酮酸依赖性糖磷酸转移酶系统(糖PTS)是主要的碳水化合物活性转运系统,其催化进入的糖底物的磷酸化及其伴随的跨细胞膜转运。

[0089] ptsG编码磷酸转移酶转运系统(PTS)的葡萄糖特异性通透酶并且描述于Meins M, 等人, Glucose permease of Escherichia coli. Purification of the IIGIc subunit and functional characterization of its oligomeric forms. *J. Biol. Chem.* 1988;263 (26) :12986-93中。结构描述于Cai M, 等人, Solution structure of the phosphoryl

transfer complex between the signal-transducing protein IIAGlucose and the cytoplasmic domain of the glucose transporter IICBGlucose of the Escherichia coli glucose phosphotransferase system.J.Biol.Chem.2003;278(27):25191-206中。

[0090] wzxE及其在分子转运中的作用由Rick PD,等人,Evidencethat the wzxE gene of Escherichia coli K-12 encodes a protein involved in the transbilayer movement of a trisaccharide-lipid intermediate in the assembly of enterobacterial common antigen.J.Biol.Chem.2003;278(19):16534-42中。

[0091] rfbx是描述于Hong Y,等人,Progress in our understanding of wzx flippase for translocation of bacterialmembranelipid-linked oligosaccharide.J.Bacteriol.2018;200(1)中的脂多糖转运蛋白。

[0092] 其他转运蛋白包括选自细菌或内源性转运蛋白的那些,其将所需的糖苷中间体转运到细胞中,和/或将所需的产物转运出细胞。例如,转运蛋白可来自宿主物种,或另一种细菌或酵母物种,并且可被工程化以调节其对特定糖苷中间体或产物的亲和力。例如,宿主细胞可过表达与选自以下的大肠杆菌转运蛋白至少约30%、或至少约40%、或至少约50%相同的转运蛋白:ampG、araE、araJ、bcr、cynX、emrA、emrB、emrD、emrE、emrK、emrY、entS、exuT、fsr、fucP、galP、garP、glpT、gudP、gudT、hcaT、hsrA、kgtP、lacY、lgoT、lplT、lptA、lptB、lptC、lptD、lptE、lptF、lptG、mdfA、mdtD、mdtG、mdtH、mdtM、mdtL、mhpT、msbA、nanT、narK、narU、nepI、nimT、nupG、proP、setA、setB、setC、shiA、tfaP、tolC、tsgA、uhpT、xapB、xylE、yaaU、yajR、ybjJ、ycaD、ydeA、ydeF、ydfJ、ydhC、ydhP、ydjE、ydjK、ydiM、ydiN、yebQ、ydcO、yegT、yfaV、yfcJ、ygaY、ygcE、ygcS、yhhS、yhjE、yhjX、yidT、yihN、yjhB和lynfM。在一些实施方案中,所述转运蛋白与大肠杆菌转运蛋白至少约60%、至少约70%、至少约80%、至少约90%或至少约95%相同。

[0093] 在一些实施方案中,微生物细胞表达与来自真核细胞如酵母、真菌或植物细胞的转运蛋白至少50%相同的转运蛋白。在一些实施方案中,转运蛋白是ABC家族转运蛋白,并且其任选地是亚类PDR(多效耐药性)转运蛋白、MDR(多药耐药性)转运蛋白、MFS家族(主要协助转运蛋白超家族)转运蛋白或SWEET(又名PQ-环、唾液或MtN3家庭)家族转运蛋白。在其他实施方案中,转运蛋白是选自以下的家族:AAAP、Su1P、LCT、APC、MOP、ZIP、MPT、VIC、CPA2、ThrE、OPT、Trk、BASS、DMT、MC、AEC、Amt、Nramp、TRP-CC、ACR3、NCS1、PiT、ArsAB、IISP、GUP、MIT、Ctr和CDF。

[0094] 在一些实施方案中,转运蛋白是ABC家族转运蛋白(又称ATP结合盒转运蛋白),其通常包含多个亚基,所述亚基中的一个或两个是跨膜蛋白,并且所述亚基中的一个或两个是膜相关ATP酶。ATP酶亚基利用三磷酸腺苷(ATP)结合和水解的能量来激发各种底物跨膜的易位,以摄取或输出底物。ABC家族转运蛋白可以是任何子类,包括但不限于:ABCA、ABCB、ABCC、ABCD、ABCE、ABCF和ABCG。

[0095] 在一些实施方案中,转运蛋白是MFS家族转运蛋白(又称主要协助转运蛋白超家族),其是能够响应于化学渗透离子梯度转运小溶质的单多肽辅助载体。通过MFS转运蛋白转运的化合物可包括单糖、寡糖、肌醇、药物、氨基酸、核苷、有机磷酸酯、克雷布斯循环代谢物以及各种有机和无机阴离子和阳离子。作为举例,MFS转运蛋白包括Xy1E(来自大肠杆菌)、QacA(来自金黄色葡萄球菌)、Bmr(来自枯草芽孢杆菌)、UhpT(来自大肠杆菌)、LacY(来

自大肠杆菌)、FucP(来自大肠杆菌)和ExtU(来自大肠杆菌)。

[0096] 在一些实施方案中,转运蛋白是转运蛋白的SWEET(糖最终将是输出转运蛋白)家族(又称PQ-环、唾液或MtN3家族),其是糖转运蛋白家族并且是TOG超家族的成员。SWEET的真核生物家族成员具有呈3+1+3重复序列排列的7个跨膜片段(TMS)。作为举例,SWEET转运蛋白包括SWEET1、SWEET2、SWEET9、SWEET12、SWEET13和SWEET14。

[0097] 在一些实施方案中,转运蛋白与来自酿酒酵母的转运蛋白至少50%相同。在一些实施方案中,转运蛋白与酿酒酵母转运蛋白至少约60%、至少约70%、至少约80%、至少约90%或至少约95%相同。示例性酿酒酵母转运蛋白包括AC1、ADP1、ANT1、AQR1、AQY3、ARN1、ARN2、ARR3、ATG22、ATP4、ATP7、ATP19、ATR1、ATX2、AUS1、AVT3、AVT5、AVT6、AVT7、AZR1、CAF16、CCH1、COT1、CRC1、CTR3、DAL4、DNF1、DNF2、DTR1、DUR3、ECM3、ECM27、ENB1、ERS1、FEN2、FLR1、FSF1、FUR4、GAP1、GET3、GEX2、GGC1、GUP1、HOL1、HCT10、HXT3、HXT5、HXT8、HXT9、HXT11、HXT15、KHA1、ITR1、LEU5、LYP1、MCH1、MCH5、MDL2、MME1、MNR2、MPH2、MPH3、MRS2、MRS3、MTM1、MUP3、NFT1、OAC1、ODC2、OPT1、ORT1、PCA1、PDR1、PDR3、PDR5、PDR8、PDR10、PDR11、PDR12、PDR15、PDR18、PDR1、PDR11、PET8、PHO89、PIC2、PMA2、PMC1、PMR1、PRM10、PUT4、QDR1、QDR2、QDR3、RCH1、SAL1、SAM3、SBH2、SE01、SGE1、SIT1、SLY41、SMF1、SNF3、SNQ2、SPF1、SRP101、SSU1、STE6、STL1、SUL1、TAT2、THI7、THI73、TIM8、TIM13、TOK1、TOM7、TOM70、TPN1、TP01、TP02、TP03、TP04、TRK2、UGA4、VBA3、VBA5、VCX1、VMA1、VMA3、VMA4、VMA6、VMR1、VPS73、YEA6、YHK8、YIA6、YMC1、YMD8、YOR1、YPK9、YVC1、ZRT1、YBR241C、YBR287W、YDR061W、YDR338C、YFR045W、YGL114W、YGR125W、YIL166C、YKL050C、YMR253C、YMR279C、YNL095C、YOL075C、YPR003C和YPR011C。

[0098] 在一些实施方案中,酿酒酵母转运蛋白选自以下中的一者或多者:ADP1、AQR1、ARN1、ARN2、ATR1、AUS1、AZR1、DAL4、DTR1、ENB1、FLR1、GEX2、HOL1、HXT3、HXT8、HXT11、NFT1、PDR1、PDR3、PDR5、PDR8、PDR10、PDR11、PDR12、PDR15、PDR18、QDR1、QDR2、QDR3、SE01、SGE1、SIT1、SNQ2、SSU1、STE6、THI7、THI73、TIM8、TPN1、TP01、TP02、TP03、TP04、YHK8、YMD8、YOR1和YVC1。在一些实施方案中,酿酒酵母转运蛋白选自FLR1、PDR1、PDR3、PDR5、PDR10、PDR15、SNQ2、TP01和YOR1中的一者或多者。

[0099] 在一些实施方案中,转运蛋白与XP\_013706116.1(来自欧洲油菜)、NP\_001288941.1(来自茺菁)、NEC1(来自矮牵牛)和SWEET13(来自乌拉尔图小麦)至少50%、至少60%相同、至少70%相同、至少80%相同或至少90%或95%相同。

[0100] 在关于RebM的生物合成的各种实施方案中,所述方法产生甜菊苷、甜菊双糖苷和RebA至RebM的至少40%转化或至少50%转化或至少75%转化。在一些实施方案中,RebM与RebD的比例是至少2:1、或至少4:1、或至少6:1、或至少8:1、或至少9:1、或至少10:1、或至少15:1、或至少20:1。

[0101] 所述方法可通过分批发酵、补料-分批发酵、连续发酵或半连续发酵进行。例如,在一些实施方案中,所述方法通过分批发酵或补料-分批发酵进行,其中孵育时间小于约72小时,或者在一些实施方案中小于约48小时或小于约24小时。

[0102] 虽然天然UGT酶通常是植物酶(其通常具有在20°C-24°C范围内的最佳温度),或源自植物酶,但在一些实施方案中,本公开使得能够在微生物细胞(例如,细菌细胞,如大肠杆菌)中以高收率产生糖基化产物,其中酶生产率在约24°C或更高的温度下,如约24°C至约37

℃、或约27℃至约37℃、或约30℃至约37℃。

[0103] 在一些实施方案中,生长期或生产期培养基可含有一种或多种洗涤剂,其量足以增强细胞渗透性,而对生长或存活力没有显著影响。示例性洗涤剂包括Tween 20、Triton X-100和SDS等。

[0104] 在一些实施方案中,所述方法可扩展用于大规模生产。例如,在一些实施方案中,培养的大小是至少约100L、至少约200L、至少约500L、至少约1,000L、或至少约10,000L。

[0105] 在各个实施方案中,方法还包括从细胞培养物或从细胞裂解物中回收糖基化产物。在一些实施方案中,培养产生至少约100mg/L、或至少约200mg/L、或至少约500mg/L、或至少约1g/L、或至少约2g/L、或至少约5g/L、或至少约10g/L、或至少约20g/L、或至少约30g/L、或至少约40g/L、或至少约50g/L的糖基化产物,其在一些实施方案中是从培养基提取的。

[0106] 在一些实施方案中,糖基化产物(例如,RebM)是从培养基组分纯化的。因此,在一些实施方案中,所述方法包括从宿主细胞分离生长培养基,并从生长培养基分离所需的糖基化产物(例如,RebM)。在一些实施方案中,从细胞材料进一步提取诸如RebM的产物。

[0107] 在一些方面,本发明提供用于制备包含糖基化产物(如RebM)的产物的方法。所述方法包括将目标甜菊醇糖苷(根据本公开产生)掺入产品,如食品、饮料、口腔护理产品、甜味剂、调味剂或其他产品中。根据本发明制备的纯化的甜菊醇糖苷可用于多种产品中,包括但不限于食品、饮料、纹理剂(例如,淀粉、纤维、树胶、脂肪和脂肪模拟物和乳化剂)、药物组合物、烟草产品、营养食品组合物、口腔卫生组合物和美容组合物。对其而言可组合使用RebM的调味剂的非限制性实例包括酸橙、柠檬、桔子、水果、香蕉、葡萄、梨、菠萝、芒果、苦杏仁、可乐果、肉桂、糖、棉花糖和香草调味剂。其它食品成分的非限制性实例包括调味剂、酸化剂和氨基酸、着色剂、增量剂、改性淀粉、树胶、调质剂、防腐剂、抗氧化剂、乳化剂、稳定剂、增稠剂和胶凝剂。

[0108] 在一些方面,本发明提供了用于制备包含多种高甜度甜味剂的甜味剂产品的方法,所述多种高甜度甜味剂如包括甜菊醇糖苷、罗汉果苷、三氯蔗糖、阿斯巴甜、纽甜、爱德万甜、乙酰磺胺酸钾、糖精、环己基氨基磺酸盐、新橙皮苷二氢查尔酮、买麻藤素E和/或白皮杉醇4'-O-β-D-吡喃葡萄糖苷中的两种或更多种。所述方法还可包括将甜味剂产品掺入食品、饮料、口腔护理产品、甜味剂、调味剂或其他产品中,包括上述那些。

[0109] 一种或多种目标甜菊醇糖苷(如RebM)和包含所述目标甜菊醇糖苷的甜味剂组合物可与各种生理活性物质或功能性成分组合使用。功能成分通常分为以下几类诸如:类胡萝卜素、膳食纤维、脂肪酸、皂苷、抗氧化剂、营养食品、类黄酮、异硫氰酸酯、酚、植物固醇和甾醇(植物甾醇和植物甾烷醇);多元醇;益生元、益生菌;植物雌激素;大豆蛋白;硫化物类/硫醇类;氨基酸;蛋白质;维生素;和矿物质。功能成分也可以基于其健康益处进行分类,诸如心血管、降胆固醇和消炎益处。

[0110] 此外,根据本发明获得的一种或多种目标甜菊醇糖苷如RebM和甜味剂组合物可作为高甜度甜味剂应用以产生口味特征改善的零卡路里、低卡路里或糖尿病饮料和食物产品。它也可用于其中不能使用糖的饮料、食品、药品和其它产品中。此外,RebM和甜味剂组合物不仅可用作饮料、食品和其它专供人消耗的产品中的甜味剂,还可用作特征改善的动物饲料和草料中的甜味剂。

[0111] 其中可使用目标甜菊醇糖苷和甜味剂组合物的产品的实例包括但不限于酒精饮

料,诸如伏特加、葡萄酒、啤酒、烈酒和清酒等;天然果汁;清凉饮料;碳酸软饮料;减肥饮料;零卡路里饮料;低卡路里饮料和食品;酸奶饮料;速溶果汁;速溶咖啡;粉状速溶饮料;罐头产品;糖浆;发酵豆瓣酱;酱油;醋;调味品;蛋黄酱;番茄酱;咖喱;汤;速食肉汤;酱油粉;醋粉;各类饼干;大米饼干;薄脆饼干;面包;巧克力;焦糖;糖果;口香糖;果冻;布丁;果蔬脯;鲜奶油;果酱;柑橘酱;花膏;奶粉;冰淇淋;果汁冰糕;瓶装果蔬;罐装煮豆;用甜酱烹煮的肉类和食品;农业蔬菜食品;海鲜;火腿;香肠;鱼肉火腿;鱼肉香肠;鱼酱;油炸鱼制品;干海鲜产品;冷冻食品;腌制海带;腌肉;烟草;药品;及许多其它产品。

[0112] 在诸如食品、饮料、药品、化妆品、桌面产品和口香糖等产品的制造过程中,可以使用常规方法诸如混合、揉捏、溶解、酸洗、渗透、渗滤、喷洒、雾化、灌注和其它方法。

[0113] 本发明的实施方案在以下非限制性实施例中展示。

[0114] 实施例

[0115] 表2示出三批甜叶菊叶提取物的甜菊醇糖苷含量。RebM途径中的两种中间体RebA和甜菊苷是批次中的两种主要糖苷。

[0116] 表2:可用甜叶菊叶提取物的甜菊醇糖苷组成

%	批次1	批次2	批次3
莱鲍迪苷A	38.2	10.5	30.3
甜菊苷	8.5	9.0	18.4
莱鲍迪苷C	12.9	4.2	16.6
莱鲍迪苷B	4.3	7.1	1.2
甜茶苷	5.0	2.2	2.0
莱鲍迪苷F	2.0	2.7	2.1
甜菊双糖苷	0.3	3.7	0.3
莱鲍迪苷D	0.2	2.1	0.9
杜克苷A	0.9	0.4	0.5

[0118] 图4示出糖基化甜菊醇糖苷中间体的生物转化。在实验中,将0.2mM甜菊双糖苷进料至96孔板中的工程化的大肠杆菌菌株。在48小时后检查产物形成。甚至早期中间体如甜菊双糖苷的全细胞生物转化是可能的,但对于天然大肠杆菌而言,UGT酶不可获得糖基化底物(菌株5显示显著活性,而菌株3和4显示可忽略的活性)。菌株的细节在图8中描述。图4中报告的值是相对于对照的化合物浓度(对于空载体对照,每种化合物的浓度除以这些化合物的总浓度)。菌株5中所含的修饰证明甜菊双糖苷至RebM的生物转化。

[0119] 菌株1-5中的每一者均包含BAC(细菌人工染色体),即单拷贝质粒,其分别表达四种UGT酶: MbUGTC13、MbUGT1.2\_2、MbUGTc19\_2和76G1\_L200A。对照含有相同的BAC主链,但不含UGT酶。

[0120] 图8示出遗传修饰以增加至UDP-葡萄糖(其是RebM途径中UGT的关键底物)的天然大肠杆菌通量。通过增加可用于UGT的底物的量,大于至UDP-葡萄糖的天然通量可允许更多的RebM形成。产生菌株5的修饰还产生细胞将进料甜菊醇糖苷转化为RebM的能力。对于菌株2和3,消耗UDP葡萄糖的酶(ushA, galETKM)缺失。通过缺失消耗UDP葡萄糖的前体,即葡萄糖-6-磷酸(G6P)的酶(pgi),从菌株3构建了菌株4。菌株5是通过过表达用于经由葡萄糖-1-磷酸(G1P)将G6P转化为UDP-葡萄糖的两种酶(pgm, galU)而从菌株4构建的。

[0121] 图5示出叶提取物中发现的两种市售中间体,甜菊苷和RebA至RebM的转化。还示出甜菊醇核心的生物转化。所述值报告为总甜菊醇糖苷组合物的百分比。所有菌株将甜菊醇转化为RebM表明,所有菌株都能够形成RebM。与甜菊双糖苷的情况一样,甜菊苷和RebA均被转化为RebM,但仅在菌株5中。很可能只有菌株5使甜菊醇糖苷可用于UGT酶。对于从甜菊苷和RebA转化,RebD:RebM的比例强烈有利于RebM(1:20)。RebM被分泌到培养基中。

[0122] 图6示出96孔板中甜菊醇糖苷的生物转化。对于这些研究,将76G1\_L200A用MbUGT1-3\_1.5替代。对于RebM途径中的五种主要化合物,在有或没有表达的UGT酶情况下示出化合物浓度。向菌株进料0.5g/L的甜菊苷/RebA叶提取物,并在取样前与底物一起孵育48小时。如图所示,所述细胞几乎只产生RebM,仅含有少量RebI和RebD。

[0123] 将所述菌株用于初始生物反应器实验。具体而言,将产生RebM的菌株从工作细胞库接种在50mL离心管中,所述离心管含有10mL的LB(Luria-Bertani)肉汤和合适的抗生素。将第一种子在37°C下在振荡孵育箱中培养20小时。在20小时后,然后通过使用第一种子在500mL锥形瓶中接种100mL发酵培养基来开始第二种子培养。在转移至各自含有170mL发酵培养基的生物反应器之前,将这种第二种子培养10小时。每10小时进行采样。使用LC-MS-MS和YSI对培养基中的相关代谢物进行定量。经由分光光度计在600nm的吸光度下测量细胞密度。图7示出在生物反应器规模上甜菊苷/RebA叶提取物的生物转化。对于RebM途径中的五种主要化合物,在整个发酵过程中的不同时间点对培养基中的化合物浓度进行采样。向菌株进料2g/L的甜菊苷/RebA叶提取物,并与底物一起孵育总共30小时。如图7所示,甜菊苷和RebA随时间推移而丢失,而高糖基化产物如RebM、RebI和RebD相应增加。

[0124] 将途径中间体(如甜菊双糖苷、甜菊苷和RebA)转化为RebM的UGT酶在大肠杆菌细胞质中表达,并且因此需要中间体可能通过转运蛋白或通过增加的膜渗透性的作用而可用于UGT酶。

[0125] 甜菊醇糖苷是可能不会被天然大肠杆菌菌株摄取的大分子。这将解释通过菌株1进行的甜菊双糖苷至RebM的可忽略的转化。菌株1-4可摄取较早的非糖基化中间体(甜菊醇)并将其转化为RebM,从而表明可忽略转化的原因是缺乏甜菊醇糖苷至细胞质的摄取,而不是在这些条件下途径中间体上UGT酶的失活。

[0126] 序列

[0127] >SrUGT85C2[甜叶菊]

[0128] (SEQ ID NO:1)

[0129] MDAMATTEKKPHVIFIPFPAQSHIKAMLKLAQLLHHKGLQITFVNTDFIHNQFLESSGPHCLDGAPGFR  
FETIPDGVSHSPEASIPRESLLRSIETNFLDRFIDLVTKLDPPTCIISDGFLSVFTIDAACKLGI PVM MYWTLAA  
CGFMGFYHIHSLIEKGFAPLKDASYLTNGYLDTVIDWVPGMEGIRLKDPLDWSTDLNDKVLMTTEAPQRSHKVS  
HIFHTFDELEPSIIKTLRLYNIHYTIGPLQLLLDQIPEEKKQTGITSLHGYSLVKEEPECFQWLQSKEPNSVYV  
FGSTTVMSLEDMEFGWGLANSNHYFLWIIIRSNLVIGENAVLPPELEEHIKKRGFIASWCSQEKVLKHPVGGFLTH  
CGWGSTIESLSAGVPMICWPYSWDQLTNCRYICKEWVGLMGTQKVRDEVKRLVQELMGEVGHKMRNKAKDWKEKA  
RIAIAPNGSSSLNIDKMVKEITVLARN

[0130] >SrUGT74G1[甜叶菊]

[0131] (SEQ ID NO:2)

[0132] MAEQKIKKSPHVLLIPFPLQGHINPFIQFGKRLISKGVKTTLVTTIHTLNSTLNHSNTTTTSIEIQAI

SDGCDEGGFMSAGESYLETFKQVGSKSLADLIKKLQSEGTTIDAI IYDSMTEWVLDVAIEFGIDGGSFFTQACVVNS  
 LYHVHVKGLISLPLGETVSVPGFPVLQRWETPLILQNHEQIQSPWSQMLFGQFANIDQARWFTNSFYKLEEEVIEW  
 TRKIWNLKVIGPTLPSMYLDRKRLDDDKDNGFNLYKANHHECMNWLDDKPKESVYVAFGSLVKHGPEQVEEITRALI  
 DSDVNFLWVIKHKEEGKLPENLSEVIKTGKGLIVAWCKQLDVLAHESVGCFTVTHCGFNSTLEAISLGVVAMPQFS  
 DQTTNAKLLDEILGVGVVKADENGIVRRGNLASC IKMIMEEERGVI IRKNAVKKDLAKVAVHEGGSSDNDIVEFV  
 SELIKA

[0133] >SrUGT76G1 [甜叶菊]

[0134] (SEQ ID NO:3)

[0135] MENKTETTIVRRRRIILFPVPFQGHINPILQLANVLYSKGFSITIFHTNFKPKTSNYPHFTFRFILDN  
 DPQDERISNLPTHGPLAGMRIPIINEHGADLRRELELLMLASEEDEEVSCLITDALWYFAQSVADSLNLRRLVLMT  
 SSLFNFAHVSPLQFDELGYLDPDDKTRLEEQASGFPMKVKDIKSAYSNWQILKEILGKMIKQTKASSGVIWNSFK  
 ELEESELETVIREIPAPSFLIPLPKHLTASSSSLLDHDRTVFQWLDQQPPSSVLVVSFGSTSEVDEKDFLEIARGLV  
 DSKQSFLWVVRPGFVKGSTWVEPLPDGFLGERGRIVKWPQQEVLAHGAIGAFWTHSGWNSTLESVCEGVPMIFSDF  
 GLDQPLNARYMSDVLKGVVYLENGWERGEIANA IRRVMVDEEGEYIRQNARVLKQKADVSLMKGSSYESLESLSVSY  
 ISSL

[0136] >SrUGT91D1 [甜叶菊]

[0137] (SEQ ID NO:4)

[0138] MYNVTYHQNSKAMATSDSIVDDRKQLHVATFPWLAFGHILPFLQLSKLIAEKGHKVSFLSTTRNIQRLS  
 SHISPLINVVQLTLPRVQELPEDAEATTDVHPEDIQYLKKAVDGLQPEVTRFLEQHSPDWI IYDFTHYWLPSIAASL  
 GISRAYFCVITPWTIAYLAPSSDAMINDSDGRTTVEDLTTPPKWFPFPTKVCWRKHDLARMEPYEAPGISDGYRMGM  
 VFKGSDCLLFKCYHEFGTQWLPLETLHQVPVVPVGLLPEIPGDEKDETWVS IKKWLGDGKQKGSVVYVALGSEALV  
 SQTEVVELALGLELSGLPFVWAYRKPKGPAKSDSVELPDGFVERTRDRGLVWTSWAPQLRILSHESVCGFLTHCGSG  
 SIVEGLMFGHPLIMLPIFCDQPLNARLLEDKQVGEIPRNEEDGCLTKESVARSLRSVVVENEGEIYKANARALSKI  
 YNDTKVEKEYVSQFVDYLEKNARAVAI DHES

[0139] >SrUGT91D2 [甜叶菊]

[0140] (SEQ ID NO:5)

[0141] MATSDSIVDDRKQLHVATFPWLAFGHILPYLQLSKLIAEKGHKVSFLSTTRNIQRLS SHISPLINVVQL  
 TLPRVQELPEDAEATTDVHPEDI PYLKKASDGLQPEVTRFLEQHSPDWI IYDYTHYWLPSIAASLGISRAHFSVTTT  
 WAIAYMGPSADAMINGS DGRTTVEDLTTPPKWFPFPTKVCWRKHDLARLVYKAPGISDGYRMGLVLKGSDCLLSKC  
 YHEFGTQWLPLETLHQVPVVPVGLLPEVPGDEKDETWVS IKKWLGDGKQKGSVVYVALGSEVLVSQTEVVELALGL  
 ELSGLPFVWAYRKPKGPAKSDSVELPDGFVERTRDRGLVWTSWAPQLRILSHESVCGFLTHCGSGSIVEGLMFGHPL  
 IMLPIFGDQPLNARLLEDKQVGEIPRNEEDGCLTKESVARSLRSVVVEKEGEIYKANARELSKIYNDTKVEKEYVS  
 QFVDYLEKNTRAVAI DHES

[0142] >SrUGT91D2e [甜叶菊]

[0143] (SEQ ID NO:6)

[0144] MATSDSIVDDRKQLHVATFPWLAFGHILPYLQLSKLIAEKGHKVSFLSTTRNIQRLS SHISPLINVVQL  
 TLPRVQELPEDAEATTDVHPEDI PYLKKASDGLQPEVTRFLEQHSPDWI IYDYTHYWLPSIAASLGISRAHFSVTTT  
 WAIAYMGPSADAMINGS DGRTTVEDLTTPPKWFPFPTKVCWRKHDLARLVYKAPGISDGYRMGLVLKGSDCLLSKC  
 YHEFGTQWLPLETLHQVPVVPVGLLPEIPGDEKDETWVS IKKWLGDGKQKGSVVYVALGSEVLVSQTEVVELALGL

ELSGLPFVWAYRKPKGPAKSDSVELPDGFVERTDRGLVWTSWAPQLRILSHESVCGFLTHCGSGSIVEGLMFGHPL  
IMLPIFGDQPLNARLLEDKQVIGIEIPRNEEDGCLTKESVARSLRSVVVEKEGEIYKANARELSKIYNDTKVEKEYVS  
QFVDYLEKNARAVAIDHES

[0145] >OsUGT1-2[水稻(*Oryza sativa*)]

[0146] (SEQ ID NO:7)

