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(54) 发明名称

植物调控元件及其应用

(57) 摘要

本发明提供可用于在植物和植物细胞中调节基因表达的DNA分子和包括其核苷酸序列的构建体。还提供包含可操作地连接异源的可转录多核苷酸的DNA分子的转基因植物、植物细胞、植物部分、种子和商品,以及其使用方法。

1. 一种显示出基因调控功能活性的DNA分子,其包含如SEQ ID NO:156所示的多核苷酸序列,

其中所述DNA分子可操作地连接异源的可转录多核苷酸分子。

2. 权利要求1所述的DNA分子,其中所述异源的可转录多核苷酸分子包含农学目的的基因。

3. 权利要求2所述的DNA分子,其中所述农学目的的基因在植物中赋予除草剂耐受性。

4. 权利要求2所述的DNA分子,其中所述农学目的的基因在植物中赋予抗害虫性。

5. 一种包含显示出基因调控功能活性的异源DNA分子的转基因植物细胞,所述异源DNA分子包含如SEQ ID NO:156所示的多核苷酸序列,

其中所述DNA分子可操作地连接异源的可转录多核苷酸分子,其中所述转基因植物细胞还是不可再生的。

6. 权利要求5所述的转基因植物细胞,其中所述转基因植物细胞是单子叶植物细胞。

7. 权利要求5所述的转基因植物细胞,其中所述转基因植物细胞是双子叶植物细胞。

8. 一种生产商品的方法,其包括:

a) 获得包含显示出基因调控功能活性的DNA分子的转基因植物或其部分,所述DNA分子包含如SEQ ID NO:156所示的多核苷酸序列,

其中所述DNA分子可操作地连接异源的可转录多核苷酸分子;和

b) 由此生产所述商品。

9. 权利要求8所述的方法,其中所述商品是蛋白质浓缩物、蛋白质分离物、谷物、淀粉、种子、粗粉、面粉、生物质或种子油。

10. 一种表达可转录的多核苷酸分子的方法,其包括:

a) 获得包含显示出基因调控功能活性的DNA分子的转基因植物,所述DNA分子包含如SEQ ID NO:156所示的多核苷酸序列,

其中所述DNA分子可操作地连接异源的可转录多核苷酸分子;和

b) 培养所述转基因植物,其中表达所述可转录的多核苷酸。

植物调控元件及其应用

[0001] 本申请是申请日为2012年5月11日和发明名称为“植物调控元件及其应用”的201280031326.X号发明专利申请的分案申请。

[0002] 相关申请的引用

[0003] 本申请要求2011年5月13日提交的美国临时申请号61/485,876的权益,其以整体通过引用方式结合到本文。

[0004] 序列表的引入

[0005] 包含在名为“MONS304W0.txt”的文件中的序列表在此通过电子递交方式提交并通过引用方式结合到本文,所述序列表为463千字节(根据Microsoft Windows®测量)并且创建于2012年5月9日。

发明领域

[0006] 本发明涉及植物分子生物和植物遗传工程以及可用于调节植物中基因表达的DNA分子的领域。

[0007] 背景

[0008] 调控元件是通过调节可操作地连接的可转录的多核苷酸分子的转录来调控基因活性的遗传元件。这种元件包括启动子、前导序列、内含子和3'非翻译区,并用于植物分子生物和植物遗传工程的领域。

[0009] 发明概述

[0010] 本发明提供来源于甜瓜(*Cucumis melo*,通常称作香瓜的植物品种)的用于植物的新型基因调控元件如启动子、前导序列和内含子。本发明还提供包含所述调控元件的DNA构建体、转基因植物细胞、植物和种子。可提供所述序列可操作地连接可转录的多核苷酸分子,所述多核苷酸分子相对于本文提供的调控序列可以是异源的。本发明还提供制备和使用所述调控元件、包含所述调控元件的DNA构建体和包含可操作地连接可转录的多核苷酸分子的调控元件的转基因植物细胞、植物和种子的方法。

[0011] 因此,在一个方面,本发明提供DNA分子,如转录的调控表达元件组、或启动子、或前导序列、或内含子,其包含选自下组的多核苷酸序列:a)与SEQ ID NO:1-199、211和212中任一个具有至少85%序列一致性的序列;b)包含SEQ ID NO:1-199、211和212中任一个的序列;和c)显示出基因调控活性的SEQ ID NO:1-199、211和212中任一个的片段,其中所述DNA分子可操作地连接异源可转录的多核苷酸分子。在具体实施方式中,转录的调控表达元件组、或启动子、或前导序列、或内含子至少90%、至少95%、至少98%或至少99%与SEQ ID NO:1-199、211和212中任一个一致。在特定的实施方式中,所述异源可转录的多核苷酸分子包含农学目的的基因、能够在植物中提供除草剂抗性的基因或能够在植物中提供植物抗害虫性的基因。

[0012] 本发明还提供含有DNA分子如转录的调控表达元件组、或启动子、或前导序列、或内含子的转基因植物细胞,所述DNA分子包含选自下组的多核苷酸序列:a)与SEQ ID NO:1-199、211和212中任一个具有至少85%序列一致性的序列;b)包含SEQ ID NO:1-199、211和

212中任一个的序列;和c)显示出基因调控活性的SEQ ID NO:1-199、211和212中任一个的片段,其中所述DNA分子可操作地连接异源的可转录的多核苷酸分子。此外,所述转录的调控表达元件组、或启动子、或前导序列、或内含子调控基因的表达。所述转基因植物细胞可以是单子叶植物或双子叶植物细胞。

[0013] 本发明进一步提供含有DNA分子如转录的调控表达元件组、或启动子、或前导序列、或内含子的转基因植物或转基因植物部分,所述DNA分子包含选自下组的多核苷酸序列:a)与SEQ ID NO:1-199、211和212中任一个具有至少85%序列一致性的序列;b)包含SEQ ID NO:1-199、211和212中任一个的序列;和c)显示出基因调控活性的SEQ ID NO:1-199、211和212中任一个的片段,其中所述DNA分子可操作地连接异源的可转录的多核苷酸分子。在具体实施方式中,所述转基因植物可以是含有所述转录的调控表达元件组、或启动子、或前导序列、或内含子的任一一代的后代植物。

[0014] 还进一步提供含有DNA分子如转录的调控表达元件组、或启动子、或前导序列、或内含子的转基因种子,所述DNA分子包含选自下组的多核苷酸序列:a)与SEQ ID NO:1-199、211和212中任一个具有至少85%序列一致性的序列;b)包含SEQ ID NO:1-199、211和212中任一个的序列;和c)显示出基因调控活性的SEQ ID NO:1-199、211和212中任一个的片段,其中所述DNA分子可操作地连接异源的可转录的多核苷酸分子。

[0015] 在另一方面,本发明提供用含有DNA分子如转录的调控表达元件组、或启动子、或前导序列、或内含子的转基因植物、转基因植物部分或转基因种子生产商品的方法,所述DNA分子包含选自下组的多核苷酸序列:a)与SEQ ID NO:1-199、211和212中任一个具有至少85%序列一致性的序列;b)包含SEQ ID NO:1-199、211和212中任一个的序列;和c)显示出基因调控活性的SEQ ID NO:1-199、211和212中任一个的片段,其中所述DNA分子可操作地连接异源的可转录的多核苷酸分子。在一个实施方式中,所述商品是蛋白质浓缩物、蛋白质分离物、谷物、淀粉、种子、粗粉、面粉、生物质或种子油。

[0016] 在另一方面,本发明提供包含DNA分子如转录的调控表达元件组、或启动子、或前导序列、或内含子的商品,所述DNA分子包含选自下组的多核苷酸序列:a)与SEQ ID NO:1-199、211和212中任一个具有至少85%序列一致性的序列;b)包含SEQ ID NO:1-199、211和212中任一个的序列;和c)显示出基因调控活性的SEQ ID NO:1-199、211和212中任一个的片段,其中所述DNA分子可操作地连接异源的可转录的多核苷酸分子。

[0017] 在另一方面,本发明提供在转基因植物中使用DNA分子如转录的调控表达元件组、或启动子、或前导序列、或内含子表达可转录的多核苷酸分子的方法,所述DNA分子具有与SEQ ID NO:1-199、211和212中任一个至少85%一致、或含有SEQ ID NO:1-199、211和212中任一个或由SEQ ID NO:1-199、211和212中任一个的片段组成的DNA序列;和栽培所述转基因植物。

[0018] 序列简要说明

[0019] SEQ ID NO:1、5、7、9、11、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49、50、51、52、53、54、55、56、57、58、59、60、61、62、63、64、65、66、67、68、69、70、71、72、73、74、75、76、77、78、79、80、81、82、83、84、85、86、87、88、89、90、91、92、93、94、95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、

122、123、124、125、126、127、128、129、130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151、152、153、154、155、156、159、162、167、168、172、175、176、177、178、181、182、183、184、185、188、189、190、191、192、193、194、195、196、197、198、199、211和212是由下述任一元件组成的甜瓜属转录的调控表达元件组或EXP序列：可操作地连接前导序列元件的启动子元件；或可操作地连接前导序列元件和内含子元件的启动子元件；或可操作地连接前导序列元件、可操作的连接内含子元件、可操作的连接前导序列元件的启动子元件。

[0020] SEQ ID NO:2、6、8、10、12、163和169是启动子元件。

[0021] SEQ ID NO:3、164、166和170是前导序列。

[0022] SEQ ID NO:4、165和171是内含子序列。

[0023] SEQ ID NO:157、160、173、179和186是其中启动子可操作地连接前导序列元件的序列。

[0024] SEQ ID NO:158、161、174、180和187是其中内含子可操作地连接前导序列元件的序列。

[0025] 附图简要说明

[0026] 图1a-1f描绘对应于从甜瓜分离的启动子元件的启动子变体片段的对比。特别是，图1a-1f显示在转录的调控表达元件组EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1) 中发现的2068bp启动子序列P-CUCme.Ubq1-1:1:15 (SEQ ID NO:2) 与经由所述启动子5'缺失衍生的启动子序列P-CUCme.Ubq1-1:1:15的对比。缺失，例如P-CUCme.Ubq1-1:1:15的5'端，产生下述启动子：在EXP-CUCme.Ubq1:1:2 (SEQ ID NO:5) 中发现的1459bp的启动子P-CUCme.Ubq1-1:1:16 (SEQ ID NO:6)；包含在EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7) 中的964bp序列P-CUCme.Ubq1-1:1:17 (SEQ ID NO:8)；包含在EXP-CUCme.Ubq1:1:4 (SEQ ID NO:9) 中的479bp序列P-CUCme.Ubq1-1:1:18 (SEQ ID NO:10)；和包含在EXP-CUCme.Ubq1:1:5 (SEQ ID NO:11) 中的173bp序列P-CUCme.Ubq1-1:1:19 (SEQ ID NO:12)。

[0027] 发明详述

[0028] 本文公开的发明提供从甜瓜中获得的具有有利的基因调控活性的多核苷酸分子。描述了这些多核苷酸分子的设计、构造和用途。在SEQ ID NO:1-199、211和212之中，提供这些多核苷酸分子的核苷酸序列。例如，这些多核苷酸分子能够影响植物组织中可操作地连接可转录的多核苷酸分子的表达，因此在转基因植物中选择性地调控基因表达或编码基因产物的活性。本发明还提供改性、制备和使用所述多核苷酸分子的方法。本发明还提供含有所述启动子和/或其它公开的核苷酸序列的组合物、转化宿主细胞、转基因植物和种子，以及制备和使用其的方法。

[0029] 提供了以下定义和方法以更好地限定本发明和在本发明的实施中指导本领域普通技术人员。除非另有说明，否则根据相关领域普通技术人员的常规用法来理解这些术语。

[0030] DNA分子

[0031] 如本文所使用，术语“DNA”或“DNA分子”是指基因组或合成来源的双链DNA分子，即脱氧核苷酸碱基的聚合物或多核苷酸分子，从5'（上游）端至3'（下游）端读取。如本文所使用的，术语“DNA序列”是指DNA分子的核苷酸序列。

[0032] 如本文所使用的，术语“分离的DNA分子”是指与通常以其自然或天然状态与其有

关的其它分子至少部分分离的DNA分子。在一个实施方式中,术语“分离的”是指与通常以其自然或天然状态与DNA分子侧面相接的一些核酸至少部分分离的DNA分子。因此,融合至通常与它们不相关的调控或编码序列的DNA分子(例如作为重组技术的结果),在本文中被认为是分离的。当被整合入宿主细胞的染色体中或者与其它DNA分子存在于核酸溶液中时,这种分子被认为是分离的,因为它们不处在其天然状态中。

[0033] 许多本领域已知的任何方法用于分离和操作本发明中公开的DNA分子或其片段。例如,PCR(聚合酶链反应)技术可用于扩增特定的起始DNA分子和/或产生原始分子的变体。如通常使用自动化寡核苷酸合成仪所进行, DNA分子或其片段也可通过其它技术例如用化学手段直接合成片段来获得。

[0034] 如本文所使用,术语“序列一致性”是指两个优化对比的多核苷酸序列或两个优化对比的多肽序列一致的程度。优化的序列对比通过手动对比两个序列例如参考序列和另一序列来产生,以最大化在序列对比中具有适当的内部核苷酸插入、缺失或间隙的核苷酸匹配数。如本文所使用,术语“参考序列”是指以SEQ ID NO:1-199、211和212的多核苷酸序列提供的序列。

[0035] 如本文所使用,术语“百分比序列一致性”或“百分比一致性”或“%一致性”为一致性分数乘以100。用参考序列优化对比的序列的“一致性分数”为优化对比中的核苷酸匹配数除以参考序列中的核苷酸总数(例如整个参考序列总长的核苷酸总数)。因此,本发明的一个实施方式为当与在本文中以SEQ ID NO:1-199、211和212提供的参考序列优化对比时,包含与所述参考序列具有至少约85%的一致性、至少约90%的一致性、至少约95%的一致性,至少约96%的一致性、至少约97%的一致性、至少约98%的一致性,或约99%的一致性的序列的DNA分子。在特定的实施方式中,这种序列可定义为具有基因调控活性或编码作用于将可操作连接的多肽定位于细胞内的肽。

[0036] 调控元件

[0037] 调控元件为具有基因调控活性的DNA分子,即具有影响可操作地连接的可转录的多核苷酸分子的转录和/或翻译的能力的DNA分子。因此,术语“基因调控活性”是指通过影响可操作地连接的可转录的多核苷酸分子的转录和/或翻译而影响可操作地连接的可转录的多核苷酸分子的表达模式的能力。如本文所使用,转录的调控表达元件组(EXP)可由可操作地连接的表达元件如增强子、启动子、前导序列和内含子组成。因此,转录的调控表达元件组可包含,例如可操作地连接5'至前导序列的启动子,所述前导序列接着可操作地连接5'至内含子序列。所述内含子序列可由从天然序列的第一内含子/外显子剪接点的点开始的序列组成,并可进一步由包含第二内含子/外显子剪接点的小前导序列片段组成,以提供适当的内含子/外显子加工以促进所形成的转录物的转录和适当的加工。前导序列和内含子可正面影响可操作地连接的可转录多核苷酸分子的转录以及所形成的转录RNA的翻译。所述加工前的RNA分子包括可影响转录的RNA的转录后加工和/或转录的RNA分子从细胞核进入细胞质的输出的前导序列和内含子。继转录的RNA分子的转录后加工之后,所述前导序列可以以最终的信使RNA部分保留,并可正面影响所述信使RNA分子的翻译。

[0038] 调控元件如启动子、前导序列、内含子和转录终止区是具有基因调控活性以及对活体细胞的基因整体表达充当整体部分的DNA分子。术语“调控元件”是指具有基因调控活性的DNA分子,即具有影响可操作地连接的可转录的多核苷酸分子的转录和/或翻译的能力

的DNA分子。因此,在植物中起作用的分离的调控元件例如启动子和前导序列可用于通过遗传工程方法修饰植物表型。

[0039] 调控元件可通过其表达模式影响(定性地和/或定量地),例如通过其时间、空间、发育、组织、环境、生理、病理、细胞周期和/或化学响应的表达模式和其任意组合以及定量或定性指标正向或负向影响和/或基本的或其它影响进行表征。启动子用作调节可操作地连接的可转录的多核苷酸分子的表达的调控元件。

[0040] 如本文所使用,“基因表达模式”为将可操作地连接的DNA分子转录为转录的RNA分子的任何模式。可翻译转录的RNA分子以产生蛋白质分子或可提供反义或其他调控RNA分子,如dsRNA、tRNA、rRNA、miRNA等。

[0041] 如本文所使用,术语“蛋白质表达”为将转录的RNA分子翻译为蛋白质分子的任何模式。蛋白质表达可通过其时间、空间、发育或形态学特性以及定量或定性指标进行表征。

[0042] 如本文所使用,术语“启动子”通常是指参与识别和结合RNA聚合酶II和其它蛋白质(反式作用转录因子)以启动转录的DNA分子。启动子可首先从基因的基因组拷贝的5'非翻译区(5'UTR)分离。可选地,启动子可为合成产生的或操控的DNA分子。启动子也可能是嵌合的,即通过两个或更多个异源DNA分子的融合产生的启动子。可用于实施本发明的启动子包括SEQ ID No:2、6、8、10、12、163和169中任一个,或包含在SEQ ID No:13-199、211和212中任一个的启动子元件,或它们的片段或变体。在本发明的具体实施方式中,本文描述的这种分子及其任何变体或衍生物进一步定义为包含启动子活性,即能够在宿主细胞如在转基因植物中作为启动子。在进一步的具体实施方式中,片段可定义为显示由衍生其的起始启动子分子所具有的启动子活性,或片段可包含提供转录基础水平的“最小启动子”并由用于识别和结合转录启动用RNA聚合酶II复合物的TATA盒或等价序列组成。

[0043] 在一个实施方式中,提供启动子分子的片段。如上所述,启动子片段提供启动子活性,并可单独或其它启动子和启动子片段组合用于例如构建嵌合的启动子。在具体实施方式中,提供了包含具有本文公开的启动子活性的多核苷酸分子的至少约50、95、150、250、500、750或至少约1000个连续核苷酸的启动子片段。

[0044] 可产生衍生自以SEQ ID No:2、6、8、10、12、163和169存在的任何启动子的组合物,或包含在SEQ ID No:13-199、211和212内的启动子元件,例如内部或5'缺失,以提高或改变表达,包括通过去除对表达具有正向或负向影响的元件;复制对表达具有正向或负向影响的元件;和/或复制或除去对表达具有组织或细胞特异性影响的元件。衍生自以SEQ ID No:2、6、8、10、12、163和169存在的任何启动子的组合物,或包含在SEQ ID No:13-199、211和212内的由'3缺失组成的启动子元件,其中去除了所述TATA盒元件或其等价序列和下游序列,例如可用于制备增强子元件。可产生进一步缺失以去除对表达具有正向或负向影响、组织特异性影响、细胞特异性影响、或时机特异性(例如,但不限于,昼夜节律)影响的所有元件。以SEQ ID No:2、6、8、10、12、163和169存在的任何启动子,或包含在SEQ ID No:13-199、211和212之内的启动子元件,和由它们衍生的片段或增强子,可用于制备嵌合的转录调控元件组合物,其由以SEQ ID No:2、6、8、10、12、163和169存在的任何启动子,或包含在SEQ ID No:13-199、211和212之内的启动子元件以及可操作地连接其它增强子和启动子的由它们衍生的片段或增强子组成。在本文描述的对特定转基因的期望表达方面的修饰、复制或缺失的功效可通过稳定且瞬时的植物试验如本文工作实施例中描述的那些凭经验测试,以

证实所述结果,其可随所做出的改变和起始分子中改变的目标而不同。

[0045] 如本文所使用,术语“前导序列”是指从基因的基因组拷贝的5'非翻译区(5'UTR)分离并且通常定义为在转录起始位点(TSS)与蛋白编码序列起始位点之间的核苷酸片段的DNA分子。或者,前导序列可为合成产生的或操控的DNA元件。前导序列可用作调节可操作地连接的可转录的多核苷酸分子的表达的5'调控元件。前导序列分子可与异源启动子或与它们的天然启动子一起使用。因此,本发明的启动子分子可以可操作地连接它们的天然前导序列或可以可操作地连接异源前导序列。可用于实施本发明的前导序列包括SEQ ID No:3、164、166和170,或包含在SEQ ID No:13-199、211和212中的前导序列元件,或它们的片段或变体。在具体实施方式中,可提供定义为能够作为包括例如转基因植物细胞的宿主细胞中的前导序列的这种序列。在一个实施方式中,这种序列解码为包含前导序列活性。

[0046] 以SEQ ID No:3、164、166和170存在的前导序列(5'UTR)或包含在SEQ ID No:13-199、211和212中任一个内的前导序列元件可由调控元件组成,或可采用能够影响转基因的转录或翻译的二级结构。以SEQ ID No:3、164、166和170存在的前导序列或包含在SEQ ID No:13-199、211和212中的前导序列元件可根据本发明用于制备影响转基因的转录或翻译的嵌合的调控元件。此外,以SEQ ID No:3、164、166和170存在的前导序列或包含在SEQ ID No:13-199、211和212中任一个内的前导序列元件可用于制备影响转基因的转录或翻译的嵌合的前导序列。

[0047] 外源基因引入新植物宿主并不总是导致所引入基因的高表达。此外,如果处理复杂性状,有时必须用空间或时间上不同的表达模式调节多个基因。内含子可主要提供这种调节。然而,已表明同一内含子在一种转基因植物中的多种用途呈现出不足。在这些情况下,必须具有一批用于构建适当重组DNA元件的基本控制元件。由于本领域已知的具有表达增强性的内含子的现有集合是有限的,因而需要替代方案。

[0048] 衍生自以SEQ ID No:4、165和171存在的任何内含子或包含在SEQ ID No:13-199、211和212内的内含子元件的组合物可由顺位调控元件的内部缺失或复制组成;和/或当可操作连接启动子+前导序列或嵌合的启动子+前导序列和编码序列时,包含内含子/外显子剪接点的5'和3'序列的改变可用于提高表达或表达特异性。也可作出进行包含内含子/外显子剪接点的5'和3'区域的改变,以在信使RNA的加工和剪接后降低所形成的转录物中产生的假启动和终止密码子的引入可能性。可如工作实施例中所描述凭经验测试所述内含子,以测定所述内含子对转基因表达的影响。

[0049] 根据本发明,可以分析启动子或启动子片段中已知启动子元件的存在,即DNA序列特性,如TATA盒和其它已知转录因子结合位点基序。本领域技术人员可使用这种已知启动子元件的识别来设计具有与原始启动子相似的表达模式的变体。

[0050] 如本文所使用的,术语“增强子”或“增强子元件”是指顺式作用(cis-acting)转录调控元件,也就是顺式元件,其提供整体表达模式的一个方面,但是通常不足以单独驱动可操作地连接的多核苷酸序列的转录。不同于启动子,增强子元件通常不包括转录起始位点(TSS)或TATA盒或等价序列。启动子可以天然包含一个或多个影响可操作地连接的多核苷酸序列的转录的增强子元件。分离的增强子元件也可与启动子融合以产生嵌合的启动子顺式元件,其提供基因表达的整体调节的一方面。启动子或启动子片段可包含一个或多个影响可操作地连接的基因的转录的增强子元件。据信许多启动子增强子元件结合DNA结合蛋

白和/或影响DNA拓扑学,产生选择性地允许或限制RNA聚合酶接触DNA模板或在转录起始位点促进双螺旋选择性打开的局部构造。增强子元件可起作用以结合调控转录的转录因子。一些增强子元件结合多于一种转录因子,并且转录因子可通过不同的亲合力与多于一个增强子结构域相互作用。增强子元件可通过许多技术进行鉴别,包括缺失分析,即从启动子5'端或内部缺失一个或多个核苷酸;DNA结合蛋白分析,使用DNase I足印法、甲基化干扰、电泳迁移率改变试验、通过连接介导PCR的体内基因组足印法和其它常规试验;或通过使用已知顺式元件基序或增强子元件作为靶序列或靶基序和常规DNA序列比较法如BLAST一起进行DNA序列相似性分析。增强子结构域的精细结构可通过一个或多个核苷酸的突变(或取代)或通过其它常规方法进一步研究。增强子元件可通过化学合成获得,或从包括这种元件的调控元件中分离获得,并且它们可与另外的含有有用的限制酶位点的侧翼核苷酸一起合成以促进随后操作。因此,本发明涵盖根据本文公开的用于调节可操作地连接的可转录的多核苷酸分子的表达的方法的增强子元件的设计、构建和使用。

[0051] 在植物中,相对于缺乏所述内含子的构建体,在基因构建体中包含一些内含子导致增加的mRNA和蛋白积聚。所述效果已称作基因表达的“内含子介导的增强”(IME)(Mascarenhas等人,(1990) *Plant Mol. Biol.* 15:913-920)。在玉米基因中(例如tubA1、Adh1、Sh1、Ubi1(Jeon等人,(2000) *Plant Physiol.* 123:1005-1014;Callis等人,(1987) *Genes Dev.* 1:1183-1200;vasil等人,(1989) *Plant Physiol.* 91:1575-1579;Christiansen等人,(1992) *Plant Mol. Biol.* 18:675-689)和在水稻基因中(例如salt、tpi;McElroy等人,*Plant Cell* 2:163-171(1990);Xu等人,*Plant Physiol.* 106:459-467(1994))已经鉴别已知在植物中刺激表达的内含子。类似地,已经发现来自双子叶植物基因如来自矮牵牛(例如rbcS)、土豆(例如st-Is1)和拟南芥(例如ubq3和pat1)的那些的内含子会提高基因表达速率(Dean等人,(1989) *Plant Cell* 1:201-208;Leon等人,(1991) *Plant Physiol.* 95:968-972;Norris等人,(1993) *Plant Mol Biol* 21:895-906;Rose和Last(1997) *Plant J.* 11:455-464)。已经证明,在内含子的剪接位点内的缺失或突变降低基因表达,这表明IME可能需要剪接(Mascarenhas等人,(1990) *Plant Mol Biol.* 15:913-920;Clancy和Hannah,(2002) *Plant Physiol.* 130:918-929)。然而,来自拟南芥的pat1基因的剪接位点内的点突变已经表明,双子叶植物中某个IME不需要剪接本身(Rose和Beliakoff(2000) *Plant Physiol.* 122:535-542)。

[0052] 通过内含子的基因表达增强不是普遍现象,因为插入重组表达盒的一些内含子不能增强表达(例如来自双子叶植物基因(来自豌豆的rbcS基因、来自豆子的云扁豆蛋白基因和来自马铃薯的st1s-1基因)的内含子和来自玉米基因(adh1基因第九内含子、hsp81基因第一内含子)的内含子)(Chee等人,(1986) *Gene* 41:47-57;Kuhlemeier等人,(1988) *Mol Gen Genet* 212:405-411;Mascarenhas等人,(1990) *Plant Mol. Biol.* 15:913-920;Sinibaldi和Mettler(1992) *In WE Cohn, K Moldave, eds, Progress in Nucleic Acid Research and Molecular Biology, Vol 42. Academic Press, New York, pp 229-257; Vancanneyt等人,1990Mol. Gen. Genet.* 220:245-250)。因此,不是每种内含子均可用于操控转基因植物中非内源性基因或内源性基因的基因表达水平。什么特性或具体序列特征必须存在于内含子序列以增强给定基因的表达速率是现有技术未知的,因此,当异源使用时,根据现有技术无法预测给定植物内含子是否会引起IME。

[0053] 如本文所使用,术语“嵌合的”是指通过将第一DNA分子与第二DNA分子融合而生成的单个DNA分子,其中第一或第二DNA分子通常都不会在该构型、即融合其它的构型中发现。因此,嵌合的DNA分子为新的DNA分子而不是通常在自然界中发现的。如本文所使用,术语“嵌合的启动子”是指通过对DNA分子进行这种操作而生成的启动子。嵌合的启动子可以结合两种或更多种DNA片段;实例是启动子与增强子元件的融合。因此,本发明涵盖根据本文公开的用于调控可操作地连接的可转录的多核苷酸分子的表达的方法的嵌合的启动子的设计、构建和使用。

[0054] 如本文所使用,术语“变体”是指与第一DNA分子组成相似但不相同的第二DNA分子,而第二DNA分子还保持常规功能性,即与第一DNA分子具有相同或相似的表达模式。变体可以是第一DNA分子的较短或截短形式和/或第一DNA分子序列的变化形式,如具有不同的限制性酶位点和/或内部缺失、取代和/或插入的DNA分子。“变体”还可以包括具有包含参考序列的一个或多个核苷酸的取代、缺失和/或插入的核苷酸序列的调控元件,其中所述衍生的调控元件具有比相应母体调控分子更强或更弱或相等的转录或翻译活性。所述调控元件“变体”还可涵盖由天然存在于细菌和植物细胞转化中的突变引起的变体。在本发明中,以SEQ ID No:1-199、211和212提供的多核苷酸序列可用于生成与原始调控元件的多核苷酸序列组成相似但不相同的变体,而它们还保持常规功能性,即与原始的调控元件相同或相似的表达模式。本领域普通技术人员根据本公开熟知本发明的这种变体的生成,其包含在本发明的范围内。嵌合的调控元件的“变体”包含与参考嵌合的调控元件序列相同的组成元件,但可通过本领域已知的各种方法如限制酶消化和连接、不依赖于克隆的连接、扩增过程中的PCR产物的模块式组装、或嵌合的调控元件的直接化学合成以及本领域已知的其它方法,可操作地连接包含所述嵌合的调控元件的组成元件。所形成的“变体”嵌合的调控元件由与所述参考序列相同但在用于可操作地连接所述组成元件的一条或多条序列方面不同的组成元件或其变体组成。在本发明中,以SEQ ID No:1-199、211和212提供的多核苷酸序列各自提供参考序列,其中所述参考序列的组成元件可通过本领域已知的方法连接,并且可由天然存在于细菌和植物细胞转化的一个或多个核苷酸或突变的取代、缺失和/或插入组成。

[0055] 构建体

[0056] 如本文所使用,术语“构建体”是指衍生自任何来源、能够进行基因组整合或自主复制、包含多核苷酸分子(其中一个或多个核苷酸分子已经以功能性操作方式连接,即可操作地连接)的任何重组多核苷酸分子,如质粒、粘粒、病毒、自主复制多核苷酸分子、噬菌体,或线状或环状单链或双链DNA或RNA多核苷酸分子。如本文所使用,术语“载体”是指任何可用于转化目的(即将异源DNA引入宿主细胞)的重组多核苷酸构建体。所述术语包括从任何上述分子分离的表达盒。

[0057] 如本文所使用的,术语“可操作地连接”是指第一分子连接到第二分子,其中所述分子如此排列以使第一分子影响第二分子的功能。所述两个分子可以是或不是单个连续分子的一部分,并且可以相邻或不相邻。例如,如果启动子调节细胞中目标可转录的多核苷酸分子的转录,则启动子可操作地连接可转录的多核苷酸分子。例如,当前导序列能够用作编码序列编码的多肽的前导序列时,其可操作地连接所述编码序列。

[0058] 在一个实施方式中,本发明的构建体可以以双Ti质粒边界(border) DNA构建体提

供,其具有从包含T-DNA的根癌土壤杆菌(*Agrobacterium tumefaciens*)分离的Ti质粒的右边界(RB或AGRtu.RB)和左边界(LB或AGRtu.LB)区域,连同根癌土壤杆菌细胞提供的转化分子一起,允许T-DNA整合至植物细胞的基因组中(参见,例如,美国专利6,603,061)。构建体也可包含在细菌细胞中提供复制功能和抗生素选择性的质粒骨架DNA片段,例如,大肠杆菌复制起点如ori322、宽宿主范围复制起点如oriV或oriRi,以及编码Tn7氨基糖苷腺苷酰转移酶(aminoglycoside adeny I transferase)(aadA)提供对壮观霉素或链霉素耐药性的可选择标记如Spec/Strp或者庆大霉素(Gm,Gent)可选择标记基因的编码区。对于植物的转化,宿主细菌菌株通常为根癌土壤杆菌ABI、C58或LBA4404;然而,植物转化领域已知的其它菌株可以在本发明中起作用。

[0059] 以将可转录的多核苷酸分子转录为翻译和表达为蛋白质产物的功能性mRNA分子的方式,将构建体组装和引入细胞中的方法是已知的。对于本发明的实施,制备和使用构建体和宿主细胞的常规组合物和方法可参见,例如,Molecular Cloning:A Laboratory Manual,3rd edition Volumes 1,2和3(2000)J.F.Sambrook,D.W.Russell和N.Irwin,Cold Spring Harbor Laboratory Press。制备特别适合于植物转化的重组载体的方法包括但不限于,以其整体描述在美国专利4,971,908、4,940,835、4,769,061和4,757,011中的那些。这些类型的载体也已在科学文献中综述过(参见,例如,Rodriguez等人,Vectors:A Survey of Molecular Cloning Vectors and Their Uses,Butterworths,Boston,(1988)和Glick等人,Methods in Plant Molecular Biology and Biotechnology,CRC Press,Boca Raton,FL.(1993))。用于在高等植物中表达核酸的典型载体是本领域众所周知的,并且包括衍生自根癌土壤杆菌的肿瘤诱导(Ti)质粒的载体(Rogers等人,Methods in Enzymology,153:253-277(1987))。用于植物转化的包括pCaMV CN转化控制载体的其它重组载体也已经描述在科学文献中(参见,例如,Fromm等人,Proc.Natl.Acad.Sci.USA,82:5824-5828(1985))。

[0060] 包括本文提供的任何那些构建体可包括不同的调控元件。任何这种调控元件可与其它调控元件组合提供。可设计或修改这种组合以产生所期望的调控特性。在一个实施方式中,本发明的构建体包含至少一个可操作地连接可转录的多核苷酸分子的调控元件,所述可转录的多核苷酸分子可操作地连接3'转录终止分子。

[0061] 本发明的构建体可包括本文提供的或本领域已知的任何启动子或前导序列。例如,本发明的启动子可以可操作地连接异源非翻译5'前导序列,例如衍生自热休克蛋白基因的异源非翻译5'前导序列(参见,例如,美国专利号5,659,122和5,362,865)。可选地,本发明的前导序列可以可操作地连接异源启动子如花椰菜花叶病毒35S转录启动子(参见,美国专利号5,352,605)。异源元件的这种可操作的连接赋予的表达特性不必然是每个启动子和前导序列所阐明特性的叠加,而是通过可操作地连接的异源启动子和前导序列驱动的表达的经验分析来确定。

[0062] 如本文所使用,术语“内含子”是指可从基因的基因组拷贝中分离或鉴别、并且通常可定义为翻译前在mRNA加工期间剪接出的区域的DNA分子。或者,内含子可为合成产生的或操控的DNA元件。内含子可包含影响可操作地连接的基因转录的增强子元件。内含子可用作调控元件以调节可操作地连接的可转录的多核苷酸分子的表达。DNA构建体可包含内含子,所述内含子与可转录的多核苷酸分子序列可以是或者不是异源的。本领域的内含子的

实例包括水稻肌动蛋白内含子(美国专利号5,641,876)以及玉米HSP70内含子(美国专利号5,859,347)。对实施本发明有用的内含子包括SEQ ID No:4、165和171或包含在SEQ ID No:13-199、211和212中任一个之内的内含子元件。

[0063] 如本文所使用,术语“3'转录终止分子”或“3'UTR”是指在转录期间用于生成mRNA分子3'非翻译区(3'UTR)的DNA分子。mRNA分子的3'非翻译区可通过特定的切割和3'多腺苷酸化(即聚A尾)产生。3'UTR可以可操作地连接至和位于可转录的多核苷酸分子的下流,并且可包括提供多腺苷酸化信号和其它能够影响转录、mRNA加工或基因表达的调控信号的多核苷酸。聚A尾被认为在mRNA稳定性和启动翻译中起作用。3'转录终止分子的实例为胭脂碱合酶3'区域(参见,Fraley等人,Proc.Natl.Acad.Sci.USA,80:4803-4807(1983));小麦hsp173'区域;豌豆核酮糖二磷酸羧化酶-加氧酶小亚基3'区域;棉花E63'区域(美国专利6,096,950);W00011200A2中公开的3'区域;以及薏苡醇溶蛋白3'UTR(美国专利号6,635,806)。

[0064] 3'UTR通常发现用于特定基因重组表达的有利用途。在动物系统中,3'UTR的机制已经完全明确(例如Zhao等人,Microbiol Mol Biol Rev 63:405-445(1999);Proudfoot,Nature 322:562-565(1986);Kim等人,Biotechnology Progress 19:1620-1622(2003);Yonaha和Proudfoot,EMBO J.19:3770-3777(2000);Cramer等人,FEBS Letters 498:179-182(2001);Kuerstem和Goodwin,Nature Reviews Genetics 4:626-637(2003))。为了防止不需要的性状无关的(下流)序列的转录(其会干涉性状的表现),要求RNA转录的有效终止。与无关的插入相比,在彼此局部邻近中(例如在一个T-DNA内)多基因表达盒的排列可导致对所述构建体内一个或多个基因的基因表达的抑制(Padidam和Cao,BioTechniques 31:328-334(2001))。例如在期望来自所有盒的强烈基因表达的情况下,这会妨碍实现足够的表达水平。