[0147] MDSGYSSSYAAAAGMHVVICPWLAFGHLLPCLDLAQLASRGHRVSFVSTPRNISRLPPVRPALAPLVA  
FVALPLPRVEGLPDGAESTNDVPHDRPDMVELHRRAFDGLAAPFSEFLGTACADWVIDVVFHHWAAAAALEHKVPCA  
MMLLGSAHMIASADRRLERAETESPAAGQGRPAAAPTFEVARMKLIRTKGSSGMSLAERFSLTLRSSSLVVGRC  
VEFEPETVPLLSTLRGKPIITFLGLMPPLHEGRREDGEDATVRWLDAQPAKSVVYVALGSEVPLGVEKVHELALGLEL  
AGTRFLWALRKPTGVSDADLLPAGFEERTRGRGVVATRWVPQMSILAAHAAVGAFLTHCGWNSTIEGLMFGHPLIMLP  
IFGDQGPNARLIEAKNAGLQVARNDGDGFSFDREGVAAAIRAVAVEEESKVFQAKAKKLQEIIVADMACHERYIDGFI  
QQLRSYKD

[0148] >MBUGTC19[环状变换体]

[0149] (SEQ ID NO:8)

[0150] MAECMNWLDDPKKESVVYVAFGSLVKHGPEQVEEITRALIDSDVNFLWVIKHKEEGKLPENLSEVIKTG  
KGLIVAWCKQLDVLAHESVGCFTVTHCGFNSTLEAISLGVVAMPQFSDQTTNAKLLDEILGVGVRVKADENGIVRR  
GNLASCIKMIMEEERGVIIIRKNAVKKWDLAKVAVHEGGSSNDIVEFVSELIKAGSGEQQKIKKSPHVLLIPFPLQG  
HINPFIQFGKRLISKGVKTTLVTTIHTLNSTLNHSNTTTTSIEIQAISDGCDEGGFMSAGESYLETFKQVGSKSLAD  
LIKKLQSEGTTIDAIYDSMTEWVLDVAIEFGIDGGSFFTQACVVNSLYYHVHKGLISLPLGETVSVPGFPVLQRWE  
TPLILQNHEQIQSPWSQMLFGQFANIDQARWVFTNSFYKLEEEVIEWTRKIWNLKVIGPTLPSMYLDRKRLDDDKDNG  
FNLYKANHH

[0151] >MBUGT1,2[环状变换体]

[0152] (SEQ ID NO:9)

[0153] MAGSSGMSLAERFSLTLRSSSLVVGRCVEFEPETVPLLSTLRGKPIITFLGLMPPLHEGRREDGEDATV  
RWLDAQPAKSVVYVALGSEVPLGVEKVHELALGLELAGTRFLWALRKPTGVSDADLLPAGFEERTRGRGVVATRWVP  
QMSILAAHAAVGAFLTHCGWNSTIEGLMFGHPLIMLPIFGDQGPNARLIEAKNAGLQVARNDGDGFSFDREGVAAAIRA  
VAVEEESKVFQAKAKKLQEIIVADMACHERYIDGFIQQLRSYKDDSGYSSSYAAAAGMHVVICPWLAFGHLLPCLDL  
AQLASRGHRVSFVSTPRNISRLPPVRPALAPLVAFVALPLPRVEGLPDGAESTNDVPHDRPDMVELHRRAFDGLAA  
PFSEFLGTACADWVIDVVFHHWAAAAALEHKVPCAMMLLGSAHMIASADRRLERAETESPAAGQGRPAAAPTFEV  
ARMKLIRTK

[0154] >MBUGT1-3[环状变换体]

[0155] (SEQ ID NO:10)

[0156] MANWQILKEILGKMIKQTKASSGVIWNSFKELEESELETVIREIPAPSFLIPLPKHLTASSSSLLDHDR  
TVFQWLDQQPPSSVLYVSFGSTSEVDEKDFLEIARGLVDSKQSFLWVVRPGFVKGSTWVEPLPDGFLGERGRIVKVV  
PQQEVLAHGAIGAFWTHSGWNSTLESVCEGVPMIFSDFGLDQPLNARYMSDVLKGVYLENGWERGEIANAIRRVMV  
DEEGEYIRQNARVLKQKADVSLMKGGSSYESLESLSVSYISSLENKTETTIVRRRRRIILFPVPFQGHINPILQLANVL  
YSKGFSTITFHTNFNKPKTSNYPHFTFRFILDNDPQDERISNLPTHGPLAGMRIPINEHGADELRRLELEMLLASE  
EDEEVSLITDALWYFAQSVADSLNLRRLVLMTSSLFNFAHVSLPQFDELGYLDPDDKTRLEEQASGFPMKVKVDI  
KSAYS

[0157] >MbugT1,2.2[环状变换体]

[0158] (SEQ ID NO:11)

[0159] MATKGSSGMSLAERFWLTLRSLLVVGRCVFEFEPETVPLLSTLRGKPIITFLGLMPPLHEGRREDGEDA  
TVRWLDAQPAKSVVYVALGSEVPLGVEKVHELALGLELAGTRFLWALRKPTGVSDADLLPAGFEERTRGRGVVATRW  
VPQMSILAHAAVGAFLTHCGWNSTIEGLMFGHPLIMLPIFGDQGPNARLIEAKNAGLQVARNDGDGSDREGVAAAI  
RAVAVEEESKVFQAKAKKLQEIVADMACHERYIDGFIQQRLRSYKDDSGYSSSYAAAAGMHVVICPWLAFGHLLPCL  
DLAQRLASRGRVSVFSTPRNISRLPPVRPALAPLVAFVALPLPRVEGLPDGAESTNDVPHDRPDMVELHRRAFDGL  
AAPFSEFLGTACADWVIVDVFHHWAAAAALEHKVPCAMMLLGSAAEMIASIADERLEHAETESPAAGQGRPAAAPT  
EVARMKLIR

[0160] >MbugTC19-2[环状变换体]

[0161] (SEQ ID NO:12)

[0162] MANHHECMNWLDDKPKESVVYVAFGSLVKHGPEQVEEITRALIDSDVNFLWVIKHKEEGKLPENLSEVI  
KTGKGLIVAWCKQLDVLAHESVGCFTVTHCGFNSTLEAISLGVPPVAMPQFSDQTTNAKLLDEILGVGRVKADENGI  
VRRGNLASCIKMIMEEERGVIIRKNAVKKDLAKVAVHEGGSSDNDIVEFVSELIKAGSGEQQKIKKSPHVLLIPFP  
LQGHINPFIQFGKRLISKGVKTTLVTTIHTLNSTLNHSNTTTSIEIQAISDGCDEGGFMSAGESYLETFKQVGSKS  
LADLIKKLQSEGTTIDAIYDSMTEWVLDVAIEFGIDGGSFFTQACVVNSLYYHVHKGLISLPLGETVSVPGFPVLQ  
RWETPLILQNHQIQSPWSQMLFGQFANIDQARWVFTNSFYKLEEEVIEWTRKIWNLKVIGPTLPSMYLDKRLDDDK  
DNGFNLYKA

[0163] >MbugTC13(甜叶菊UGT85C2,P215T)

[0164] (SEQ ID NO:13)

[0165] MADAMATTEKKPHVIFIPFPAQSHIKAMLKLAQLLHHKGLQITFVNTDFIHNQFLESSGPHCLDGAPGF  
RFETIPDGVSHSPEASIPRESLLRSIETNFLDRFIDLVTKLPDPPTCIISDGFSLVFTIDAACKLGI PVMMYWT  
LACGFMGFYHIHSLIEKGFAPLKDASYLTNGYLDTVIDWVPGMEGIRLKDPLDWSTDLNDKVLMTTEATQRSHKVS  
HHIFHTFDELEPSIIKTLSLRYNHIYTI GPLQLLLDQIPEEKKQTGITSLHGYSLVKEEPECQWLQSKPENSVVYV  
NFGSTTVMSLEDMTEFGWGLANSNHYFLWIRSNLVIGENAVLPPELEEHIKKRGFIASWCSQEKVLKHPVGGFLT  
HCGWGSTIESLSAGVPMICWPYSWDQLTNCRYICKEWVGLMGTKVKRDEVKRLVQELMGEQGHKMRNKAKDWKEK  
ARIAIAPNGSSSLNIDKMVKEITVLARN

[0166] >76G1\_L200A[甜叶菊,L200A]

[0167] (SEQ ID NO:14)

[0168] MAENKTETT VRRRRRIILFPVPFQGHINPILQLANVLYSKGFSITIFHTNFKPKTSNYPHFTFRFILD  
NDPQDERISNLPHGPLAGMRIPIINEHGADELRRLELLMLASEEDEVSLITDALWYFAQSVADSLNLRRLVLM  
TSSLFNFAHVSLPQFDELGYLDPDDKTRLEEQASGFPMKVKDIKSAYSNWQIAKEILGKMIKQTKASSGVIWNSF  
KELEESELETVIREIPAPSFLIPLPKHLTASSSSLLDHRTVFQWLDQQPPSSVLYVSGSTSEVDEKDFLEIARGL  
VDSKQSFLWVVRPGFVKGSTWVEPLPDGFLGERGRIVKWPQQEVLAHGAIGAFWTHSGWNSTLESVCEGVPMIFSD  
FGLDQPLNARYMSDVLKGVYLENGWERGEIANAIRRVMVDEEGEYIRQNARVLKQKADVSLMKGSSYESLESLSV  
YISSL

[0169] >MbugT1-3\_1[环状变换体]

[0170] (SEQ ID NO:15)

[0171] MAFLWVVRPGFVKGSTWVEPLPDGFLGERGRIVKWPQQEVLAHGAIGAFWTHGGWNSTLESVCEGVPM

IFSDFGLDQPLNARYMSDVLKGVVYLENGWERGEIANAIRRLMVDEEGEYIRQNARVLKQKADVSLMKGGSSYESLE  
SLVSYISSLGGSGGSGRRRRIILFPVPFQGHINPMLQLANVLYSKGFSITIFHTNFKPKTSNYPHFTFRFILDN  
DPQDERISNLPTHGLAGMRIPINEHGADELRRLELMLASEEDEEVSLITDALWYFAQSVADSLNLRRLVLMT  
SSLFNFHAHVSLPQFDELGYLDPDDKTRLEEQASGFPMKVKDIKSAYSNWQIAKEILGKMIKQTKASSGVIWNSFK  
ELEESELETVIREIPAPSFLIPLPKHLTASSSSLLDHDRTVFQWLDQQPPSSVLYVSFGSTSEVDEKDFLEIARGLV  
DSQS

[0172] >MBUGT1-3\_1.5[环状变换体]

[0173] (SEQ ID NO:16)

[0174] MAFLWVVRPGFVKGSTWVEPLPDGFLGERGRIVKWVPPQEVLAHGAIGAFWTHGGWNSTLESVCEGVPM  
IFSDFGLDQPLNARYMSDVLKGVVYLENGWERGEIANAIRRLMVDEEGEYIRQNARVLKQKADVSLMKGGSSYESLE  
SLVSYISSLGGSGGSGRRRRIILFPVPFQGHINPMLQLANVLYSKGFSITIFHTNFKPKTSNYPHFTFRFILDN  
DPQTTHGPLAGMRIPINEHGADELRRLELQMLASEEDEEVSLITDALWYFAQSVADSLNLRRLVLMTSSLFNFH  
AHVSLPQFDELGYLDPDDKTRLEEQASGFPMKVKDIKSAYSNWQIAKEILGKMIKQTKASSGVIWNSFKLEESELE  
ETVIREIPAPSFLIPLPKHLTASSSSLLDHDRTVFQWLDQQPPSSVLYVSFGSTSEVDEKDFLEIARGLVDSQS

[0175] >MBUGT1-3\_2[环状变换体]

[0176] (SEQ ID NO:17)

[0177] MAFLWVVRPGFVKGSTWVEPLPDGFLGERGRIVKWVPPQEVLAHGAIGAFWTHGGWNSTLESVCEGVPM  
IFSDFGLDQPLNARYMSDVLKGVVYLENGWERGEIANAIRRLMVDEEGEYIRQNARVLKQKADVSLMKGGSSYESLE  
SLVSYISSLGGSGGSGRRRRIILFPVPFQGHINPMLQLANVLYSKGFSITIFHTNFKPKTSNYPHFTFRFILDNDPQ  
DERISNLPTHGPLAGMRIPINEHGADELRRLELQMLASEEDEEVSLITDALWYFAQSVADSLNLRRLVLMTSSL  
FNFHAHVSLPQFDELGYLDPDDKTRLEEQASGFPMKVKDIKSAYSNWQIAKEILGKMIKQTKASSGVIWNSFKLE  
ESELETVIREIPAPSFLIPLPKHLTASSSSLLEHDRTVFQWLDQQPPSSVLYVSFGSTSEVDEKDFLEIARGLVDSQ  
S

[0178] >SgUGT720-269-1(罗汉果)

[0179] (SEQ ID NO:18)

[0180] MEDRNAMDMSRIKYRPQPLRPASMVQPRVLLFPFPALGHVKPFLSLAELLS DAGIDVVFLSTEYNHRR  
SNTEALASRFPTLHFETIPDGLPPNESRALADGPLYFSMREGTKPRFRQLIQSLNDGRWPITCIITDIMLSSPIEVA  
EEFGIPVIAFCPCSARYLSIHFFIPKLVEEGQIPYADDDPIGEIQGVPLFEGLLRRNHLPGSWSKADISFSHGLI  
NQTLAAGRASALILNTFDELEAPFLTHLSSIFNKIYTIGPLHALSKSRLGDSSSSASALSGFWKEDRACMSWLDCQP  
PRSVVVSFGSTMKMADELREFWYGLVSSGKPFVLRSDVVS GGEEAELIEQMAEEEGAGGKLG MVVEWAAQEKV  
LSHPAVGGFLTHCGWNSTVESIAAGVPMWCWPILGDQPSNATWIDRVWVIGVERNREWDRLTVEKMRALMEGQKR  
VEIQRSMEKLSKLANEKVVRGINLHPTISLKKDTPTTSEHPRHEFENMRGMNYEMLVGNAIKSPTLTKK

[0181] >SgUGT94-289-3(罗汉果)

[0182] (SEQ ID NO:19)

[0183] MTIFFSVEILVLGIAEFAAIAMDAAQQGDTTTILMLPWLGYGHLSAFLELAKSLSRNFHIYFCSTSVN  
LDAIKPKLPSSFSDSIQFVELHLPSSPEFPHLHTNGLPPTLMPALHQAFSMAAQHFESILQTLAPHLIYDSLQP  
WAPRVASSLKIPAINFNTTGVFVVISQGLHPHYPHSKFPFSEFVLHNHWKAMYSTADGASTERTRKRGEAFLYCLHA  
SCSVILINSFRELEGKYMDYLSVLLNKKVVPVGPLVYEPNQDGEDEGYSSIKNWLKKEPSSVTVVSFGSEYFPSKE  
EMEEIAHGLEASEVNFIVVVRFPQGDNTSGIEDALPKGFLERAGERGMVVKGWAPQAKILKHSTGGFVSHCGWNSV

MESMMFGVPIIGVPMHVDQPFNAGLVEEAGVGVEAKRDPDGKIQRDEVAKLKEVVVEKTREDVRKKAREMSEILRS  
KGEEKFDEMVAEISLLLLKI

[0184] >SgUGT74-345-2 (罗汉果)

[0185] (SEQ ID NO:20)

[0186] MDETTVNGGRRASDVVVFAPRHHMSPMLQFSKRLVSKGLRVTFLLITTSATESLRLNLPPSSSLDLQV  
ISDVPESNDIATLEGYLRFSKATVSKTLADFIDGIGNPPKFIVYDSVMPWVQEVARGRGLDAAPFFTQSSAVNHILN  
HVIYGGSLIPAPENTAVSLPSMPVLQAEDLPAFPDDPEVVMNFMTSQFSNFQDAKWIFFNTFDQLECKKQSQVWNW  
ADRWPIKTVGPTIPSAYLDDGRLEDDRAFGLNLLKPEDGKNTRQWQLDSKDTASVLYISFGSLAILQEEQVKELAY  
FLKDTNLSFLWVLRDSELQKLPNHFVQETSHRGLVWNWCSQLQVLSHRAVSCFVTHCGWNSTLEALSLGVPMAIPIQ  
WVDQTTNAKFVADVWRVGVVKKKDERIVTKEELEASIRQVVQGEGRNEFKHNAIKWKKLAKEAVDEGGSSDKNIEE  
FVKTIA

[0187] >SgUGT75-281-2 (罗汉果)

[0188] (SEQ ID NO:21)

[0189] MGDNGDGGEKKELKENVKKGKELGRQAIGEGYINPSLQLARRLISLGVNVTFATTVLAGRMMKNKTHQT  
ATTPGLSFATFSDGFDETLKPNGLTHYFSELRRCGSESLTHLITSAANEGRPITFVIYSLLSWAADIASTYDIP  
SALFFAQPATVLALYFYFHHGYGDTICSKLQDPSSYIELPGLPLLSQDMPSFFSPSGPHAFILPPMREQAFLGRQ  
SQPKVLVNTFDALADLRAIDKLMALIGPLIPSALLGGNDSSDASFCGDLFQVSSSEDIWLNKPKDSSVVYISV  
GSICVLSDEQEDELVHALLNSGHTFLWVKRSKENNEGKQETDEEKLLKLEEQGMVSWCRQVEVLKHPALGCFLTH  
CGWNSTIESLVSLPVVAFPPQIDQATNAKLIEDVWKTGVRVKANTEGIVEREEIRCLDLVMGSRDGQKEEIERNA  
KKWELARQAIGEGGSSDSNLKTFLWEIDLEI

[0190] >SgUGT720-269-4 (罗汉果)

[0191] (SEQ ID NO:22)

[0192] MAEQAHDLLHVLLFPFPAEGHIKPFCLAEELCNAGFHVTFNLTDYNNRRLHNLHLLAARFPSLHFESI  
SDGLPPDQPRDILDPKFFISICQVTKPLFRELLLSYKRISVQTGRPPITCVITDVIFRFPIDVAEELDIPVFSFCT  
FSARFMFLYFWIPKLIEDGQLPYPNGNINQKLYGVAPEAEGLLRCKDLPGHWAFADDELKDDQLNFVDQTTASSRSSG  
LILNTFDDLEAPFLGRLSTIFKKIYAVGPIHSLNNSHHCGLWKEDHSCLAWLDSRAAKSVVVFVSGSLVKITSRQLM  
EFWHGLLNSGKSFLFVLRSDVVEGDDEKQVVEIYETKAEGKWL VVGWAPQEKVLAHEAVGGFLTHSGWNSILESIA  
AGVPMISCPKIGDQSSNCTWISKVWKIGLEMEDRYDRVSVETMVRSIMEQEGEKMQKTIAELAKQAKYKVSXKDGTSY  
QNLECLIQDIKLNQIEGFINPNFSDLLRV

[0193] >SgUGT94-289-2 (罗汉果)

[0194] (SEQ ID NO:23)

[0195] MDAQQGHTTTILMLPWVGYGHLLPFLELAKSLSRRKLFHIYFCSTSVSLDAIKPKLPPSISDDSIQLV  
ELRLPSSPELPPHLHTTNGLP SHLMPALHQAFVMAAQHFQVILQTLAPHLIYDILQPWAPQVASSLNIPAINFSTT  
GASMLSRTLHPTHYPSSKFPISEFVLHNHWRAMYTTADGALTEEGHKIEETLANCLHTSCGVVLVNSFRELETKYID  
YLSVLLNKKVVPVGPLVYEPNQEGEDEGYSSIKNWLDKKEPSSTVFVSGTEYFPSKEEMEEIAYGLELSEVNFIIWV  
LRFPPQGDSTSTIEDALPKGFLERAGERAMVVKGWAPQAKILKHWSTGGLVSHCGWNSMMEGMMFGVPIIAVPMHLDQ  
PFNAGLVEEAGVGVEAKRSDGKIQREEVAKSKEVVIEKTREDVRKKAREMDTKHGPTYFSRSKVSSFGRLYKINR  
PTTLTVGRFWSKQIKMKRE

[0196] >SgUGT94-289-1 (罗汉果)

[0197] (SEQ ID NO:24)

[0198] MDAQRGHTTTILMFPWLGYGHLSAFLELAKSLSRNFHIYFCSTSVNLDAIKPKLPSSSSSDSIQLVEL  
CLPSSPDQLPPLHTTNALPPLMPTLHQAFSMAAQHFAAILHTLAPHLIIYDSFQPWAPQLASSLNIPAINFNTTG  
ASVLRMLHATHYPSSKFPISSEFVLHDYWKAMYSAAGGAVTKDKHIGETLANCLHASCVILINSFRELEEKYMDY  
LSVLLNKKVVPVGPLVYEPNQDGEDEGYSSIKNWLDKKEPSSVTFVVSFGSEYFPSKEEMEEIAHGLEASEVHFIWVV  
RFPQGDNTSAIEDALPKGFLEVRGERGMVVKWAPQAKILKHSTGGFVSHCGWNSVMESMMFGVPIIGVPMHLDQP  
FNAGLAEAGVGVKAKRDPDGKIQRDEVAKLIEVVVEKTREDVRKKAREMSEILRSKGEEKMDEMVAASLFLKI

[0199] >XP\_022151474.1 7-脱氧番木鳖苷酸 (7-deoxyloganetic acid) 葡萄糖基转移酶样  
[苦瓜]

[0200] (SEQ ID NO:25)

[0201] MAQPQTQARVLVFPYPTVGHIKPFLSLAELLADGGLDVVFLSTEYNHRRIPNLEALASRFPTLHFDTIP  
DGLPIDKPRVIGGELYTSMRDGVKQRLRQVLQSYNDGSSPITCVICDVMLSGPIEAAEELGIPVVTFCPYSARYLC  
AHFVMPKLIIEGQIPFTDGNLAGEIQGVPLFGGLLRDHLPGFWFKVLSLSEVWVSHAFNLQTLAVGRTSALIINTLD  
ELEAPFLAHLSSFTDKIYPIGPLDALSKSRLGDSSSSSTVLTAFWKEDQACMSWLSQPPKSVIFVSFGSTMRMTAD  
KLVEFWHGLVNSGTRFLCVLRSDIVEGGGAADLIKQVGETGNGIVVEWAAQEKVLAHRAVGGFLTHCGWNSTMESIA  
AGVPMMCWQIYGDQMINATWIGKVVKIGIERDDKWRSTVEKMIKELMEGEKGAIEIQRSMKFSKLANDKVVKGGS  
FENLELIVEYLKCLKPSN

[0202] >XP\_022151546.1 7-脱氧番木鳖苷酸葡萄糖基转移酶样[苦瓜]

[0203] (SEQ ID NO:26)

[0204] MAQPRVLLFPFPAMGHVKPFLSLAELLSDAGVEVVFLSTEYNHRRIPDIGALAARFPTLHFETIPDGLP  
PDQPRVLADGHLYFSMLDGTKPRFRQLIQSLNGNPRPITCIINDVMLSSPIEVAEEFGIPVIAFCPCSARFLSVHFF  
MPNFIEEAQIPYTDENPMGKIEEATVFEGLLRRKDLPLWCAKSSNISFSHRFINQTTAAGRASALILNTFDELESP  
FLNHLSSIFPKIYCIPLNALSRSRLGKSSSSSSSALAGFWKEDQAYMSWLESQPPRSVIFVSFGSTMKMEAWKLAEF  
WYGLVNSGSPFLFVFRPDCVINSGDAAEVMEGRGRGMVVEWASQEKVLAHPAVGGFLTHCGWNSTVESIVAGVPMMC  
CPIVADQLSNATWIHKVWKIGIEGDEKWRSTVEMMIKELMESQKGTERTSIEMLSKLANEKVVKGGTSLNMFELL  
VEDIKTLRRPYT

[0205] >XP\_022151514.1 7-脱氧番木鳖苷酸葡萄糖基转移酶样[苦瓜]

[0206] (SEQ ID NO:27)

[0207] MEQSDSNSDDHQHHVLLFPFPAKGHIKPFCLLAQLLCCGAGLQVTFNLTDHNHRRIDDRHRRLATQFPM  
LHFKSISDGLPPDHPDLLDGKLIASMRRVTESLFRQLLSYNGYGNGTNNVSNRPPISCVITDVIFSFPVEVA  
EELGIPVFSFATFSARFLFLYFWIPKLIQEGQLPFPDGTNQLYGVPGAEGIRCKDLPGSWSVEAVAKNDPMNFV  
KQTLASSRSSGLILNTFEDLEAPFVTHLSNTFDKIYTIPIHSLGTSGLWKEDYACLAWLDARPRKSVVVSFG  
SLVKTTRELMELWHGLVSSGKSFLVLRSDVVEGEDEEQVKEILESNGEGKWL VVGWAPQEEVLAHEAIGGFLTH  
SGWNSTMESIAAGVPMVCWPKIGDQPSNCTWVSRVWVGLMEERYDRSTVARMARSMMEQEGKEMERRIAELAKRV  
KYRVGKDGESYRNLESLIRDIKITKSSN

[0208] >XP\_004147933.2预测的:7-脱氧番木鳖苷酸葡萄糖基转移酶样[黄瓜]

[0209] (SEQ ID NO:28)

[0210] MGLSPTDHVLLFPFPAKGHIKPFCLAHLLCNAGLRVTFVFLSTEHHHQKLHNLTHLAAQIPSLHFQSISD  
GLSLDHPRNLLDGQLFKSMPQVTKPLFRQLLSYKDGTSPTCVITDLILRFPMDVAQELDIPVFCFSTFSARFLFL

YFSIPKILLEDGQIPYPEGNSNQVLHGIPGAEGLLRCKDLPGYWSVEAVANYNPMNFVNQTIATSKSHGLILNTFDEL  
 EVPFITNLSKIYKKVYTI GPIHSLKKSQVQTQYEFWKEDHSCLAWLDSQPPRSVMFVSFGSIVKLSQQLKEFWNGL  
 VDSGKAFLLVLRSDALVEETGEEDEKQKELVIKEIMETKEEGRWVIVNWAPQEKVLEHKAIGGFLTHSGWNSTLESV  
 AVGVPMVSWPQIGDQPSNATWLSKVWKIGVEMEDSYDRSTVESKVRSEMEHEDKKMENAIVELAKRVDVRSKEGTS  
 YQNLQRLIEDIEGFKLN

[0211] >XP\_022978164.1 7-脱氧番木鳖苷酸葡萄糖基转移酶样[笋瓜]

[0212] (SEQ ID NO:29)

[0213] MELSHTHVLLFPFPAKGHIKPFSLAQLLCNAGLRVTFNLTDHHHRIHDLNRLAAQLPTLHFDSVSD  
 GLPPDEPRNVFDGKLYESIRQVTSSLFRELLVSYNNGTSSGRPPITCVITDVMFRFPIDIAEELGIPVFTFSTFSAR  
 FLFLIFWIPKLEDGQLRYPEQELHGVPGAEGIRWKDLPGFWSVEDVADWDPNMFVNQTLATSRSSGLILNTFDEL  
 EAPFLTSLSKIYKKIYSLGPINSLKKNFQSQPQYNLWKEDHSCMAWLDSQPRKSVFVSFGSVVKLTSRQLMEFWNG  
 LVNSGMPFLLVLRSDVIEAGEEVVREIMERKAEGRWVIVSWAPQEEVLAHDAVGGFLTHSGWNSTLES LAAGVPMIS  
 WPQIGDQTSNSTWISKVWRIGLQLEDGFDSSITETMVRSIMDQTMKETAELAERAKNRASKNGTSYRNFQTLIQDI  
 TNIIETHI

[0214] >XP\_022950128.1 7-脱氧番木鳖苷酸葡萄糖基转移酶样[南瓜]

[0215] (SEQ ID NO:30)

[0216] MELSPTHLLLFPFPAKGHIKPFSLAQLLCNAGARVTFNLTDHHHRIHDLRLAAQLPTLHFDSVSD  
 GLPPDESARNVFDGKLYESIRQVTSSLFRELLVSYNNGTSSGRPPITCVITDCMFRFPIDIAEELGIPVFTFSTFSAR  
 FLFLFFWIPKLEDGQLRYPEQELHGVPGAEGIRCKDLPGFLSDEDVAHWKPINFVNQILATSRSSGLILNTFDEL  
 EAPFLTSLSKIYKKIYSLGPINSLKKNFQSQPQYNLWKEDHSCMAWLDSQPPKSVFVSFGSVVKL TNRQLVEFWNG  
 LVNSGKPFLLVLRSDVIEAGEEVVRENMERKAEGRWVIVSWAPQEEVLAHDAVGGFLTHSGWNSTLES LAAGVPMIS  
 WTQIGDQTSNSTWVSKVWRIGLQLEDGFDSSITETMVRSVMDQTMKETAELAERAKNRASKNGTSYRNFQTLIQDI  
 TNIIETHI