[0065] 在植物中,明确定义的多腺苷酸化信号序列是未知的。Hasegawa等人,Plant J.33:1063-1072,(2003)不能鉴别在林烟草(Nicotiana glauca)中体外和体内系统中保守的多腺苷酸化信号序列并测定一级(非多腺苷酸化的)转录物的实际长度。弱的3'UTR具有产生通读的可能性,这可影响位于相邻表达盒中的基因表达(Padidam和Cao,BioTechniques 31:328-334(2001))。转录终止的适当控制可以防止位于下流的序列(例如其它表达盒)中的通读(read-through),并可以进一步允许RNA聚合酶的有效再循环,从而提高基因表达。转录的有效终止(来自DNA的RNA聚合酶释放)对于转录的再启动是必须的,并由此直接影响整个转录物水平。转录终止之后,从细胞质的合成和模板位点释放成熟的mRNA。真核mRNA以多(A)形式在体内积聚,因此难以通过常规方法检测转录的终止位点。然而,通过生物信息学方法的功能和有效3'UTR的预测是困难的,因为不存在使有效3'UTR容易预测的保守的序列。

[0066] 从实践的观点,在转基因盒中使用的3'UTR具有下述特征通常是有利的。3'UTR将能高效且有效地终止转基因的转录并防止转录物通读任何相邻DNA序列中或T-DNA已经插入的相邻染色体的DNA,如在多个盒残留在一个T-DNA中的情况下,所述相邻DNA序列可由另一转基因盒组成。所述3'UTR不应引起用于驱动转基因表达的启动子、前导序列和内含子赋予的转录活性的降低。在植物生物工艺学中,所述3'UTR通常用于启动从所述转化的植物提取的反转录RNA的扩增反应,并用于(1)评估曾经整合入所述植物染色体的转基因盒的转录

活性或表达；(2) 评估所述植物DNA内插入的拷贝数；和(3) 评估育种后所形成的种子的接合性。所述3'UTR也用于从所转化的植物中提取的DNA的扩增反应，以表征所插入的盒的完整性。

[0067] 可基于由从衍生自小米 (*Setaria italica* (L.) Beauv) 的种子、花和其它组织分离的信使RNA构成的cDNA文库中表达的序列标签 (EST) 的表达鉴别对在植物中提供转基因表达有用的3'UTR。cDNA文库由使用花组织、种子、叶和根从所选择的植物品种分离的组织构成。使用不同的测序方法测序所得到的cDNA。使用生物信息学软件如clc_ref_assemble_complete版2.01.37139 (CLC bio USA, Cambridge, Massachusetts 02142), 将所得到的EST组装成簇。通过计数每个簇的cDNA读取数目来测定每个簇的转录物丰度。所识别的3'UTR可以由衍生自cDNA序列以及衍生自基因组DNA的序列组成。cDNA序列用于设计引物，其然后与按照制造商的方案构建的GenomeWalkerTM (Clontech Laboratories, Inc, Mountain View, CA) 文库一起用于克隆相应基因组DNA序列的3'区域，以提供较长的终止序列。通过每个组织文库观察到的序列读取的直接计数或标准化计数的相对转录物丰度分析可用于推断关于表达模式的特性。例如，与叶相反，可在根组织中观察到的较高丰度的转录物中发现一些3'UTR。这表明所述转录物在根中高度表达，且根表达的所述特性可归因于启动子、前导序列、内含子或3'UTR的转录调控。根据在特定器官、组织或细胞类型内表达的所述特性鉴别3'UTR的经验性测试可导致在那些特定器官、组织或细胞类型中增强表达的3'UTR的鉴别。

[0068] 构建体和载体也可包括表达连接的肽类的转运肽编码序列，所述连接的肽用于将蛋白质产物靶向特别是叶绿体、白色体或其它质体细胞器；线粒体；过氧化物酶体；液泡或细胞外部位。使用叶绿体转运肽的描述，参见美国专利号5,188,642和美国专利号5,728,925。许多叶绿体-定位 (chloroplast-localized) 蛋白质从核基因中表达为前体，并且通过叶绿体转运肽 (CTP) 靶向叶绿体。这种分离的叶绿体蛋白质的实例包括但不限于，在美国专利号7,193,133中描述的与核酮糖-1,5,-二磷酸羧化酶的小亚基 (SSU)、铁氧还蛋白、铁氧还蛋白氧化还原酶、捕光复合物蛋白I和蛋白II、硫氧还蛋白F、烯醇丙酮酸莽草酸磷酸合成酶 (EPSPS) 和转运肽类相关的那些。已在体内和体外证明，通过使用与异源CTP的蛋白质融合可将非-叶绿体蛋白质靶向叶绿体，并且所述CTP足以将蛋白质靶向叶绿体。适合的叶绿体转运肽如拟南芥EPSPS CTP (CTP2) (参见, Klee等人, Mol. Gen. Genet., 210:437-442 (1987)) 或矮牵牛EPSPS CTP (CTP4) (参见, della-Cioppa等人, Proc. Natl. Acad. Sci. USA, 83:6873-6877 (1986)) 的引入已显示在转基因植物中将异源EPSPS蛋白质序列靶向叶绿体 (参见, 美国专利号5,627,061、5,633,435和5,312,910以及EP 0218571、EP 189707、EP 508909和EP 924299)。

[0069] 可转录的多核苷酸分子

[0070] 如本文所使用，术语“可转录的多核苷酸分子”是指任何能够被转录为RNA分子的DNA分子，包括但不限于具有蛋白质编码序列的那些以及生成具有用于基因抑制的序列的RNA分子的那些。“转基因”是指至少相对于其在基因组中的位置与宿主细胞异源的可转录的多核苷酸分子和/或人工引入目前或任何先代细胞中宿主细胞基因组的可转录的多核苷酸分子。

[0071] 本发明的启动子可以可操作地连接相对于启动子分子为异源的可转录的多核苷酸分子。如本文所使用，术语“异源的”是指当这种组合通常不存在于自然界时两个或更多

个多核苷酸分子的组合。例如,两个分子可衍生自不同物种和/或两个分子可衍生自不同基因,例如,来自相同物种的不同基因或来自不同物种的相同基因。因此,如果这种组合通常不存在于自然界,则启动子相对于可操作地连接的可转录的多核苷酸分子为异源的,即所述可转录的多核苷酸分子与所述启动子分子的组合不是天然存在的可操作地连接的。

[0072] 可转录的多核苷酸分子通常可为表达RNA转录物所需的任何DNA分子。RNA转录物的这种表达可导致所得到的mRNA分子的翻译以及蛋白质的表达。可选地,例如,可转录的多核苷酸分子可设计为最终引起特定基因或蛋白质的表达降低。在一个实施方式中,这可通过使用以反义方向定向的可转录的多核苷酸分子来实现。简而言之,当转录反义可转录的多核苷酸分子时,RNA产物在细胞中与互补RNA分子杂交并且隔离(sequesters)互补RNA分子。这种双螺旋RNA分子不能通过细胞的翻译机制翻译为蛋白质,并且在细胞中降解。可以这种方式负性调控任何基因。

[0073] 因此,本发明的一个实施方式是本发明的调控元件,例如以SEQ ID NO:1-199、211和212提供的那些,其可操作地连接可转录的多核苷酸分子,使得当将所述构建体引入植物细胞的基因组中时以期望水平或以期望模式调控可转录的多核苷酸分子的转录。在一个实施方式中,可转录的多核苷酸分子包含基因的蛋白质编码区域,并且启动子影响被翻译和表达为蛋白质产物的RNA分子的转录。在另一个实施方式中,可转录的多核苷酸分子包含基因的反义区域,并且启动子影响反义RNA分子、双链RNA或其它相似抑制性RNA分子的转录以抑制靶宿主细胞中目的特定RNA分子的表达。

[0074] 农学目的基因

[0075] 可转录的多核苷酸分子可以是农学目的基因。如本文所使用,术语“农学目的基因”是指当在特定的植物组织、细胞或细胞类型中表达时赋予与植物形态、生理、生长、发育、产量、产物、营养分布、疾病或抗害虫性和/或环境或化学耐受性相关的期望特征的可转录的多核苷酸分子。农学目的基因包括,但不限于,编码产量蛋白、应激抗性蛋白、发育控制蛋白、组织分化蛋白、分生组织蛋白、环境响应蛋白、衰老蛋白、激素响应蛋白、脱落蛋白(abscission protein)、来源蛋白、sink蛋白、花控制蛋白、种子蛋白、除草剂抗性蛋白、疾病抗性蛋白、脂肪酸生物合成酶、生育酚生物合成酶、氨基酸生物合成酶、杀虫蛋白或例如靶向抑制特定基因的反义或RNAi分子的任何其它试剂的那些基因。农学目的基因的产物可在植物内起作用以影响植物生理或代谢,或可作为以植物为食的害虫的食物的杀虫剂起作用。

[0076] 在本发明的一个实施方式中,将本发明的启动子引入构建体中,以使启动子可操作地连接作为农学目的基因的可转录的多核苷酸分子。为了赋予农学有益性状,农学目的基因的表达是所期望的。有益农学性状可以是例如,但不限于,除草剂耐受性、昆虫控制、改良的产量、真菌疾病抗性、病毒抗性、线虫抗性、细菌疾病抗性、植物生长和发育、淀粉生产、改良的油生产、高的油生产、改良的脂肪酸含量、高的蛋白质生产、果实成熟、提高的动物和人类营养、生物聚合物、环境压力抗性、药用肽和可分泌肽、改良的加工性状、改良的消化性、酶生产、味道、固氮、杂交制种、纤维生产和生物燃料生产。农学目的基因的实例包括具有除草剂抗性(美国专利号6,803,501、6,448,476、6,248,876、6,225,114、6,107,549、5,866,775、5,804,425、5,633,435、和5,463,175)、提高的产量(美国专利号USRE38,446、6,716,474、6,663,906、6,476,295、6,441,277、6,423,828、6,399,330、6,372,211、6,235,

971、6,222,098和5,716,837)、昆虫控制(美国专利号6,809,078、6,713,063、6,686,452、6,657,046、6,645,497、6,642,030、6,639,054、6,620,988、6,593,293、6,555,655、6,538,109、6,537,756、6,521,442、6,501,009、6,468,523、6,326,351、6,313,378、6,284,949、6,281,016、6,248,536、6,242,241、6,221,649、6,177,615、6,156,573、6,153,814、6,110,464、6,093,695、6,063,756、6,063,597、6,023,013、5,959,091、5,942,664、5,942,658、5,880,275、5,763,245和5,763,241)、真菌疾病抗性(美国专利号6,653,280、6,573,361、6,506,962、6,316,407、6,215,048、5,516,671、5,773,696、6,121,436、6,316,407和6,506,962)、病毒抗性(美国专利号6,617,496、6,608,241、6,015,940、6,013,864、5,850,023和5,304,730)、线虫抗性(美国专利号6,228,992)、细菌疾病抗性(美国专利号5,516,671)、植物生长和发育(美国专利号6,723,897和6,518,488)、淀粉生产(美国专利号6,538,181、6,538,179、6,538,178、5,750,876、6,476,295)、改良的油生产(美国专利号6,444,876、6,426,447和6,380,462)、高的油生产(美国专利号6,495,739、5,608,149、6,483,008和6,476,295)、改良的脂肪酸含量(美国专利号6,828,475、6,822,141、6,770,465、6,706,950、6,660,849、6,596,538、6,589,767、6,537,750、6,489,461和6,459,018)、高的蛋白质生产(美国专利号6,380,466)、果实成熟(美国专利号5,512,466)、提高的动物和人类营养(美国专利号6,723,837、6,653,530、6,5412,59、5,985,605和6,171,640)、生物聚合物(美国专利号USRE37,543、6,228,623、5,958,745和6,946,588)、环境压力抗性(美国专利号6,072,103)、药用肽和可分泌肽(美国专利号6,812,379、6,774,283、6,140,075和6,080,560)、改良的加工性状(美国专利号6,476,295)、改良的消化性(美国专利号6,531,648)、低棉子糖(美国专利号6,166,292)、工业酶生产(美国专利号5,543,576)、改良的味道(美国专利号6,011,199)、固氮(美国专利号5,229,114)、杂交制种(美国专利号5,689,041)、纤维生产(美国专利号6,576,818、6,271,443、5,981,834和5,869,720)和生物燃料生产(美国专利号5,998,700)。

[0077] 可选地,农学目的基因可以通过编码引起内源基因的基因表达的靶向调节的RNA分子而影响上述的植物特性或表型,例如通过反义(参见,例如,美国专利5,107,065)、抑制性RNA("RNAi",包括通过miRNA-、siRNA-、反式作用siRNA-和相控sRNA-介导机制调节基因表达,例如,如公开的申请US2006/0200878和US2008/0066206和美国专利申请11/974,469中所描述的),或共抑制-介导机制。RNA也可以是经工程化以切割期望的内源性mRNA产物的催化的RNA分子(例如,核酶或核糖开关;参见例如,US2006/0200878)。因此,任何编码影响农学重要表型或目的形态学变化的转录RNA分子的可转录的多核苷酸分子可用于实施本发明。通过将可转录的多核苷酸分子转录为能够引起基因抑制的分子的方式,构建构建体并将构建体引入细胞内的方法在本领域是已知的。例如,在植物细胞中使用构建体和反义定向的可转录的多核苷酸分子以调控基因表达的转录后基因抑制在美国专利号5,107,065和5,759,829中公开,并且在植物中使用构建体和有义定向的可转录的多核苷酸分子以调控基因表达的转录后基因抑制在美国专利号5,283,184和5,231,020中公开。在植物细胞中表达可转录的多核苷酸也可用于抑制食用植物细胞的植物害虫,例如,从鞘翅目害虫分离的组合物(美国专利公开号US20070124836)和从线虫害虫分离的组合物(美国专利公开号US20070250947)。植物害虫包括,但不限于节肢类害虫、线虫害虫和真菌或微生物害虫。引入本发明的构建体的示例性的可转录的多核苷酸分子包括,例如,来自不同于靶物种的物

种的DNA分子或基因,或起始于或存在于相同物种但通过遗传工程方法而不是传统的繁殖或育种技术而引入受体细胞中的基因。多核苷酸分子的类型包括但不限于,已经存在于植物细胞中的多核苷酸分子、来自另一种植物的多核苷酸分子、来自不同生物体的多核苷酸分子或外部产生的多核苷酸分子,例如含有基因反义消息的多核苷酸分子,或编码人工、合成或另外的改良形式的转基因的多核苷酸分子。

[0078] 可选择性标记

[0079] 如在本文中所用,术语“标记”是指其表达或不表达可以以某种方式筛选或评分的任何可转录的多核苷酸分子。用于实施本发明的标记基因包括但不限于,编码 β -葡萄糖醛酸酶(在美国专利号5,599,670中描述的GUS)、绿色荧光蛋白及其变体(在美国专利号5,491,084和6,146,826中描述的GFP)、赋予抗生素耐药性的蛋白质或赋予除草剂耐受性的蛋白质的可转录的多核苷酸分子。可用的抗生素耐药性标记包括编码赋予卡那霉素(nptII)、潮霉素B(aph IV)、链霉素或壮观霉素(aad,spec/strep)和庆大霉素(aac3和aacC4)耐药性的蛋白质的那些标记。已证实转基因植物耐受性并且可应用本发明方法的除草剂包括但不限于:氨基-甲基-膦酸、草甘膦、草胺膦(glufosinate)、磺酰脲、咪唑啉酮、溴苯腈、delapon、麦草畏、环己二酮、原卟啉原氧化酶抑制剂和异噁唑草酮(isoxafIutole)除草剂。编码涉及除草剂耐受性的蛋白质的可转录的多核苷酸分子包括,但不限于,编码5-烯醇丙酮酸莽草酸-3-磷酸合成酶(在美国专利号5,627,061、5,633,435、6,040,497和5,094,945中描述的用于草甘膦耐受性的EPSPS)的可转录的多核苷酸分子;编码草甘膦氧化还原酶和草甘膦-N-乙酰基转移酶(在美国专利号5,463,175中描述的GOX、在美国专利公开号20030083480中描述的GAT,和美国专利公开号20030135879中的麦草畏单加氧酶)的可转录的多核苷酸分子;编码溴苯腈腈水解酶(在美国专利号4,810,648中描述的用于溴苯腈耐受性的Bxn)的可转录的多核苷酸分子;在Misawa等人,Plant Journal 4:833-840(1993)和Misawa等人,Plant Journal6:481-489(1994)中描述的用于达草灭耐受性的编码八氢番茄红素去饱和酶(crtI)的可转录的多核苷酸分子;在Sathasiivan等人,Nucl.Acids Res.18:2188-2193(1990)中描述的用于磺酰脲类除草剂耐受性的编码乙酰羟酸合成酶(AHAS,也就是ALS)的可转录的多核苷酸分子;以及在DeBlock等人,embo EMBO Journal6:2513-2519(1987)中描述的用于草胺膦和双丙氨膦耐受性的bar基因。本发明的启动子分子可表达连接的可转录的多核苷酸分子,其编码草胺膦(phosphinothricin)乙酰基转移酶、草甘膦抗性EPSPS、氨基糖苷磷酸转移酶、羟苯基丙酮酸脱氢酶、潮霉素磷酸转移酶、新霉素磷酸转移酶、茅草枯脱卤素酶、溴苯腈抗性腈水解酶、邻氨基苯甲酸酯合成酶、芳氧基链烷酸酯双加氧酶、乙酰辅酶A羧化酶、草甘膦氧化还原酶和草甘膦-N-乙酰基转移酶。

[0080] 包括在术语“可选择性标记”中的还有编码可分泌的标记的基因,其分泌可作为鉴别或选择转化细胞的手段。实例包括编码可通过抗体相互作用鉴别的可分泌的抗原的标记或甚至编码可催化检测的可分泌酶的标记。可选择的分泌的标记蛋白质分为几种类型,包括可检测的(例如通过ELISA)小的可扩散的蛋白质、可在胞外溶液中检测到的小的活性酶(例如, α -淀粉酶、 β -内酰胺酶、草胺膦转移酶)或插入或陷入细胞壁中的蛋白质(例如包括在例如延伸表达单元中发现的前导序列的蛋白质或者又称为烟草PR-S的与烟草致病相关的蛋白质)。其它可能的可选择性标记基因对于本领域技术人员来说是显而易见的并且涵盖在本发明中。

[0081] 细胞转化

[0082] 本发明还涉及生产包含可操作地连接可转录的多核苷酸分子的启动子的转化的细胞和植物的方法。

[0083] 术语“转化”是指将核苷酸引入受体宿主。如本文所使用,术语“宿主”是指细菌、真菌或植物,包括细菌、真菌或植物的任何细胞、组织、器官或后代。特定的目标植物组织和细胞包括原生质体、愈伤组织、根、块茎、种子、茎、叶、幼苗、胚芽和花粉。

[0084] 如本文所使用,术语“转化的”是指已引入外源多核苷酸分子如构建体的细胞、组织、器官或生物体。引入的多核苷酸分子可被整合至受体细胞、组织、器官或生物体的基因组DNA,因此引入的多核苷酸分子由后续的后代继承。“转基因的”或“转化的”细胞或生物体还包括细胞或生物体的后代,以及由使用这种转基因生物体作为母代杂交并显示由外源多核苷酸分子的存在所引起的改变的表型的育种程序生成的后代。术语“转基因”是指含有一个或多个异源多核苷酸分子的细菌、真菌或植物。