[0217] >XP\_020422423.1 7-脱氧番木鳖苷酸葡萄糖基转移酶[碧桃]

[0218] (SEQ ID NO:31)

[0219] MAMKQPHVIFPFPLQGHMKPLLCLAEELLCHAGLHVTVNTHNHQRLANRQALSTHFPTLHFESISDG  
 LPEDDPRTLNSQLLIALKTSIRPHFRELLKTI SLKAESNDTLVPPSPSCIMTDGLVTFADFVAEELGLPILSFNVPCP  
 RYLWTCLCLPKLIENGQLPFQDDMNVEITGVPGMEGLLHRQDLPGFCRVKQADHPSLQFAINETQTLKRASALILD  
 TVYELDAPCISHMALMFPKIYTLGPLHALLNSQIGMSRGLASHGSLWKS DLNLCMTWLD SQSKSIIYVSFGTLVHL  
 TRAQVIEFWYGLVNSGHPFLWVMRSDITSGDHI PAELENKTKERGCIVDWVSQEEVLAHKS VGGFLTHSGWNSTLE  
 SIVAGLPMICWPKLGDHYIISSTVCRQWKIGLQLNENCDRSNIESMVQTLMGSKREEIQSSMDAISKLSRDSVAEGG  
 SSHNNLEQLIEYIRNLQHQN

[0220] >EOY07351.1 UDP-葡萄糖基转移酶85A3,推定[可可]

[0221] (SEQ ID NO:32)

[0222] MRQPHVLVLPFPAQGHKIPMLCLAEELLQAGLRVTFNLTHSHRRLNQLDLSTRFPTLHFESVSDGLP  
 EDHPRNLVHFMHLVHSIKNVTKPLLRDLLTSLSLKTDIPPVSCI IADGILSFAIDVAEELQIKVIFRTISSCLWS  
 YLCVPKLIQQGELQFSDSDMGQKVSSVPEMKGSLRLHDPYSGFLKQLEDPNFQFFVSETQAMTRASAVIFNTFDSL  
 EAPVLSQMIPLPKVYTI GPLHALRKARLGDLSQHSSFNGLREADHNCITWLD SQPLRSVVYVSFGSHVLTSEEL  
 LEFWHGLVNSGKRFLWVLRPDI IAGEKDHNQIIAREPDLGTKEKGLLDVWAPQEEVLAHPSVGGFLTHCGWNSTLES

MVAGVPMCLCWPKLPDQLVNSSCVSEVWKIGLDLKDMDRSTVEKMVRALMEDRREEVMRSVDGISKLARESVMHGGSSSSNLEMLIQELET

[0223] >XP\_022155979.1β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[苦瓜]

[0224] (SEQ ID NO:33)

[0225] MDAHQQAEHTTTILMLPWVGYGHLTAYLELAKALSRRNFHIYYCSTPVNIESIKPKLTIPCSSIQFVELHLPSSDDLPPNLHTTNGLP SHLMPTLHQAFSAAAPLFEEILQTLCPHLLIYDSLQPWAPKIASLKI PALNFNTSGVSVIAQALHAIHHPDSKFPLSDFILHNYWKSTYTTADGGASEKTRRAREAFLYCLNSSGNAILINTFRELEGEYIDYLSLLLNNKKVIPIGPLVYEPNQDEEDQDEEYRSIKNWLDKKEPCSTVFSFGSEYFPSNEEMEEIAPGLEESGANFIWVVRFPKLENRNGIIEEGLLERAGERGMVIKEWAPQARILRHGSIGGFVSHCGWNSVMESIICGVPVIGVPMRVDQPYNAGLVEEAGVGVEAKRDPDGKIQRHEVSKLIKQVVVEKTRDDVRKKVAQMSEILRRKGDEKIDEMVALISLLPKG

[0226] >XP\_022986080.1β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[笋瓜]

[0227] (SEQ ID NO:34)

[0228] MDAQKAVDTPPTTVLMLPWIGYGHLSAYLELAKALSRRNFHVYFCSTPVNLDISKPNLIPPPSSIQFVDLHLPSSPELPPHLHTTNGLP SHLKPTLHQAFSAAAQHFEEILQTLSPHLLIYDSLQPWAPRIASSLNIPAINFNNTTAVSIIAHALHSVHYPDSKFPFSDFLHDYWKAKYTTADGATSEKIRRGAEAFLYCLNASCDDVVLVNSFRELEGEYMDYLSVLLKKKVVSVGPLVYEPSEGEDEEYWRICKWLDEKEALSTVLVSGSEYFPSKEEMEEIAHGLEESEANFIWVVRFPKGEESCRGIEEALPKGFVERAGERAMVVKWAPQGGKILKHGSIGGFVSHCGWNSVLESIRFGVPVIGVPMHLDQPYNAGLLEEAGIGVEAKRDADGKIQRDQVASLIKRVVVEKTREDIWKTVREMREVLRRRDDDDMIDEMVAEISVVLKI

[0229] >XP\_022156002.1β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[苦瓜]

[0230] (SEQ ID NO:35)

[0231] MDARQQAEHTTTILMLPWVGYGHLSAYLELAKALSRRNFHIYYCSTPVNIESIKPKLTIPCSSIQFVELHLPFSDDLPPNLHTTNGLP SHLMPALHQAFSAAAPLFEEILQTLCPHLLIYDSLQPWAPQIASLKI PALNFNTTGVSVIARALHTIHHPDSKFPLSEIVLHNYWKATHATADGANPEKFRRDLEALLCCLHSSCNAILINTFRELEGEYIDYLSLLLNNKKVTPIGPLVYEPNQDEEQDEEYRSIKNWLDKKEPYSTIFVSGSEYFPSNEEMEEIARGLEESGANFIWVVRFHKLENGNGITEEGLLERAGERGMVIQGWAPQARILRHGSIGGFVSHCGWNSVMESIICGVPVIGVPMGLDQPYNAGLVEEAGVGVEAKRDPDGKIQRHEVSKLIKQVVVEKTRDDVRKKVAQMSEILRRKGDEKIDEMVALISLLLKG

[0232] >XP\_022943327.1β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[南瓜]

[0233] (SEQ ID NO:36)

[0234] MDAQKAVDTPPTTVLMLPWIGYGHLSAYLELAKALSRRNFHVYFCSTPVNLDISKPNLIPPPPSIQFVDLHLPSSPELPPHLHTTNGLP SHLKPTLHQAFSAAAQHFEEILQTLSPHLLIYDSLQPWAPRIASSLNIPAINFNNTTAVSIIAHALHSVHYPDSKFPFSDFLHDYWKAKYTTADGATSEKTRRGVEAFLYCLNASCDDVVLVNSFRELEGEYMDYLSVLLKKKVVSVGPLVYEPSEGEDEEYWRICKWLDEKEALSTVLVSGSEYFPPKEEMEEIAHGLEESEANFIWVVRFPKGEESSSRGIEEALPKGFVERAGERAMVVKWAPQGGKILKHGSIGGFVSHCGWNSVLESIRFGVPVIGAPMHLDDQPYNAGLLEEAGIGVEAKRDADGKIQRDQVASLIKQVVVEKTREDIWKVREMREVLRRRDDDDMMIDEMVAVISVVLKI

[0235] >XP\_022996307.1β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[笋瓜]

[0236] (SEQ ID NO:37)

[0237] MSSNLFLKISIPFGRLRDSALNCSVFHCKLHLAIAIAMDAQQAANKSPTATTIFMLPWAGYGHLSAYLELAKALSTRNFHIYFCSTPVSLASIKPRLIPSCSSIQFVELHLPSSDEFPPHLHTTNGLP SRLVPTFHQAFSEAAQTF

EAFQLTLRPHLLIYDSLQPWAPRIASSLNIPAINFFTAGAFVSHVLR AFHYPDSQFPSSDFVLHSRWKIKNTTAES  
PTQAKLPKIGEAIGYCLNASRGVILTNSFRELEGKYIDYLSVILKKRVFPIGPLVYQPNQDEEDEDYSRIKNWDRK  
EASSTVLVSFGSEFFLSKEETEAIAHGLEQSEANFIWGIRFPKGAKKNAIEEALPEGFLERAGGRAMVVEEWVPGK  
ILKHGSIGGFVSHCGWNSAMESIVCGVPIIGIPMQVDQPFNAGILEEAGVGVEAKRSDGKIQRDEVAKLIKEVVVE  
RTREDIRNKLEKINEILRSRREEKLDELATEISLLSRN

[0238] >XP\_022957664.1  $\beta$ -D-葡萄糖基西红花酸 $\beta$ -1,6-葡萄糖基转移酶样[南瓜]

[0239] (SEQ ID NO:38)

[0240] MDAQQAANKSPTASTIFMLPWVGYGHL SAYLELAKALSTRNFHVYFCSTPVS LASIKPRLIPSCSSIQF  
VELHLPSSDEFPPHLHTTNGLPAHLVPTIHQAFAAAAQTFEAFLQLTRPHLLIYDSLQPWAPRIASSLNIPAINFF  
AGAFVSHVLR AFHYPDSQFPSSDFVLHSRWKIKNTTAESPTQVKIPKIGEAIGYCLNASRGVILTNSFRELEGKYI  
DYLSVILKKRVLP IGPLVYQPNQDEEDEDYSRIKNWDRKEASSTVLVSFGSEFFLSKEETEAIAHGLEQSEANFIW  
GIRFPKGAKKNAIEEALPEGFLERVGGRAMVVEEWVPGKILKHGNIGGFVSHCGWNSAMESIMCGVPIIGIPMQVD  
QPFNAGILEEAGVGVEAKRSDGKIQRDEVAKLIKEVVVERTREDIRNKLEEINEILRTRREEKLDELATEISLLCK  
N

[0241] >OM057892.1 UDP-葡萄糖苷酸基/UDP-葡萄糖基转移酶[圆果种黄麻]

[0242] (SEQ ID NO:39)

[0243] MDSKQKKMSVLMFPWLAYGHISPFLELAKKLSKRNFTFFFSTPINLNSIKSKLSPKYAQSIQFVELHL  
PSLPDLPPHYHTTNGLPPHLMNTLKKAFDMSSLQFSKILKTLNPDLLVYDFIQPWAPLLALS NKIPAVHFLCTSAAM  
SSFSVHAFKKPCEDFPFPNIYVHGNFMNAKFN NMENCSSDDSIDQDRVLQCFERSTKIILVKT FEELEGKFMDYLS  
VLLNKKIVPTGPLTQDPNEDEGDDERTKLLLEWLNKKSSTV FVSFGSEYFLSKEEREEIAYGLELSKVNFIWVI  
RFPLGENKTNLEEALPQGFLQRVSE RGLVVENWAPQAKILQHSSIGGFVSHCGWSSVMESLKFVPIIAIPMHLDQP  
LNARLVVDVGVGLEVIRNHGSLEREEIAKLIKEVVLGNGNDGEIVRRKAREMSNHIKKKGKMDDELVEELMLICKM  
KPNSCHLS

[0244] >XP\_015886141.1 预测的: $\beta$ -D-葡萄糖基西红花酸 $\beta$ -1,6-葡萄糖基转移酶样同种型X1  
[枣]

[0245] (SEQ ID NO:40)

[0246] MMERQRSIKVLMFPWLAHGHI SPFLELAKRLTDRNFQIYFCSTPVNLT SVKPKLSQKYSSSIKLVELHL  
PSLPDLPPHYHTTNGLALNLIPTLKKAFDMSSSSSFSTILSTIKPDLLIYDFLQPWAPQLASCMNIPAVNFLSAGASM  
VSFVLHSIKYNGDDHDDEFLLTELHLSDSMEAKFAEMTESSPDEHIDRAVTCLERSNLILIKSFRELEGKYLDYLS  
LSFAKKVVP IGPLVAQDTNPEDDSMDI INWLDKKEKSSTV FVSFGSEYYLTNEEMEEIAYGLELSKVNFIWVVRFP  
GQKMAVEEALPKGFLERVGEKGMVVEDWAPQMKILGHSSIGGFVSHCGWSSLMESLKLGVPIIAMPMLDQPINAKL  
VERSGVLEVKRDKNGRIEREYLAKVIREIVVEKARQDIEKKAREMSNIITEKGEE IDNVVEELAKLCGM

[0247] >XP\_002271587.3 预测的: $\beta$ -D-葡萄糖基西红花酸 $\beta$ -1,6-葡萄糖基转移酶样[葡萄]

[0248] (SEQ ID NO:41)

[0249] MDARQSDGISVLMFPWLAHGHI SPFLQLAKKLSKRNFSIYFCSTPVNLDPIKGLSESYLSIQLVKLH  
LPSLPPELPPQYHTTNGLPPHLMPTLKM AFDMASPNF SNILKTLHPDLLIYDFLQPWAPAAASSLNIPAVQFLSTGAT  
LQSFLAHRHRKPGIEFPFQEIHL PDYEIGRLNRFLEPSAGRISDRDRANQCLERSRSLIKTFREIEAKYLDYVSD  
LTKKKMVTVGPLLQDPEDEDEATDIVEWLNKKCEASAVFVSFGSEYFVSKEEMEEIAHGLELSNVDFIWVVRFP  
MGEKIRLEDALPPGFLHRLGDRGMVVEGWAPQRKILGHSSIGGFVSHCGWSSVMEGMKFGVPIIAMPMLDQPINAKLVE

AVGVGREVKRDENRKLREEEIAKVIKEVVGEKNGENVRRKARELSETLRKKGDEEIDVVVEELKQLCSY

[0250] >XP\_018840205.1预测的:β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[核桃]

[0251] (SEQ ID NO:42)

[0252] MDTARKRIRVVMLPWLAHGHISPFLELSKKLAKRNFHIYFCSTPVNLSSIKPKLSGKYSRSIQLVELHL  
PSLPELPPQYHTTKGLPPLNATLKRAFDMAGPHFSNLIKTLSPDLLIYDFLQPWAPAIASQNIPAINFLSTGAAM  
TSFVLHAMKKPGDEFPFPEIHLDECMKTRFVDLPEDHSPSDDHNHISDKDRALKCFERSSGFVMMKTFEELEGKYIN  
FLSHLMQKKIIVPGPLVQNPVRGDHEKAKTLEWLDKRRQSSAVFVSFGTEYFLSKEEMEEIAYGLELSNVNFIWVVR  
FPEGEKVKLEEALPEGFLQRVGEKGMVVEGWAPQAKILMHP SIGGFVSHCGWSSVMESIDFGVPIVAIPMQLDQPVN  
AKVVEQAGVGVEVKRDRDGKLEREEVATVIREVVMGNIGESVRKKEREMRDNIRKKGEEKMDGVAQELVQLYNGGIK  
NV

[0253] >XP\_021652171.1β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[橡胶树]

[0254] (SEQ ID NO:43)

[0255] METLQRRKISVLMFPWLAHGHLSPFLELSKKLNKRNFHVYFCSTPVNLDSIKPKLSAEYSFSIQLVELH  
LPSSPELPLHYHTTNGLPPLMKNLKNAFDMASSSFFNLIKTLKPDLLIYDFIQPWAPALASSLNIPAVNFLCTSMA  
MSCFGLHLNNQEAKFPPFGIYPRDYMRMKVFGALESSNDIKDGERAGRCMDQSFHLILAKTFRELEGKYIDYLSVK  
LMKKIIVPGPLVQDPIFEDDEKIMDHHQVIKWLEKKERLSTVFSFGTEYFLSTEEMEEIAYGLELSKAHFIWVVR  
PTGEKINLEESLPKRYLERVQERGIIVEGWAPQQKILRHSSIGGFVSHCGWSSIMESMKFGVPIIAMPNLDQPVNS  
RIVEDAGVGIEVRRNKSGELEREEIAKTIKRVVVEKDGNVSRKAREMSDTRKKGEEEIDGVVDELLQLCDVKTNY  
LQ

[0256] >XP\_021619073.1β-D-葡萄糖基西红花酸β-1,6-葡萄糖基转移酶样[木薯]

[0257] (SEQ ID NO:44)

[0258] MATAQTRKISVLMFPWLAHGHLSPFLELSKKLANRNFHVYFCSTPVNLDSIKPKLSPEYHFSIQFVELH  
LPSSPELPSHYHTTNGLPPLMKTLKKAFFDMASSSFFNLIKTLNPDLLIYDFLQPWAPALASSLNIPAVNFLCSSMA  
MSCFGLNLNKNKEIKFLFPEIYPRDYMEMKLFVFESSNQIKDGERAGRCIDQSFHVLAKTFRELEGKYIDYVSV  
KCNKKIIVPGPLVEDTIHEDDEKTMDDHHHHHDEVIKWLEKKERSTTVFSFGSEYFLSKEEMEEIAHGLELSKVN  
FIWVVRFPKGEKINLEESLPEGLERIQERGIIVEGWAPQRKILGHSSIGGFVSHCGWSSIMESMKLGVPIIAMPN  
LDQPINSRIVEAAGVGIEVSRNQSGELEREEMAKTIRKVVVEREGVYVRRKAREMSDVLKKGEEEIDGVVDELVQLC  
DMKTNYL

[0259] >GAV83746.1含UDPGT结构域蛋白[土瓶草]

[0260] (SEQ ID NO:45)

[0261] MDLKRRSIRVLMFPWLAHGHISPFLELAKKLTNRNFLIYFCSTPINLNSIKPKLSSKYSFSIQLVELHL  
PSLPELPPHYHTTNGLPPLHMLTKTAFDMASPSFLNLIKTLKPDLLICDHLQPWAPSLASSLNIPAIIFPTNSAIM  
MAFSLHHAKNPGEFPFPSININDDMVKSINFLHSASNGLTMDRVLQCLERSNTMLLKTFRQLEAKYVDYSSALL  
KKKIVLAGPLVQVPDNEDEKIEIKWLDSRQSSSTVFSFGSEYFLSKEEREDIAHGLELSKVNFIWVVRFPVGEKV  
KLEEALPNGFAERIGERGLVVEGWAPQAMILSHSSIGGFVSHCGWSSMMESMKFGVPIIAMPMHIDQPLNARLVEDV  
GVGLEIKRNKDGFREREELARVIKEVLVYKNGDAVRSKAREMSEHIKKNGDQEIDGVADALVKLCMKTNSLNQD

[0262] >小粒咖啡 (Coffea Arabica) UGT\_1,6

[0263] (SEQ ID NO:46)

[0264] MENHATFNVLMFPWLAHGHVSPYLELAKKLTARNFNVYLCSSPATLSSVRSKLTEKFSQSIHLVELHLP

KLPELPAEYHTTNGLPPLMPTLKDAFDMAKPNFCNVLKSLKPDLLIYDLLQPWAPEAASAFNIPAVVFISSSATMT  
SFGLHFFKNPGTKYPYGNAIFYRDYESVFVENLTRDRDRTYRVINCMERSSKIILIKGFNEIEGKYFDYFSCLTGKK  
VVPVGPLVQDPVLDDDEDCRIMQWLNKKEKGSTVFVVSFGSEYFLSKKDMEEIAHGLEVSNDVFIWVVRFPKGENIVIE  
ETLPKGGFFERVGERGLVVNGWAPQAKILTHPNVGGFVSHCGWNSVMESMKFGLPIIAMPMLDQPINARLIEEVGAG  
VEVLRDSKGLHRERMAETINKVMKEASGESVRKKARELQEKLELKGDEEIDDVVKELVQLCATKNKRNGLHYY

[0265] >环状变换体接头序列

[0266] (SEQ ID NO:47)

[0267] GSGGSG

[0268] >环状变换体接头序列

[0269] (SEQ ID NO:48)

[0270] GSGGSGGSG

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[0048]	Leu Asp Gln Ile Pro Glu Glu Lys Lys Gln Thr Gly Ile Thr Ser Leu		
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[0126]	Thr His Cys Gly Phe Asn Ser Thr Leu Glu Ala Ile Ser Leu Gly Val		
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[0128]	Pro Val Val Ala Met Pro Gln Phe Ser Asp Gln Thr Thr Asn Ala Lys		
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[0130]	Leu Leu Asp Glu Ile Leu Gly Val Gly Val Arg Val Lys Ala Asp Glu		
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[0132]	Asn Gly Ile Val Arg Arg Gly Asn Leu Ala Ser Cys Ile Lys Met Ile		
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[0134]	Met Glu Glu Glu Arg Gly Val Ile Ile Arg Lys Asn Ala Val Lys Trp		
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[0136]	Lys Asp Leu Ala Lys Val Ala Val His Glu Gly Gly Ser Ser Asp Asn		
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[0155]	Thr His Gly Pro Leu Ala Gly Met Arg Ile Pro Ile Ile Asn Glu His		

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[0165]	Phe Asp Glu Leu Gly Tyr Leu Asp Pro Asp Asp Lys Thr Arg Leu Glu					
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[0167]	Glu Gln Ala Ser Gly Phe Pro Met Leu Lys Val Lys Asp Ile Lys Ser					
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[0173]	Leu Glu Glu Ser Glu Leu Glu Thr Val Ile Arg Glu Ile Pro Ala Pro					
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[0175]	Ser Phe Leu Ile Pro Leu Pro Lys His Leu Thr Ala Ser Ser Ser Ser					
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[0177]	Leu Leu Asp His Asp Arg Thr Val Phe Gln Trp Leu Asp Gln Gln Pro					
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[0179]	Pro Ser Ser Val Leu Tyr Val Ser Phe Gly Ser Thr Ser Glu Val Asp					
[0180]		275		280		285
[0181]	Glu Lys Asp Phe Leu Glu Ile Ala Arg Gly Leu Val Asp Ser Lys Gln					
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[0183]	Ser Phe Leu Trp Val Val Arg Pro Gly Phe Val Lys Gly Ser Thr Trp					
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[0185]	Val Glu Pro Leu Pro Asp Gly Phe Leu Gly Glu Arg Gly Arg Ile Val					
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[0187]	Lys Trp Val Pro Gln Gln Glu Val Leu Ala His Gly Ala Ile Gly Ala					
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[0189]	Phe Trp Thr His Ser Gly Trp Asn Ser Thr Leu Glu Ser Val Cys Glu					
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[0193]	Ala Arg Tyr Met Ser Asp Val Leu Lys Val Gly Val Tyr Leu Glu Asn					
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[0195]	Gly Trp Glu Arg Gly Glu Ile Ala Asn Ala Ile Arg Arg Val Met Val
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[0197]	Asp Glu Glu Gly Glu Tyr Ile Arg Gln Asn Ala Arg Val Leu Lys Gln
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[0212]	Trp Leu Ala Phe Gly His Ile Leu Pro Phe Leu Gln Leu Ser Lys Leu
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[0214]	Ile Ala Glu Lys Gly His Lys Val Ser Phe Leu Ser Thr Thr Arg Asn
[0215]	50 55 60
[0216]	Ile Gln Arg Leu Ser Ser His Ile Ser Pro Leu Ile Asn Val Val Gln
[0217]	65 70 75 80
[0218]	Leu Thr Leu Pro Arg Val Gln Glu Leu Pro Glu Asp Ala Glu Ala Thr
[0219]	85 90 95
[0220]	Thr Asp Val His Pro Glu Asp Ile Gln Tyr Leu Lys Lys Ala Val Asp
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[0222]	Gly Leu Gln Pro Glu Val Thr Arg Phe Leu Glu Gln His Ser Pro Asp
[0223]	115 120 125
[0224]	Trp Ile Ile Tyr Asp Phe Thr His Tyr Trp Leu Pro Ser Ile Ala Ala
[0225]	130 135 140
[0226]	Ser Leu Gly Ile Ser Arg Ala Tyr Phe Cys Val Ile Thr Pro Trp Thr
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[0228]	Ile Ala Tyr Leu Ala Pro Ser Ser Asp Ala Met Ile Asn Asp Ser Asp
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[0230]	Gly Arg Thr Thr Val Glu Asp Leu Thr Thr Pro Pro Lys Trp Phe Pro
[0231]	180 185 190
[0232]	Phe Pro Thr Lys Val Cys Trp Arg Lys His Asp Leu Ala Arg Met Glu
[0233]	195 200 205

[0234]	Pro Tyr Glu Ala Pro Gly Ile Ser Asp Gly Tyr Arg Met Gly Met Val
[0235]	210 215 220
[0236]	Phe Lys Gly Ser Asp Cys Leu Leu Phe Lys Cys Tyr His Glu Phe Gly
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[0238]	Thr Gln Trp Leu Pro Leu Leu Glu Thr Leu His Gln Val Pro Val Val
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[0240]	Pro Val Gly Leu Leu Pro Pro Glu Ile Pro Gly Asp Glu Lys Asp Glu
[0241]	260 265 270
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[0243]	275 280 285
[0244]	Val Val Tyr Val Ala Leu Gly Ser Glu Ala Leu Val Ser Gln Thr Glu
[0245]	290 295 300
[0246]	Val Val Glu Leu Ala Leu Gly Leu Glu Leu Ser Gly Leu Pro Phe Val
[0247]	305 310 315 320
[0248]	Trp Ala Tyr Arg Lys Pro Lys Gly Pro Ala Lys Ser Asp Ser Val Glu
[0249]	325 330 335
[0250]	Leu Pro Asp Gly Phe Val Glu Arg Thr Arg Asp Arg Gly Leu Val Trp
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[0252]	Thr Ser Trp Ala Pro Gln Leu Arg Ile Leu Ser His Glu Ser Val Cys
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[0254]	Gly Phe Leu Thr His Cys Gly Ser Gly Ser Ile Val Glu Gly Leu Met
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[0256]	Phe Gly His Pro Leu Ile Met Leu Pro Ile Phe Cys Asp Gln Pro Leu
[0257]	385 390 395 400
[0258]	Asn Ala Arg Leu Leu Glu Asp Lys Gln Val Gly Ile Glu Ile Pro Arg
[0259]	405 410 415
[0260]	Asn Glu Glu Asp Gly Cys Leu Thr Lys Glu Ser Val Ala Arg Ser Leu
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[0262]	Arg Ser Val Val Val Glu Asn Glu Gly Glu Ile Tyr Lys Ala Asn Ala
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[0265]	450 455 460
[0266]	Val Ser Gln Phe Val Asp Tyr Leu Glu Lys Asn Ala Arg Ala Val Ala
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 [0279] Leu Ser Lys Leu Ile Ala Glu Lys Gly His Lys Val Ser Phe Leu Ser  
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 [0281] Thr Thr Arg Asn Ile Gln Arg Leu Ser Ser His Ile Ser Pro Leu Ile  
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 [0283] Asn Val Val Gln Leu Thr Leu Pro Arg Val Gln Glu Leu Pro Glu Asp  
 [0284] 65 70 75 80  
 [0285] Ala Glu Ala Thr Thr Asp Val His Pro Glu Asp Ile Pro Tyr Leu Lys  
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 [0287] Lys Ala Ser Asp Gly Leu Gln Pro Glu Val Thr Arg Phe Leu Glu Gln  
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 [0289] His Ser Pro Asp Trp Ile Ile Tyr Asp Tyr Thr His Tyr Trp Leu Pro  
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 [0291] Ser Ile Ala Ala Ser Leu Gly Ile Ser Arg Ala His Phe Ser Val Thr  
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 [0297] Lys Trp Phe Pro Phe Pro Thr Lys Val Cys Trp Arg Lys His Asp Leu  
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 [0299] Ala Arg Leu Val Pro Tyr Lys Ala Pro Gly Ile Ser Asp Gly Tyr Arg  
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 [0301] Met Gly Leu Val Leu Lys Gly Ser Asp Cys Leu Leu Ser Lys Cys Tyr  
 [0302] 210 215 220  
 [0303] His Glu Phe Gly Thr Gln Trp Leu Pro Leu Leu Glu Thr Leu His Gln  
 [0304] 225 230 235 240  
 [0305] Val Pro Val Val Pro Val Gly Leu Leu Pro Pro Glu Val Pro Gly Asp  
 [0306] 245 250 255  
 [0307] Glu Lys Asp Glu Thr Trp Val Ser Ile Lys Lys Trp Leu Asp Gly Lys  
 [0308] 260 265 270  
 [0309] Gln Lys Gly Ser Val Val Tyr Val Ala Leu Gly Ser Glu Val Leu Val  
 [0310] 275 280 285  
 [0311] Ser Gln Thr Glu Val Val Glu Leu Ala Leu Gly Leu Glu Leu Ser Gly

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[0315]	Asp Ser Val Glu Leu Pro Asp Gly Phe Val Glu Arg Thr Arg Asp Arg		
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[0317]	Gly Leu Val Trp Thr Ser Trp Ala Pro Gln Leu Arg Ile Leu Ser His		
[0318]		340	345
[0319]	Glu Ser Val Cys Gly Phe Leu Thr His Cys Gly Ser Gly Ser Ile Val		
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[0321]	Glu Gly Leu Met Phe Gly His Pro Leu Ile Met Leu Pro Ile Phe Gly		
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[0323]	Asp Gln Pro Leu Asn Ala Arg Leu Leu Glu Asp Lys Gln Val Gly Ile		
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[0325]	Glu Ile Pro Arg Asn Glu Glu Asp Gly Cys Leu Thr Lys Glu Ser Val		
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[0327]	Ala Arg Ser Leu Arg Ser Val Val Val Glu Lys Glu Gly Glu Ile Tyr		
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[0329]	Lys Ala Asn Ala Arg Glu Leu Ser Lys Ile Tyr Asn Asp Thr Lys Val		
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[0331]	Glu Lys Glu Tyr Val Ser Gln Phe Val Asp Tyr Leu Glu Lys Asn Thr		
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[0344]	Leu Ser Lys Leu Ile Ala Glu Lys Gly His Lys Val Ser Phe Leu Ser		
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[0346]	Thr Thr Arg Asn Ile Gln Arg Leu Ser Ser His Ile Ser Pro Leu Ile		
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[0348]	Asn Val Val Gln Leu Thr Leu Pro Arg Val Gln Glu Leu Pro Glu Asp		
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[0350]	Ala Glu Ala Thr Thr Asp Val His Pro Glu Asp Ile Pro Tyr Leu Lys		

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[0356]	Ser	Ile	Ala	Ala	Ser	Leu	Gly	Ile	Ser	Arg	Ala	His	Phe	Ser	Val	Thr
[0357]				130					135					140		
[0358]	Thr	Pro	Trp	Ala	Ile	Ala	Tyr	Met	Gly	Pro	Ser	Ala	Asp	Ala	Met	Ile
[0359]				145					150					155		160
[0360]	Asn	Gly	Ser	Asp	Gly	Arg	Thr	Thr	Val	Glu	Asp	Leu	Thr	Thr	Pro	Pro
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[0362]	Lys	Trp	Phe	Pro	Phe	Pro	Thr	Lys	Val	Cys	Trp	Arg	Lys	His	Asp	Leu
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[0364]	Ala	Arg	Leu	Val	Pro	Tyr	Lys	Ala	Pro	Gly	Ile	Ser	Asp	Gly	Tyr	Arg
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[0366]	Met	Gly	Leu	Val	Leu	Lys	Gly	Ser	Asp	Cys	Leu	Leu	Ser	Lys	Cys	Tyr
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[0368]	His	Glu	Phe	Gly	Thr	Gln	Trp	Leu	Pro	Leu	Leu	Glu	Thr	Leu	His	Gln
[0369]					225					230					235	240
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[0372]	Glu	Lys	Asp	Glu	Thr	Trp	Val	Ser	Ile	Lys	Lys	Trp	Leu	Asp	Gly	Lys
[0373]						260						265				270
[0374]	Gln	Lys	Gly	Ser	Val	Val	Tyr	Val	Ala	Leu	Gly	Ser	Glu	Val	Leu	Val
[0375]						275									280	285
[0376]	Ser	Gln	Thr	Glu	Val	Val	Glu	Leu	Ala	Leu	Gly	Leu	Glu	Leu	Ser	Gly
[0377]						290									295	300
[0378]	Leu	Pro	Phe	Val	Trp	Ala	Tyr	Arg	Lys	Pro	Lys	Gly	Pro	Ala	Lys	Ser
[0379]						305									310	315
[0380]	Asp	Ser	Val	Glu	Leu	Pro	Asp	Gly	Phe	Val	Glu	Arg	Thr	Arg	Asp	Arg
[0381]															325	330
[0382]	Gly	Leu	Val	Trp	Thr	Ser	Trp	Ala	Pro	Gln	Leu	Arg	Ile	Leu	Ser	His
[0383]															340	345
[0384]	Glu	Ser	Val	Cys	Gly	Phe	Leu	Thr	His	Cys	Gly	Ser	Gly	Ser	Ile	Val
[0385]															355	360
[0386]	Glu	Gly	Leu	Met	Phe	Gly	His	Pro	Leu	Ile	Met	Leu	Pro	Ile	Phe	Gly
[0387]															370	375
[0388]	Asp	Gln	Pro	Leu	Asn	Ala	Arg	Leu	Leu	Glu	Asp	Lys	Gln	Val	Gly	Ile
[0389]						385									390	395
																400

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[0391]	405 410 415
[0392]	Ala Arg Ser Leu Arg Ser Val Val Val Glu Lys Glu Gly Glu Ile Tyr
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[0394]	Lys Ala Asn Ala Arg Glu Leu Ser Lys Ile Tyr Asn Asp Thr Lys Val
[0395]	435 440 445
[0396]	Glu Lys Glu Tyr Val Ser Gln Phe Val Asp Tyr Leu Glu Lys Asn Ala
[0397]	450 455 460
[0398]	Arg Ala Val Ala Ile Asp His Glu Ser
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[0407]	Val Val Ile Cys Pro Trp Leu Ala Phe Gly His Leu Leu Pro Cys Leu
[0408]	20 25 30
[0409]	Asp Leu Ala Gln Arg Leu Ala Ser Arg Gly His Arg Val Ser Phe Val
[0410]	35 40 45
[0411]	Ser Thr Pro Arg Asn Ile Ser Arg Leu Pro Pro Val Arg Pro Ala Leu
[0412]	50 55 60
[0413]	Ala Pro Leu Val Ala Phe Val Ala Leu Pro Leu Pro Arg Val Glu Gly
[0414]	65 70 75 80
[0415]	Leu Pro Asp Gly Ala Glu Ser Thr Asn Asp Val Pro His Asp Arg Pro
[0416]	85 90 95
[0417]	Asp Met Val Glu Leu His Arg Arg Ala Phe Asp Gly Leu Ala Ala Pro
[0418]	100 105 110
[0419]	Phe Ser Glu Phe Leu Gly Thr Ala Cys Ala Asp Trp Val Ile Val Asp
[0420]	115 120 125
[0421]	Val Phe His His Trp Ala Ala Ala Ala Ala Leu Glu His Lys Val Pro
[0422]	130 135 140
[0423]	Cys Ala Met Met Leu Leu Gly Ser Ala His Met Ile Ala Ser Ile Ala
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[0425]	Asp Arg Arg Leu Glu Arg Ala Glu Thr Glu Ser Pro Ala Ala Ala Gly
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[0427]	Gln Gly Arg Pro Ala Ala Ala Pro Thr Phe Glu Val Ala Arg Met Lys
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[0429]	Leu Ile Arg Thr Lys Gly Ser Ser Gly Met Ser Leu Ala Glu Arg Phe
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[0431]	Ser Leu Thr Leu Ser Arg Ser Ser Leu Val Val Gly Arg Ser Cys Val
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[0433]	Glu Phe Glu Pro Glu Thr Val Pro Leu Leu Ser Thr Leu Arg Gly Lys
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[0435]	Pro Ile Thr Phe Leu Gly Leu Met Pro Pro Leu His Glu Gly Arg Arg
[0436]	245 250 255
[0437]	Glu Asp Gly Glu Asp Ala Thr Val Arg Trp Leu Asp Ala Gln Pro Ala
[0438]	260 265 270
[0439]	Lys Ser Val Val Tyr Val Ala Leu Gly Ser Glu Val Pro Leu Gly Val
[0440]	275 280 285
[0441]	Glu Lys Val His Glu Leu Ala Leu Gly Leu Glu Leu Ala Gly Thr Arg
[0442]	290 295 300
[0443]	Phe Leu Trp Ala Leu Arg Lys Pro Thr Gly Val Ser Asp Ala Asp Leu
[0444]	305 310 315 320
[0445]	Leu Pro Ala Gly Phe Glu Glu Arg Thr Arg Gly Arg Gly Val Val Ala
[0446]	325 330 335
[0447]	Thr Arg Trp Val Pro Gln Met Ser Ile Leu Ala His Ala Ala Val Gly
[0448]	340 345 350
[0449]	Ala Phe Leu Thr His Cys Gly Trp Asn Ser Thr Ile Glu Gly Leu Met
[0450]	355 360 365
[0451]	Phe Gly His Pro Leu Ile Met Leu Pro Ile Phe Gly Asp Gln Gly Pro
[0452]	370 375 380
[0453]	Asn Ala Arg Leu Ile Glu Ala Lys Asn Ala Gly Leu Gln Val Ala Arg
[0454]	385 390 395 400
[0455]	Asn Asp Gly Asp Gly Ser Phe Asp Arg Glu Gly Val Ala Ala Ala Ile
[0456]	405 410 415
[0457]	Arg Ala Val Ala Val Glu Glu Glu Ser Ser Lys Val Phe Gln Ala Lys
[0458]	420 425 430
[0459]	Ala Lys Lys Leu Gln Glu Ile Val Ala Asp Met Ala Cys His Glu Arg
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[0461]	Tyr Ile Asp Gly Phe Ile Gln Gln Leu Arg Ser Tyr Lys Asp
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[0474]	Glu Glu Ile Thr Arg Ala Leu Ile Asp Ser Asp Val Asn Phe Leu Trp	
[0475]		35 40 45
[0476]	Val Ile Lys His Lys Glu Glu Gly Lys Leu Pro Glu Asn Leu Ser Glu	
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[0478]	Val Ile Lys Thr Gly Lys Gly Leu Ile Val Ala Trp Cys Lys Gln Leu	
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[0480]	Asp Val Leu Ala His Glu Ser Val Gly Cys Phe Val Thr His Cys Gly	
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[0482]	Phe Asn Ser Thr Leu Glu Ala Ile Ser Leu Gly Val Pro Val Val Ala	
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[0484]	Met Pro Gln Phe Ser Asp Gln Thr Thr Asn Ala Lys Leu Leu Asp Glu	
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[0486]	Ile Leu Gly Val Gly Val Arg Val Lys Ala Asp Glu Asn Gly Ile Val	
[0487]		130 135 140
[0488]	Arg Arg Gly Asn Leu Ala Ser Cys Ile Lys Met Ile Met Glu Glu Glu	
[0489]	145	150 155 160
[0490]	Arg Gly Val Ile Ile Arg Lys Asn Ala Val Lys Trp Lys Asp Leu Ala	
[0491]		165 170 175
[0492]	Lys Val Ala Val His Glu Gly Gly Ser Ser Asp Asn Asp Ile Val Glu	
[0493]		180 185 190
[0494]	Phe Val Ser Glu Leu Ile Lys Ala Gly Ser Gly Glu Gln Gln Lys Ile	
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[0496]	Lys Lys Ser Pro His Val Leu Leu Ile Pro Phe Pro Leu Gln Gly His	
[0497]		210 215 220
[0498]	Ile Asn Pro Phe Ile Gln Phe Gly Lys Arg Leu Ile Ser Lys Gly Val	
[0499]	225	230 235 240
[0500]	Lys Thr Thr Leu Val Thr Thr Ile His Thr Leu Asn Ser Thr Leu Asn	
[0501]		245 250 255
[0502]	His Ser Asn Thr Thr Thr Thr Ser Ile Glu Ile Gln Ala Ile Ser Asp	
[0503]		260 265 270
[0504]	Gly Cys Asp Glu Gly Gly Phe Met Ser Ala Gly Glu Ser Tyr Leu Glu	
[0505]		275 280 285
[0506]	Thr Phe Lys Gln Val Gly Ser Lys Ser Leu Ala Asp Leu Ile Lys Lys	

[0507]	290	295	300
[0508]	Leu Gln Ser Glu Gly Thr Thr Ile Asp Ala Ile Ile Tyr Asp Ser Met		
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[0510]	Thr Glu Trp Val Leu Asp Val Ala Ile Glu Phe Gly Ile Asp Gly Gly		
[0511]	325	330	335
[0512]	Ser Phe Phe Thr Gln Ala Cys Val Val Asn Ser Leu Tyr Tyr His Val		
[0513]	340	345	350
[0514]	His Lys Gly Leu Ile Ser Leu Pro Leu Gly Glu Thr Val Ser Val Pro		
[0515]	355	360	365
[0516]	Gly Phe Pro Val Leu Gln Arg Trp Glu Thr Pro Leu Ile Leu Gln Asn		
[0517]	370	375	380
[0518]	His Glu Gln Ile Gln Ser Pro Trp Ser Gln Met Leu Phe Gly Gln Phe		
[0519]	385	390	395
[0520]	Ala Asn Ile Asp Gln Ala Arg Trp Val Phe Thr Asn Ser Phe Tyr Lys		
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[0522]	Leu Glu Glu Glu Val Ile Glu Trp Thr Arg Lys Ile Trp Asn Leu Lys		
[0523]	420	425	430
[0524]	Val Ile Gly Pro Thr Leu Pro Ser Met Tyr Leu Asp Lys Arg Leu Asp		
[0525]	435	440	445
[0526]	Asp Asp Lys Asp Asn Gly Phe Asn Leu Tyr Lys Ala Asn His His		
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[0539]	Pro Glu Thr Val Pro Leu Leu Ser Thr Leu Arg Gly Lys Pro Ile Thr		
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[0541]	Phe Leu Gly Leu Met Pro Pro Leu His Glu Gly Arg Arg Glu Asp Gly		
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[0543]	Glu Asp Ala Thr Val Arg Trp Leu Asp Ala Gln Pro Ala Lys Ser Val		
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[0545]	Val Tyr Val Ala Leu Gly Ser Glu Val Pro Leu Gly Val Glu Lys Val		

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[0549]	Ala	Leu	Arg	Lys	Pro	Thr	Gly	Val	Ser	Asp	Ala	Asp	Leu	Leu	Pro	Ala			
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[0553]	Val	Pro	Gln	Met	Ser	Ile	Leu	Ala	His	Ala	Ala	Val	Gly	Ala	Phe	Leu			
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[0558]					180						185					190			
[0559]	Leu	Ile	Glu	Ala	Lys	Asn	Ala	Gly	Leu	Gln	Val	Ala	Arg	Asn	Asp	Gly			
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[0561]	Asp	Gly	Ser	Phe	Asp	Arg	Glu	Gly	Val	Ala	Ala	Ala	Ile	Arg	Ala	Val			
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[0573]	Ala	Ser	Arg	Gly	His	Arg	Val	Ser	Phe	Val	Ser	Thr	Pro	Arg	Asn	Ile			
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[0575]	Ser	Arg	Leu	Pro	Pro	Val	Arg	Pro	Ala	Leu	Ala	Pro	Leu	Val	Ala	Phe			
[0576]							325					330				335			
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[0579]	Ser	Thr	Asn	Asp	Val	Pro	His	Asp	Arg	Pro	Asp	Met	Val	Glu	Leu	His			
[0580]							355					360				365			
[0581]	Arg	Arg	Ala	Phe	Asp	Gly	Leu	Ala	Ala	Pro	Phe	Ser	Glu	Phe	Leu	Gly			
[0582]							370					375				380			
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[0584]						385						390				395			400

[0585]	Ala Ala Ala Ala Leu Glu His Lys Val Pro Cys Ala Met Met Leu Leu
[0586]	405 410 415
[0587]	Gly Ser Ala His Met Ile Ala Ser Ile Ala Asp Arg Arg Leu Glu Arg
[0588]	420 425 430
[0589]	Ala Glu Thr Glu Ser Pro Ala Ala Ala Gly Gln Gly Arg Pro Ala Ala
[0590]	435 440 445
[0591]	Ala Pro Thr Phe Glu Val Ala Arg Met Lys Leu Ile Arg Thr Lys
[0592]	450 455 460
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[0604]	Glu Glu Ser Glu Leu Glu Thr Val Ile Arg Glu Ile Pro Ala Pro Ser
[0605]	35 40 45
[0606]	Phe Leu Ile Pro Leu Pro Lys His Leu Thr Ala Ser Ser Ser Ser Leu
[0607]	50 55 60
[0608]	Leu Asp His Asp Arg Thr Val Phe Gln Trp Leu Asp Gln Gln Pro Pro
[0609]	65 70 75 80
[0610]	Ser Ser Val Leu Tyr Val Ser Phe Gly Ser Thr Ser Glu Val Asp Glu
[0611]	85 90 95
[0612]	Lys Asp Phe Leu Glu Ile Ala Arg Gly Leu Val Asp Ser Lys Gln Ser
[0613]	100 105 110
[0614]	Phe Leu Trp Val Val Arg Pro Gly Phe Val Lys Gly Ser Thr Trp Val
[0615]	115 120 125
[0616]	Glu Pro Leu Pro Asp Gly Phe Leu Gly Glu Arg Gly Arg Ile Val Lys
[0617]	130 135 140
[0618]	Trp Val Pro Gln Gln Glu Val Leu Ala His Gly Ala Ile Gly Ala Phe
[0619]	145 150 155 160
[0620]	Trp Thr His Ser Gly Trp Asn Ser Thr Leu Glu Ser Val Cys Glu Gly
[0621]	165 170 175
[0622]	Val Pro Met Ile Phe Ser Asp Phe Gly Leu Asp Gln Pro Leu Asn Ala
[0623]	180 185 190

[0624]	Arg Tyr Met Ser Asp Val Leu Lys Val Gly Val Tyr Leu Glu Asn Gly
[0625]	195 200 205
[0626]	Trp Glu Arg Gly Glu Ile Ala Asn Ala Ile Arg Arg Val Met Val Asp
[0627]	210 215 220
[0628]	Glu Glu Gly Glu Tyr Ile Arg Gln Asn Ala Arg Val Leu Lys Gln Lys
[0629]	225 230 235 240
[0630]	Ala Asp Val Ser Leu Met Lys Gly Gly Ser Ser Tyr Glu Ser Leu Glu
[0631]	245 250 255
[0632]	Ser Leu Val Ser Tyr Ile Ser Ser Leu Glu Asn Lys Thr Glu Thr Thr
[0633]	260 265 270
[0634]	Val Arg Arg Arg Arg Arg Ile Ile Leu Phe Pro Val Pro Phe Gln Gly
[0635]	275 280 285
[0636]	His Ile Asn Pro Ile Leu Gln Leu Ala Asn Val Leu Tyr Ser Lys Gly
[0637]	290 295 300
[0638]	Phe Ser Ile Thr Ile Phe His Thr Asn Phe Asn Lys Pro Lys Thr Ser
[0639]	305 310 315 320
[0640]	Asn Tyr Pro His Phe Thr Phe Arg Phe Ile Leu Asp Asn Asp Pro Gln
[0641]	325 330 335
[0642]	Asp Glu Arg Ile Ser Asn Leu Pro Thr His Gly Pro Leu Ala Gly Met
[0643]	340 345 350
[0644]	Arg Ile Pro Ile Ile Asn Glu His Gly Ala Asp Glu Leu Arg Arg Glu
[0645]	355 360 365
[0646]	Leu Glu Leu Leu Met Leu Ala Ser Glu Glu Asp Glu Glu Val Ser Cys
[0647]	370 375 380
[0648]	Leu Ile Thr Asp Ala Leu Trp Tyr Phe Ala Gln Ser Val Ala Asp Ser
[0649]	385 390 395 400
[0650]	Leu Asn Leu Arg Arg Leu Val Leu Met Thr Ser Ser Leu Phe Asn Phe
[0651]	405 410 415
[0652]	His Ala His Val Ser Leu Pro Gln Phe Asp Glu Leu Gly Tyr Leu Asp
[0653]	420 425 430
[0654]	Pro Asp Asp Lys Thr Arg Leu Glu Glu Gln Ala Ser Gly Phe Pro Met
[0655]	435 440 445
[0656]	Leu Lys Val Lys Asp Ile Lys Ser Ala Tyr Ser
[0657]	450 455
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 [0667] Leu Thr Leu Ser Arg Ser Ser Leu Val Val Gly Arg Ser Cys Val Glu  
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 [0669] Phe Glu Pro Glu Thr Val Pro Leu Leu Ser Thr Leu Arg Gly Lys Pro  
 [0670] 35 40 45  
 [0671] Ile Thr Phe Leu Gly Leu Met Pro Pro Leu His Glu Gly Arg Arg Glu  
 [0672] 50 55 60  
 [0673] Asp Gly Glu Asp Ala Thr Val Arg Trp Leu Asp Ala Gln Pro Ala Lys  
 [0674] 65 70 75 80  
 [0675] Ser Val Val Tyr Val Ala Leu Gly Ser Glu Val Pro Leu Gly Val Glu  
 [0676] 85 90 95  
 [0677] Lys Val His Glu Leu Ala Leu Gly Leu Glu Leu Ala Gly Thr Arg Phe  
 [0678] 100 105 110  
 [0679] Leu Trp Ala Leu Arg Lys Pro Thr Gly Val Ser Asp Ala Asp Leu Leu  
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 [0681] Pro Ala Gly Phe Glu Glu Arg Thr Arg Gly Arg Gly Val Val Ala Thr  
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 [0683] Arg Trp Val Pro Gln Met Ser Ile Leu Ala His Ala Ala Val Gly Ala  
 [0684] 145 150 155 160  
 [0685] Phe Leu Thr His Cys Gly Trp Asn Ser Thr Ile Glu Gly Leu Met Phe  
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 [0687] Gly His Pro Leu Ile Met Leu Pro Ile Phe Gly Asp Gln Gly Pro Asn  
 [0688] 180 185 190  
 [0689] Ala Arg Leu Ile Glu Ala Lys Asn Ala Gly Leu Gln Val Ala Arg Asn  
 [0690] 195 200 205  
 [0691] Asp Gly Asp Gly Ser Phe Asp Arg Glu Gly Val Ala Ala Ala Ile Arg  
 [0692] 210 215 220  
 [0693] Ala Val Ala Val Glu Glu Glu Ser Ser Lys Val Phe Gln Ala Lys Ala  
 [0694] 225 230 235 240  
 [0695] Lys Lys Leu Gln Glu Ile Val Ala Asp Met Ala Cys His Glu Arg Tyr  
 [0696] 245 250 255  
 [0697] Ile Asp Gly Phe Ile Gln Gln Leu Arg Ser Tyr Lys Asp Asp Ser Gly  
 [0698] 260 265 270  
 [0699] Tyr Ser Ser Ser Tyr Ala Ala Ala Ala Gly Met His Val Val Ile Cys  
 [0700] 275 280 285  
 [0701] Pro Trp Leu Ala Phe Gly His Leu Leu Pro Cys Leu Asp Leu Ala Gln

[0702]	290	295	300
[0703]	Arg Leu Ala Ser Arg Gly His Arg Val Ser Phe Val Ser Thr Pro Arg		
[0704]	305	310	315 320
[0705]	Asn Ile Ser Arg Leu Pro Pro Val Arg Pro Ala Leu Ala Pro Leu Val		
[0706]	325	330	335
[0707]	Ala Phe Val Ala Leu Pro Leu Pro Arg Val Glu Gly Leu Pro Asp Gly		
[0708]	340	345	350
[0709]	Ala Glu Ser Thr Asn Asp Val Pro His Asp Arg Pro Asp Met Val Glu		
[0710]	355	360	365
[0711]	Leu His Arg Arg Ala Phe Asp Gly Leu Ala Ala Pro Phe Ser Glu Phe		
[0712]	370	375	380
[0713]	Leu Gly Thr Ala Cys Ala Asp Trp Val Ile Val Asp Val Phe His His		
[0714]	385	390	395 400
[0715]	Trp Ala Ala Ala Ala Ala Leu Glu His Lys Val Pro Cys Ala Met Met		
[0716]	405	410	415
[0717]	Leu Leu Gly Ser Ala Glu Met Ile Ala Ser Ile Ala Asp Glu Arg Leu		
[0718]	420	425	430
[0719]	Glu His Ala Glu Thr Glu Ser Pro Ala Ala Ala Gly Gln Gly Arg Pro		
[0720]	435	440	445
[0721]	Ala Ala Ala Pro Thr Phe Glu Val Ala Arg Met Lys Leu Ile Arg		
[0722]	450	455	460
[0723]	<210>	12	
[0724]	<211>	463	
[0725]	<212>	PRT	
[0726]	<213>	Artificial Sequence	
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[0733]	20	25	30
[0734]	Glu Gln Val Glu Glu Ile Thr Arg Ala Leu Ile Asp Ser Asp Val Asn		
[0735]	35	40	45
[0736]	Phe Leu Trp Val Ile Lys His Lys Glu Glu Gly Lys Leu Pro Glu Asn		
[0737]	50	55	60
[0738]	Leu Ser Glu Val Ile Lys Thr Gly Lys Gly Leu Ile Val Ala Trp Cys		
[0739]	65	70	75 80
[0740]	Lys Gln Leu Asp Val Leu Ala His Glu Ser Val Gly Cys Phe Val Thr		



[0780]	Gly Gln Phe Ala Asn Ile Asp Gln Ala Arg Trp Val Phe Thr Asn Ser
[0781]	405 410 415
[0782]	Phe Tyr Lys Leu Glu Glu Glu Val Ile Glu Trp Thr Arg Lys Ile Trp
[0783]	420 425 430
[0784]	Asn Leu Lys Val Ile Gly Pro Thr Leu Pro Ser Met Tyr Leu Asp Lys
[0785]	435 440 445
[0786]	Arg Leu Asp Asp Asp Lys Asp Asn Gly Phe Asn Leu Tyr Lys Ala
[0787]	450 455 460
[0788]	<210> 13
[0789]	<211> 482
[0790]	<212> PRT
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[0792]	<400> 13
[0793]	Met Ala Asp Ala Met Ala Thr Thr Glu Lys Lys Pro His Val Ile Phe
[0794]	1 5 10 15
[0795]	Ile Pro Phe Pro Ala Gln Ser His Ile Lys Ala Met Leu Lys Leu Ala
[0796]	20 25 30
[0797]	Gln Leu Leu His His Lys Gly Leu Gln Ile Thr Phe Val Asn Thr Asp
[0798]	35 40 45
[0799]	Phe Ile His Asn Gln Phe Leu Glu Ser Ser Gly Pro His Cys Leu Asp
[0800]	50 55 60
[0801]	Gly Ala Pro Gly Phe Arg Phe Glu Thr Ile Pro Asp Gly Val Ser His
[0802]	65 70 75 80
[0803]	Ser Pro Glu Ala Ser Ile Pro Ile Arg Glu Ser Leu Leu Arg Ser Ile
[0804]	85 90 95
[0805]	Glu Thr Asn Phe Leu Asp Arg Phe Ile Asp Leu Val Thr Lys Leu Pro
[0806]	100 105 110
[0807]	Asp Pro Pro Thr Cys Ile Ile Ser Asp Gly Phe Leu Ser Val Phe Thr
[0808]	115 120 125
[0809]	Ile Asp Ala Ala Lys Lys Leu Gly Ile Pro Val Met Met Tyr Trp Thr
[0810]	130 135 140
[0811]	Leu Ala Ala Cys Gly Phe Met Gly Phe Tyr His Ile His Ser Leu Ile
[0812]	145 150 155 160
[0813]	Glu Lys Gly Phe Ala Pro Leu Lys Asp Ala Ser Tyr Leu Thr Asn Gly
[0814]	165 170 175
[0815]	Tyr Leu Asp Thr Val Ile Asp Trp Val Pro Gly Met Glu Gly Ile Arg
[0816]	180 185 190
[0817]	Leu Lys Asp Phe Pro Leu Asp Trp Ser Thr Asp Leu Asn Asp Lys Val
[0818]	195 200 205