[0085] 存在许多将多核苷酸分子引入植物细胞的方法。所述方法通常包括选择合适的宿主细胞、使用重组载体转化宿主细胞以及获得转化的宿主细胞的步骤。合适的方法包括细菌感染(例如土壤杆菌属)、二元细菌人工染色体载体、DNA的直接递送(例如,通过PEG-介导的转化)、干燥/抑制-介导的DNA摄取、电穿孔、用碳化硅纤维搅拌以及DNA涂层颗粒的加速等(在Potrykus等人,Ann.Rev.Plant Physiol.Plant Mol.Biol.,42:205(1991)中综述)。

[0086] 任何转化方法均可以用于用本发明的一个或多个启动子和/或构建体转化宿主细胞。宿主细胞可以是诸如植物细胞、藻类细胞、藻类、真菌细胞、真菌、细菌细胞或昆虫细胞的任何细胞或生物体。优选的宿主和转化的细胞包括来自于植物、曲霉属(*Aspergillus*)、酵母、昆虫、细菌和藻类的细胞。

[0087] 再生的转基因植物可以自花授粉以提供纯合的转基因植物。可选地,从再生的转基因植物获得的花粉可与非转基因植物,优选农学重要物种的自交系杂交。通常用于不同性状和作物的育种方法的描述可见于几本参考书之一,参见,例如,Allard,Principles of Plant Breeding,John Wiley&Sons,NY,U.of CA,Davis,CA,50-98(1960);Simmonds,Principles of crop improvement,Longman,Inc.,NY,369-399(1979);Sneep和Hendriksen,Plant breeding perspectives,Wageningen(ed),Center for Agricultural Publishing and Documentation(1979);Fehr,Soybeans:Improvement,Production and Uses,2nd Edition,Monograph,16:249(1987);Fehr,Principles of variety development,Theory and Technique,(Vol.1)and Crop Species Soybean(Vol 2),Iowa State Univ.,Macmillan Pub.Co.,NY,360-376(1987)。相反地,来自非转基因植物的花粉可用于为再生的转基因植物授粉。

[0088] 可以分析转化的植物的目标基因的存在以及本发明调控元件所赋予的表达水平和/或分布。本领域技术人员了解许多可用于分析转化植物的方法。例如,植物分析方法包括,但不限于Southern印迹法或northern印迹法、基于PCR的方法、生化分析、表型筛选法、现场评估以及免疫诊断试验。可转录的多核苷酸分子的表达可用TaqMan® (Applied Biosystems,Foster City,CA)试剂和制造商描述的方法和使用TaqMan® Testing Matrix测定的PCR周期时间来测量。或者,Invader® (Third WaveTechnologies,

Madison, WI) 试剂和制造商描述的方法可用于转基因表达。

[0089] 本发明的植物的种子可从能繁殖的转基因植物中收获并且用于生长本发明的转化的植物后代,包括包含本发明构建体并表达农学目标基因的植物杂交系。

[0090] 本发明还提供本发明所述植物的部分。植物部分包括但不限于叶、茎、根、块茎、种子、胚乳、胚珠和花粉。本发明还包括和提供包含本发明的核酸分子的转化的植物细胞。

[0091] 转基因植物可将转基因多核苷酸分子传递至其后代。后代包括任何可再生的包含衍生自祖代植物的转基因的植物部分或种子。对于转化的多核苷酸分子,转基因植物优选是纯合的,并且作为有性繁殖的结果将所述序列传递至所有后代。后代可从转基因植物生成的种子生长。然后,这些另外的植物可以自花授粉,以生成植物的真正育种系。在其他事项中,评价来自这些植物的后代的基因表达。基因表达可通过若干常规方法如western印迹法、northern印迹法、免疫沉淀和ELISA来检测。

[0092] 现已大体上描述了本发明,通过参考以下通过举例说明的方式提供的实施例将会更加容易地理解本发明,除非规定,其并不意图限制本发明。本领域技术人员应理解,以下实施例中公开的技术代表本发明人发现的很好地实施本发明的技术。然而,本领域技术人员应理解,根据本发明公开的内容,可以对公开的具体实施方式进行许多改变,并仍然会得到类似或相似的结果,而不会偏离本发明的精神和范围,因此列于或显示于所附附图中的所有内容应被理解为示例性的,而不是限制性的。

具体实施方式

[0093] 实施例1:调控元件的鉴别和克隆

[0094] 从双子叶植物物种甜瓜WSH-39-1070AN的基因组DNA中鉴别并分离新型转录的调控元件或转录的调控表达元件组 (EXP) 序列。

[0095] 基于专有的和公共的微阵列数据选择转录的调控元件,所述微阵列数据来源于在大豆 (*Glycine max*) 和拟南芥中进行的转录谱实验以及使用已知的双子叶植物序列对专有的甜瓜序列查询的基于同源性的搜索。

[0096] 使用所鉴别的序列,进行生物信息学分析以鉴别扩增DNA中的调控元件,接着鉴别转录的起始位点 (TSS) 以及序列中存在的任何双向性、内含子或上游编码序列。使用该分析结果,在所述DNA序列内确定调控元件,并设计引物以扩增所述调控元件。使用标准的聚合酶链反应条件、使用含有独特的限制性酶位点的引物和从甜瓜分离的基因组DNA来扩增每个调控元件的相应DNA分子。使用相容限制性位点的标准限制酶消化和DNA连接方法将所得到的DNA片段连接至基础植物表达载体中。

[0097] 可使用转化的植物原生质体进行所述调控元件TSS和内含子/外显子剪接点的分析。简而言之,用包含可操作地连接异源可转录的多核苷酸分子的克隆DNA片段的所述植物表达载体转化所述原生质体,并且通过分析由此生成的所述mRNA转录物的序列,使用用于cDNA末端快速扩增的5' RACE系统,2.0版 (Invitrogen, Carlsbad, California 92008) 确认所述调控元件TSS和内含子/外显子剪接点。

[0098] 如上所述分析编码泛素1转录的调控表达元件组 (EXP) 的序列,并将每个转录的调控表达元件组 ("EXP") 分解为包含每个转录的调控表达元件组的相应启动子、前导序列和内含子。在本文中以SEQ ID No:1、5、7、9和11提供所鉴别的泛素1转录的调控表达元件组 ("

EXP”)的序列,并列于下表1中。在本文中SEQ ID No:2、6、8、10和12提供相应的泛素1启动子。在本文中分别以SEQID No:3和4提供所述泛素1前导序列和内含子。

[0099] 以SEQ ID No:13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49、50、51、52、53、54、55、56、57、58、59、60、61、62、63、64、65、66、67、68、69、70、71、72、73、74、75、76、77、78、79、80、81、82、83、84、85、86、87、88、89、90、91、92、93、94、95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151、152、153、154、155、156、159、162、167、168、172、175、176、177、178、181、182、183、184、185、188、189、190、191、192、193、194、195、196、197、198、199、211和212提供编码其它甜瓜属转录的调控表达元件组或EXP序列的序列,并且所述序列也列于下表1中,其中所述EXP序列由可操作地连接前导序列元件的启动子元件;或可操作地连接前导序列元件和内含子元件的启动子元件;或可操作地连接前导序列元件、可操作地连接内含子元件、可操作地连接前导序列元件的启动子元件组成。以SEQ ID No:163和169提供额外的启动子元件。以SEQ ID No:164、166和170提供额外的前导序列元件。以SEQ ID No:165和171提供额外的内含子元件。以SEQ ID No:157、160、173、179和186提供其中启动子可操作地连接前导序列元件的元件。以SEQ ID No:158、161、174、180和187提供其中内含子可操作地连接前导序列元件的元件。相对于以SEQ ID No:13-199、211和212提供的序列的子集,基于实验的结果如由来自不同物种的同系基因提出期望表达模式如组成型表达、根表达、地上表达(above ground expression)或种子表达的启动子驱动的转录物分布或表达来选择并克隆这些序列。所述甜瓜属序列赋予的实际活性凭经验来确定,并且当用于转化的植物宿主细胞和整个转基因植物时,其不必然与衍生自来自不同于甜瓜的物种的同系基因的调控元件的活性相同。

[0100]

表 1.从甜瓜分离的转录的调控表达元件组、启动子、前导序列和内含子

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
EXP-CUCme.Ubq1:1:1	1	泛素 1	EXP	2611	启动子; 前导序列; 内含子	1-2068;2069-2150;2151-2608
P-CUCme.Ubq1-1:1:15	2	泛素 1	P	2068	启动子	
L-CUCme.Ubq1-1:1:1	3	泛素 1	L	82	前导序列	
I-CUCme.Ubq1-1:1:1	4	泛素 1	I	461	内含子	
EXP-CUCme.Ubq1:1:2	5	泛素 1	EXP	2002	启动子; 前导序列; 内含子	1-1459;1460-1541;1542-1999
P-CUCme.Ubq1-1:1:16	6	泛素 1	P	1459	启动子	
EXP-CUCme.Ubq1:1:3	7	泛素 1	EXP	1507	启动子; 前导序列; 内含子	1-964;965-1046;1047-1504
P-CUCme.Ubq1-1:1:17	8	泛素 1	P	964	启动子	
EXP-CUCme.Ubq1:1:4	9	泛素 1	EXP	1022	启动子; 前导序列; 内含子	1-479;480-561;562-1019
P-CUCme.Ubq1-1:1:18	10	泛素 1	P	479	启动子	
EXP-CUCme.Ubq1:1:5	11	泛素 1	EXP	716	启动子; 前导序列; 内含子	1-173;174-255;256-713
P-CUCme.Ubq1-1:1:19	12	泛素 1	P	173	启动子	
P-CUCme.1-1:1:1	13	磷酸酶 2A	EXP	2000	启动子; 前导序列; 内含子; 前导序列	反向互补; 参见 SEQ ID NO: 155
P-CUCme.2-1:1:1	14	肌动蛋白 1	EXP	2000	启动子; 前导序列; 内含子; 前导序列	1-964;965-1028;1029-1991;1992-2003
P-CUCme.3-1:1:3	15	肌动蛋白 2	EXP	1990	启动子; 前导序列; 内含子; 前导序列	1-1243;1244-1319;1320-1982;1983-1990

[0101]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
P-CUCMe.4-1:1:2	16	泛素 2	EXP	2005	启动子; 前导序列; 内含子; 前导序列	1-1646;1647-1704;1705-2005;2006-2008
P-CUCMe.5-1:1:2	17	泛素 3	EXP	2004	启动子; 前导序列; 内含子	1-748;749-819;820-2004
P-CUCMe.6-1:1:1	18	微管蛋白 β 链	EXP	1935	启动子; 前导序列; 内含子; 前导序列	1-1436;1437-1482;1483-1919;1920-1935
P-CUCMe.8-1:1:2	19	微管蛋白 β 链	EXP	1606	启动子; 前导序列	1-1527;1528-1606
P-CUCMe.9-1:1:2	20	微管蛋白 β 链	EXP	1487	启动子; 前导序列	1-1384;1385-1487
P-CUCMe.10-1:1:1	21	微管蛋白 β 链	EXP	1448	启动子; 前导序列	1-1363;1364-1448
P-CUCMe.11-1:1:2	22	延长因子 1α	EXP	1235	启动子; 前导序列; 内含子	1-617;618-677;678-1213;1214-1235
P-CUCMe.15-1:1:2	23	延长因子 1α	EXP	2003	启动子; 前导序列; 内含子; 前导序列	1-1330;1331-1435;1430-1975;1976-2002
P-CUCMe.16a-1:1:2	24	泛素 7	EXP	2015	启动子; 前导序列	
P-CUCMe.16b-1:1:1	25	泛素 6	EXP	2006	启动子; 前导序列	
P-CUCMe.17-1:1:2	26	泛素-40S 核糖体蛋白 S27a	EXP	2017	启动子; 前导序列	1-1969;1970-2017
P-CUCMe.18-1:1:2	27	泛素-40S 核糖体蛋白 S27a	EXP	1353	启动子; 前导序列	1-1308;1309-1353
P-CUCMe.19-1:1:2	28	叶绿素 a/b 结合蛋白	EXP	2005	启动子; 前导序列	1-1960;1961-2005
P-CUCMe.20-1:1:2	29	叶绿素 a/b 结合蛋白	EXP	1445	启动子; 前导序列	1-1390;1391-1445
P-CUCMe.21-1:1:1	30	叶绿素 a/b 结合蛋白	EXP	1282	启动子; 前导序列	1-1233;1234-1282
P-CUCMe.22-1:1:3	31	延长因子 4α	EXP	2002		

[0102]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
P-CUCme.24-1:1:2	32	S-腺苷甲硫氨酸合成酶	EXP	2003	启动子; 前导序列; 内含子; 前导序列	1-1067;1068-1165;1166-2001;2002-2003
P-CUCme.26-1:1:2	33	应激反应蛋白	EXP	1372	启动子; 前导序列; 内含子; 前导序列	1-577;578-654;655-1366;1367-1372
P-CUCme.28-1:1:2	34	核糖体蛋白 S5a	EXP	1122		
P-CUCme.29-1:1:2	35	核糖体蛋白 S5a	EXP	2017	启动子; 前导序列; 内含子; 前导序列	1-490;491-571;572-2012;2013-2017
CumMe_WSM_SF1439 81.G5150	36	LHCB6 (捕光复合物 PSII 亚基 6)	EXP	2000		
CumMe_WSM_SF1448 39.G5080	37	EIF2 γ 翻译起始因子	EXP	1760		
CumMe_WSM_SF1460 40.G5050	38	EIF2 翻译起始因子	EXP	1767		
CumMe_WSM_SF1640 8.G5350	39	延长因子 Tu	EXP	2000		
CumMe_WSM_SF1642 9.G5670	40	未知蛋白	EXP	2000		
CumMe_WSM_SF1644 4.G5140	41	组织蛋白 H4	EXP	2000	启动子; 前导序列	1-1947;1948-2000
CumMe_WSM_SF1653 0.G6000	42	HMGB2(高迁移率组 B2) 转录因子	EXP	2000		
CumMe_WSM_SF1655 3.G5090	43	PBG1; 苏氨酸型肽链内切酶	EXP	1115		
CumMe_WSM_SF1656 3.G5560	44	ATARFB1A (ADP-核糖基化因子 B1A)	EXP	2000	启动子; 前导序列; 内含子; 前导序列	1-1329;1330-1427;1428-1988;1989-2000

[0103]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
CumMe_WSM_SF1667 5.G5720	45	染色质蛋白家族	EXP	2000		
CumMe_WSM_SF1692 0.G5650	46	CSD1(铜/锌超氧化物歧 化酶 1)	EXP	2000		
CumMe_WSM_SF1695 3.G5180	47	SCE1 (SUMO 缀合酶 1); SUMO 连接酶	EXP	2000		
CumMe_WSM_SF1705 1.G5470	48	60S 核糖体蛋白 L9 (RPL90D)	EXP	2000		
CumMe_WSM_SF1711 1.G5790	49	泛醇-细胞色素 C 还原酶 复合物 辅酶结合蛋白	EXP	2000	启动子; 前导序列	1-1895;1896-2000
CumMe_WSM_SF1714 2.G5920	50	肽基脯氨酰基顺反异构 酶, 叶绿体	EXP	2000		
CumMe_WSM_SF1719 0.G6200	51	PRK (磷酸核酮糖激酶)	EXP	2000		
CumMe_WSM_SF1725 0.G5910	52	LHCB5 (光系统 II 5 的捕 光复合物)	EXP	2000		
CumMe_WSM_SF1725 2.G7330	53	新生多肽结合复合物 (NAC) 含结构域蛋白	EXP	2000	启动子; 前导序列; 内 含子	1-1195;1196-1297; 1298-2000
CumMe_WSM_SF1725 3.G5150	54	RPS9(核糖体蛋白 S9)	EXP	1547		
CumMe_WSM_SF1732 2.G5110	55	60S 核糖体蛋白 L22 (RPL22A)	EXP	2000		
CumMe_WSM_SF1734 9.G5770	56	PGRL1B (PGR5-样 B)	EXP	2000		
CumMe_WSM_SF1735	57	40S 核糖体蛋白 S10	EXP	2000		

[0104]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
7.G5630		(RPS10B)				
CumMe_WSM_SF1749 4.G5140	58	MEE34 (母体效应胚胎发育停滞 34)	EXP	1591		
CumMe_WSM_SF1752 4.G6410	59	SUS2 (异常胚胎 2)	EXP	2000		
CumMe_WSM_SF1767 2.G5610	60	PSAK (光系统 I 亚基 K)	EXP	2000		
CumMe_WSM_SF1777 3.G6620	61	顺乌头酸酶 C-末端含结 构域蛋白	EXP	2000		
CumMe_WSM_SF1786 6.G6050	62	ATPDIL5-1 (PDI-样 5-1)	EXP	2000		
CumMe_WSM_SF1800 4.G6600	63	富含羟脯氨酸糖蛋白家 族蛋白	EXP	2000		
CumMe_WSM_SF1804 5.G6670	64		EXP	2000		
CumMe_WSM_SF1805 3.G5410	65	内膜蛋白 70	EXP	2000		
CumMe_WSM_SF1828 7.G5380	66	CP12-1	EXP	2000		
CumMe_WSM_SF1848 8.G5340	67	咖啡酰 CoA 3-O-甲基转 移酶	EXP	2000	启动子; 前导序列	1-1923;1924-2000
CumMe_WSM_SF1850 4.G5090	68	膜泡 ATP 合成酶亚基 H 家族蛋白	EXP	2000		
CumMe_WSM_SF1853 0.G5750	69	GUN5 (基因组解除偶联 5); 镁螯合酶	EXP	2000		
CumMe_WSM_SF1853 6.G6480	70	MBF1A (多蛋白桥梁因子 1A) 转录共激活因子	EXP	2000		

[0105]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
CumMe_WSM_SF1857 5.G6410	71	未知蛋白	EXP	2000		
CumMe_WSM_SF1863 4.G5190	72	60S 核糖体蛋白 L23 (RPL23A)	EXP	2000	启动子; 前导序列	1-1971;1972-2000
CumMe_WSM_SF1864 5.G5380	73	GS2 (谷氨酰氨合成酶 2)	EXP	2000		
CumMe_WSM_SF1871 6.G5860	74	40S 核糖体蛋白 S12 (RPS12A); 反向互补: 生长素诱导蛋白 x10A-样	EXP	2000	启动子; 前导序列	反向互补; 参见 SEQ ID NO: 184
CumMe_WSM_SF1880 1.G5040	75		EXP	2000		
CumMe_WSM_SF1880 6.G6220	76	未知蛋白	EXP	2000		
CumMe_WSM_SF1885 0.G5630	77	PAC1; 苏氨酸型肽链内切酶	EXP	2000		
CumMe_WSM_SF1886 3.G7550	78	ATP 合酶 γ 链, 线粒体 (ATPC)	EXP	2000		
CumMe_WSM_SF1898 6.G6110	79	GER1 (类朊蛋白 1); 草酸氧化酶	EXP	2000		
CumMe_WSM_SF1906 4.G5690	80	组织蛋白 H3.2	EXP	2000	启动子; 前导序列; 内含子	1-1581;1582-1670; 1671-2000
CumMe_WSM_SF1932 3.G5120	81	叶绿体外膜 GTP-结合蛋白, 假定的	EXP	2000		
CumMe_WSM_SF1945 2.G5090	82	葡聚糖磷酸化酶, 假定的	EXP	1072		
CumMe_WSM_SF1963 1.G5170	83	RuBisCO 阿替普酶, 假定	EXP	1730		

[0106]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
		的				
CumMe_WSM_SF1964 7.G5760	84	6-磷酸葡萄糖酸脱氢酶家族蛋白	EXP	2000	启动子; 前导序列; 内含子; 前导序列	1-936;937-1021;1022-1992;1993-2000
CumMe_WSM_SF1983 9.G5090	85	ATPDX1.1(吡哆醇生物合成 1.1)	EXP	1020	启动子; 前导序列	1-928;929-1020
CumMe_WSM_SF1985 0.G5130	86	HMGB2 (高迁移率组 B 2) 转录因子	EXP	2000		
CumMe_WSM_SF1990 2.G5260	87	普遍胁迫蛋白(USP) 家族蛋白 / 早期结瘤素 ENOD18 家族蛋白	EXP	2000		
CumMe_WSM_SF1999 2.G6100	88	未知蛋白	EXP	2000		
CumMe_WSM_SF2013 2.G5560	89	过氧化物酶 21	EXP	2000	启动子; 前导序列	1-1962;1963-2000
CumMe_WSM_SF2014 7.G7910	90	CSD1 (铜/锌超氧化物歧化酶 1)	EXP	2000		
CumMe_WSM_SF2035 5.G5130	91	ATP 合酶家族	EXP	2000		
CumMe_WSM_SF2035 9.G5870	92	NADH-泛醌氧化还原酶 20 kDa 亚基、线粒体	EXP	2000		
CumMe_WSM_SF2036 8.G5700	93	PGR5 (质子梯度调控蛋白 5)	EXP	2000		
CumMe_WSM_SF2040 9.G5240	94	延长因子 1B α -亚基 1 (eEF1B α 1)	EXP	2000		
CumMe_WSM_SF2043 1.G6340	95	DHS2 (3-脱氧-d-阿拉伯庚酮糖酸 7 磷酸合成酶)	EXP	2000		

[0107]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
CumMe_WSM_SF2050 5.G5440	96	THIC (硫酸素 C); ADP-核糖焦磷酸水解酶	EXP	1373		
CumMe_WSM_SF2050 9.G5920	97	Y14; RNA 结合/蛋白结合	EXP	2000		
CumMe_WSM_SF2064 58.G5970	98	FAD2 (脂肪酸去饱和酶 2)	EXP	2000	启动子	1-2000
CumMe_WSM_SF2065 34.G5200	99	未知蛋白	EXP	2000		
CumMe_WSM_SF2099 7.G6990	100	ALD1 (AGD2-样防御反应蛋白 1)	EXP	2000		
CumMe_WSM_SF2103 5.G5090	101	钠/钙交换家族蛋白	EXP	1078		
CumMe_WSM_SF2111 7.G5370	102	30S 核糖体蛋白, 假定的	EXP	2000		
CumMe_WSM_SF2114 1.G5630	103	40S 核糖体蛋白 S24 (RPS24A)	EXP	2000		
CumMe_WSM_SF2119 8.G5180	104		EXP	1974		
CumMe_WSM_SF2136 6.G5980	105	GRF12 (通用调控因子 12)	EXP	2000		
CumMe_WSM_SF2182 8.G5150	106	cpHsc70-1 (叶绿体热休克蛋白 70-1)	EXP	1643		
CumMe_WSM_SF2188 6.G5080	107	NPQ4 (非光化学猝灭)	EXP	2000		
CumMe_WSM_SF2200 8.G5670	108	NAP1; 2(核小体组蛋白 1; 2)	EXP	2000		
CumMe_WSM_SF2207	109	果糖-二磷酸醛缩酶, 假	EXP	2000		

[0108]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
0.G5280		定的				
CumMe_WSM_SF2209 7.G5540	110	APX3 (抗坏血酸过氧化物酶 3)	EXP	2000		
CumMe_WSM_SF2225 4.G5760	111	40S 核糖体蛋白 S7 (RPS7B)	EXP	2000		
CumMe_WSM_SF2227 5.G5780	112	核糖体蛋白 L17 家族蛋白	EXP	1027		
CumMe_WSM_SF2235 5.G5310	113		EXP	2000		
CumMe_WSM_SF2253 1.G5120	114	真核翻译起始因子 1A, 假定的	EXP	2000	启动子; 前导序列; 内含子; 前导序列	1-759;760-858;859-1979;1980-2000
CumMe_WSM_SF2287 0.G5370	115	ATSARA1A (拟南芥分泌相关的 RAS 超家族 1)	EXP	2000		
CumMe_WSM_SF2293 4.G5290	116	T-复合蛋白 1 ε 亚基, 假定的	EXP	2000		
CumMe_WSM_SF2318 1.G5100	117	CEV1 (VSP 1 的组成型表达)	EXP	1025		
CumMe_WSM_SF2318 6.G6160	118	泛醇-细胞色素 C 还原酶复合物 14 kDa 蛋白, 假定的	EXP	2000		
CumMe_WSM_SF2339 7.G5210	119	RPL27 (核糖体蛋白大亚基 27)	EXP	2000		
CumMe_WSM_SF2376 0.G5200	120	NDPK1; ATP 结合/核苷二磷酸激酶	EXP	2000	启动子; 前导序列	1-1901;1902-2000
CumMe_WSM_SF2390	121	PSBX(光系统 II 亚基 X)	EXP	2000		

[0109]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
6.G6180						
CumMe_WSM_SF2404 0.G5450	122	RPS17 (核糖体蛋白 S17)	EXP	2000		
CumMe_WSM_SF2404 5.G5400	123	EXL3 (EXORDIUM 样 3)	EXP	2000		
CumMe_WSM_SF2411 7.G5600	124	60S 核糖体蛋白 L26 (RPL26A)	EXP	2000		
CumMe_WSM_SF2508 4.G5580	125		EXP	2000		
CumMe_WSM_SF2514 1.G5160	126	异柠檬酸脱氢酶, 假定的	EXP	1397	启动子; 前导序列	1-1322;1323-1397
CumMe_WSM_SF2535 5.G5000	127	LOS1; 铜离子结合翻译延长因子	EXP	2000	启动子; 前导序列; 内含子; 前导序列; CDS	1-734;735-811;812-1340;1341-1360;1361-2000
CumMe_WSM_SF2537 0.G5000	128	PSBP-1 (光系统 II 亚基 P-1)	EXP	1657		
CumMe_WSM_SF2545 5.G5370	129	GLY3 (乙二醛酶 II 3)	EXP	2000		
CumMe_WSM_SF2593 6.G5450	130	线粒体基质载体家族蛋白	EXP	2000	启动子; 前导序列	1-1878;1879-2000
CumMe_WSM_SF2708 0.G5510	131	LIP1 (硫辛酸合成酶 1)	EXP	2000		
CumMe_WSM_SF2722 2.G5150	132	DRT112; 铜离子结合/电子载体	EXP	2000		
CumMe_WSM_SF2795 7.G5450	133	SMAP1 (小酸性蛋白 1)	EXP	2000		
CumMe_WSM_SF2872	134	RNA-结合蛋白 cp29, 假	EXP	1696		

[0110]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
9.G5340		定的				
CumMe_WSM_SF2880 5.G6200	135	未知蛋白	EXP	2000		
CumMe_WSM_SF3126 4.G5380	136	ATPH1 (拟南芥普列克底物蛋白同源物 1)	EXP	2000		
CumMe_WSM_SF3585 6.G5150	137	TIP4;1(液泡膜内在蛋白 4;1)	EXP	1575		
CumMe_WSM_SF4085 9.G5250	138	SMT2 (固醇甲基转移酶 2)	EXP	2000		
CumMe_WSM_SF4112 4.G5080	139	40S 核糖体蛋白 S2 (RPS2C)	EXP	1006	启动子; 前导序列	1-883;884-1006
CumMe_WSM_SF4112 8.G5410	140	CRY2 (隐花色素 2)	EXP	2000		
CumMe_WSM_SF4125 4.G5160	141	GDP-D-葡萄糖磷酸化酶	EXP	1556		
CumMe_WSM_SF4158 8.G5470	142	PRPL11 (质体核糖体蛋白 L11)	EXP	2000		
CumMe_WSM_SF4164 4.G6400	143	SHD (SHEPHERD)	EXP	2000		
CumMe_WSM_SF4198 3.G5000	144	催化/辅酶结合	EXP	1337		
CumMe_WSM_SF4207 5.G5100	145	CPN60B (伴侣蛋白 60 β)	EXP	2000		
CumMe_WSM_SF4214 1.G5110	146	组织蛋白酶B样半胱氨酸蛋白酶, 假定的	EXP	1212		
CumMe_WSM_SF4493 3.G5290	147	EBF1 (EIN3-结合 F 盒蛋白 1) 泛素蛋白连接酶	EXP	2000		

[0111]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
CumMe_WSM_SF4497 7.G5000	148	PAP26 (紫色酸性磷酸酯酶 26)	EXP	1254		
CumMe_WSM_SF4544 1.G5510	149	GAPA-2 (甘油醛-3-磷酸脱氢酶 A 亚基 2)	EXP	2000		
CumMe_WSM_SF4588 2.G5120	150	果糖-1,6-二磷酸酶, 假定的	EXP	1680		
CumMe_WSM_SF4780 6.G5070	151	ATP 合酶ε链, 线粒体	EXP	1524		
CumMe_WSM_SF5310 6.G5190	152	CPN60A (伴侣蛋白-60α)	EXP	1851		
CumMe_WSM_SF6558 8.G5230	153	液泡钙结合蛋白相关的	EXP	2000		
CumMe_WSM_SF9060 .G5120	154	APE2 (光合作用对环境 的适应 2)	EXP	1288		
P-CUCme.1-1:1:1rc	155	磷酸酶 2A	EXP	2000	启动子; 前导序列; 内含子; 前导序列	1-1135;1136-1249; 1250-1990;1991-2000
EXP-CUCme.4:1:1	156	泛素 2	EXP	2011	启动子; 前导序列; 内含子; 前导序列	1-1646;1647-1704; 1705-2005;2006-2008
P-CUCme.4-1:1:4	157	泛素 2	P;L	1698	启动子; 前导序列	
I-CUCme.4-1:1:1	158	泛素 2	I;L	313	内含子; 前导序列	
EXP-CUCme.5:1:1	159	泛素 3	EXP	2010	启动子; 前导序列; 内含子; 前导序列	1-748;749-819;820-2004;2005-2007
P-CUCme.5-1:1:3	160	泛素 3	P;L	1107	启动子; 前导序列	
I-CUCme.5-1:1:1	161	泛素 3	I;L	903	内含子; 前导序列	

[0112]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
EXP-CUCme.cEF1a:1:1	162	延长因子 1 α	EXP	1235	启动子; 前导序列; 内含子; 前导序列	1-617;618-677;678-1213;1214-1235
P-CUCme.cEF1a-1:1:1	163	延长因子 1 α	P	617	启动子	
L-CUCme.cEF1a-1:1:1	164	延长因子 1 α	L	54	前导序列	
I-CUCme.cEF1a-1:1:1	165	延长因子 1 α	I	545	内含子	
L-CUCme.cEF1a-1:1:2	166	延长因子 1 α	L	19	前导序列	
P-CUCme.19-1:1:3	167	叶绿素 a/b 结合蛋白	EXP	2003	启动子; 前导序列	1-1958;1959-2003
EXP-CUCme.SAMS2:1:1	168	S-腺苷甲硫氨酸合成酶	EXP	2004	启动子; 前导序列; 内含子	1-1067;1068-1165;1166-2003
P-CUCme.SAMS2-1:1:1	169	S-腺苷甲硫氨酸合成酶	P	1067	启动子	
L-CUCme.SAMS2-1:1:1	170	S-腺苷甲硫氨酸合成酶	L	92	前导序列	
I-CUCme.SAMS2-1:1:1	171	S-腺苷甲硫氨酸合成酶	I	845	内含子	
EXP-CUCme.29:1:1	172	核糖体蛋白 S5a	EXP	2018	启动子; 前导序列; 内含子; 前导序列	1-490;491-571;572-2012;2013-2018
P-CUCme.29-1:1:4	173	核糖体蛋白 S5a	P;L	565	启动子; 前导序列	
I-CUCme.29-1:1:1	174	核糖体蛋白 S5a	I;L	1453	内含子; 前导序列	
P-CUCme.CumMe_WS M_SF16444.G5140-1:1:1	175	组织蛋白 H4	EXP	1999	启动子; 前导序列; 内含子	1-1946;947-1999
P-CUCme.CumMe_WS M_SF16563.G5560-1:1:1	176	ATARFB1A (ADP-核糖基化因子 B1A)	EXP	2004	启动子; 前导序列; 内含子; 前导序列	1-1331;1332-1429;1430-1992;1993-2004
P-CUCme.CumMe_WS M_SF17111.G5790-1:1:1	177	泛醇-细胞色素 C 还原酶复合物泛醌-结合蛋白	EXP	2005	启动子; 前导序列	1-1901;1902-2005

[0113]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
:1						
EXP-CumMe.WSM_SF17252.G7330:1:1	178	新生多肽结合复合物(NAC)含结构域蛋白	EXP	1978	启动子; 前导序列; 内含子; 前导序列	1-1167;1168-1269;1270-1972;1973-1975
P-CUCme.WSM_SF17252.G7330-1:1:1	179	新生多肽结合复合物(NAC)含结构域蛋白	P;L	1263	启动子; 前导序列	
I-CUCme.WSM_SF17252.G7330-1:1:1	180	新生多肽结合复合物(NAC)含结构域蛋白	I;L	715	内含子; 前导序列	
P-CUCme.CumMe_WSM_SF18488.G5340-1:1:1	181	咖啡酰 CoA 3-O-甲基转移酶	EXP	2000	启动子; 前导序列	1-923;1924-2000
P-CUCme.CumMe_WSM_SF18536.G6480-1:1:1	182	MBF1A (多蛋白桥梁因子1A)转录共激活因子	EXP	2000	启动子; 前导序列; 内含子	
P-CUCme.CumMe_WSM_SF18634.G5190-1:1:1	183	60S 核糖体蛋白 L23 (RPL23A)	EXP	1989	启动子; 前导序列	1-1960;1961-1989
P-CUCme.CumMe_WSM_SF18716.G5860-1:1:1	184	生长素诱导蛋白 X10A-样	EXP	1463	启动子; 前导序列	1-1392;1393-1463
EXP-CUCme.WSM_SF19064.G5690:1:1	185	组织蛋白 H3.2	EXP	2006	启动子; 前导序列; 内含子; 前导序列	1-1581;1582-1670;1671-2000;2001-2003
P-CUCme.WSM_SF19064.G5690-1:1:1	186	组织蛋白 H3.2	P;L	1664	启动子; 前导序列	
I-CUCme.WSM_SF19064.G5690-1:1:1	187	组织蛋白 H3.2	I;L	342	内含子; 前导序列	

[0114]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
P-CUCme.CumMe_WS M_SF19647.G5760-1:1 :1	188	6-磷酸葡萄糖酸脱氢酶 家族蛋白	EXP	2003	启动子; 前导序列; 内 含子; 前导序列	1-939;940-1024;10 25-1995;1996-2003
P-CUCme.CumMe_WS M_SF19839.G5090-1:1 :1	189	ATPDX1.1(吡哆醇生物合 成 1.1)	EXP	1024	启动子; 前导序列	1-904;905-1024
P-CUCme.CumMe_WS M_SF20132.G5560-1:1 :1	190	过氧化物酶 21	EXP	2001	启动子; 前导序列	1-1962;1963-2001
P-CUCme.CumMe_WS M_SF206458.G5970-1: 1:1	191	FAD2(脂肪酸去饱和酶 2)	EXP	4175	启动子; 前导序列; 内 含子; 前导序列	1-2171;2172-2325; 2326-4155;4156-41 75
P-CUCme.CumMe_WS M_SF22531.G5120-1:1 :1	192	真核翻译起始因子 1A, 假定的	EXP	1999	启动子; 前导序列; 内 含子; 前导序列	1-759;760-858;859- 1978;1979-1999
P-CUCme.CumMe_WS M_SF23760.G5200-1:1 :1	193	NDPK1; ATP 结合/核苷 二磷酸激酶	EXP	2000	启动子; 前导序列	1-1901;1902-2000
P-CUCme.CumMe_WS M_SF23906.G6180-1:1 :1	194	PSBX (光系统 II 亚基 X)	EXP	2000	启动子; 前导序列	
P-CUCme.CumMe_WS M_SF25141.G5160-1:1 :2	195	异柠檬酸脱氢酶, 假定的	EXP	1400	启动子; 前导序列	1-1325;1326-1400
P-CUCme.CumMe_WS M_SF25355.G5000-1:1 :1	196	LOS1; 铜离子结合翻译 延长因子	EXP	2019	启动子; 前导序列; 内 含子; 前导序列; CDS	1-734;735-811;812- 1340;1341-1360;13 61-2019

[0115]

注释	SEQ ID NO:	说明	组成类型	大小(bp)	组成	EXP内元件的坐标
P-CUCme.CumMe_WS M_SF25936.G5450-1:1	197	线粒体基质载体家族蛋白	EXP	1999	启动子; 前导序列	1-1877;1878-1999
P-CUCme.CumMe_WS M_SF35856.G5150-1:1	198	TIP4;1(液泡膜内在蛋白4;1)	EXP	1578		
P-CUCme.CumMe_WS M_SF41124.G5080-1:1	199	40S 核糖体蛋白 S2 (RPS2C)	EXP	1023	启动子; 前导序列	1-945;946-1023
P-CUCme.20-1:3	211	叶绿素 a/b 结合蛋白	EXP	1446	启动子; 前导序列	1-1390;1391-1446
EXP-CUCme.29:1:2	212	核糖体蛋白 S5a	EXP	2018	启动子; 前导序列; 内含子; 前导序列	1-490;491-571;572-2011;2013-2018

[0116] 如表1所示,例如,具有从甜瓜分离的组件的EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1) 表示的转录的调控表达元件组 (EXP) 包含可操作地连接5'至前导序列元件L-CUCme.Ubq1-1