[0819]	Leu Met Phe Thr Thr Glu Ala Thr Gln Arg Ser His Lys Val Ser His
[0820]	210 215 220
[0821]	His Ile Phe His Thr Phe Asp Glu Leu Glu Pro Ser Ile Ile Lys Thr
[0822]	225 230 235 240
[0823]	Leu Ser Leu Arg Tyr Asn His Ile Tyr Thr Ile Gly Pro Leu Gln Leu
[0824]	245 250 255
[0825]	Leu Leu Asp Gln Ile Pro Glu Glu Lys Lys Gln Thr Gly Ile Thr Ser
[0826]	260 265 270
[0827]	Leu His Gly Tyr Ser Leu Val Lys Glu Glu Pro Glu Cys Phe Gln Trp
[0828]	275 280 285
[0829]	Leu Gln Ser Lys Glu Pro Asn Ser Val Val Tyr Val Asn Phe Gly Ser
[0830]	290 295 300
[0831]	Thr Thr Val Met Ser Leu Glu Asp Met Thr Glu Phe Gly Trp Gly Leu
[0832]	305 310 315 320
[0833]	Ala Asn Ser Asn His Tyr Phe Leu Trp Ile Ile Arg Ser Asn Leu Val
[0834]	325 330 335
[0835]	Ile Gly Glu Asn Ala Val Leu Pro Pro Glu Leu Glu Glu His Ile Lys
[0836]	340 345 350
[0837]	Lys Arg Gly Phe Ile Ala Ser Trp Cys Ser Gln Glu Lys Val Leu Lys
[0838]	355 360 365
[0839]	His Pro Ser Val Gly Gly Phe Leu Thr His Cys Gly Trp Gly Ser Thr
[0840]	370 375 380
[0841]	Ile Glu Ser Leu Ser Ala Gly Val Pro Met Ile Cys Trp Pro Tyr Ser
[0842]	385 390 395 400
[0843]	Trp Asp Gln Leu Thr Asn Cys Arg Tyr Ile Cys Lys Glu Trp Glu Val
[0844]	405 410 415
[0845]	Gly Leu Glu Met Gly Thr Lys Val Lys Arg Asp Glu Val Lys Arg Leu
[0846]	420 425 430
[0847]	Val Gln Glu Leu Met Gly Glu Gly Gly His Lys Met Arg Asn Lys Ala
[0848]	435 440 445
[0849]	Lys Asp Trp Lys Glu Lys Ala Arg Ile Ala Ile Ala Pro Asn Gly Ser
[0850]	450 455 460
[0851]	Ser Ser Leu Asn Ile Asp Lys Met Val Lys Glu Ile Thr Val Leu Ala
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[0854]	<210> 14
[0855]	<211> 459
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[0857]	<213> Stevia rebaudiana

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 [0864] 35 40 45  
 [0865] Thr Asn Phe Asn Lys Pro Lys Thr Ser Asn Tyr Pro His Phe Thr Phe  
 [0866] 50 55 60  
 [0867] Arg Phe Ile Leu Asp Asn Asp Pro Gln Asp Glu Arg Ile Ser Asn Leu  
 [0868] 65 70 75 80  
 [0869] Pro Thr His Gly Pro Leu Ala Gly Met Arg Ile Pro Ile Ile Asn Glu  
 [0870] 85 90 95  
 [0871] His Gly Ala Asp Glu Leu Arg Arg Glu Leu Glu Leu Leu Met Leu Ala  
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 [0873] Ser Glu Glu Asp Glu Glu Val Ser Cys Leu Ile Thr Asp Ala Leu Trp  
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 [0875] Tyr Phe Ala Gln Ser Val Ala Asp Ser Leu Asn Leu Arg Arg Leu Val  
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 [0877] Leu Met Thr Ser Ser Leu Phe Asn Phe His Ala His Val Ser Leu Pro  
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 [0879] Gln Phe Asp Glu Leu Gly Tyr Leu Asp Pro Asp Asp Lys Thr Arg Leu  
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 [0882] 180 185 190  
 [0883] Ser Ala Tyr Ser Asn Trp Gln Ile Ala Lys Glu Ile Leu Gly Lys Met  
 [0884] 195 200 205  
 [0885] Ile Lys Gln Thr Lys Ala Ser Ser Gly Val Ile Trp Asn Ser Phe Lys  
 [0886] 210 215 220  
 [0887] Glu Leu Glu Glu Ser Glu Leu Glu Thr Val Ile Arg Glu Ile Pro Ala  
 [0888] 225 230 235 240  
 [0889] Pro Ser Phe Leu Ile Pro Leu Pro Lys His Leu Thr Ala Ser Ser Ser  
 [0890] 245 250 255  
 [0891] Ser Leu Leu Asp His Asp Arg Thr Val Phe Gln Trp Leu Asp Gln Gln  
 [0892] 260 265 270  
 [0893] Pro Pro Ser Ser Val Leu Tyr Val Ser Phe Gly Ser Thr Ser Glu Val  
 [0894] 275 280 285  
 [0895] Asp Glu Lys Asp Phe Leu Glu Ile Ala Arg Gly Leu Val Asp Ser Lys  
 [0896] 290 295 300

[0897]	Gln Ser Phe Leu Trp Val Val Arg Pro Gly Phe Val Lys Gly Ser Thr
[0898]	305 310 315 320
[0899]	Trp Val Glu Pro Leu Pro Asp Gly Phe Leu Gly Glu Arg Gly Arg Ile
[0900]	325 330 335
[0901]	Val Lys Trp Val Pro Gln Gln Glu Val Leu Ala His Gly Ala Ile Gly
[0902]	340 345 350
[0903]	Ala Phe Trp Thr His Ser Gly Trp Asn Ser Thr Leu Glu Ser Val Cys
[0904]	355 360 365
[0905]	Glu Gly Val Pro Met Ile Phe Ser Asp Phe Gly Leu Asp Gln Pro Leu
[0906]	370 375 380
[0907]	Asn Ala Arg Tyr Met Ser Asp Val Leu Lys Val Gly Val Tyr Leu Glu
[0908]	385 390 395 400
[0909]	Asn Gly Trp Glu Arg Gly Glu Ile Ala Asn Ala Ile Arg Arg Val Met
[0910]	405 410 415
[0911]	Val Asp Glu Glu Gly Glu Tyr Ile Arg Gln Asn Ala Arg Val Leu Lys
[0912]	420 425 430
[0913]	Gln Lys Ala Asp Val Ser Leu Met Lys Gly Gly Ser Ser Tyr Glu Ser
[0914]	435 440 445
[0915]	Leu Glu Ser Leu Val Ser Tyr Ile Ser Ser Leu
[0916]	450 455
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[0925]	1 5 10 15
[0926]	Trp Val Glu Pro Leu Pro Asp Gly Phe Leu Gly Glu Arg Gly Arg Ile
[0927]	20 25 30
[0928]	Val Lys Trp Val Pro Gln Gln Glu Val Leu Ala His Gly Ala Ile Gly
[0929]	35 40 45
[0930]	Ala Phe Trp Thr His Gly Gly Trp Asn Ser Thr Leu Glu Ser Val Cys
[0931]	50 55 60
[0932]	Glu Gly Val Pro Met Ile Phe Ser Asp Phe Gly Leu Asp Gln Pro Leu
[0933]	65 70 75 80
[0934]	Asn Ala Arg Tyr Met Ser Asp Val Leu Lys Val Gly Val Tyr Leu Glu
[0935]	85 90 95

[0936]	Asn Gly Trp Glu Arg Gly Glu Ile Ala Asn Ala Ile Arg Arg Leu Met
[0937]	100 105 110
[0938]	Val Asp Glu Glu Gly Glu Tyr Ile Arg Gln Asn Ala Arg Val Leu Lys
[0939]	115 120 125
[0940]	Gln Lys Ala Asp Val Ser Leu Met Lys Gly Gly Ser Ser Tyr Glu Ser
[0941]	130 135 140
[0942]	Leu Glu Ser Leu Val Ser Tyr Ile Ser Ser Leu Gly Ser Gly Gly Ser
[0943]	145 150 155 160
[0944]	Gly Gly Ser Gly Arg Arg Arg Arg Ile Ile Leu Phe Pro Val Pro Phe
[0945]	165 170 175
[0946]	Gln Gly His Ile Asn Pro Met Leu Gln Leu Ala Asn Val Leu Tyr Ser
[0947]	180 185 190
[0948]	Lys Gly Phe Ser Ile Thr Ile Phe His Thr Asn Phe Asn Lys Pro Lys
[0949]	195 200 205
[0950]	Thr Ser Asn Tyr Pro His Phe Thr Phe Arg Phe Ile Leu Asp Asn Asp
[0951]	210 215 220
[0952]	Pro Gln Asp Glu Arg Ile Ser Asn Leu Pro Thr His Gly Pro Leu Ala
[0953]	225 230 235 240
[0954]	Gly Met Arg Ile Pro Ile Ile Asn Glu His Gly Ala Asp Glu Leu Arg
[0955]	245 250 255
[0956]	Arg Glu Leu Glu Leu Leu Met Leu Ala Ser Glu Glu Asp Glu Glu Val
[0957]	260 265 270
[0958]	Ser Cys Leu Ile Thr Asp Ala Leu Trp Tyr Phe Ala Gln Ser Val Ala
[0959]	275 280 285
[0960]	Asp Ser Leu Asn Leu Arg Arg Leu Val Leu Met Thr Ser Ser Leu Phe
[0961]	290 295 300
[0962]	Asn Phe His Ala His Val Ser Leu Pro Gln Phe Asp Glu Leu Gly Tyr
[0963]	305 310 315 320
[0964]	Leu Asp Pro Asp Asp Lys Thr Arg Leu Glu Glu Gln Ala Ser Gly Phe
[0965]	325 330 335
[0966]	Pro Met Leu Lys Val Lys Asp Ile Lys Ser Ala Tyr Ser Asn Trp Gln
[0967]	340 345 350
[0968]	Ile Ala Lys Glu Ile Leu Gly Lys Met Ile Lys Gln Thr Lys Ala Ser
[0969]	355 360 365
[0970]	Ser Gly Val Ile Trp Asn Ser Phe Lys Glu Leu Glu Glu Ser Glu Leu
[0971]	370 375 380
[0972]	Glu Thr Val Ile Arg Glu Ile Pro Ala Pro Ser Phe Leu Ile Pro Leu
[0973]	385 390 395 400
[0974]	Pro Lys His Leu Thr Ala Ser Ser Ser Ser Leu Leu Asp His Asp Arg

[0975]		405		410		415
[0976]	Thr Val Phe Gln Trp Leu Asp Gln Gln Pro Pro Ser Ser Val Leu Tyr					
[0977]		420		425		430
[0978]	Val Ser Phe Gly Ser Thr Ser Glu Val Asp Glu Lys Asp Phe Leu Glu					
[0979]		435		440		445
[0980]	Ile Ala Arg Gly Leu Val Asp Ser Gln Ser					
[0981]		450		455		
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[0983]	<211>	451				
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[0985]	<213>	Artificial Sequence				
[0986]	<220>					
[0987]	<223>	Synthetic sequence				
[0988]	<400>	16				
[0989]	Met Ala Phe Leu Trp Val Val Arg Pro Gly Phe Val Lys Gly Ser Thr					
[0990]	1	5		10		15
[0991]	Trp Val Glu Pro Leu Pro Asp Gly Phe Leu Gly Glu Arg Gly Arg Ile					
[0992]		20		25		30
[0993]	Val Lys Trp Val Pro Gln Gln Glu Val Leu Ala His Gly Ala Ile Gly					
[0994]		35		40		45
[0995]	Ala Phe Trp Thr His Gly Gly Trp Asn Ser Thr Leu Glu Ser Val Cys					
[0996]		50		55		60
[0997]	Glu Gly Val Pro Met Ile Phe Ser Asp Phe Gly Leu Asp Gln Pro Leu					
[0998]	65	70		75		80
[0999]	Asn Ala Arg Tyr Met Ser Asp Val Leu Lys Val Gly Val Tyr Leu Glu					
[1000]		85		90		95
[1001]	Asn Gly Trp Glu Arg Gly Glu Ile Ala Asn Ala Ile Arg Arg Leu Met					
[1002]		100		105		110
[1003]	Val Asp Glu Glu Gly Glu Tyr Ile Arg Gln Asn Ala Arg Val Leu Lys					
[1004]		115		120		125
[1005]	Gln Lys Ala Asp Val Ser Leu Met Lys Gly Gly Ser Ser Tyr Glu Ser					
[1006]		130		135		140
[1007]	Leu Glu Ser Leu Val Ser Tyr Ile Ser Ser Leu Gly Ser Gly Gly Ser					
[1008]	145	150		155		160
[1009]	Gly Gly Ser Gly Arg Arg Arg Arg Ile Ile Leu Phe Pro Val Pro Phe					
[1010]		165		170		175
[1011]	Gln Gly His Ile Asn Pro Met Leu Gln Leu Ala Asn Val Leu Tyr Ser					
[1012]		180		185		190
[1013]	Lys Gly Phe Ser Ile Thr Ile Phe His Thr Asn Phe Asn Lys Pro Lys					

[1014]	195	200	205
[1015]	Thr Ser Asn Tyr Pro His Phe Thr Phe Arg Phe Ile Leu Asp Asn Asp		
[1016]	210	215	220
[1017]	Pro Gln Thr Thr His Gly Pro Leu Ala Gly Met Arg Ile Pro Ile Ile		
[1018]	225	230	235
[1019]	Asn Glu His Gly Ala Asp Glu Leu Arg Arg Glu Leu Glu Leu Gln Met		
[1020]	245	250	255
[1021]	Leu Ala Ser Glu Glu Asp Glu Glu Val Ser Cys Leu Ile Thr Asp Ala		
[1022]	260	265	270
[1023]	Leu Trp Tyr Phe Ala Gln Ser Val Ala Asp Ser Leu Asn Leu Pro Arg		
[1024]	275	280	285
[1025]	Leu Val Leu Met Thr Ser Ser Leu Phe Asn Phe His Ala His Val Ser		
[1026]	290	295	300
[1027]	Leu Pro Gln Phe Asp Glu Leu Gly Tyr Leu Asp Pro Asp Asp Lys Thr		
[1028]	305	310	315
[1029]	Arg Leu Glu Glu Gln Ala Ser Gly Phe Pro Met Leu Lys Val Lys Asp		
[1030]	325	330	335
[1031]	Ile Lys Ser Ala Tyr Ser Asn Trp Gln Ile Ala Lys Glu Ile Leu Gly		
[1032]	340	345	350
[1033]	Lys Met Ile Lys Gln Thr Lys Ala Ser Ser Gly Val Ile Trp Asn Ser		
[1034]	355	360	365
[1035]	Phe Lys Glu Leu Glu Glu Ser Glu Leu Glu Thr Val Ile Arg Glu Ile		
[1036]	370	375	380
[1037]	Pro Ala Pro Ser Phe Leu Ile Pro Leu Pro Lys His Leu Thr Ala Ser		
[1038]	385	390	395
[1039]	Ser Ser Ser Leu Leu Asp His Asp Arg Thr Val Phe Gln Trp Leu Asp		
[1040]	405	410	415
[1041]	Gln Gln Pro Pro Ser Ser Val Leu Tyr Val Ser Phe Gly Ser Thr Ser		
[1042]	420	425	430
[1043]	Glu Val Asp Glu Lys Asp Phe Leu Glu Ile Ala Arg Gly Leu Val Asp		
[1044]	435	440	445
[1045]	Ser Gln Ser		
[1046]	450		
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[1048]	<211> 455		
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[1125]	Asn His Arg Arg Ile Ser Asn Thr Glu Ala Leu Ala Ser Arg Phe Pro
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[1129]	Arg Ala Leu Ala Asp Gly Pro Leu Tyr Phe Ser Met Arg Glu Gly Thr
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[1166]	385 390 395 400
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[1334]	Ile Pro Ser Ala Leu Phe Phe Ala Gln Pro Ala Thr Val Leu Ala Leu		
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[1336]	Tyr Phe Tyr Tyr Phe His Gly Tyr Gly Asp Thr Ile Cys Ser Lys Leu		
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[1340]	Ser Gln Asp Met Pro Ser Phe Phe Ser Pro Ser Gly Pro His Ala Phe		
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[1344]	Gln Pro Lys Val Leu Val Asn Thr Phe Asp Ala Leu Glu Ala Asp Ala		
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[1346]	Leu Arg Ala Ile Asp Lys Leu Lys Met Leu Ala Ile Gly Pro Leu Ile		
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[1350]	Gly Asp Leu Phe Gln Val Ser Ser Glu Asp Tyr Ile Glu Trp Leu Asn		
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[1352]	Ser Lys Pro Asp Ser Ser Val Val Tyr Ile Ser Val Gly Ser Ile Cys		
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[1356]	Ser Gly His Thr Phe Leu Trp Val Lys Arg Ser Lys Glu Asn Asn Glu		
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[1362]	Pro Ala Leu Gly Cys Phe Leu Thr His Cys Gly Trp Asn Ser Thr Ile		
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[1374]	Ile Gly Glu Gly Gly Ser Ser Asp Ser Asn Leu Lys Thr Phe Leu Trp			
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[1393]	Ile Leu Asp Pro Lys Phe Phe Ile Ser Ile Cys Gln Val Thr Lys Pro			
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[1409]	Leu Asn Phe Val Asp Gln Thr Thr Ala Ser Ser Arg Ser Ser Gly Leu					
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[1413]	Ser Thr Ile Phe Lys Lys Ile Tyr Ala Val Gly Pro Ile His Ser Leu					
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[1417]	Ala Trp Leu Asp Ser Arg Ala Ala Lys Ser Val Val Phe Val Ser Phe					
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[1421]	Gly Leu Leu Asn Ser Gly Lys Ser Phe Leu Phe Val Leu Arg Ser Asp					
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[1425]	Thr Lys Ala Glu Gly Lys Trp Leu Val Val Gly Trp Ala Pro Gln Glu					
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[1427]	Lys Val Leu Ala His Glu Ala Val Gly Gly Phe Leu Thr His Ser Gly					
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[1433]	Val Trp Lys Ile Gly Leu Glu Met Glu Asp Arg Tyr Asp Arg Val Ser					
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[1435]	Val Glu Thr Met Val Arg Ser Ile Met Glu Gln Glu Gly Glu Lys Met					
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[1437]	Gln Lys Thr Ile Ala Glu Leu Ala Lys Gln Ala Lys Tyr Lys Val Ser					
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[1439]	Lys Asp Gly Thr Ser Tyr Gln Asn Leu Glu Cys Leu Ile Gln Asp Ile					
[1440]		450		455		460
[1441]	Lys Lys Leu Asn Gln Ile Glu Gly Phe Ile Asn Asn Pro Asn Phe Ser					
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[1456]	Leu Asp Ala Ile Lys Pro Lys Leu Pro Pro Ser Ile Ser Ser Asp Asp		
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[1458]	Ser Ile Gln Leu Val Glu Leu Arg Leu Pro Ser Ser Pro Glu Leu Pro		
[1459]		65	70 75 80
[1460]	Pro His Leu His Thr Thr Asn Gly Leu Pro Ser His Leu Met Pro Ala		
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[1462]	Leu His Gln Ala Phe Val Met Ala Ala Gln His Phe Gln Val Ile Leu		
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[1464]	Gln Thr Leu Ala Pro His Leu Leu Ile Tyr Asp Ile Leu Gln Pro Trp		
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[1466]	Ala Pro Gln Val Ala Ser Ser Leu Asn Ile Pro Ala Ile Asn Phe Ser		
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[1468]	Thr Thr Gly Ala Ser Met Leu Ser Arg Thr Leu His Pro Thr His Tyr		
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[1474]	Lys Ile Glu Glu Thr Leu Ala Asn Cys Leu His Thr Ser Cys Gly Val		
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[1476]	Val Leu Val Asn Ser Phe Arg Glu Leu Glu Thr Lys Tyr Ile Asp Tyr		
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[1478]	Leu Ser Val Leu Leu Asn Lys Lys Val Val Pro Val Gly Pro Leu Val		
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[1480]	Tyr Glu Pro Asn Gln Glu Gly Glu Asp Glu Gly Tyr Ser Ser Ile Lys		
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[1483]	260 265 270
[1484]	Gly Thr Glu Tyr Phe Pro Ser Lys Glu Glu Met Glu Glu Ile Ala Tyr
[1485]	275 280 285
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[1487]	290 295 300
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[1490]	Leu Glu Arg Ala Gly Glu Arg Ala Met Val Val Lys Gly Trp Ala Pro
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[1492]	Gln Ala Lys Ile Leu Lys His Trp Ser Thr Gly Gly Leu Val Ser His
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[1494]	Cys Gly Trp Asn Ser Met Met Glu Gly Met Met Phe Gly Val Pro Ile
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[1496]	Ile Ala Val Pro Met His Leu Asp Gln Pro Phe Asn Ala Gly Leu Val
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[1502]	Lys Thr Arg Glu Asp Val Arg Lys Lys Ala Arg Glu Met Asp Thr Lys
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[1504]	His Gly Pro Thr Tyr Phe Ser Arg Ser Lys Val Ser Ser Phe Gly Arg
[1505]	435 440 445
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[1517]	Leu Gly Tyr Gly His Leu Ser Ala Phe Leu Glu Leu Ala Lys Ser Leu
[1518]	20 25 30
[1519]	Ser Arg Arg Asn Phe His Ile Tyr Phe Cys Ser Thr Ser Val Asn Leu
[1520]	35 40 45

[1521]	Asp Ala Ile Lys Pro Lys Leu Pro Ser Ser Ser Ser Ser Asp Ser Ile
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[1523]	Gln Leu Val Glu Leu Cys Leu Pro Ser Ser Pro Asp Gln Leu Pro Pro
[1524]	65 70 75 80
[1525]	His Leu His Thr Thr Asn Ala Leu Pro Pro His Leu Met Pro Thr Leu
[1526]	85 90 95
[1527]	His Gln Ala Phe Ser Met Ala Ala Gln His Phe Ala Ala Ile Leu His
[1528]	100 105 110
[1529]	Thr Leu Ala Pro His Leu Leu Ile Tyr Asp Ser Phe Gln Pro Trp Ala
[1530]	115 120 125
[1531]	Pro Gln Leu Ala Ser Ser Leu Asn Ile Pro Ala Ile Asn Phe Asn Thr
[1532]	130 135 140
[1533]	Thr Gly Ala Ser Val Leu Thr Arg Met Leu His Ala Thr His Tyr Pro
[1534]	145 150 155 160
[1535]	Ser Ser Lys Phe Pro Ile Ser Glu Phe Val Leu His Asp Tyr Trp Lys
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[1537]	Ala Met Tyr Ser Ala Ala Gly Gly Ala Val Thr Lys Lys Asp His Lys
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[1539]	Ile Gly Glu Thr Leu Ala Asn Cys Leu His Ala Ser Cys Ser Val Ile
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[1541]	Leu Ile Asn Ser Phe Arg Glu Leu Glu Glu Lys Tyr Met Asp Tyr Leu
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[1543]	Ser Val Leu Leu Asn Lys Lys Val Val Pro Val Gly Pro Leu Val Tyr
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[1545]	Glu Pro Asn Gln Asp Gly Glu Asp Glu Gly Tyr Ser Ser Ile Lys Asn
[1546]	245 250 255
[1547]	Trp Leu Asp Lys Lys Glu Pro Ser Ser Thr Val Phe Val Ser Phe Gly
[1548]	260 265 270
[1549]	Ser Glu Tyr Phe Pro Ser Lys Glu Glu Met Glu Glu Ile Ala His Gly
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[1552]	290 295 300
[1553]	Gly Asp Asn Thr Ser Ala Ile Glu Asp Ala Leu Pro Lys Gly Phe Leu
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[1555]	Glu Arg Val Gly Glu Arg Gly Met Val Val Lys Gly Trp Ala Pro Gln
[1556]	325 330 335
[1557]	Ala Lys Ile Leu Lys His Trp Ser Thr Gly Gly Phe Val Ser His Cys
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[1559]	Gly Trp Asn Ser Val Met Glu Ser Met Met Phe Gly Val Pro Ile Ile

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[1563]	Glu Ala Gly Val Gly Val Glu Ala Lys Arg Asp Pro Asp Gly Lys Ile		
[1564]	385	390	395
[1565]	Gln Arg Asp Glu Val Ala Lys Leu Ile Lys Glu Val Val Val Glu Lys		
[1566]	405	410	415
[1567]	Thr Arg Glu Asp Val Arg Lys Lys Ala Arg Glu Met Ser Glu Ile Leu		
[1568]	420	425	430
[1569]	Arg Ser Lys Gly Glu Glu Lys Met Asp Glu Met Val Ala Ala Ile Ser		
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[1571]	Leu Phe Leu Lys Ile		
[1572]	450		
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[1582]	Asp Gly Gly Leu Asp Val Val Phe Leu Ser Thr Glu Tyr Asn His Arg		
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[1586]	Phe Asp Thr Ile Pro Asp Gly Leu Pro Ile Asp Lys Pro Arg Val Ile		
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[1588]	Ile Gly Gly Glu Leu Tyr Thr Ser Met Arg Asp Gly Val Lys Gln Arg		
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[1590]	Leu Arg Gln Val Leu Gln Ser Tyr Asn Asp Gly Ser Ser Pro Ile Thr		
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[1592]	Cys Val Ile Cys Asp Val Met Leu Ser Gly Pro Ile Glu Ala Ala Glu		
[1593]	115	120	125
[1594]	Glu Leu Gly Ile Pro Val Val Thr Phe Cys Pro Tyr Ser Ala Arg Tyr		
[1595]	130	135	140
[1596]	Leu Cys Ala His Phe Val Met Pro Lys Leu Ile Glu Glu Gly Gln Ile		
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[1682]	305 310 315 320
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[1687]	Val Gly Gly Phe Leu Thr His Cys Gly Trp Asn Ser Thr Val Glu Ser
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[1697]	Leu Ser Lys Leu Ala Asn Glu Lys Val Val Lys Gly Gly Thr Ser Leu
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[1712]	Ala Gln Leu Leu Cys Gly Ala Gly Leu Gln Val Thr Phe Leu Asn Thr
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[1714]	Asp His Asn His Arg Arg Ile Asp Asp Arg His Arg Arg Leu Leu Ala
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[1716]	Thr Gln Phe Pro Met Leu His Phe Lys Ser Ile Ser Asp Gly Leu Pro
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[1718]	Pro Asp His Pro Arg Asp Leu Leu Asp Gly Lys Leu Ile Ala Ser Met
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[1720]	Arg Arg Val Thr Glu Ser Leu Phe Arg Gln Leu Leu Leu Ser Tyr Asn
[1721]	100 105 110
[1722]	Gly Tyr Gly Asn Gly Thr Asn Asn Val Ser Asn Ser Gly Arg Arg Pro
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[1726]	Val Ala Glu Glu Leu Gly Ile Pro Val Phe Ser Phe Ala Thr Phe Ser
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[1728]	Ala Arg Phe Leu Phe Leu Tyr Phe Trp Ile Pro Lys Leu Ile Gln Glu
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[1732]	Val Pro Gly Ala Glu Gly Ile Ile Arg Cys Lys Asp Leu Pro Gly Ser
[1733]	195 200 205
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[1735]	210 215 220
[1736]	Gln Thr Leu Ala Ser Ser Arg Ser Ser Gly Leu Ile Leu Asn Thr Phe
[1737]	225 230 235 240
[1738]	Glu Asp Leu Glu Ala Pro Phe Val Thr His Leu Ser Asn Thr Phe Asp
[1739]	245 250 255
[1740]	Lys Ile Tyr Thr Ile Gly Pro Ile His Ser Leu Leu Gly Thr Ser His
[1741]	260 265 270
[1742]	Cys Gly Leu Trp Lys Glu Asp Tyr Ala Cys Leu Ala Trp Leu Asp Ala
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[1744]	Arg Pro Arg Lys Ser Val Val Phe Val Ser Phe Gly Ser Leu Val Lys
[1745]	290 295 300
[1746]	Thr Thr Ser Arg Glu Leu Met Glu Leu Trp His Gly Leu Val Ser Ser
[1747]	305 310 315 320
[1748]	Gly Lys Ser Phe Leu Leu Val Leu Arg Ser Asp Val Val Glu Gly Glu
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[1752]	Lys Trp Leu Val Val Gly Trp Ala Pro Gln Glu Glu Val Leu Ala His
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[1754]	Glu Ala Ile Gly Gly Phe Leu Thr His Ser Gly Trp Asn Ser Thr Met

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[1758]	Asp Gln Pro Ser Asn Cys Thr Trp Val Ser Arg Val Trp Lys Val Gly		
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[1784]	Asp Gly Gln Leu Phe Lys Ser Met Pro Gln Val Thr Lys Pro Leu Phe		
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[1786]	Arg Gln Leu Leu Leu Ser Tyr Lys Asp Gly Thr Ser Pro Ile Thr Cys		
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			160