:1:1 (SEQ ID NO:3)、可操作地连接5'至内含子元件I-CUCme.Ubq1-1:1:1 (SEQ ID NO:4)的大小 (bp) 为2068个碱基对的启动子元件P-CUCme.Ubq1-1:1:15 (SEQ ID NO:2)。具有从甜瓜分离的组件的EXP-CUCme.Ubq1:1:2 (SEQ ID NO:5) 表示的转录的调控表达元件组 (EXP) 包含可操作地连接5'至前导序列元件L-CUCme.Ubq1-1:1:1 (SEQ ID NO:3)、可操作地连接5'至内含子元件I-CUCme.Ubq1-1:1:1 (SEQ ID NO:4) 的1459bp的启动子元件P-CUCme.Ubq1-1:1:16 (SEQ ID NO:6)。具有从甜瓜分离的组件的EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7) 表示的转录的调控表达元件组 (EXP) 包含可操作地连接5'至前导序列元件L-CUCme.Ubq1-1:1:1 (SEQ ID NO:3)、可操作地连接5'至内含子元件I-CUCme.Ubq1-1:1:1 (SEQ ID NO:4) 的964bp的启动子元件P-CUCme.Ubq1-1:1:17 (SEQ ID NO:8)。具有从甜瓜分离的组件的EXP-CUCme.Ubq1:1:4 (SEQ ID NO:9) 表示的转录的调控表达元件组 (EXP) 包含可操作地连接5'至前导序列元件L-CUCme.Ubq1-1:1:1 (SEQ ID NO:3)、可操作地连接5'至内含子元件I-CUCme.Ubq1-1:1:1 (SEQ ID NO:4) 的479bp的启动子元件P-CUCme.Ubq1-1:1:18 (SEQ ID NO:10)。具有从甜瓜分离的组件的EXP-CUCme.Ubq1:1:5 (SEQ ID NO:11) 表示的转录的调控表达元件组 (EXP) 包含可操作地连接5'至前导序列元件L-CUCme.Ubq1-1:1:1 (SEQ ID NO:3)、可操作地连接5'至内含子元件I-CUCme.Ubq1-1:1:1 (SEQ ID NO:4) 的173bp的启动子元件P-CUCme.Ubq1-1:1:19 (SEQ ID NO:12)。

[0117] 所述泛素1启动子序列的对比提供于图1a-1f中。通过引入来自所述启动子P-CUCme.Ubq1-1:1:15 (SEQ ID NO:2) 的5'端的不同长度的缺失,构造启动子元件P-CUCme.Ubq1-1:1:16 (SEQ ID NO:6)、P-CUCme.Ubq1-1:1:17 (SEQ ID NO:8)、P-CUCme.Ubq1-1:1:18 (SEQ ID NO:10) 和P-CUCme.Ubq1-1:1:19 (SEQ ID NO:12)。

[0118] 实施例2:大豆子叶原生质体中驱动GUS的调控元件的分析

[0119] 用含有驱动所述 β -葡糖醛酸酶 (GUS) 转基因表达的测试转录调控表达元件组的植物表达载体转化大豆子叶原生质体,并与叶原生质体中的GUS表达进行比较,其中GUS的表达由已知的组成型启动子驱动。

[0120] 将EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1)、EXP-CUCme.Ubq1:1:2 (SEQ ID NO:5)、EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7)、EXP-CUCme.Ubq1:1:4 (SEQ ID NO:9) 和EXP-CUCme.Ubq1:1:5 (SEQ ID NO:11) 驱动的转基因表达与来自已知的组成型启动子的表达进行比较。每种植物表达载体由来自根癌土壤杆菌的右边界区域、第一转基因盒、第二转基因选择盒和来自根癌土壤杆菌的左边界区域组成,其中所述第一转基因盒由可操作地连接5'至含衍生自土豆光诱导组织特异性ST-LS1基因 (基因库登记号:X04753) 的可加工内含子的 β -葡糖醛酸酶 (GUS, SEQ ID NO:206) 用编码序列的、可操作地连接5'至来自海岛棉 (*Gossypium barbadense*) E6基因 (T-Gb.E6-3b:1:1, SEQ ID NO:204)、豌豆 (*Pisum sativum*) RbcS2-E9基因 (T-Ps.RbcS2-E9-1:1:6, SEQ ID NO:203) 或海岛棉FbLate-2基因 (T-Gb.FbL2-1:1:1, SEQ ID NO:205) 的3'终止区域的EXP序列或已知的组成型启动子组成;所述第二转基因选择盒用于选择赋予除草剂草甘膦 (由拟南芥肌动蛋白7启动子驱动) 或抗生素、卡那霉素抗性的转化的植物细胞。无启动子的对照植物表达载体 (pMON124912) 作为表达的阴性对照。将上述测试和组成型表达元件组克隆成如下表2所示的植物表达载体。

[0121] 表2.植物表达载体和相应的表达元件组以及3'UTR

[0122]

表达载体	调控元件	SEQ ID NO:	3' UTR
pMON80585	EXP-At.Atntt1:1:2	200	T-Ps.RbcS2-E9-1:1:6
pMON109584	EXP-CaMV.35S-enh + Ph.DnaK:1:3	201	T-Gb.E6-3b:1:1
pMON118756	EXP-At.Act7:1:11	202	T-Gb.E6-3b:1:1

[0123]

表达载体	调控元件	SEQ ID NO:	3' UTR
pMON124912	无启动子		T-Gb.FbL2-1:1:1
pMON138776	EXP-CUCme.Ubq1:1:1	1	T-Gb.FbL2-1:1:1
pMON138777	EXP-CUCme.Ubq1:1:2	5	T-Gb.FbL2-1:1:1
pMON138778	EXP-CUCme.Ubq1:1:3	7	T-Gb.FbL2-1:1:1
pMON138779	EXP-CUCme.Ubq1:1:4	9	T-Gb.FbL2-1:1:1
pMON138780	EXP-CUCme.Ubq1:1:5	11	T-Gb.FbL2-1:1:1

[0124] 此外,构建用于共-转化和数据标准化的两个质粒。一个转化对照质粒由驱动萤火虫 (*Photinus pyralis*) 荧光素酶编码序列 (FLuc, SEQ ID NO:207) 表达的组成型启动子组成,所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因 (T-AGRtu.nos-1:1:13, SEQ ID NO:209) 的3'终止区域。另一个转化对照质粒由驱动海肾 (*Renilla reniformis*) 荧光素酶编码序列 (RLuc, SEQ ID NO:208) 表达的组成型启动子组成,所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因的3'终止区域。

[0125] 使用PEG转化方法,使用植物表达载体pMON80585、pMON109584、pMON118756、pMON124912、pMON138776、pMON138777、pMON138778、pMON138779和pMON138780转化大豆子叶原生质体细胞。用等摩尔量的所述两个转化对照质粒中每一个和测试植物表达载体转化原生质体细胞。测定GUS和荧光素酶活性。通过将等分的如上转化的细胞的裂解制剂放入两个不同的小孔托盘中来进行GUS和荧光素酶的测量。一个托盘用于GUS测量,而另一个托盘用于进行使用双荧光素酶报告基因检测系统 (Promega Corp., Madison, WI; 参见例如, Promega Notes Magazine, No:57, 1996, p.02) 的双荧光素酶检测。每次转化使用3或4个复制物来进行试样测量。GUS和荧光素酶的平均值显示于下表3中。

[0126] 表3.GUS和荧光素酶表达平均值和GUS/荧光素酶比值

[0127]

构建体	调控元件	SEQ ID NO:	平均 GUS	平均 FLuc	平均 RLuc	GUS/ FLuc	GUS/ RLuc
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[0128]

构建体	调控元件	SEQ ID NO:	平 均 GUS	平 均 FLuc	平 均 RLuc	GUS/ FLuc	GUS/ RLuc
pMON80585	EXP-At.Atnnt1:1:2	200	55173	6498	30503	8.49	1.81
pMON10958 4	EXP-CaMV.35S-e nh+Ph.DnaK:1:3	200	24940	5050.75	35495	4.94	0.70
pMON11875 6	EXP-At.Act7:1:11	201	9871	6880	40850	1.43	0.24
pMON12491 2	无启动子		2000	11670	73187	0.17	0.03
pMON13877 6	EXP-CUCme.Ubq 1:1:1	1	26972	6467.25	37200	4.17	0.73
pMON13877 7	EXP-CUCme.Ubq 1:1:2	5	41307	5902.5	24396	7.00	1.69
pMON13877 8	EXP-CUCme.Ubq 1:1:3	7	90140	10710.5	60983	8.42	1.48
pMON13877 9	EXP-CUCme.Ubq 1:1:4	9	35526	5590	28001	6.36	1.27
pMON13878 0	EXP-CUCme.Ubq 1:1:5	11	23298	4483.25	19075	5.20	1.22

[0129] 为了比较大豆子叶原生质体中每个启动子的相对活性,将GUS值表示为GUS与荧光素酶活性的比例,并且根据观察到的组成型表达元件组EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3的表达水平进行标准化。下表4显示根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例。下表5显示根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与海肾荧光素酶 (RLuc) 的比例。

[0130] 表4. 根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例

[0131]

构建体	调控元件	SEQ ID NO:	根据 EXP-At.Act7:1:11 标准化的 GUS/FLuc	根据 EXP-CaMV.35S-enh + Ph.DnaK:1:3 标准化的 GUS/FLuc
pMON80585	EXP-At.Atntt1:1:2	200	5.92	1.72
pMON109584	EXP-CaMV.35S-enh+ Ph.DnaK:1:3	201	3.44	1.00
pMON118756	EXP-At.Act7:1:11	202	1.00	0.29
pMON124912	无启动子		0.12	0.03
pMON138776	EXP-CUCme.Ubq1:1: 1	1	2.91	0.84
pMON138777	EXP-CUCme.Ubq1:1: 2	5	4.88	1.42
pMON138778	EXP-CUCme.Ubq1:1: 3	7	5.87	1.70
pMON138779	EXP-CUCme.Ubq1:1: 4	9	4.43	1.29
pMON138780	EXP-CUCme.Ubq1:1: 5	11	3.62	1.05

[0132] 表5. 根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与海肾荧光素酶(RLuc)的比例

[0133]

构建体	调控元件	SEQ ID NO:	根据 EXP-At.Act7:1:11 标准化的 GUS/RLuc	根据 EXP-CaMV.35S-enh+ Ph.DnaK:1:3 标准化的 GUS/RLuc
pMON80585	EXP-At.Atntt1:1:2	200	7.49	2.57
pMON109584	EXP-CaMV.35S-enh+ Ph.DnaK:1:3	201	2.91	1.00

[0134]

pMON118756	EXP-At.Act7:1:11	202	1.00	0.34
pMON124912	无启动子		0.11	0.04
pMON138776	EXP-CUCme.Ubq1:1:1 1	1	3.00	1.03
pMON138777	EXP-CUCme.Ubq1:1:1 2	5	7.01	2.41
pMON138778	EXP-CUCme.Ubq1:1:1 3	7	6.12	2.10
pMON138779	EXP-CUCme.Ubq1:1:1 4	9	5.25	1.81
pMON138780	EXP-CUCme.Ubq1:1:1 5	11	5.05	1.74

[0135] 从上表4和5中可以看出,表达元件组EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1)、EXP-CUCme.Ubq1:1:2 (SEQ ID NO:5)、EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7)、EXP-CUCme.Ubq1:1:4 (SEQ ID NO:9) 和EXP-CUCme.Ubq1:1:5 (SEQ ID NO:11) 中每一个均显示在大豆子叶原生质体中驱动转基因表达的能力。其在该检测中的表达水平高于EXP-At.Act7:1:11的表达水平,并且比EXP-At.Act7:1:11高2.9至5.8倍 (FLuc) 或3至7倍 (RLuc)。所述表达与观察到的EXP-CaMV.35S-enh+Ph.DnaK:1:3的表达相当,或高于观察到的EXP-CaMV.35S-enh+Ph.DnaK:1:3的表达。所述表达水平比观察到的EXP-CaMV.35S-enh+Ph.DnaK:1:3的表达高0.8至1.7倍 (FLuc) 或1至2.4倍 (RLuc)。

[0136] 实施例3:在轰击的大豆叶和根中驱动GUS的调控元件的分析

[0137] 用含有驱动所述 β -葡糖醛酸酶 (GUS) 转基因表达的测试转录调控表达元件组的植物表达载体转化大豆叶和根,并与根和叶中的GUS表达进行比较,其中GUS的表达由已知的组成型启动子驱动。

[0138] 将在粒子轰击的大豆叶和根中由EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1)、EXP-CUCme.Ubq1:1:2 (SEQ ID NO:5)、EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7)、EXP-CUCme.Ubq1:1:4 (SEQ ID NO:9) 和EXP-CUCme.Ubq1:1:5 (SEQ ID NO:11) 驱动的转基因表达与来自己知的组成型启动子的表达进行比较。用于转化叶和根的植物表达载体与以上实施例2的表2中所示的那些相同。

[0139] 使用粒子轰击转化方法,将植物表达载体pMON80585、pMON109584、pMON118756、pMON124912、pMON138776、pMON138777、pMON138778、pMON138779和pMON138780用于转化大豆叶和根。

[0140] 简而言之,对A3244大豆种子进行表面消毒,并用16小时光照和8小时黑暗的光周期使其在托盘中发芽。大约13天后,在无菌条件下从所述幼苗收获叶和根组织,并用于轰击。将所述组织试样随机放置在含植物培养基的陪替氏培养皿上。使用10微克的质粒DNA包被用于轰击的0.6微米金粒子 (目录#165-2262Bio-Rad,Hercules,CA)。用所述DNA包被的金粒子 (目录#165-2335Bio-Rad,Hercules CA) 装载大载体。使用PDS 1000/He基因枪进行转

化(目录#165-2257Bio-Rad,Hercules CA)。使所轰击的根和叶组织在26摄氏度下在黑暗中培育24小时。继该过夜培育之后,在用于GUS表达的溶液中,在37摄氏度下过夜染色所述组织。过夜染色后,在70%的乙醇中过夜浸泡所述组织,以除去叶绿素并显示GUS染色。然后,对所述组织拍照,并将反映GUS表达水平的“0”、“+”至“+++++”的等级量表分配给每个构建体(0为不表达,+至+++++分别为低至高)。

[0141] 在每个组织中显示的GUS转基因的表达用来推断每个元件在稳定转化的玉米植物中驱动转基因表达的能力的相对潜在水平和特异性。GUS表达平均等级提供于下表6中。

[0142] 表6. 粒子轰击的叶和根的GUS表达等级

	构建体	调控元件	SEQ ID NO:	叶表达等级	根表达等级
[0143]	pMON80585	EXP-At.Atn1:1:2	200	++++	++
	pMON109584	EXP-CaMV.35S-enh+ Ph.DnaK:1:3	201	+++++	+++
[0144]	pMON118756	EXP-At.Act7:1:11	202	++++	++
	pMON124912	无启动子		0	0
	pMON138776	EXP-CUCme.Ubq1:1:1	1	++++	+++
	pMON138777	EXP-CUCme.Ubq1:1:2	5	+++	++
	pMON138778	EXP-CUCme.Ubq1:1:3	7	+++	++
	pMON138779	EXP-CUCme.Ubq1:1:4	9	+++	++
	pMON138780	EXP-CUCme.Ubq1:1:5	11	++	+

[0145] 从上表6中可以看出,表达元件组EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1)、EXP-CUCme.Ubq1:1:2 (SEQ ID NO:5)、EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7)、EXP-CUCme.Ubq1:1:4 (SEQ ID NO:9)和EXP-CUCme.Ubq1:1:5 (SEQ ID NO:11)中每一个均显示在粒子轰击的转化的叶和根组织中驱动转基因表达的能力。

[0146] 实施例4:大豆子叶原生质体中驱动GUS的调控元件的分析

[0147] 用含有驱动所述 β -葡萄糖醛酸酶(GUS)转基因表达的测试转录调控表达元件组的植物表达载体转化大豆子叶原生质体,并与叶原生质体中的GUS表达进行比较,其中GUS的表达由已知的组成型启动子驱动。

[0148] 将由P-CUCme.1-1:1:1rc (SEQ ID NO:155)、P-CUCme.2-1:1:1 (SEQ ID NO:156)、P-CUCme.3-1:1:1 (SEQ ID NO:157)、EXP-CUCme.4-1:1:1 (SEQ ID NO:158)、EXP-CUCme.5-1:1:1 (SEQ ID NO:159)、P-CUCme.6-1:1:1 (SEQ ID NO:160)、P-CUCme.8-1:1:2 (SEQ ID NO:161)、P-CUCme.9-1:1:2 (SEQ ID NO:162)、P-CUCme.10-1:1:1 (SEQ ID NO:163)、EXP-CUCme.eEF:a:1:1 (SEQ ID NO:164)、P-CUCme.15-1:1:2 (SEQ ID NO:165)、P-CUCme.16a-1:1:2 (SEQ ID NO:166)、P-CUCme.17-1:1:2 (SEQ ID NO:167)、P-CUCme.18-1:1:2 (SEQ ID NO:168)、P-CUCme.19-1:1:3 (SEQ ID NO:169)、P-CUCme.20-1:3 (SEQ ID NO:210)、P-CUCme.21-1:1:1 (SEQ ID NO:30)、P-CUCme.22-1:1:3 (SEQ ID NO:31)、EXP-CUCme.SAMS2:1:1 (SEQ ID NO:

168)、P-CUCme.26-1:1:2 (SEQ ID NO:33)、P-CUCme.28-1:1:2 (SEQ ID NO:34) 和EXP-CUCme.29:1:2 (SEQ ID NO:212) 驱动的转基因的表达与来自己知的组成型表达元件组的表达进行比较。每种植物表达载体由来自根癌土壤杆菌的右边界区域、第一转基因盒、第二转基因选择盒和来自根癌土壤杆菌的左边界区域组成,其中所述第一转基因盒由可操作地连接5'至含有衍生自土豆光诱导组织特异性ST-LS1基因(基因库登记号:X04753)的可加工内含子的 β -葡萄糖醛酸酶(GUS,SEQ ID NO:206)用编码序列的、可操作地连接5'至来自海岛棉E6基因(T-Gb.E6-3b:1:1,SEQ ID NO:204)、豌豆RbcS2-E9基因(T-Ps.RbcS2-E9-1:1:6,SEQ ID NO:203)或海岛棉FbLate-2基因(T-Gb.FbL2-1:1:1,SEQ ID NO:205)的3'终止区域的测试启动子或已知的组成型启动子组成;所述第二转基因选择盒用于选择赋予除草剂草甘膦(由拟南芥肌动蛋白7启动子驱动)或抗生素、卡那霉素抗性的转化的植物细胞。无启动子的对照植物表达载体(pMON124912)作为表达的阴性对照。将上述测试和组成型表达元件组克隆成如下表7所示的植物表达载体。

[0149] 表7. 植物表达载体和相应的表达元件组以及3'UTR

[0150]