[1794]	Tyr Pro Glu Gly Asn Ser Asn Gln Val Leu His Gly Ile Pro Gly Ala
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[1796]	Glu Gly Leu Leu Arg Cys Lys Asp Leu Pro Gly Tyr Trp Ser Val Glu
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[1798]	Ala Val Ala Asn Tyr Asn Pro Met Asn Phe Val Asn Gln Thr Ile Ala
[1799]	195 200 205
[1800]	Thr Ser Lys Ser His Gly Leu Ile Leu Asn Thr Phe Asp Glu Leu Glu
[1801]	210 215 220
[1802]	Val Pro Phe Ile Thr Asn Leu Ser Lys Ile Tyr Lys Lys Val Tyr Thr
[1803]	225 230 235 240
[1804]	Ile Gly Pro Ile His Ser Leu Leu Lys Lys Ser Val Gln Thr Gln Tyr
[1805]	245 250 255
[1806]	Glu Phe Trp Lys Glu Asp His Ser Cys Leu Ala Trp Leu Asp Ser Gln
[1807]	260 265 270
[1808]	Pro Pro Arg Ser Val Met Phe Val Ser Phe Gly Ser Ile Val Lys Leu
[1809]	275 280 285
[1810]	Lys Ser Ser Gln Leu Lys Glu Phe Trp Asn Gly Leu Val Asp Ser Gly
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[1812]	Lys Ala Phe Leu Leu Val Leu Arg Ser Asp Ala Leu Val Glu Glu Thr
[1813]	305 310 315 320
[1814]	Gly Glu Glu Asp Glu Lys Gln Lys Glu Leu Val Ile Lys Glu Ile Met
[1815]	325 330 335
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[1817]	340 345 350
[1818]	Glu Lys Val Leu Glu His Lys Ala Ile Gly Gly Phe Leu Thr His Ser
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[1820]	Gly Trp Asn Ser Thr Leu Glu Ser Val Ala Val Gly Val Pro Met Val
[1821]	370 375 380
[1822]	Ser Trp Pro Gln Ile Gly Asp Gln Pro Ser Asn Ala Thr Trp Leu Ser
[1823]	385 390 395 400
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[1828]	Met Glu Asn Ala Ile Val Glu Leu Ala Lys Arg Val Asp Asp Arg Val
[1829]	435 440 445
[1830]	Ser Lys Glu Gly Thr Ser Tyr Gln Asn Leu Gln Arg Leu Ile Glu Asp
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[1843]	Ala Gly Leu Arg Val Thr Phe Leu Asn Thr Asp His His His Arg Arg	
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[1845]	Ile His Asp Leu Asn Arg Leu Ala Ala Gln Leu Pro Thr Leu His Phe	
[1846]		50 55 60
[1847]	Asp Ser Val Ser Asp Gly Leu Pro Pro Asp Glu Pro Arg Asn Val Phe	
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[1849]	Asp Gly Lys Leu Tyr Glu Ser Ile Arg Gln Val Thr Ser Ser Leu Phe	
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[1851]	Arg Glu Leu Leu Val Ser Tyr Asn Asn Gly Thr Ser Ser Gly Arg Pro	
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[1853]	Pro Ile Thr Cys Val Ile Thr Asp Val Met Phe Arg Phe Pro Ile Asp	
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[1857]	Ala Arg Phe Leu Phe Leu Ile Phe Trp Ile Pro Lys Leu Leu Glu Asp	
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[1861]	Glu Gly Leu Ile Arg Trp Lys Asp Leu Pro Gly Phe Trp Ser Val Glu	
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[1865]	Thr Ser Arg Ser Ser Gly Leu Ile Leu Asn Thr Phe Asp Glu Leu Glu	
[1866]		210 215 220
[1867]	Ala Pro Phe Leu Thr Ser Leu Ser Lys Ile Tyr Lys Lys Ile Tyr Ser	
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[1869]	Leu Gly Pro Ile Asn Ser Leu Leu Lys Asn Phe Gln Ser Gln Pro Gln	
[1870]		245 250 255
[1871]	Tyr Asn Leu Trp Lys Glu Asp His Ser Cys Met Ala Trp Leu Asp Ser	

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[1891]	Ile	Met	Asp	Gln	Thr	Met	Glu	Lys	Thr	Val	Ala	Glu	Leu	Ala	Glu	Arg				
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[1895]	Thr	Leu	Ile	Gln	Asp	Ile	Thr	Asn	Ile	Ile	Glu	Thr	His	Ile						
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[1906]	Ala	Gly	Ala	Arg	Val	Thr	Phe	Leu	Asn	Thr	Asp	His	His	His	Arg	Arg				
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[1916]	Pro Ile Thr Cys Val Ile Thr Asp Cys Met Phe Arg Phe Pro Ile Asp			
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[1922]	Gly Gln Leu Arg Tyr Pro Glu Gln Glu Leu His Gly Val Pro Gly Ala			
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[1924]	Glu Gly Leu Ile Arg Cys Lys Asp Leu Pro Gly Phe Leu Ser Asp Glu			
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[1926]	Asp Val Ala His Trp Lys Pro Ile Asn Phe Val Asn Gln Ile Leu Ala			
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[1928]	Thr Ser Arg Ser Ser Gly Leu Ile Leu Asn Thr Phe Asp Glu Leu Glu			
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[1930]	Ala Pro Phe Leu Thr Ser Leu Ser Lys Ile Tyr Lys Lys Ile Tyr Ser			
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[1932]	Leu Gly Pro Ile Asn Ser Leu Leu Lys Asn Phe Gln Ser Gln Pro Gln			
[1933]		245	250	255
[1934]	Tyr Asn Leu Trp Lys Glu Asp His Ser Cys Met Ala Trp Leu Asp Ser			
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[1936]	Gln Pro Pro Lys Ser Val Val Phe Val Ser Phe Gly Ser Val Val Lys			
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[1946]	Val Gly Gly Phe Leu Thr His Ser Gly Trp Asn Ser Thr Leu Glu Ser			
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[1952]	Leu Glu Asp Gly Phe Asp Ser Phe Thr Ile Glu Thr Met Val Arg Ser
[1953]	405 410 415
[1954]	Val Met Asp Gln Thr Met Glu Lys Thr Val Ala Glu Leu Ala Glu Arg
[1955]	420 425 430
[1956]	Ala Lys Asn Arg Ala Ser Lys Asn Gly Thr Ser Tyr Arg Asn Phe Gln
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[1967]	Gly His Met Lys Pro Leu Leu Cys Leu Ala Glu Leu Leu Cys His Ala
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[1969]	Gly Leu His Val Thr Tyr Val Asn Thr His His Asn His Gln Arg Leu
[1970]	35 40 45
[1971]	Ala Asn Arg Gln Ala Leu Ser Thr His Phe Pro Thr Leu His Phe Glu
[1972]	50 55 60
[1973]	Ser Ile Ser Asp Gly Leu Pro Glu Asp Asp Pro Arg Thr Leu Asn Ser
[1974]	65 70 75 80
[1975]	Gln Leu Leu Ile Ala Leu Lys Thr Ser Ile Arg Pro His Phe Arg Glu
[1976]	85 90 95
[1977]	Leu Leu Lys Thr Ile Ser Leu Lys Ala Glu Ser Asn Asp Thr Leu Val
[1978]	100 105 110
[1979]	Pro Pro Pro Ser Cys Ile Met Thr Asp Gly Leu Val Thr Phe Ala Phe
[1980]	115 120 125
[1981]	Asp Val Ala Glu Glu Leu Gly Leu Pro Ile Leu Ser Phe Asn Val Pro
[1982]	130 135 140
[1983]	Cys Pro Arg Tyr Leu Trp Thr Cys Leu Cys Leu Pro Lys Leu Ile Glu
[1984]	145 150 155 160
[1985]	Asn Gly Gln Leu Pro Phe Gln Asp Asp Asp Met Asn Val Glu Ile Thr
[1986]	165 170 175
[1987]	Gly Val Pro Gly Met Glu Gly Leu Leu His Arg Gln Asp Leu Pro Gly
[1988]	180 185 190

[1989]	Phe Cys Arg Val Lys Gln Ala Asp His Pro Ser Leu Gln Phe Ala Ile
[1990]	195 200 205
[1991]	Asn Glu Thr Gln Thr Leu Lys Arg Ala Ser Ala Leu Ile Leu Asp Thr
[1992]	210 215 220
[1993]	Val Tyr Glu Leu Asp Ala Pro Cys Ile Ser His Met Ala Leu Met Phe
[1994]	225 230 235 240
[1995]	Pro Lys Ile Tyr Thr Leu Gly Pro Leu His Ala Leu Leu Asn Ser Gln
[1996]	245 250 255
[1997]	Ile Gly Asp Met Ser Arg Gly Leu Ala Ser His Gly Ser Leu Trp Lys
[1998]	260 265 270
[1999]	Ser Asp Leu Asn Cys Met Thr Trp Leu Asp Ser Gln Pro Ser Lys Ser
[2000]	275 280 285
[2001]	Ile Ile Tyr Val Ser Phe Gly Thr Leu Val His Leu Thr Arg Ala Gln
[2002]	290 295 300
[2003]	Val Ile Glu Phe Trp Tyr Gly Leu Val Asn Ser Gly His Pro Phe Leu
[2004]	305 310 315 320
[2005]	Trp Val Met Arg Ser Asp Ile Thr Ser Gly Asp His Gln Ile Pro Ala
[2006]	325 330 335
[2007]	Glu Leu Glu Asn Gly Thr Lys Glu Arg Gly Cys Ile Val Asp Trp Val
[2008]	340 345 350
[2009]	Ser Gln Glu Glu Val Leu Ala His Lys Ser Val Gly Gly Phe Leu Thr
[2010]	355 360 365
[2011]	His Ser Gly Trp Asn Ser Thr Leu Glu Ser Ile Val Ala Gly Leu Pro
[2012]	370 375 380
[2013]	Met Ile Cys Trp Pro Lys Leu Gly Asp His Tyr Ile Ile Ser Ser Thr
[2014]	385 390 395 400
[2015]	Val Cys Arg Gln Trp Lys Ile Gly Leu Gln Leu Asn Glu Asn Cys Asp
[2016]	405 410 415
[2017]	Arg Ser Asn Ile Glu Ser Met Val Gln Thr Leu Met Gly Ser Lys Arg
[2018]	420 425 430
[2019]	Glu Glu Ile Gln Ser Ser Met Asp Ala Ile Ser Lys Leu Ser Arg Asp
[2020]	435 440 445
[2021]	Ser Val Ala Glu Gly Gly Ser Ser His Asn Asn Leu Glu Gln Leu Ile
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[2023]	Glu Tyr Ile Arg Asn Leu Gln His Gln Asn
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[2027]	<212> PRT

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[2032]	Ile Lys Pro Met Leu Cys Leu Ala Glu Leu Leu Cys Gln Ala Gly Leu	
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[2034]	Arg Val Thr Phe Leu Asn Thr His His Ser His Arg Arg Leu Asn Asn	
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[2036]	Leu Gln Asp Leu Ser Thr Arg Phe Pro Thr Leu His Phe Glu Ser Val	
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[2038]	Ser Asp Gly Leu Pro Glu Asp His Pro Arg Asn Leu Val His Phe Met	
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[2040]	His Leu Val His Ser Ile Lys Asn Val Thr Lys Pro Leu Leu Arg Asp	
[2041]		85 90 95
[2042]	Leu Leu Thr Ser Leu Ser Leu Lys Thr Asp Ile Pro Pro Val Ser Cys	
[2043]		100 105 110
[2044]	Ile Ile Ala Asp Gly Ile Leu Ser Phe Ala Ile Asp Val Ala Glu Glu	
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[2046]	Leu Gln Ile Lys Val Ile Ile Phe Arg Thr Ile Ser Ser Cys Cys Leu	
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[2048]	Trp Ser Tyr Leu Cys Val Pro Lys Leu Ile Gln Gln Gly Glu Leu Gln	
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[2050]	Phe Ser Asp Ser Asp Met Gly Gln Lys Val Ser Ser Val Pro Glu Met	
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[2052]	Lys Gly Ser Leu Arg Leu His Asp Arg Pro Tyr Ser Phe Gly Leu Lys	
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[2054]	Gln Leu Glu Asp Pro Asn Phe Gln Phe Phe Val Ser Glu Thr Gln Ala	
[2055]		195 200 205
[2056]	Met Thr Arg Ala Ser Ala Val Ile Phe Asn Thr Phe Asp Ser Leu Glu	
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[2058]	Ala Pro Val Leu Ser Gln Met Ile Pro Leu Leu Pro Lys Val Tyr Thr	
[2059]		225 230 235 240
[2060]	Ile Gly Pro Leu His Ala Leu Arg Lys Ala Arg Leu Gly Asp Leu Ser	
[2061]		245 250 255
[2062]	Gln His Ser Ser Phe Asn Gly Asn Leu Arg Glu Ala Asp His Asn Cys	
[2063]		260 265 270
[2064]	Ile Thr Trp Leu Asp Ser Gln Pro Leu Arg Ser Val Val Tyr Val Ser	
[2065]		275 280 285
[2066]	Phe Gly Ser His Val Val Leu Thr Ser Glu Glu Leu Leu Glu Phe Trp	

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[2069]	305	310	315 320
[2070]	Asp Ile Ile Ala Gly Glu Lys Asp His Asn Gln Ile Ile Ala Arg Glu		
[2071]		325	330 335
[2072]	Pro Asp Leu Gly Thr Lys Glu Lys Gly Leu Leu Val Asp Trp Ala Pro		
[2073]		340	345 350
[2074]	Gln Glu Glu Val Leu Ala His Pro Ser Val Gly Gly Phe Leu Thr His		
[2075]		355	360 365
[2076]	Cys Gly Trp Asn Ser Thr Leu Glu Ser Met Val Ala Gly Val Pro Met		
[2077]		370	375 380
[2078]	Leu Cys Trp Pro Lys Leu Pro Asp Gln Leu Val Asn Ser Ser Cys Val		
[2079]		385	390 395 400
[2080]	Ser Glu Val Trp Lys Ile Gly Leu Asp Leu Lys Asp Met Cys Asp Arg		
[2081]		405	410 415
[2082]	Ser Thr Val Glu Lys Met Val Arg Ala Leu Met Glu Asp Arg Arg Glu		
[2083]		420	425 430
[2084]	Glu Val Met Arg Ser Val Asp Gly Ile Ser Lys Leu Ala Arg Glu Ser		
[2085]		435	440 445
[2086]	Val Ser His Gly Gly Ser Ser Ser Ser Asn Leu Glu Met Leu Ile Gln		
[2087]		450	455 460
[2088]	Glu Leu Glu Thr		
[2089]	465		
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[2092]	<212> PRT		
[2093]	<213> Momordica charantia		
[2094]	<400> 33		
[2095]	Met Asp Ala His Gln Gln Ala Glu His Thr Thr Thr Ile Leu Met Leu		
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[2097]	Pro Trp Val Gly Tyr Gly His Leu Thr Ala Tyr Leu Glu Leu Ala Lys		
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[2099]	Ala Leu Ser Arg Arg Asn Phe His Ile Tyr Tyr Cys Ser Thr Pro Val		
[2100]		35	40 45
[2101]	Asn Ile Glu Ser Ile Lys Pro Lys Leu Thr Ile Pro Cys Ser Ser Ile		
[2102]		50	55 60
[2103]	Gln Phe Val Glu Leu His Leu Pro Ser Ser Asp Asp Leu Pro Pro Asn		
[2104]		65	70 75 80
[2105]	Leu His Thr Thr Asn Gly Leu Pro Ser His Leu Met Pro Thr Leu His		



[2145]	Glu Val Ser Lys Leu Ile Lys Gln Val Val Val Glu Lys Thr Arg Asp
[2146]	405 410 415
[2147]	Asp Val Arg Lys Lys Val Ala Gln Met Ser Glu Ile Leu Arg Arg Lys
[2148]	420 425 430
[2149]	Gly Asp Glu Lys Ile Asp Glu Met Val Ala Leu Ile Ser Leu Leu Pro
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[2152]	450
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[2156]	<213> Cucurbita maxima
[2157]	<400> 34
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[2162]	Lys Ala Leu Ser Arg Arg Asn Phe His Val Tyr Phe Cys Ser Thr Pro
[2163]	35 40 45
[2164]	Val Asn Leu Asp Ser Ile Lys Pro Asn Leu Ile Pro Pro Pro Ser Ser
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[2166]	Ile Gln Phe Val Asp Leu His Leu Pro Ser Ser Pro Glu Leu Pro Pro
[2167]	65 70 75 80
[2168]	His Leu His Thr Thr Asn Gly Leu Pro Ser His Leu Lys Pro Thr Leu
[2169]	85 90 95
[2170]	His Gln Ala Phe Ser Ala Ala Ala Gln His Phe Glu Ala Ile Leu Gln
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[2172]	Thr Leu Ser Pro His Leu Leu Ile Tyr Asp Ser Leu Gln Pro Trp Ala
[2173]	115 120 125
[2174]	Pro Arg Ile Ala Ser Ser Leu Asn Ile Pro Ala Ile Asn Phe Asn Thr
[2175]	130 135 140
[2176]	Thr Ala Val Ser Ile Ile Ala His Ala Leu His Ser Val His Tyr Pro
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[2178]	Asp Ser Lys Phe Pro Phe Ser Asp Phe Val Leu His Asp Tyr Trp Lys
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[2180]	Ala Lys Tyr Thr Thr Ala Asp Gly Ala Thr Ser Glu Lys Ile Arg Arg
[2181]	180 185 190
[2182]	Gly Ala Glu Ala Phe Leu Tyr Cys Leu Asn Ala Ser Cys Asp Val Val
[2183]	195 200 205

[2184]	Leu Val Asn Ser Phe Arg Glu Leu Glu Gly Glu Tyr Met Asp Tyr Leu
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[2186]	Ser Val Leu Leu Lys Lys Lys Val Val Ser Val Gly Pro Leu Val Tyr
[2187]	225 230 235 240
[2188]	Glu Pro Ser Glu Gly Glu Glu Asp Glu Glu Tyr Trp Arg Ile Lys Lys
[2189]	245 250 255
[2190]	Trp Leu Asp Glu Lys Glu Ala Leu Ser Thr Val Leu Val Ser Phe Gly
[2191]	260 265 270
[2192]	Ser Glu Tyr Phe Pro Ser Lys Glu Glu Met Glu Glu Ile Ala His Gly
[2193]	275 280 285
[2194]	Leu Glu Glu Ser Glu Ala Asn Phe Ile Trp Val Val Arg Phe Pro Lys
[2195]	290 295 300
[2196]	Gly Glu Glu Ser Cys Arg Gly Ile Glu Glu Ala Leu Pro Lys Gly Phe
[2197]	305 310 315 320
[2198]	Val Glu Arg Ala Gly Glu Arg Ala Met Val Val Lys Lys Trp Ala Pro
[2199]	325 330 335
[2200]	Gln Gly Lys Ile Leu Lys His Gly Ser Ile Gly Gly Phe Val Ser His
[2201]	340 345 350
[2202]	Cys Gly Trp Asn Ser Val Leu Glu Ser Ile Arg Phe Gly Val Pro Val
[2203]	355 360 365
[2204]	Ile Gly Val Pro Met His Leu Asp Gln Pro Tyr Asn Ala Gly Leu Leu
[2205]	370 375 380
[2206]	Glu Glu Ala Gly Ile Gly Val Glu Ala Lys Arg Asp Ala Asp Gly Lys
[2207]	385 390 395 400
[2208]	Ile Gln Arg Asp Gln Val Ala Ser Leu Ile Lys Arg Val Val Val Glu
[2209]	405 410 415
[2210]	Lys Thr Arg Glu Asp Ile Trp Lys Thr Val Arg Glu Met Arg Glu Val
[2211]	420 425 430
[2212]	Leu Arg Arg Arg Asp Asp Asp Met Ile Asp Glu Met Val Ala Glu Ile
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[2214]	Ser Val Val Leu Lys Ile
[2215]	450
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[2225]	Ala Leu Ser Arg Arg Asn Phe His Ile Tyr Tyr Cys Ser Thr Pro Val
[2226]	35 40 45
[2227]	Asn Ile Glu Ser Ile Lys Pro Lys Leu Thr Ile Pro Cys Ser Ser Ile
[2228]	50 55 60
[2229]	Gln Phe Val Glu Leu His Leu Pro Phe Ser Asp Asp Leu Pro Pro Asn
[2230]	65 70 75 80
[2231]	Leu His Thr Thr Asn Gly Leu Pro Ser His Leu Met Pro Ala Leu His
[2232]	85 90 95
[2233]	Gln Ala Phe Ser Ala Ala Ala Pro Leu Phe Glu Ala Ile Leu Gln Thr
[2234]	100 105 110
[2235]	Leu Cys Pro His Leu Leu Ile Tyr Asp Ser Leu Gln Pro Trp Ala Pro
[2236]	115 120 125
[2237]	Gln Ile Ala Ser Ser Leu Lys Ile Pro Ala Leu Asn Phe Asn Thr Thr
[2238]	130 135 140
[2239]	Gly Val Ser Val Ile Ala Arg Ala Leu His Thr Ile His His Pro Asp
[2240]	145 150 155 160
[2241]	Ser Lys Phe Pro Leu Ser Glu Ile Val Leu His Asn Tyr Trp Lys Ala
[2242]	165 170 175
[2243]	Thr His Ala Thr Ala Asp Gly Ala Asn Pro Glu Lys Phe Arg Arg Asp
[2244]	180 185 190
[2245]	Leu Glu Ala Leu Leu Cys Cys Leu His Ser Ser Cys Asn Ala Ile Leu
[2246]	195 200 205
[2247]	Ile Asn Thr Phe Arg Glu Leu Glu Gly Glu Tyr Ile Asp Tyr Leu Ser
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[2249]	Leu Leu Leu Asn Lys Lys Val Thr Pro Ile Gly Pro Leu Val Tyr Glu
[2250]	225 230 235 240
[2251]	Pro Asn Gln Asp Glu Glu Gln Asp Glu Glu Tyr Arg Ser Ile Lys Asn
[2252]	245 250 255
[2253]	Trp Leu Asp Lys Lys Glu Pro Tyr Ser Thr Ile Phe Val Ser Phe Gly
[2254]	260 265 270
[2255]	Ser Glu Tyr Phe Pro Ser Asn Glu Glu Met Glu Glu Ile Ala Arg Gly
[2256]	275 280 285
[2257]	Leu Glu Glu Ser Gly Ala Asn Phe Ile Trp Val Val Arg Phe His Lys
[2258]	290 295 300
[2259]	Leu Glu Asn Gly Asn Gly Ile Thr Glu Glu Gly Leu Leu Glu Arg Ala
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[2272]				405					410					415					
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[2275]	Gly	Asp	Glu	Lys	Ile	Asp	Glu	Met	Val	Ala	Leu	Ile	Ser	Leu	Leu	Leu			
[2276]				435					440					445					
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[2278]				450															
[2279]	<210>	36																	
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[2281]	<212>	PRT																	
[2282]	<213>	Cucurbita moschata																	
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[2288]	Lys	Ala	Leu	Ser	Arg	Arg	Asn	Phe	His	Val	Tyr	Phe	Cys	Ser	Thr	Pro			
[2289]				35					40					45					
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[2292]	Ile	Gln	Phe	Val	Asp	Leu	His	Leu	Pro	Ser	Ser	Pro	Glu	Leu	Pro	Pro			
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[2295]				85					90					95					
[2296]	His	Gln	Ala	Phe	Ser	Ala	Ala	Ala	Gln	His	Phe	Glu	Ala	Ile	Leu	Gln			
[2297]				100					105					110					
[2298]	Thr	Leu	Ser	Pro	His	Leu	Leu	Ile	Tyr	Asp	Ser	Leu	Gln	Pro	Trp	Ala			
[2299]				115					120					125					
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[2302]	Thr Ala Val Ser Ile Ile Ala His Ala Leu His Ser Val His Tyr Pro		
[2303]	145	150	155
[2304]	Asp Ser Lys Phe Pro Phe Ser Asp Phe Val Leu His Asp Tyr Trp Lys		
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[2306]	Ala Lys Tyr Thr Thr Ala Asp Gly Ala Thr Ser Glu Lys Thr Arg Arg		
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[2308]	Gly Val Glu Ala Phe Leu Tyr Cys Leu Asn Ala Ser Cys Asp Val Val		
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[2310]	Leu Val Asn Ser Phe Arg Glu Leu Glu Gly Glu Tyr Met Asp Tyr Leu		
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[2312]	Ser Val Leu Leu Lys Lys Lys Val Val Ser Val Gly Pro Leu Val Tyr		
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[2314]	Glu Pro Ser Glu Gly Glu Glu Asp Glu Glu Tyr Trp Arg Ile Lys Lys		
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[2316]	Trp Leu Asp Glu Lys Glu Ala Leu Ser Thr Val Leu Val Ser Phe Gly		
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[2318]	Ser Glu Tyr Phe Pro Pro Lys Glu Glu Met Glu Glu Ile Ala His Gly		
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[2320]	Leu Glu Glu Ser Glu Ala Asn Phe Ile Trp Val Val Arg Phe Pro Lys		
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[2322]	Gly Glu Glu Ser Ser Ser Arg Gly Ile Glu Glu Ala Leu Pro Lys Gly		
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[2324]	Phe Val Glu Arg Ala Gly Glu Arg Ala Met Val Val Lys Lys Trp Ala		
[2325]		325	330
[2326]	Pro Gln Gly Lys Ile Leu Lys His Gly Ser Ile Gly Gly Phe Val Ser		
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[2328]	His Cys Gly Trp Asn Ser Val Leu Glu Ser Ile Arg Phe Gly Val Pro		
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[2330]	Val Ile Gly Ala Pro Met His Leu Asp Gln Pro Tyr Asn Ala Gly Leu		
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[2332]	Leu Glu Glu Ala Gly Ile Gly Val Glu Ala Lys Arg Asp Ala Asp Gly		
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[2334]	Lys Ile Gln Arg Asp Gln Val Ala Ser Leu Ile Lys Gln Val Val Val		
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[2336]	Glu Lys Thr Arg Glu Asp Ile Trp Lys Lys Val Arg Glu Met Arg Glu		
[2337]		420	425
[2338]	Val Leu Arg Arg Arg Asp Asp Asp Asp Met Met Ile Asp Glu Met Val		
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			445

[2340]	Ala Val Ile Ser Val Val Leu Lys Ile
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[2351]	Ala Ile Ala Ile Ala Met Asp Ala Gln Gln Ala Ala Asn Lys Ser Pro
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[2355]	Ser Ala Tyr Leu Glu Leu Ala Lys Ala Leu Ser Thr Arg Asn Phe His
[2356]	65 70 75 80
[2357]	Ile Tyr Phe Cys Ser Thr Pro Val Ser Leu Ala Ser Ile Lys Pro Arg
[2358]	85 90 95
[2359]	Leu Ile Pro Ser Cys Ser Ser Ile Gln Phe Val Glu Leu His Leu Pro
[2360]	100 105 110
[2361]	Ser Ser Asp Glu Phe Pro Pro His Leu His Thr Thr Asn Gly Leu Pro
[2362]	115 120 125
[2363]	Ser Arg Leu Val Pro Thr Phe His Gln Ala Phe Ser Glu Ala Ala Gln
[2364]	130 135 140
[2365]	Thr Phe Glu Ala Phe Leu Gln Thr Leu Arg Pro His Leu Leu Ile Tyr
[2366]	145 150 155 160
[2367]	Asp Ser Leu Gln Pro Trp Ala Pro Arg Ile Ala Ser Ser Leu Asn Ile
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[2369]	Pro Ala Ile Asn Phe Phe Thr Ala Gly Ala Phe Ala Val Ser His Val
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[2371]	Leu Arg Ala Phe His Tyr Pro Asp Ser Gln Phe Pro Ser Ser Asp Phe
[2372]	195 200 205
[2373]	Val Leu His Ser Arg Trp Lys Ile Lys Asn Thr Thr Ala Glu Ser Pro
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[2375]	Thr Gln Ala Lys Leu Pro Lys Ile Gly Glu Ala Ile Gly Tyr Cys Leu
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[2377]	Asn Ala Ser Arg Gly Val Ile Leu Thr Asn Ser Phe Arg Glu Leu Glu
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[2379]	Gly Lys Tyr Ile Asp Tyr Leu Ser Val Ile Leu Lys Lys Arg Val Phe
[2380]	260 265 270
[2381]	Pro Ile Gly Pro Leu Val Tyr Gln Pro Asn Gln Asp Glu Glu Asp Glu
[2382]	275 280 285
[2383]	Asp Tyr Ser Arg Ile Lys Asn Trp Leu Asp Arg Lys Glu Ala Ser Ser
[2384]	290 295 300
[2385]	Thr Val Leu Val Ser Phe Gly Ser Glu Phe Phe Leu Ser Lys Glu Glu
[2386]	305 310 315 320
[2387]	Thr Glu Ala Ile Ala His Gly Leu Glu Gln Ser Glu Ala Asn Phe Ile
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[2389]	Trp Gly Ile Arg Phe Pro Lys Gly Ala Lys Lys Asn Ala Ile Glu Glu
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[2391]	Ala Leu Pro Glu Gly Phe Leu Glu Arg Ala Gly Gly Arg Ala Met Val
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[2393]	Val Glu Glu Trp Val Pro Gln Gly Lys Ile Leu Lys His Gly Ser Ile
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[2395]	Gly Gly Phe Val Ser His Cys Gly Trp Asn Ser Ala Met Glu Ser Ile
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[2397]	Val Cys Gly Val Pro Ile Ile Gly Ile Pro Met Gln Val Asp Gln Pro
[2398]	405 410 415
[2399]	Phe Asn Ala Gly Ile Leu Glu Glu Ala Gly Val Gly Val Glu Ala Lys
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[2401]	Arg Asp Ser Asp Gly Lys Ile Gln Arg Asp Glu Val Ala Lys Leu Ile
[2402]	435 440 445
[2403]	Lys Glu Val Val Val Glu Arg Thr Arg Glu Asp Ile Arg Asn Lys Leu
[2404]	450 455 460
[2405]	Glu Lys Ile Asn Glu Ile Leu Arg Ser Arg Arg Glu Glu Lys Leu Asp
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[2416]	Phe Met Leu Pro Trp Val Gly Tyr Gly His Leu Ser Ala Tyr Leu Glu
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[2418]	Leu Ala Lys Ala Leu Ser Thr Arg Asn Phe His Val Tyr Phe Cys Ser
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[2420]	Thr Pro Val Ser Leu Ala Ser Ile Lys Pro Arg Leu Ile Pro Ser Cys
[2421]	50 55 60
[2422]	Ser Ser Ile Gln Phe Val Glu Leu His Leu Pro Ser Ser Asp Glu Phe
[2423]	65 70 75 80
[2424]	Pro Pro His Leu His Thr Thr Asn Gly Leu Pro Ala His Leu Val Pro
[2425]	85 90 95
[2426]	Thr Ile His Gln Ala Phe Ala Ala Ala Ala Gln Thr Phe Glu Ala Phe
[2427]	100 105 110
[2428]	Leu Gln Thr Leu Arg Pro His Leu Leu Ile Tyr Asp Ser Leu Gln Pro
[2429]	115 120 125
[2430]	Trp Ala Pro Arg Ile Ala Ser Ser Leu Asn Ile Pro Ala Ile Asn Phe
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[2432]	Phe Thr Ala Gly Ala Phe Ala Val Ser His Val Leu Arg Ala Phe His
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[2434]	Tyr Pro Asp Ser Gln Phe Pro Ser Ser Asp Phe Val Leu His Ser Arg
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[2436]	Trp Lys Ile Lys Asn Thr Thr Ala Glu Ser Pro Thr Gln Val Lys Ile
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[2438]	Pro Lys Ile Gly Glu Ala Ile Gly Tyr Cys Leu Asn Ala Ser Arg Gly
[2439]	195 200 205
[2440]	Val Ile Leu Thr Asn Ser Phe Arg Glu Leu Glu Gly Lys Tyr Ile Asp
[2441]	210 215 220
[2442]	Tyr Leu Ser Val Ile Leu Lys Lys Arg Val Leu Pro Ile Gly Pro Leu
[2443]	225 230 235 240
[2444]	Val Tyr Gln Pro Asn Gln Asp Glu Glu Asp Glu Asp Tyr Ser Arg Ile
[2445]	245 250 255
[2446]	Lys Asn Trp Leu Asp Arg Lys Glu Ala Ser Ser Thr Val Leu Val Ser
[2447]	260 265 270
[2448]	Phe Gly Ser Glu Phe Phe Leu Ser Lys Glu Glu Thr Glu Ala Ile Ala
[2449]	275 280 285
[2450]	His Gly Leu Glu Gln Ser Glu Ala Asn Phe Ile Trp Gly Ile Arg Phe
[2451]	290 295 300
[2452]	Pro Lys Gly Ala Lys Lys Asn Ala Ile Glu Glu Ala Leu Pro Glu Gly
[2453]	305 310 315 320
[2454]	Phe Leu Glu Arg Val Gly Gly Arg Ala Met Val Val Glu Glu Trp Val
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[2456]	Pro Gln Gly Lys Ile Leu Lys His Gly Asn Ile Gly Gly Phe Val Ser

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[2463]	385						390					395				400		
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[2465]					405					410						415		
[2466]	Glu	Arg	Thr	Arg	Glu	Asp	Ile	Arg	Asn	Lys	Leu	Glu	Glu	Ile	Asn	Glu		
[2467]					420					425						430		
[2468]	Ile	Leu	Arg	Thr	Arg	Arg	Glu	Glu	Lys	Leu	Asp	Glu	Leu	Ala	Thr	Glu		
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[2493]	Ala	Leu	Ser	Asn	Lys	Ile	Pro	Ala	Val	His	Phe	Leu	Cys	Thr	Ser	Ala		
[2494]					130						135					140		
[2495]	Ala	Met	Ser	Ser	Phe	Ser	Val	His	Ala	Phe	Lys	Lys	Pro	Cys	Glu	Asp		

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[2497]	Phe Pro Phe Pro Asn Ile Tyr Val His Gly Asn Phe Met Asn Ala Lys			
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[2499]	Phe Asn Asn Met Glu Asn Cys Ser Ser Asp Asp Ser Ile Ser Asp Gln			
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[2501]	Asp Arg Val Leu Gln Cys Phe Glu Arg Ser Thr Lys Ile Ile Leu Val			
[2502]		195	200	205
[2503]	Lys Thr Phe Glu Glu Leu Glu Gly Lys Phe Met Asp Tyr Leu Ser Val			
[2504]		210	215	220
[2505]	Leu Leu Asn Lys Lys Ile Val Pro Thr Gly Pro Leu Thr Gln Asp Pro			
[2506]		225	230	240
[2507]	Asn Glu Asp Glu Gly Asp Asp Asp Glu Arg Thr Lys Leu Leu Leu Glu			
[2508]		245	250	255
[2509]	Trp Leu Asn Lys Lys Ser Lys Ser Ser Thr Val Phe Val Ser Phe Gly			
[2510]		260	265	270
[2511]	Ser Glu Tyr Phe Leu Ser Lys Glu Glu Arg Glu Glu Ile Ala Tyr Gly			
[2512]		275	280	285
[2513]	Leu Glu Leu Ser Lys Val Asn Phe Ile Trp Val Ile Arg Phe Pro Leu			
[2514]		290	295	300
[2515]	Gly Glu Asn Lys Thr Asn Leu Glu Glu Ala Leu Pro Gln Gly Phe Leu			
[2516]		305	310	320
[2517]	Gln Arg Val Ser Glu Arg Gly Leu Val Val Glu Asn Trp Ala Pro Gln			
[2518]		325	330	335
[2519]	Ala Lys Ile Leu Gln His Ser Ser Ile Gly Gly Phe Val Ser His Cys			
[2520]		340	345	350
[2521]	Gly Trp Ser Ser Val Met Glu Ser Leu Lys Phe Gly Val Pro Ile Ile			
[2522]		355	360	365
[2523]	Ala Ile Pro Met His Leu Asp Gln Pro Leu Asn Ala Arg Leu Val Val			
[2524]		370	375	380
[2525]	Asp Val Gly Val Gly Leu Glu Val Ile Arg Asn His Gly Ser Leu Glu			
[2526]		385	390	400
[2527]	Arg Glu Glu Ile Ala Lys Leu Ile Lys Glu Val Val Leu Gly Asn Gly			
[2528]		405	410	415
[2529]	Asn Asp Gly Glu Ile Val Arg Arg Lys Ala Arg Glu Met Ser Asn His			
[2530]		420	425	430
[2531]	Ile Lys Lys Lys Gly Glu Lys Asp Met Asp Glu Leu Val Glu Glu Leu			
[2532]		435	440	445
[2533]	Met Leu Ile Cys Lys Met Lys Pro Asn Ser Cys His Leu Ser			
[2534]		450	455	460



[2574]	Leu Thr Asn Glu Glu Met Glu Glu Ile Ala Tyr Gly Leu Glu Leu Ser
[2575]	275 280 285
[2576]	Lys Val Asn Phe Ile Trp Val Val Arg Phe Pro Leu Gly Gln Lys Met
[2577]	290 295 300
[2578]	Ala Val Glu Glu Ala Leu Pro Lys Gly Phe Leu Glu Arg Val Gly Glu
[2579]	305 310 315 320
[2580]	Lys Gly Met Val Val Glu Asp Trp Ala Pro Gln Met Lys Ile Leu Gly
[2581]	325 330 335
[2582]	His Ser Ser Ile Gly Gly Phe Val Ser His Cys Gly Trp Ser Ser Leu
[2583]	340 345 350
[2584]	Met Glu Ser Leu Lys Leu Gly Val Pro Ile Ile Ala Met Pro Met Gln
[2585]	355 360 365
[2586]	Leu Asp Gln Pro Ile Asn Ala Lys Leu Val Glu Arg Ser Gly Val Gly
[2587]	370 375 380
[2588]	Leu Glu Val Lys Arg Asp Lys Asn Gly Arg Ile Glu Arg Glu Tyr Leu
[2589]	385 390 395 400
[2590]	Ala Lys Val Ile Arg Glu Ile Val Val Glu Lys Ala Arg Gln Asp Ile
[2591]	405 410 415
[2592]	Glu Lys Lys Ala Arg Glu Met Ser Asn Ile Ile Thr Glu Lys Gly Glu
[2593]	420 425 430
[2594]	Glu Glu Ile Asp Asn Val Val Glu Glu Leu Ala Lys Leu Cys Gly Met
[2595]	435 440 445
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[2603]	Leu Ala His Gly His Ile Ser Pro Phe Leu Gln Leu Ala Lys Lys Leu
[2604]	20 25 30
[2605]	Ser Lys Arg Asn Phe Ser Ile Tyr Phe Cys Ser Thr Pro Val Asn Leu
[2606]	35 40 45
[2607]	Asp Pro Ile Lys Gly Lys Leu Ser Glu Ser Tyr Ser Leu Ser Ile Gln
[2608]	50 55 60
[2609]	Leu Val Lys Leu His Leu Pro Ser Leu Pro Glu Leu Pro Pro Gln Tyr
[2610]	65 70 75 80
[2611]	His Thr Thr Asn Gly Leu Pro Pro His Leu Met Pro Thr Leu Lys Met
[2612]	85 90 95

[2613]	Ala Phe Asp Met Ala Ser Pro Asn Phe Ser Asn Ile Leu Lys Thr Leu
[2614]	100 105 110
[2615]	His Pro Asp Leu Leu Ile Tyr Asp Phe Leu Gln Pro Trp Ala Pro Ala
[2616]	115 120 125
[2617]	Ala Ala Ser Ser Leu Asn Ile Pro Ala Val Gln Phe Leu Ser Thr Gly
[2618]	130 135 140
[2619]	Ala Thr Leu Gln Ser Phe Leu Ala His Arg His Arg Lys Pro Gly Ile
[2620]	145 150 155 160
[2621]	Glu Phe Pro Phe Gln Glu Ile His Leu Pro Asp Tyr Glu Ile Gly Arg
[2622]	165 170 175
[2623]	Leu Asn Arg Phe Leu Glu Pro Ser Ala Gly Arg Ile Ser Asp Arg Asp
[2624]	180 185 190
[2625]	Arg Ala Asn Gln Cys Leu Glu Arg Ser Ser Arg Phe Ser Leu Ile Lys
[2626]	195 200 205
[2627]	Thr Phe Arg Glu Ile Glu Ala Lys Tyr Leu Asp Tyr Val Ser Asp Leu
[2628]	210 215 220
[2629]	Thr Lys Lys Lys Met Val Thr Val Gly Pro Leu Leu Gln Asp Pro Glu
[2630]	225 230 235 240
[2631]	Asp Glu Asp Glu Ala Thr Asp Ile Val Glu Trp Leu Asn Lys Lys Cys
[2632]	245 250 255
[2633]	Glu Ala Ser Ala Val Phe Val Ser Phe Gly Ser Glu Tyr Phe Val Ser
[2634]	260 265 270
[2635]	Lys Glu Glu Met Glu Glu Ile Ala His Gly Leu Glu Leu Ser Asn Val
[2636]	275 280 285
[2637]	Asp Phe Ile Trp Val Val Arg Phe Pro Met Gly Glu Lys Ile Arg Leu
[2638]	290 295 300
[2639]	Glu Asp Ala Leu Pro Pro Gly Phe Leu His Arg Leu Gly Asp Arg Gly
[2640]	305 310 315 320
[2641]	Met Val Val Glu Gly Trp Ala Pro Gln Arg Lys Ile Leu Gly His Ser
[2642]	325 330 335
[2643]	Ser Ile Gly Gly Phe Val Ser His Cys Gly Trp Ser Ser Val Met Glu
[2644]	340 345 350
[2645]	Gly Met Lys Phe Gly Val Pro Ile Ile Ala Met Pro Met His Leu Asp
[2646]	355 360 365
[2647]	Gln Pro Ile Asn Ala Lys Leu Val Glu Ala Val Gly Val Gly Arg Glu
[2648]	370 375 380
[2649]	Val Lys Arg Asp Glu Asn Arg Lys Leu Glu Arg Glu Glu Ile Ala Lys
[2650]	385 390 395 400
[2651]	Val Ile Lys Glu Val Val Gly Glu Lys Asn Gly Glu Asn Val Arg Arg

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[2667]		35		40		45										
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[2670]	Val	Glu	Leu	His	Leu	Pro	Ser	Leu	Pro	Glu	Leu	Pro	Pro	Gln	Tyr	His
[2671]	65		70		75		80									
[2672]	Thr	Thr	Lys	Gly	Leu	Pro	Pro	His	Leu	Asn	Ala	Thr	Leu	Lys	Arg	Ala
[2673]		85		90		95										
[2674]	Phe	Asp	Met	Ala	Gly	Pro	His	Phe	Ser	Asn	Ile	Leu	Lys	Thr	Leu	Ser
[2675]		100		105		110										
[2676]	Pro	Asp	Leu	Leu	Ile	Tyr	Asp	Phe	Leu	Gln	Pro	Trp	Ala	Pro	Ala	Ile
[2677]		115		120		125										
[2678]	Ala	Ala	Ser	Gln	Asn	Ile	Pro	Ala	Ile	Asn	Phe	Leu	Ser	Thr	Gly	Ala
[2679]		130		135		140										
[2680]	Ala	Met	Thr	Ser	Phe	Val	Leu	His	Ala	Met	Lys	Lys	Pro	Gly	Asp	Glu
[2681]	145		150		155		160									
[2682]	Phe	Pro	Phe	Pro	Glu	Ile	His	Leu	Asp	Glu	Cys	Met	Lys	Thr	Arg	Phe
[2683]		165		170		175										
[2684]	Val	Asp	Leu	Pro	Glu	Asp	His	Ser	Pro	Ser	Asp	Asp	His	Asn	His	Ile
[2685]		180		185		190										
[2686]	Ser	Asp	Lys	Asp	Arg	Ala	Leu	Lys	Cys	Phe	Glu	Arg	Ser	Ser	Gly	Phe
[2687]		195		200		205										
[2688]	Val	Met	Met	Lys	Thr	Phe	Glu	Glu	Leu	Glu	Gly	Lys	Tyr	Ile	Asn	Phe
[2689]		210		215		220										
[2690]	Leu	Ser	His	Leu	Met	Gln	Lys	Lys	Ile	Val	Pro	Val	Gly	Pro	Leu	Val

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[2692]	Gln Asn Pro Val Arg Gly Asp His Glu Lys Ala Lys Thr Leu Glu Trp			
[2693]		245	250	255
[2694]	Leu Asp Lys Arg Lys Gln Ser Ser Ala Val Phe Val Ser Phe Gly Thr			
[2695]		260	265	270
[2696]	Glu Tyr Phe Leu Ser Lys Glu Glu Met Glu Glu Ile Ala Tyr Gly Leu			
[2697]		275	280	285
[2698]	Glu Leu Ser Asn Val Asn Phe Ile Trp Val Val Arg Phe Pro Glu Gly			
[2699]		290	295	300
[2700]	Glu Lys Val Lys Leu Glu Glu Ala Leu Pro Glu Gly Phe Leu Gln Arg			
[2701]		305	310	315
[2702]	Val Gly Glu Lys Gly Met Val Val Glu Gly Trp Ala Pro Gln Ala Lys			
[2703]		325	330	335
[2704]	Ile Leu Met His Pro Ser Ile Gly Gly Phe Val Ser His Cys Gly Trp			
[2705]		340	345	350
[2706]	Ser Ser Val Met Glu Ser Ile Asp Phe Gly Val Pro Ile Val Ala Ile			
[2707]		355	360	365
[2708]	Pro Met Gln Leu Asp Gln Pro Val Asn Ala Lys Val Val Glu Gln Ala			
[2709]		370	375	380
[2710]	Gly Val Gly Val Glu Val Lys Arg Asp Arg Asp Gly Lys Leu Glu Arg			
[2711]		385	390	395
[2712]	Glu Glu Val Ala Thr Val Ile Arg Glu Val Val Met Gly Asn Ile Gly			
[2713]		405	410	415
[2714]	Glu Ser Val Arg Lys Lys Glu Arg Glu Met Arg Asp Asn Ile Arg Lys			
[2715]		420	425	430
[2716]	Lys Gly Glu Glu Lys Met Asp Gly Val Ala Gln Glu Leu Val Gln Leu			
[2717]		435	440	445
[2718]	Tyr Gly Asn Gly Ile Lys Asn Val			
[2719]		450	455	
[2720]	<210>	43		
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[2723]	<213>	Hevea brasiliensis		
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[2727]	Leu Ala His Gly His Leu Ser Pro Phe Leu Glu Leu Ser Lys Lys Leu			
[2728]		20	25	30
[2729]	Asn Lys Arg Asn Phe His Val Tyr Phe Cys Ser Thr Pro Val Asn Leu			

[2730]	35	40	45
[2731]	Asp Ser Ile Lys Pro Lys Leu Ser Ala Glu Tyr Ser Phe Ser Ile Gln		
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[2733]	Leu Val Glu Leu His Leu Pro Ser Ser Pro Glu Leu Pro Leu His Tyr		
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[2735]	His Thr Thr Asn Gly Leu Pro Pro His Leu Met Lys Asn Leu Lys Asn		
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[2737]	Ala Phe Asp Met Ala Ser Ser Ser Phe Phe Asn Ile Leu Lys Thr Leu		
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[2739]	Lys Pro Asp Leu Leu Ile Tyr Asp Phe Ile Gln Pro Trp Ala Pro Ala		
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[2741]	Leu Ala Ser Ser Leu Asn Ile Pro Ala Val Asn Phe Leu Cys Thr Ser		
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[2743]	Met Ala Met Ser Cys Phe Gly Leu His Leu Asn Asn Gln Glu Ala Lys		
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[2745]	Phe Pro Phe Pro Gly Ile Tyr Pro Arg Asp Tyr Met Arg Met Lys Val		
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[2747]	Phe Gly Ala Leu Glu Ser Ser Ser Asn Asp Ile Lys Asp Gly Glu Arg		
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[2749]	Ala Gly Arg Cys Met Asp Gln Ser Phe His Leu Ile Leu Ala Lys Thr		
[2750]	195	200	205
[2751]	Phe Arg Glu Leu Glu Gly Lys Tyr Ile Asp Tyr Leu Ser Val Lys Leu		
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[2753]	Met Lys Lys Ile Val Pro Val Gly Pro Leu Val Gln Asp Pro Ile Phe		
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[2755]	Glu Asp Asp Glu Lys Ile Met Asp His His Gln Val Ile Lys Trp Leu		
[2756]	245	250	255
[2757]	Glu Lys Lys Glu Arg Leu Ser Thr Val Phe Val Ser Phe Gly Thr Glu		
[2758]	260	265	270
[2759]	Tyr Phe Leu Ser Thr Glu Glu Met Glu Glu Ile Ala Tyr Gly Leu Glu		
[2760]	275	280	285
[2761]	Leu Ser Lys Ala His Phe Ile Trp Val Val Arg Phe Pro Thr Gly Glu		
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[2763]	Lys Ile Asn Leu Glu Glu Ser Leu Pro Lys Arg Tyr Leu Glu Arg Val		
[2764]	305	310	315
[2765]	Gln Glu Arg Gly Lys Ile Val Glu Gly Trp Ala Pro Gln Gln Lys Ile		
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[2767]	Leu Arg His Ser Ser Ile Gly Gly Phe Val Ser His Cys Gly Trp Ser		
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[2771]	Met Asn Leu Asp Gln Pro Val Asn Ser Arg Ile Val Glu Asp Ala Gly
[2772]	370 375 380
[2773]	Val Gly Ile Glu Val Arg Arg Asn Lys Ser Gly Glu Leu Glu Arg Glu
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[2775]	Glu Ile Ala Lys Thr Ile Arg Lys Val Val Val Glu Lys Asp Gly Lys
[2776]	405 410 415
[2777]	Asn Val Ser Arg Lys Ala Arg Glu Met Ser Asp Thr Ile Arg Lys Lys
[2778]	420 425 430
[2779]	Gly Glu Glu Glu Ile Asp Gly Val Val Asp Glu Leu Leu Gln Leu Cys
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[2781]	Asp Val Lys Thr Asn Tyr Leu Gln
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[2792]	Ala Asn Arg Asn Phe His Val Tyr Phe Cys Ser Thr Pro Val Asn Leu
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[2794]	Asp Ser Ile Lys Pro Lys Leu Ser Pro Glu Tyr His Phe Ser Ile Gln
[2795]	50 55 60
[2796]	Phe Val Glu Leu His Leu Pro Ser Ser Pro Glu Leu Pro Ser His Tyr
[2797]	65 70 75 80
[2798]	His Thr Thr Asn Gly Leu Pro Pro His Leu Met Lys Thr Leu Lys Lys
[2799]	85 90 95
[2800]	Ala Phe Asp Met Ala Ser Ser Ser Phe Phe Asn Ile Leu Lys Thr Leu
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[2802]	Asn Pro Asp Leu Leu Ile Tyr Asp Phe Leu Gln Pro Trp Ala Pro Ala
[2803]	115 120 125
[2804]	Leu Ala Ser Ser Leu Asn Ile Pro Ala Val Asn Phe Leu Cys Ser Ser
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[2806]	Met Ala Met Ser Cys Phe Gly Leu Asn Leu Asn Lys Asn Lys Glu Ile
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[2808]	Lys Phe Leu Phe Pro Glu Ile Tyr Pro Arg Asp Tyr Met Glu Met Lys
[2809]	165 170 175
[2810]	Leu Phe Arg Val Phe Glu Ser Ser Ser Asn Gln Ile Lys Asp Gly Glu
[2811]	180 185 190
[2812]	Arg Ala Gly Arg Cys Ile Asp Gln Ser Phe His Val Ile Leu Ala Lys
[2813]	195 200 205
[2814]	Thr Phe Arg Glu Leu Glu Gly Lys Tyr Ile Asp Tyr Val Ser Val Lys
[2815]	210 215 220
[2816]	Cys Asn Lys Lys Ile Val Pro Val Gly Pro Leu Val Glu Asp Thr Ile
[2817]	225 230 235 240
[2818]	His Glu Asp Asp Glu Lys Thr Met Asp His His His His His His Asp
[2819]	245 250 255
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[2821]	260 265 270
[2822]	Val Ser Phe Gly Ser Glu Tyr Phe Leu Ser Lys Glu Glu Met Glu Glu
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[2853]	Ala His Gly His Ile Ser Pro Phe Leu Glu Leu Ala Lys Lys Leu Thr	
[2854]	20	30
[2855]	Asn Arg Asn Phe Leu Ile Tyr Phe Cys Ser Thr Pro Ile Asn Leu Asn	
[2856]	35	45
[2857]	Ser Ile Lys Pro Lys Leu Ser Ser Lys Tyr Ser Phe Ser Ile Gln Leu	
[2858]	50	60
[2859]	Val Glu Leu His Leu Pro Ser Leu Pro Glu Leu Pro Pro His Tyr His	
[2860]	65	80
[2861]	Thr Thr Asn Gly Leu Pro Leu His Leu Met Asn Thr Leu Lys Thr Ala	
[2862]	85	95
[2863]	Phe Asp Met Ala Ser Pro Ser Phe Leu Asn Ile Leu Lys Thr Leu Lys	
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[2865]	Pro Asp Leu Leu Ile Cys Asp His Leu Gln Pro Trp Ala Pro Ser Leu	
[2866]	115	125
[2867]	Ala Ser Ser Leu Asn Ile Pro Ala Ile Ile Phe Pro Thr Asn Ser Ala	
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[2869]	Ile Met Met Ala Phe Ser Leu His His Ala Lys Asn Pro Gly Glu Glu	
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[2871]	Phe Pro Phe Pro Ser Ile Asn Ile Asn Asp Asp Met Val Lys Ser Ile	
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[2875]	Leu Gln Cys Leu Glu Arg Ser Ser Asn Thr Met Leu Leu Lys Thr Phe	
[2876]	195	205
[2877]	Arg Gln Leu Glu Ala Lys Tyr Val Asp Tyr Ser Ser Ala Leu Leu Lys	
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[2879]	Lys Lys Ile Val Leu Ala Gly Pro Leu Val Gln Val Pro Asp Asn Glu	
[2880]	225	240
[2881]	Asp Glu Lys Ile Glu Ile Ile Lys Trp Leu Asp Ser Arg Gly Gln Ser	
[2882]	245	255
[2883]	Ser Thr Val Phe Val Ser Phe Gly Ser Glu Tyr Phe Leu Ser Lys Glu	
[2884]	260	270
[2885]	Glu Arg Glu Asp Ile Ala His Gly Leu Glu Leu Ser Lys Val Asn Phe	

[2886]	275	280	285
[2887]	Ile Trp Val Val Arg Phe Pro Val Gly Glu Lys Val Lys Leu Glu Glu		
[2888]	290	295	300
[2889]	Ala Leu Pro Asn Gly Phe Ala Glu Arg Ile Gly Glu Arg Gly Leu Val		
[2890]	305	310	315
[2891]	Val Glu Gly Trp Ala Pro Gln Ala Met Ile Leu Ser His Ser Ser Ile		
[2892]	325	330	335
[2893]	Gly Gly Phe Val Ser His Cys Gly Trp Ser Ser Met Met Glu Ser Met		
[2894]	340	345	350
[2895]	Lys Phe Gly Val Pro Ile Ile Ala Met Pro Met His Ile Asp Gln Pro		
[2896]	355	360	365
[2897]	Leu Asn Ala Arg Leu Val Glu Asp Val Gly Val Gly Leu Glu Ile Lys		
[2898]	370	375	380
[2899]	Arg Asn Lys Asp Gly Arg Phe Glu Arg Glu Glu Leu Ala Arg Val Ile		
[2900]	385	390	395
[2901]	Lys Glu Val Leu Val Tyr Lys Asn Gly Asp Ala Val Arg Ser Lys Ala		
[2902]	405	410	415
[2903]	Arg Glu Met Ser Glu His Ile Lys Lys Asn Gly Asp Gln Glu Ile Asp		
[2904]	420	425	430
[2905]	Gly Val Ala Asp Ala Leu Val Lys Leu Cys Glu Met Lys Thr Asn Ser		
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[2908]	450		
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[2914]	Met Glu Asn His Ala Thr Phe Asn Val Leu Met Leu Pro Trp Leu Ala		
[2915]	1	5	10
[2916]	His Gly His Val Ser Pro Tyr Leu Glu Leu Ala Lys Lys Leu Thr Ala		
[2917]	20	25	30
[2918]	Arg Asn Phe Asn Val Tyr Leu Cys Ser Ser Pro Ala Thr Leu Ser Ser		
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[2920]	Val Arg Ser Lys Leu Thr Glu Lys Phe Ser Gln Ser Ile His Leu Val		
[2921]	50	55	60
[2922]	Glu Leu His Leu Pro Lys Leu Pro Glu Leu Pro Ala Glu Tyr His Thr		
[2923]	65	70	75
[2924]	Thr Asn Gly Leu Pro Pro His Leu Met Pro Thr Leu Lys Asp Ala Phe		