构建体	调控元件	SEQ ID NO:	3' UTR
pMON80585	EXP-At.Atnnt1:1:2	200	T-Ps.RbcS2-E9-1:1:6
pMON109584	EXP-CaMV.35S-enh+ Ph.DnaK:1:3	201	T-Gb.E6-3b:1:1
pMON118756	EXP-At.Act7:1:11	202	T-Gb.E6-3b:1:1
pMON124912	无启动子		T-Gb.FbL2-1:1:1
pMON140818	P-CUCme.1-1:1:1rc	155	T-Gb.FbL2-1:1:1
pMON140819	P-CUCme.2-1:1:1	14	T-Gb.FbL2-1:1:1
pMON140820	P-CUCme.3-1:1:3	15	T-Gb.FbL2-1:1:1
pMON140821	EXP-CUCme.4:1:1	156	T-Gb.FbL2-1:1:1
pMON140822	EXP-CUCme.5:1:1	159	T-Gb.FbL2-1:1:1
pMON140823	P-CUCme.6-1:1:1	18	T-Gb.FbL2-1:1:1
pMON140824	P-CUCme.8-1:1:2	19	T-Gb.FbL2-1:1:1
pMON140825	P-CUCme.9-1:1:2	20	T-Gb.FbL2-1:1:1
pMON140826	P-CUCme.10-1:1:1	21	T-Gb.FbL2-1:1:1
pMON140827	EXP-CUCme.eEF1a:1:1	162	T-Gb.FbL2-1:1:1
pMON140828	P-CUCme.15-1:1:2	23	T-Gb.FbL2-1:1:1
pMON140829	P-CUCme.16a-1:1:2	24	T-Gb.FbL2-1:1:1
pMON140830	P-CUCme.17-1:1:2	26	T-Gb.FbL2-1:1:1

[0151]

构建体	调控元件	SEQ ID NO:	3' UTR
pMON140831	P-CUCme.18-1:1:2	27	T-Gb.FbL2-1:1:1
pMON140832	P-CUCme.19-1:1:3	167	T-Gb.FbL2-1:1:1
pMON140833	P-CUCme.20-1:3	211	T-Gb.FbL2-1:1:1
pMON140834	P-CUCme.21-1:1:1	30	T-Gb.FbL2-1:1:1
pMON140835	P-CUCme.22-1:1:3	31	T-Gb.FbL2-1:1:1
pMON140836	EXP-CUCme.SAMS2:1:1	168	T-Gb.FbL2-1:1:1
pMON140837	P-CUCme.26-1:1:2	33	T-Gb.FbL2-1:1:1
pMON140838	P-CUCme.28-1:1:2	34	T-Gb.FbL2-1:1:1
pMON140839	EXP-CUCme.29:1:2	212	T-Gb.FbL2-1:1:1

[0152] 此外,构建用于共-转化和数据标准化的两个质粒。一个转化对照质粒由驱动萤火虫(*Photinus pyralis*)荧光素酶编码序列(FLuc,SEQ ID NO:207)表达的组成型启动子组成,所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因(T-AGRtu.nos-1:1:13,SEQ ID NO:209)的3'终止区域。另一个转化对照质粒由驱动海肾(*Renilla reniformis*)荧光素酶编码序列(RLuc,SEQ ID NO:208)表达的组成型启动子组成,所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因的3'终止区域。

[0153] 使用PEG转化法,使用植物表达载体pMON80585、pMON109584、pMON118756、pMON124912、pMON140818、pMON140819、pMON140820、pMON140821pMON140822、pMON140823、pMON140824、pMON140825、pMON140826、pMON140827、pMON140828、pMON140829、pMON140830、pMON140831、pMON140832、pMON140833、pMON140834、pMON140835、pMON140836、pMON140837、pMON140838和pMON140839转化大豆子叶原生质体细胞。用等摩尔量的所述两个转化对照质粒中每一个和测试植物表达载体转化原生质体细胞。测定GUS和荧光素酶活性。通过将等分的如上转化的细胞的裂解制剂放入两个不同的小孔托盘中来进行GUS和荧光素酶的测量。一个托盘用于GUS测量,而另一个托盘用于进行使用双荧光素酶报告基因检测系统(Promega Corp.,Madison,WI;参见例如,Promega Notes Magazine,No:57,1996,p.02)的双荧光素酶检测。每次转化使用3或4个复制物来进行试样测量。GUS和荧光素酶的平均值显示于下表8中。

[0154] 表8.GUS和荧光素酶表达平均值和GUS/荧光素酶比例

[0155]

构建体	调控元件	SEQ ID NO:	平均 GUS	平均 FLuc	平均 RLuc	GUS/FLuc	GUS/RLuc
pMON80585	EXP-At.Atnnt1:1:2	200	586	5220.7	8323	0.1100	0.0700
pMON109584	EXP-CaMV.35S-enh+ Ph.DnaK:1:3	201	5768	4275	15098	1.3500	0.3800
pMON118756	EXP-At.Act7:1:11	202	773	7722	10545	0.1000	0.0700
pMON124912	无启动子		48	9746.5	13905	0.0000	0.0000
pMON140818	P-CUCme.1-1:1:1rc	155	194	4772	6363	0.0400	0.0300
pMON140819	P-CUCme.2-1:1:1	14	171	6855	10123	0.0200	0.0200
pMON140820	P-CUCme.3-1:1:3	15	37	7089.3	9593	0.0100	0.0000
pMON140821	EXP-CUCme.4:1:1	156	4211	7626.8	13935	0.5500	0.3000
pMON140822	EXP-CUCme.5:1:1	159	626	15609.3	21140	0.0400	0.0300
pMON140823	P-CUCme.6-1:1:1	18	331	15178.5	22818	0.0200	0.0100
pMON140824	P-CUCme.8-1:1:2	19	238	17514.5	28429	0.0100	0.0100
pMON140825	P-CUCme.9-1:1:2	20	510	13208	19567	0.0400	0.0300
pMON140826	P-CUCme.10-1:1:1	21	352	14805.3	22200	0.0200	0.0200
pMON140827	EXP-CUCme.eEF1a:1:1	162	724	9326.8	14476	0.0800	0.0500
pMON140828	P-CUCme.15-1:1:2	23	304	11798	17486	0.0300	0.0200
pMON140829	P-CUCme.16a-1:1:2	24	88	5429	9596	0.0200	0.0100
pMON140830	P-CUCme.17-1:1:2	26	180	10477.8	15291	0.0200	0.0100
pMON140831	P-CUCme.18-1:1:2	27	111	5059.3	6778	0.0200	0.0200
pMON140832	P-CUCme.19-1:1:3	167	121	3765	6032	0.0300	0.0200
pMON140833	P-CUCme.20-1:3	211	155	10458.8	14748	0.0100	0.0100
pMON140834	P-CUCme.21-1:1:1	30	582	7760	11440	0.0800	0.0500
pMON140835	P-CUCme.22-1:1:3	31	400	11393.8	18654	0.0400	0.0200
pMON140836	EXP-CUCme.SAMS2:1:1	168	568	9466.3	13962	0.0600	0.0400
pMON140837	P-CUCme.26-1:1:2	33	87	6683	8494	0.0100	0.0100

[0156]

构建体	调控元件	SEQ ID NO:	平均 GUS	平均 FLuc	平均 RLuc	GUS/FLuc	GUS/RLuc
pMON140838	P-CUCme.28-1:1:2	34	171	19104.8	29619	0.0100	0.0100
pMON140839	EXP-CUCme.29:1:2	212	90	11247.3	15919	0.0100	0.0057

[0157] 为了比较大豆制品原生质体中每个启动子的相对活性,将GUS值表示为GUS与荧光素酶活性的比例,并且根据观察到的组成型表达元件组EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3的表达水平进行标准化。下表9显示根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例。下表10显示根据EXP-At.Act7:1:11和EXP-CaMV.310S-enh+Ph.DnaK:1:3标准化的GUS与海肾荧光素

酶(RLuc)的比例。

[0158] 表9. 根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与萤火虫荧光素酶(FLuc)的比例

[0159]

构建体	调控元件	SEQ ID NO:	根据 EXP-At.Act7:1:11 标准化的 GUS/FLuc	根据 EXP-CaMV.35S-enh +Ph.DnaK:1:3 标准化的 GUS/FLuc
pMON80585	EXP-At.Atnnt1:1:2	200	1.12	0.08
pMON109584	EXP-CaMV.35S-enh+Ph.DnaK:1:3	201	13.48	1.00
pMON118756	EXP-At.Act7:1:11	202	1.00	0.07
pMON124912	无启动子		0.05	0.00
pMON140818	P-CUCme.1-1:1:1rc	155	0.41	0.03
pMON140819	P-CUCme.2-1:1:1	14	0.25	0.02
pMON140820	P-CUCme.3-1:1:3	15	0.05	0.00
pMON140821	EXP-CUCme.4:1:1	156	5.52	0.41
pMON140822	EXP-CUCme.5:1:1	159	0.40	0.03
pMON140823	P-CUCme.6-1:1:1	18	0.22	0.02
pMON140824	P-CUCme.8-1:1:2	19	0.14	0.01
pMON140825	P-CUCme.9-1:1:2	20	0.39	0.03
pMON140826	P-CUCme.10-1:1:1	21	0.24	0.02
pMON140827	EXP-CUCme.eEF1a:1:1	162	0.78	0.06
pMON140828	P-CUCme.15-1:1:2	23	0.26	0.02
pMON140829	P-CUCme.16a-1:1:2	24	0.16	0.01
pMON140830	P-CUCme.17-1:1:2	26	0.17	0.01

[0160]

构建体	调控元件	SEQ ID NO:	根据 EXP-At.Act7:1:11 标准化的 GUS/FLuc	根据 EXP-CaMV.35S-enh +Ph.DnaK:1:3 标准化的 GUS/FLuc
pMON140831	P-CUCme.18-1:1:2	27	0.22	0.02
pMON140832	P-CUCme.19-1:1:3	167	0.32	0.02
pMON140833	P-CUCme.20-1:3	211	0.15	0.01
pMON140834	P-CUCme.21-1:1:1	30	0.75	0.06
pMON140835	P-CUCme.22-1:1:3	31	0.35	0.03
pMON140836	EXP-CUCme.SAMS2:1:1	168	0.60	0.04
pMON140837	P-CUCme.26-1:1:2	33	0.13	0.01
pMON140838	P-CUCme.28-1:1:2	34	0.09	0.01
pMON140839	EXP-CUCme.29:1:2	212	0.08	0.01

[0161] 表10. 根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与海肾荧光素酶(RLuc)的比例

[0162]

构建体	调控元件	SEQ ID NO:	根 EXP-At.Act7:1:1 1 标准化的 GUS/RLuc	根 EXP-CaMV.35S-enh + Ph.DnaK:1:3 标准 化的 GUS/RLuc
pMON80585	EXP-At.Atntt1:1:2	200	0.96	0.18
pMON109584	EXP-CaMV.35S-enh+ Ph.DnaK:1:3	201	5.21	1.00
pMON118756	EXP-At.Act7:1:11	202	1.00	0.19
pMON124912	无启动子		0.05	0.01
pMON140818	P-CUCme.1-1:1:1rc	155	0.42	0.08
pMON140819	P-CUCme.2-1:1:1	14	0.23	0.04
pMON140820	P-CUCme.3-1:1:3	15	0.05	0.01
pMON140821	EXP-CUCme.4:1:1	156	4.12	0.79
pMON140822	EXP-CUCme.5:1:1	159	0.40	0.08
pMON140823	P-CUCme.6-1:1:1	18	0.20	0.04
pMON140824	P-CUCme.8-1:1:2	19	0.11	0.02
pMON140825	P-CUCme.9-1:1:2	20	0.36	0.07
pMON140826	P-CUCme.10-1:1:1	21	0.22	0.04
pMON140827	EXP-CUCme.eEF1a:1:1	162	0.68	0.13
pMON140828	P-CUCme.15-1:1:2	23	0.24	0.05
pMON140829	P-CUCme.16a-1:1:2	24	0.13	0.02
pMON140830	P-CUCme.17-1:1:2	26	0.16	0.03
pMON140831	P-CUCme.18-1:1:2	27	0.22	0.04
pMON140832	P-CUCme.19-1:1:3	167	0.27	0.05

[0163]

构建体	调控元件	SEQ ID NO:	根 EXP-At.Act7:1:1 1 标准化的 GUS/RLuc	根 EXP-CaMV.35S-enh + Ph.DnaK:1:3 标准 化的 GUS/RLuc
pMON140833	P-CUCme.20-1:3	211	0.14	0.03
pMON140834	P-CUCme.21-1:1:1	30	0.69	0.13
pMON140835	P-CUCme.22-1:1:3	31	0.29	0.06
pMON140836	EXP-CUCme.SAMS2:1: 1	168	0.55	0.11
pMON140837	P-CUCme.26-1:1:2	33	0.14	0.03
pMON140838	P-CUCme.28-1:1:2	34	0.08	0.02
pMON140839	EXP-CUCme.29:1:2	212	0.08	0.01

[0164] 从表9和10中可以看出,大部分所测试的表达元件组显示在大豆子叶原生质体细胞中驱动转基因表达的能力。在该检测中,一个表达元件组EXP-CUCme.4:1:1 (SEQ ID NO: 156) 显示高于EXP-At.Act7:1:11的转基因表达水平。

[0165] 实施例5:在轰击的大豆叶和根中驱动GUS的调控元件的分析

[0166] 用含有驱动所述 β -葡萄糖醛酸酶 (GUS) 转基因表达的测试转录调控表达元件组的植物表达载体转化大豆叶和根,并与根和叶中的GUS表达进行比较,其中GUS的表达由已知的组成型启动子驱动。

[0167] 将在粒子轰击的大豆叶和根中由P-CUCme.1-1:1:1rc (SEQ ID NO:155)、P-CUCme.2-1:1:1 (SEQ ID NO:14)、P-CUCme.3-1:1:3 (SEQ ID NO:15)、EXP-CUCme.4:1:1 (SEQ ID NO:156)、EXP-CUCme.5:1:1 (SEQ ID NO:159)、P-CUCme.6-1:1:1 (SEQ ID NO:18)、P-CUCme.8-1:1:2 (SEQ ID NO:19)、P-CUCme.9-1:1:2 (SEQ ID NO:20)、P-CUCme.10-1:1:1

(SEQ ID NO:21)、EXP-CUCme.eEF1a:1:1 (SEQ ID NO:162)、P-CUCme.15-1:1:2 (SEQ ID NO:23)、P-CUCme.16a-1:1:2 (SEQ ID NO:24)、P-CUCme.17-1:1:2 (SEQ ID NO:26)、P-CUCme.18-1:1:2 (SEQ ID NO:27)、P-CUCme.19-1:1:3 (SEQ ID NO:167)、P-CUCme.20-1:3 (SEQ ID NO:211)、P-CUCme.21-1:1:1 (SEQ ID NO:30)、P-CUCme.22-1:1:3 (SEQ ID NO:31)、EXP-CUCme.SAMS2:1:1 (SEQ ID NO:168)、P-CUCme.26-1:1:2 (SEQ ID NO:33)、P-CUCme.28-1:1:2 (SEQ ID NO:34) 和 EXP-CUCme.29:1:2 (SEQ ID NO:212) 驱动的转基因的表达与来自已知的组成型表达元件组的表达进行比较。用于转化叶和根的植物表达载体与以上实施例4的表7中所示的那些相同。

[0168] 使用粒子轰击转化法,使用植物表达载体pMON80585、pMON109584、pMON1 18756、pMON124912、pMON140818、pMON140819、pMON140820、pMON140821、pMON140822、pMON140823、pMON140824、pMON140825、pMON140826、pMON140827、pMON140828、pMON140829、pMON140830、pMON140831、pMON140832、pMON140833、pMON140834、pMON140835、pMON140836、pMON140837、pMON140838和pMON140839转化大豆叶和根。

[0169] 简而言之,对A3244大豆种子进行表面消毒,并用16小时光照和8小时黑暗的光周期使其在托盘中发芽。大约13天后,在无菌条件下从所述幼苗收获叶和根组织,并用于轰击。将所述组织试样随机放置在含植物培养基的培替氏培养皿上。使用10微克的质粒DNA包被用于轰击的0.6微米金粒子(目录#165-2262Bio-Rad,Hercules,CA)。用所述DNA包被的金粒子(目录#165-2335Bio-Rad,Hercules CA)装载大载体。使用PDS 1000/He基因枪进行转化(目录#165-2257Bio-Rad,Hercules CA)。使所轰击的根和叶组织在26摄氏度下在黑暗中培育24小时。继该过夜培育之后,在用于GUS表达的溶液中,在37摄氏度下过夜染色所述组织。过夜染色后,在70%的乙醇中过夜浸泡所述组织,以除去叶绿素并显示GUS染色。然后,对所述组织拍照,并将反映GUS表达水平的“0”、“+”至“+++++”的等级量表分配给每个构建体(0为不表达,+至+++++分别为低至高)。

[0170] 在每个组织中显示的GUS转基因的表达用来推断每个元件在稳定转化的玉米植物中驱动转基因表达的能力的相对潜在水平和特异性。GUS表达平均等级提供于下表11中。

[0171] 表11. 粒子轰击的叶和根的GUS表达等级

[0172]

构建体	调控元件	SEQ ID NO:	叶表达	根表达
pMON80585	EXP-At.Atnnt1:1:2	200	+++	+++
pMON109584	EXP-CaMV.35S-enh+ Ph.DnaK:1:3	201	+++++	++
pMON118756	EXP-At.Act7:1:11	202	++++	+++
pMON124912	无启动子		0	0
pMON140818	P-CUCme.1-1:1:1rc	155	+++	+
pMON140819	P-CUCme.2-1:1:1	14	++	+
pMON140820	P-CUCme.3-1:1:3	15	0	0
pMON140821	EXP-CUCme.4:1:1	156	+++++	+++
pMON140822	EXP-CUCme.5:1:1	159	++	+
pMON140823	P-CUCme.6-1:1:1	18	++	+
pMON140824	P-CUCme.8-1:1:2	19	+	+
pMON140825	P-CUCme.9-1:1:2	20	++	+
pMON140826	P-CUCme.10-1:1:1	21	+++	+++
pMON140827	EXP-CUCme.eEF1a:1:1	162	++++	+++
pMON140828	P-CUCme.15-1:1:2	23	+	+
pMON140829	P-CUCme.16a-1:1:2	24	+	-
pMON140830	P-CUCme.17-1:1:2	26	++++	+
pMON140831	P-CUCme.18-1:1:2	27	+++	+
pMON140832	P-CUCme.19-1:1:3	167	+	+
pMON140833	P-CUCme.20-1:3	211	+	+
pMON140834	P-CUCme.21-1:1:1	30	+	+
pMON140835	P-CUCme.22-1:1:3	31	++++	+
pMON140836	EXP-CUCme.SAMS2:1:1	168	+++++	+++
pMON140837	P-CUCme.26-1:1:2	33	+	+
pMON140838	P-CUCme.28-1:1:2	34	+	+
pMON140839	EXP-CUCme.29:1:2	212	+	+

[0173] 从上表11中可以看出,所述表达元件组除一个以外都显示在粒子轰击的大豆叶和根组织中驱动转基因表达的能力。在该检测中,相对于由EXP-CaMV.35S-enh+Ph.DnaK:1:3驱动的表达,两个表达元件组P-CUCme.28-1:1:2 (SEQ ID NO:34) 和EXP-CUCme.4:1:1 (SEQ ID NO:156) 显示相似的或更高的表达水平。