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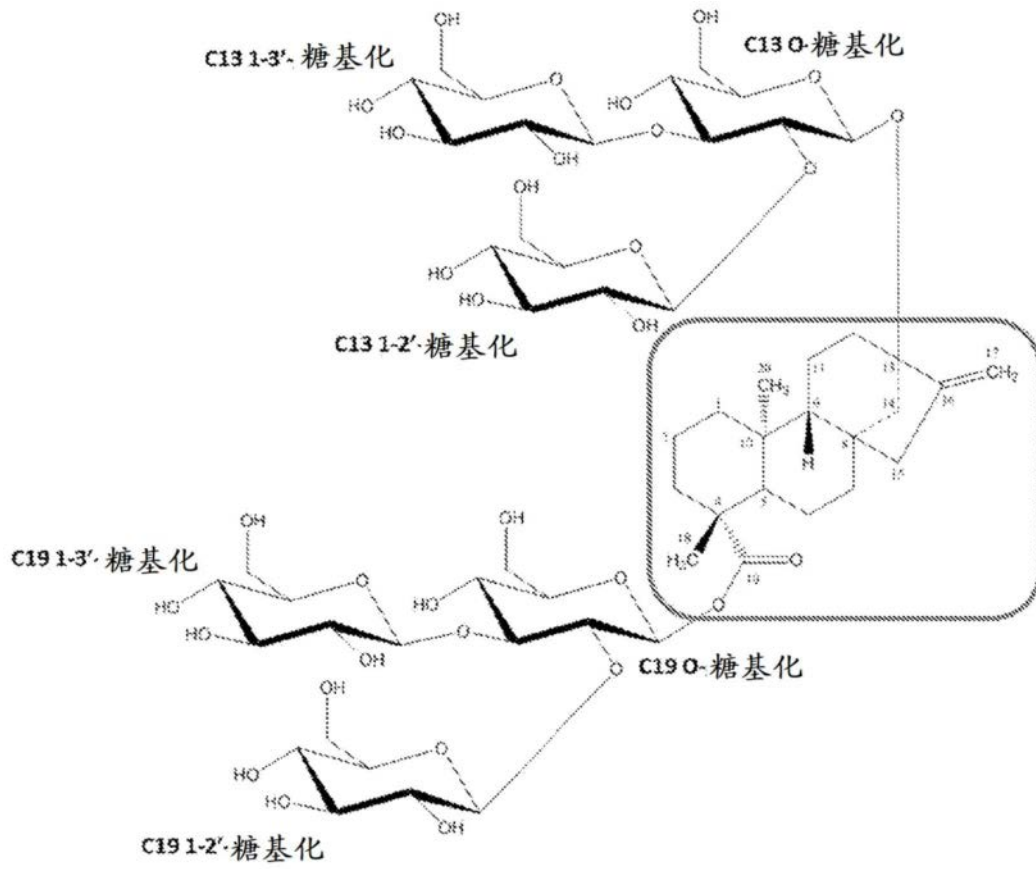


图1

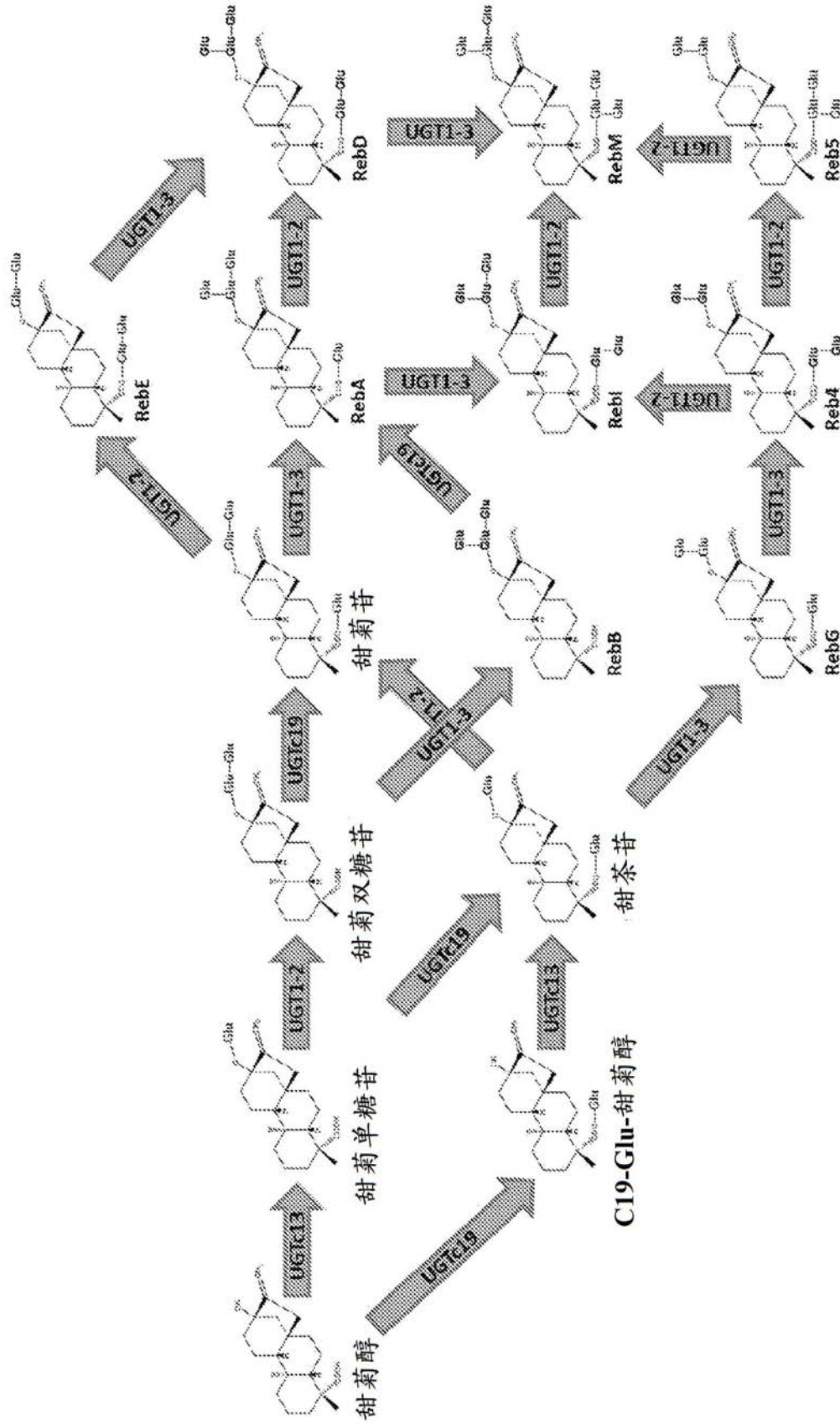


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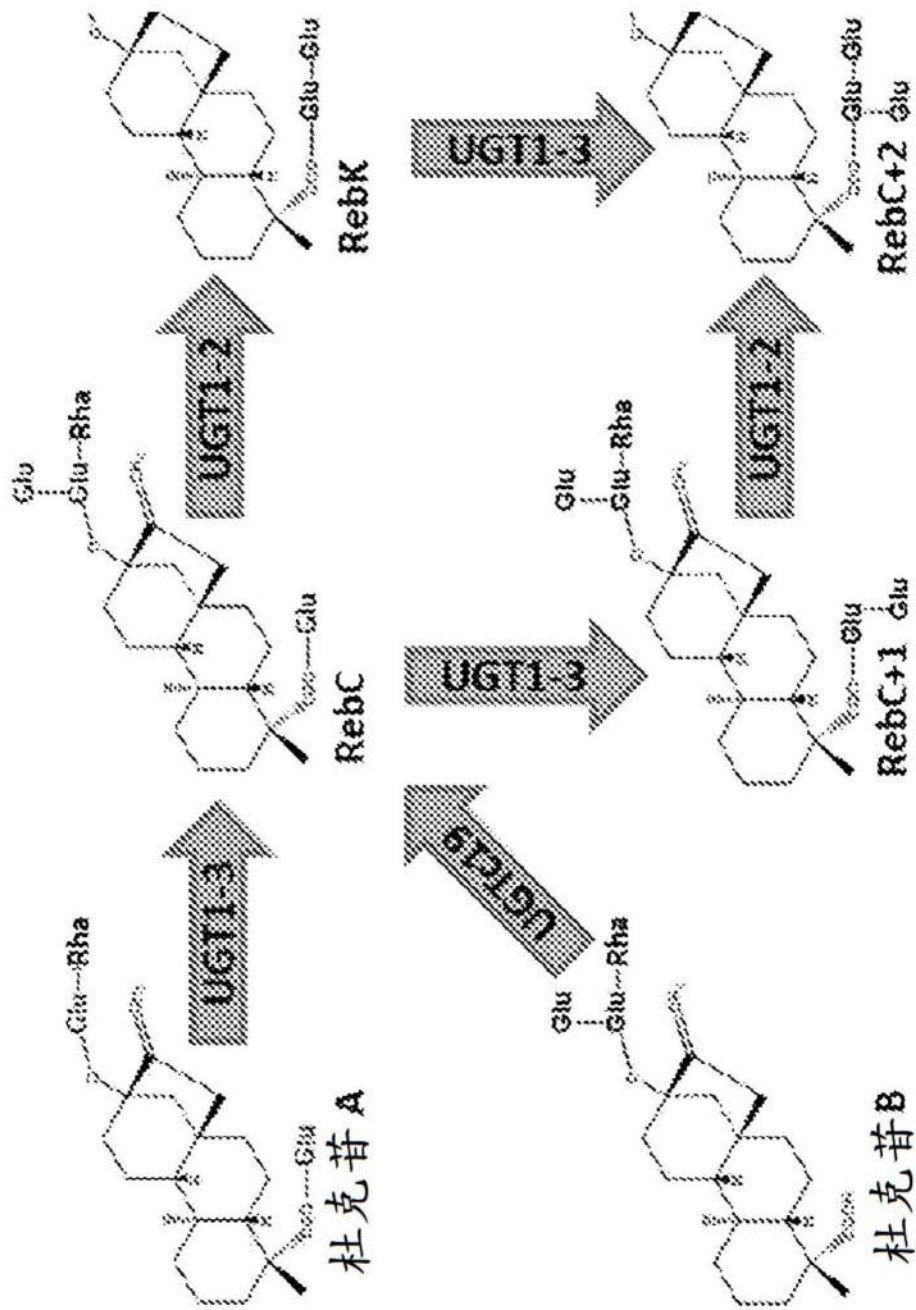


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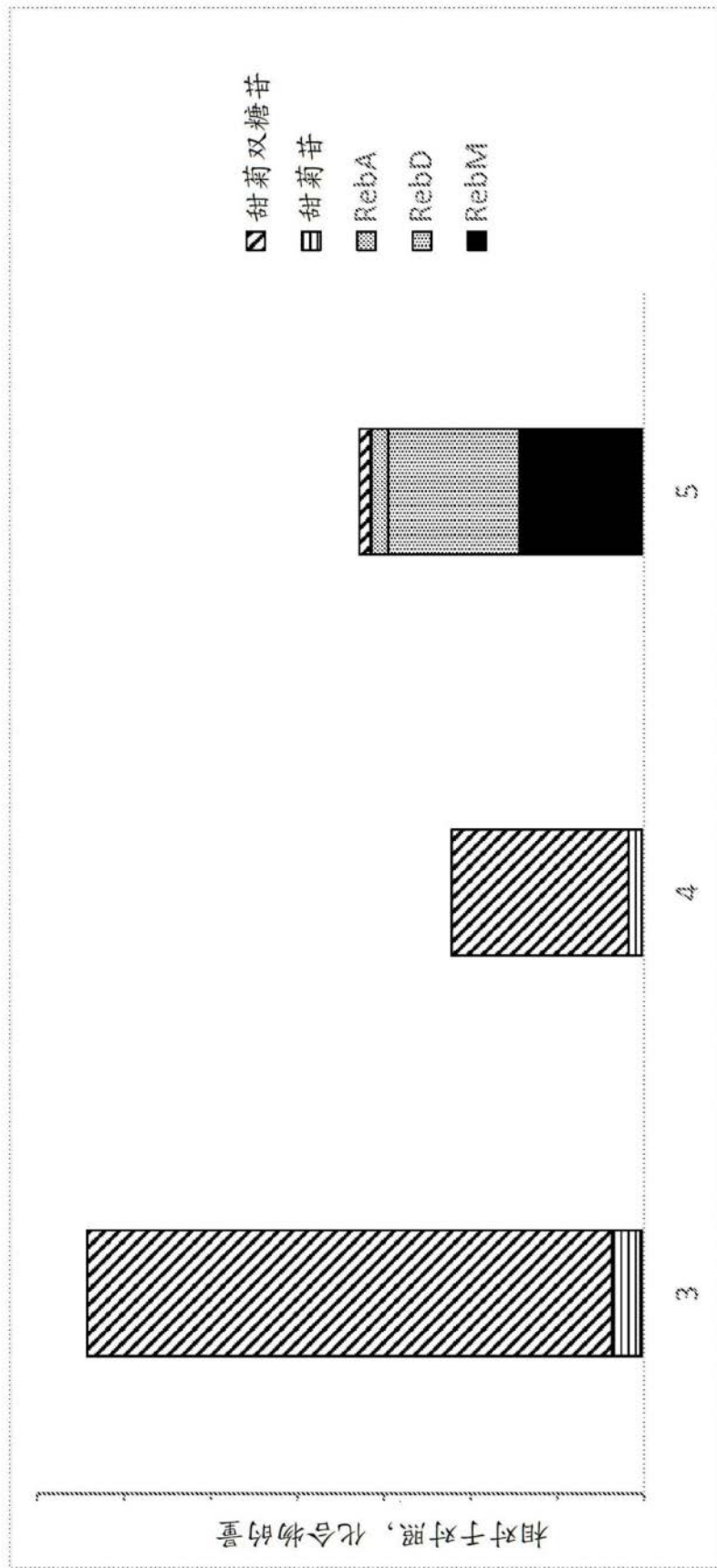


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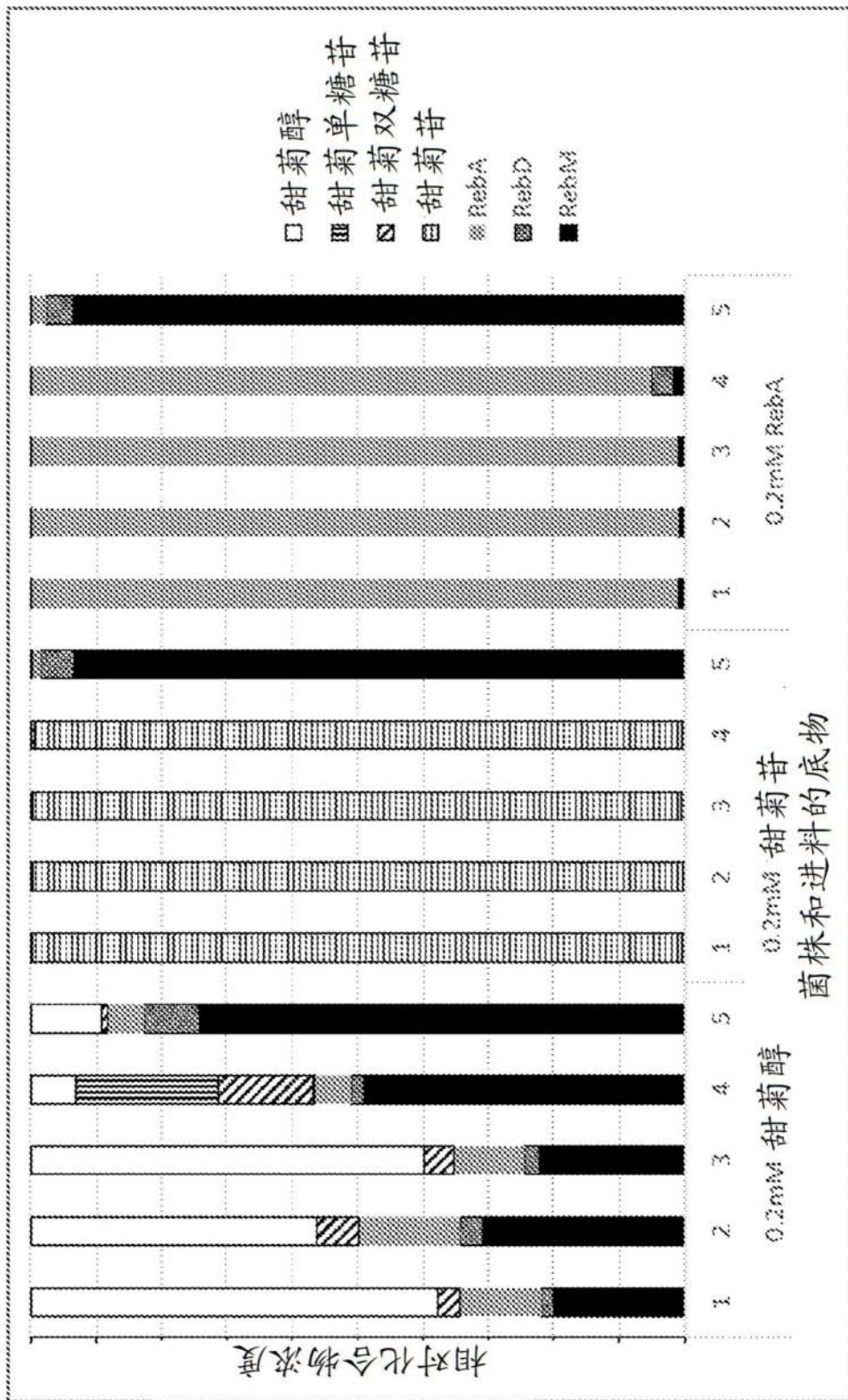


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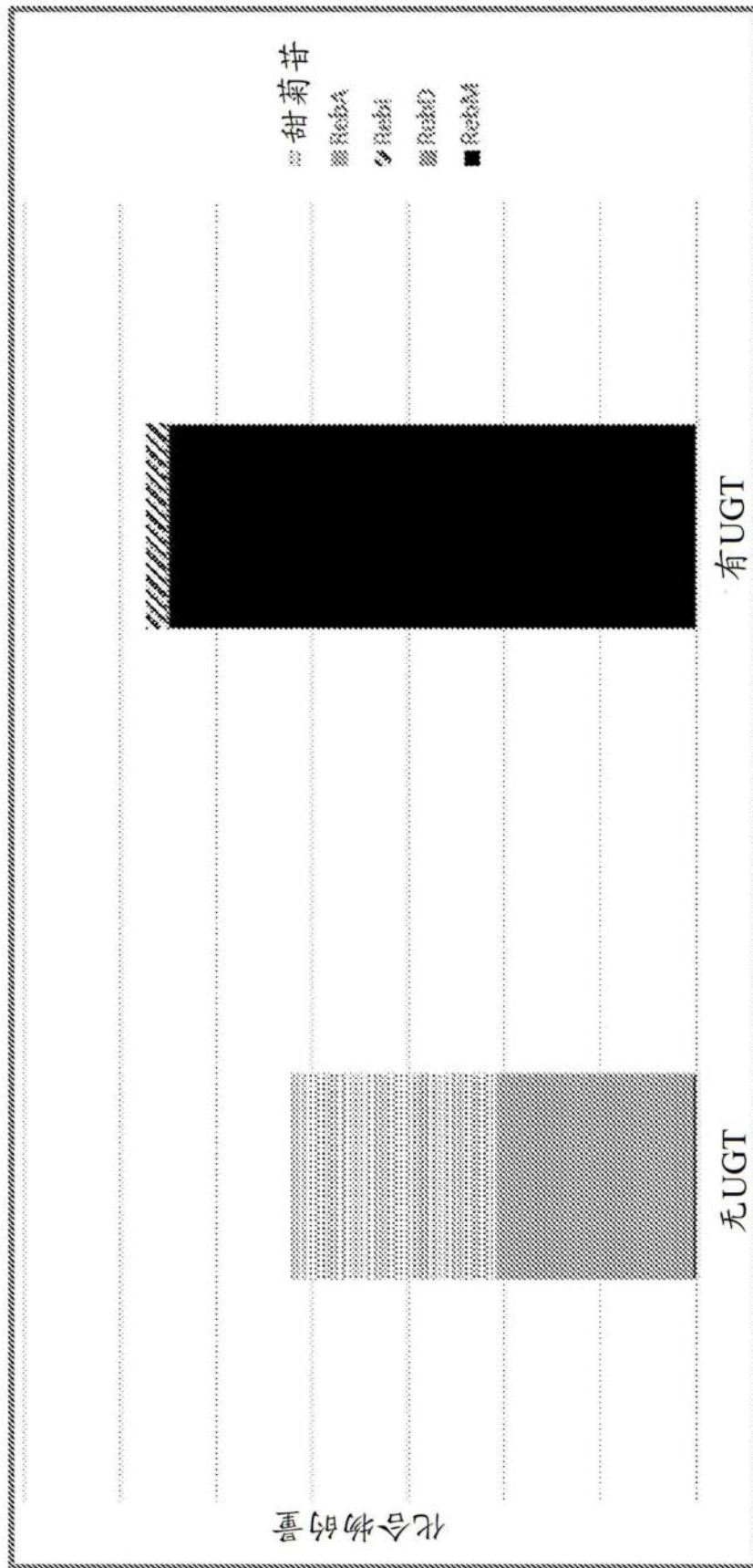


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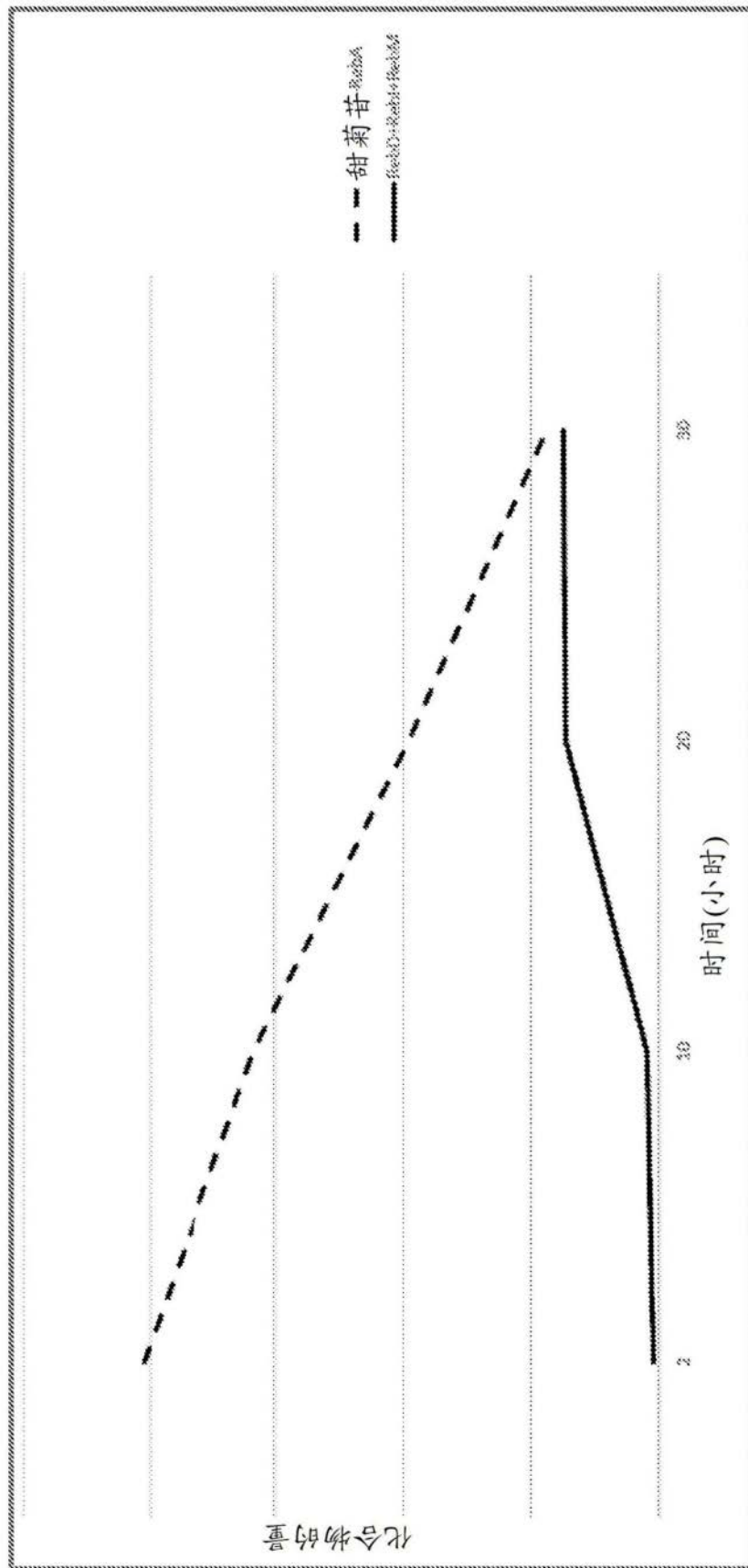


图7

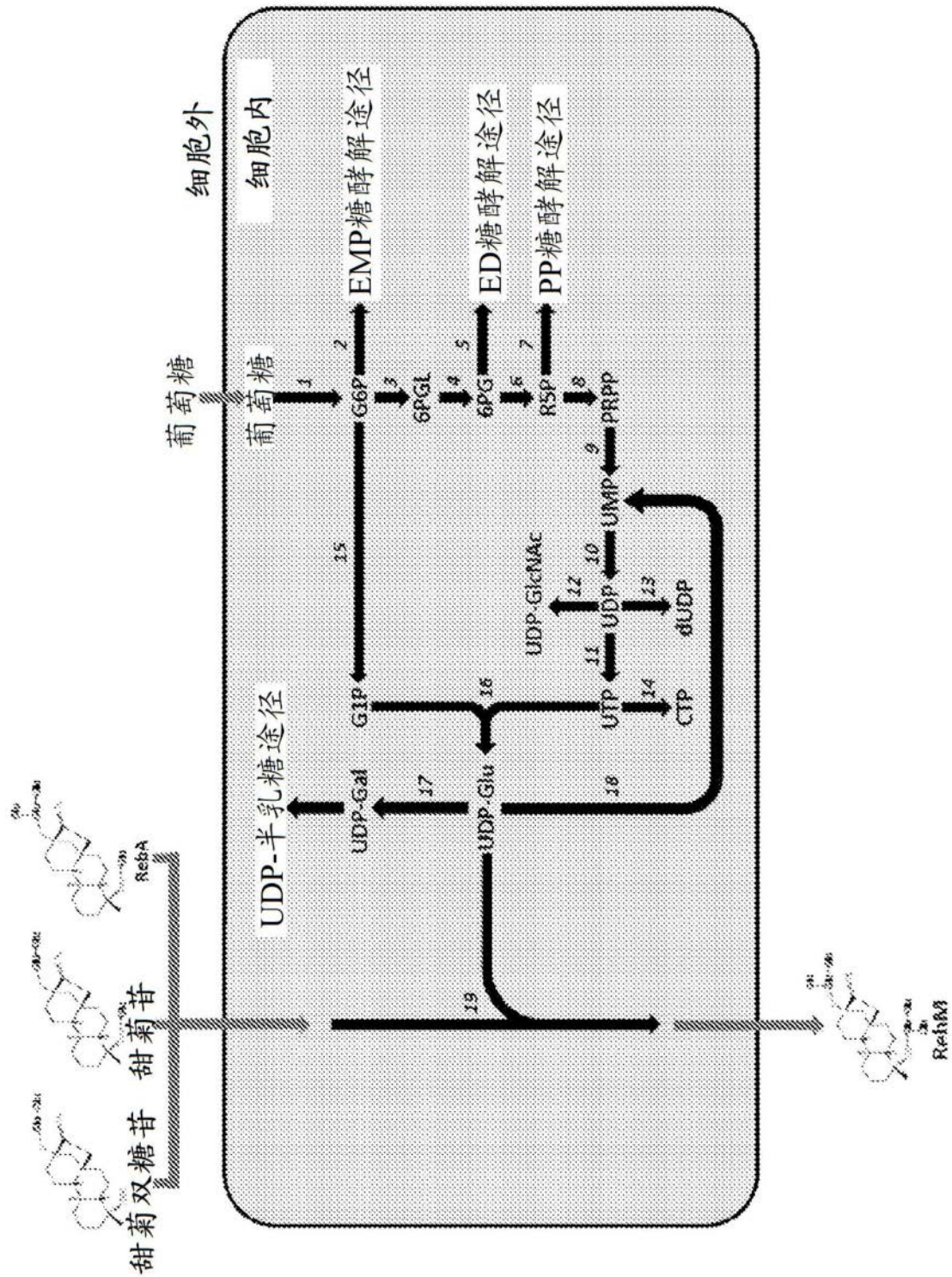


图8