[0174] 实施例6:使用转基因盒扩增子在大豆子叶原生质体中驱动GUS的调控元件的分析

[0175] 用含有驱动所述 β -葡糖醛酸酶 (GUS) 转基因表达的转录调控表达元件组的转基因盒扩增子转化大豆子叶原生质体,并与叶原生质体中的GUS表达进行比较,其中GUS的表达由已知的组成型启动子驱动。所述转基因盒扩增子由可操作地连接GUS编码序列 (GUS, SEQ ID NO:206)、可操作地连接3'UTR (T-Gb.FbL2-1:1:1, SEQ ID NO:205) 的EXP序列组成。将GUS平均表达与所述对照EXP元件P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2 (SEQ ID NO:210) 和EXP-At.Atnnt1:1:2 (SEQ ID NO:200) 进行比较。

[0176] 此外,以与如上在实施例2中所描述的相似方式使用用于共-转化和数据标准化的质粒。所述转化对照质粒由驱动萤火虫 (Photinus pyralis) 荧光素酶编码序列 (FLuc, SEQ ID NO:205) 表达的组成型启动子组成,所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因 (T-AGRtu.nos-1:1:13, SEQ ID NO:209) 的3'终止区域。

[0177] 下表12显示由每个转基因扩增子赋予的GUS表达平均值。下表13显示根据EXP-At.Atnntt1:1:2和P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例。

[0178]

表 12. GUS 和荧光素酶表达平均值和 GUS/荧光素酶比例

扩增子 ID	调控元件	SEQ ID NO:	平均 GUS	平均 Fluc	GUS/Fluc
无 DNA			0.00	0.00	0.00
pMON124912	无启动子		54.67	34905.00	0.00
pMON33449	P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2	210	107064.67	21757.67	4.92
pMON80585	EXP-At.Atnntt1:1:2	200	4962.33	40778.67	0.12
56969	CumMe_WSM_SF16429.G5670	40	283.67	53452.00	0.01
56877	P-CUCme.CumMe_WSM_SF16444.G5140-1:1:1	175	5297.67	46576.67	0.11
56749	P-CUCme.CumMe_WSM_SF16563.G5560-1:1:1	176	280.67	41958.33	0.01
56918	CumMe_WSM_SF17051.G5470	48	1088.00	36321.00	0.03
56849	P-CUCme.CumMe_WSM_SF17111.G5790-1:1:1	177	196.00	48128.00	0.00
56754	P-CUCme.WSM_SF17252.G7330-1:1:1	179	175.67	45427.00	0.00
56892	CumMe_WSM_SF17349.G5770	56	34.00	38016.00	0.00
56477	CumMe_WSM_SF17866.G6050	62	862.00	52203.33	0.02
56842	P-CUCme.CumMe_WSM_SF18488.G5340-1:1:1	181	2892.67	49144.33	0.06
56852	P-CUCme.CumMe_WSM_SF18536.G6480-1:1:1	182	3462.67	46549.33	0.07
56497	CumMe_WSM_SF18575.G6410	71	92.67	47628.33	0.00
56847	P-CUCme.CumMe_WSM_SF18634.G5190-1:1:1	183	122.33	36815.33	0.00
56746	P-CUCme.CumMe_WSM_SF18716.G5860-1:1:1	184	14.33	62483.33	0.00
56883	CumMe_WSM_SF18986.G6110	79	863.33	54379.33	0.02
56734	EXP-CUCme.WSM_SF19064.G5690:1:1	185	142.00	46962.67	0.00
56912	P-CUCme.CumMe_WSM_SF19647.G5760-1:1:1	188	7659.00	46935.67	0.16
56482	P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1	189	3279.00	37070.67	0.09
56963	CumMe_WSM_SF19902.G5260	87	1629.00	55649.00	0.03
56747	P-CUCme.CumMe_WSM_SF20132.G5560-1:1:1	190	340.33	40577.00	0.01
56479	CumMe_WSM_SF20359.G5870	92	192.00	61341.67	0.00
56744	CumMe_WSM_SF206458.G5970	98	154.67	33139.33	0.00

[0179]

扩增子 ID	调控元件	SEQ ID NO:	平均 GUS	平均 Fluc	GUS/Fluc
56948	CumMe_WSM_SF206534.G5200	99	62.00	52118.00	0.00
56896	CumMe_WSM_SF22008.G5670	108	1585.00	53540.00	0.03
56919	CumMe_WSM_SF22275.G5780	112	8.33	48546.33	0.00
56967	CumMe_WSM_SF22355.G5310	113	74.33	36202.67	0.00
56837	P-CUCme.CumMe_WSM_SF22531.G5120-1:1:1	192	1526.67	52799.33	0.03
56940	CumMe_WSM_SF22870.G5370	115	14.67	53663.33	0.00
56495	P-CUCme.CumMe_WSM_SF23760.G5200-1:1:1	193	196.33	49870.67	0.00
56868	P-CUCme.CumMe_WSM_SF23906.G6180-1:1:1	194	1584.33	42532.33	0.04
56998	CumMe_WSM_SF24045.G5400	123	80.67	47553.00	0.00
56976	P-CUCme.CumMe_WSM_SF25141.G5160-1:1:2	195	4506.00	57213.00	0.08
56742	P-CUCme.CumMe_WSM_SF25355.G5000-1:1:1	196	4.00	41114.33	0.00
56915	P-CUCme.CumMe_WSM_SF25936.G5450-1:1:1	197	965.33	34494.67	0.03
56854	CumMe_WSM_SF28729.G5340	134	208.33	53956.00	0.00
56936	CumMe_WSM_SF31264.G5380	136	292.67	42320.67	0.01
56863	P-CUCme.CumMe_WSM_SF35856.G5150-1:1:1	198	125.00	48705.33	0.00
56751	P-CUCme.CumMe_WSM_SF41124.G5080-1:1:1	199	31.33	53595.00	0.00
56921	CumMe_WSM_SF41254.G5160	141	11.67	52643.67	0.00
56884	CumMe_WSM_SF42141.G5110	146	48.33	40556.67	0.00

表 13.根据 EXP-At.Atmnt1:1:2 和 P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2 标准化的 GUS 与萤火虫荧光素酶(FLuc)的比例

[0180]

扩增子 ID	调控元件	SEQ ID NO:	根据 EXP-At.Atn1:1:2 标准化的 GUS/Fluc	根据 P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2 标准化的 GUS/Fluc
无 DNA			0.00	0.00
pMON124912	无启动子		0.01	0.00
pMON33449	P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2	210	40.44	1.00
pMON80585	EXP-At.Atn1:1:2	200	1.00	0.02
56969	CumMe WSM SF16429.G5670	40	0.04	0.00
56877	P-CUCme.CumMe WSM SF16444.G5140-1:1:1	175	0.93	0.02
56749	P-CUCme.CumMe WSM SF16563.G5560-1:1:1	176	0.05	0.00
56918	CumMe WSM SF17051.G5470	48	0.25	0.01
56849	P-CUCme.CumMe WSM SF17111.G5790-1:1:1	177	0.03	0.00
56754	P-CUCme.WSM SF17252.G7330-1:1:1	179	0.03	0.00
56892	CumMe WSM SF17349.G5770	56	0.01	0.00
56477	CumMe WSM SF17866.G6050	62	0.14	0.00
56842	P-CUCme.CumMe WSM SF18488.G5340-1:1:1	181	0.48	0.01
56852	P-CUCme.CumMe WSM SF18536.G6480-1:1:1	182	0.61	0.02
56497	CumMe WSM SF18575.G6410	71	0.02	0.00
56847	P-CUCme.CumMe WSM SF18634.G5190-1:1:1	183	0.03	0.00
56746	P-CUCme.CumMe WSM SF18716.G5860-1:1:1	184	0.00	0.00
56883	CumMe WSM SF18986.G6110	79	0.13	0.00
56734	EXP-CUCme.WSM SF19064.G5690:1:1	185	0.02	0.00
56912	P-CUCme.CumMe WSM SF19647.G5760-1:1:1	188	1.34	0.03
56482	P-CUCme.CumMe WSM SF19839.G5090-1:1:1	189	0.73	0.02
56963	CumMe WSM SF19902.G5260	87	0.24	0.01
56747	P-CUCme.CumMe WSM SF20132.G5560-1:1:1	190	0.07	0.00
56479	CumMe WSM SF20359.G5870	92	0.03	0.00
56744	CumMe WSM SF206458.G5970	98	0.04	0.00

[0181]

扩增子 ID	调控元件	SEQ ID NO:	根据 EXP-At.Atntt1:1:2 标准化的 GUS/Fluc	根据 P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2 标准化的 GUS/Fluc
56948	CumMe_WSM_SF206534.G5200	99	0.01	0.00
56896	CumMe_WSM_SF22008.G5670	108	0.24	0.01
56919	CumMe_WSM_SF22275.G5780	112	0.00	0.00
56967	CumMe_WSM_SF22355.G5310	113	0.02	0.00
56837	P-CUCme.CumMe_WSM_SF22531.G5120-1:1:1	192	0.24	0.01
56940	CumMe_WSM_SF22870.G5370	115	0.00	0.00
56495	P-CUCme.CumMe_WSM_SF23760.G5200-1:1:1	193	0.03	0.00
56868	P-CUCme.CumMe_WSM_SF23906.G6180-1:1:1	194	0.31	0.01
56998	CumMe_WSM_SF24045.G5400	123	0.01	0.00
56976	P-CUCme.CumMe_WSM_SF25141.G5160-1:1:2	195	0.65	0.02
56742	P-CUCme.CumMe_WSM_SF25355.G5000-1:1:1	196	0.00	0.00
56915	P-CUCme.CumMe_WSM_SF25936.G5450-1:1:1	197	0.23	0.01
56854	CumMe_WSM_SF28729.G5340	134	0.03	0.00
56936	CumMe_WSM_SF31264.G5380	136	0.06	0.00
56863	P-CUCme.CumMe_WSM_SF35856.G5150-1:1:1	198	0.02	0.00
56751	P-CUCme.CumMe_WSM_SF41124.G5080-1:1:1	199	0.00	0.00
56921	CumMe_WSM_SF41254.G5160	141	0.00	0.00
56884	CumMe_WSM_SF42141.G5110	146	0.01	0.00

[0182] 从上表12中可以看出,当与所述无启动子的对照相比时,不是所有的EXP序列都显示驱动转基因表达的能力。然而,EXP序列CumMe_WSM_SF16429.G5670 (SEQ ID NO:40)、P-

CUCme.CumMe_WSM_SF16444.G5140-1:1:1 (SEQ ID NO:175)、P-CUCme.CumMe_WSM_SF16563.G5560-1:1:1 (SEQ ID NO:176)、CumMe_WSM_SF17051.G5470 (SEQ ID NO:48)、P-CUCme.CumMe_WSM_SF17111.G5790-1:1:1 (SEQ ID NO:177)、P-CUCme.WSM_SF17252.G7330-1:1:1 (SEQ ID NO:179)、CumMe_WSM_SF17866.G6050 (SEQ ID NO:62)、P-CUCme.CumMe_WSM_SF18488.G5340-1:1:1 (SEQ ID NO:181)、P-CUCme.CumMe_WSM_SF18536.G6480-1:1:1 (SEQ ID NO:182)、CumMe_WSM_SF18575.G6410 (SEQ ID NO:71)、P-CUCme.CumMe_WSM_SF18634.G5190-1:1:1 (SEQ ID NO:183)、CumMe_WSM_SF18986.G6110 (SEQ ID NO:79)、EXP-CUCme.WSM_SF19064.G5690:1:1 (SEQ ID NO:185)、P-CUCme.CumMe_WSM_SF19647.G5760-1:1:1 (SEQ ID NO:188)、P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1 (SEQ ID NO:189)、CumMe_WSM_SF19902.G5260 (SEQ ID NO:87)、P-CUCme.CumMe_WSM_SF20132.G5560-1:1:1 (SEQ ID NO:190)、CumMe_WSM_SF20359.G5870 (SEQ ID NO:92)、CumMe_WSM_SF206458.G5970 (SEQ ID NO:98)、CumMe_WSM_SF206534.G5200 (SEQ ID NO:99)、CumMe_WSM_SF22008.G5670 (SEQ ID NO:108)、CumMe_WSM_SF22355.G5310 (SEQ ID NO:113)、P-CUCme.CumMe_WSM_SF22531.G5120-1:1:1 (SEQ ID NO:192)、EXP-CUCme.WSM_SF19064.G5690:1:1 (SEQ ID NO:193)、P-CUCme.CumMe_WSM_SF23906.G6180-1:1:1 (SEQ ID NO:194)、CumMe_WSM_SF24045.G5400 (SEQ ID NO:123)、P-CUCme.CumMe_WSM_SF25141.G5160-1:1:2 (SEQ ID NO:195)、P-CUCme.CumMe_WSM_SF25936.G5450-1:1:1 (SEQ ID NO:197)、CumMe_WSM_SF28729.G5340 (SEQ ID NO:134)、CumMe_WSM_SF31264.G5380 (SEQ ID NO:136) 和 P-CUCme.CumMe_WSM_SF35856.G5150-1:1:1 (SEQ ID NO:198) 显示以与 EXP-At.Atnnt1:1:2 相似或更高的水平驱动大豆子叶原生质体中转基因表达的能力。如上表 13 所示,在该检测中,EXP 序列 P-CUCme.CumMe_WSM_SF 19647.G5760-1:1:1 (SEQ ID NO:188) 显示以高于 EXP-At.Atnnt1:1:2 的水平驱动转基因表达的能力。

[0183] 实施例7:棉花叶原生质体中驱动GUS的调控元件的分析

[0184] 用含有驱动所述 β -葡糖醛酸酶 (GUS) 转基因表达的测试转录调控表达元件组的植物表达载体转化棉花叶原生质体,并与叶原生质体中的GUS表达进行比较,其中GUS表达由已知的组成型启动子驱动。

[0185] 将由 P-CUCme.1-1:1:1rc (SEQ ID NO:155)、P-CUCme.2-1:1:1 (SEQ ID NO:14)、P-CUCme.3-1:1:3 (SEQ ID NO:15)、EXP-CUCme.4:1:1 (SEQ ID NO:156)、P-CUCme.6-1:1:1 (SEQ ID NO:18)、P-CUCme.8-1:1:2 (SEQ ID NO:19)、P-CUCme.9-1:1:2 (SEQ ID NO:20)、P-CUCme.10-1:1:1 (SEQ ID NO:21)、EXP-CUCme.eEF1a:1:1 (SEQ ID NO:162)、P-CUCme.15-1:1:2 (SEQ ID NO:23)、P-CUCme.16a-1:1:2 (SEQ ID NO:24)、P-CUCme.17-1:1:2 (SEQ ID NO:26)、P-CUCme.18-1:1:2 (SEQ ID NO:27)、P-CUCme.19-1:1:3 (SEQ ID NO:167)、P-CUCme.20-1:3 (SEQ ID NO:211)、P-CUCme.21-1:1:1 (SEQ ID NO:30)、P-CUCme.22-1:1:3 (SEQ ID NO:31)、EXP-CUCme.SAMS2:1:1 (SEQ ID NO:168)、P-CUCme.26-1:1:2 (SEQ ID NO:33)、P-CUCme.28-1:1:2 (SEQ ID NO:34) 和 EXP-CUCme.29:1:2 (SEQ ID NO:212) 驱动的转基因表达与来自已知的组成型表达元件组的表达进行比较。每种植物表达载体由来自根癌土壤杆菌的右边界区域、第一转基因盒、第二转基因选择盒和来自根癌土壤杆菌的左边界区域组成,其中所述第一转基因盒由可操作地连接5'至含有衍生自土豆光诱导组织特异性 ST-LS1 基因 (基因库登记号:X04753) 的可加工内含子的 β -葡糖醛酸酶 (GUS, SEQ ID NO:

206) 用编码序列的、可操作地连接5'至来自海岛棉E6基因 (T-Gb.E6-3b:1:1, SEQ ID NO: 204)、豌豆RbcS2-E9基因 (T-Ps.RbcS2-E9-1:1:6, SEQ ID NO: 203) 或海岛棉FbLate-2基因 (T-Gb.FbL2-1:1:1, SEQ ID NO: 205) 的3'终止区域的测试启动子或已知的组成型启动子组成; 所述第二转基因选择盒用于选择赋予除草剂草甘膦(由拟南芥肌动蛋白7启动子驱动) 或抗生素、卡那霉素抗性的转化的植物细胞。无启动子的对照植物表达载体 (pMON124912) 作为表达的阴性对照。将上述测试和组成型表达元件组克隆成如下表14所示的植物表达载体。

[0186] 表14. 植物表达载体和相应的表达元件组以及3'UTR

构建体	调控元件	SEQ ID NO:	3' UTR
pMON109584	EXP-CaMV.35S-enh+Ph.DnaK:1:3	201	T-Gb.E6-3b:1:1
pMON118756	EXP-At.Act7:1:11	202	T-Gb.E6-3b:1:1
pMON124912	无启动子		T-Gb.FbL2-1:1:1
pMON140818	P-CUCme.1-1:1:1rc	155	T-Gb.FbL2-1:1:1
pMON140819	P-CUCme.2-1:1:1	14	T-Gb.FbL2-1:1:1
pMON140820	P-CUCme.3-1:1:3	15	T-Gb.FbL2-1:1:1
pMON140821	EXP-CUCme.4:1:1	156	T-Gb.FbL2-1:1:1
pMON140823	P-CUCme.6-1:1:1	18	T-Gb.FbL2-1:1:1
pMON140824	P-CUCme.8-1:1:2	19	T-Gb.FbL2-1:1:1
pMON140825	P-CUCme.9-1:1:2	20	T-Gb.FbL2-1:1:1
pMON140826	P-CUCme.10-1:1:1	21	T-Gb.FbL2-1:1:1
pMON140827	EXP-CUCme.eEF1a:1:1	162	T-Gb.FbL2-1:1:1
pMON140828	P-CUCme.15-1:1:2	23	T-Gb.FbL2-1:1:1
pMON140829	P-CUCme.16a-1:1:2	24	T-Gb.FbL2-1:1:1
pMON140830	P-CUCme.17-1:1:2	26	T-Gb.FbL2-1:1:1
pMON140831	P-CUCme.18-1:1:2	27	T-Gb.FbL2-1:1:1
pMON140832	P-CUCme.19-1:1:3	167	T-Gb.FbL2-1:1:1
pMON140833	P-CUCme.20-1:3	211	T-Gb.FbL2-1:1:1
pMON140834	P-CUCme.21-1:1:1	30	T-Gb.FbL2-1:1:1
pMON140835	P-CUCme.22-1:1:3	31	T-Gb.FbL2-1:1:1
pMON140836	EXP-CUCme.SAMS2:1:1	168	T-Gb.FbL2-1:1:1
pMON140837	P-CUCme.26-1:1:2	33	T-Gb.FbL2-1:1:1
pMON140838	P-CUCme.28-1:1:2	34	T-Gb.FbL2-1:1:1
pMON140839	EXP-CUCme.29:1:2	212	T-Gb.FbL2-1:1:1

[0188] 此外, 构建用于共-转化和数据标准化的两个质粒。一个转化对照质粒由驱动萤火虫 (*Photinus pyralis*) 荧光素酶编码序列 (FLuc, SEQ ID NO: 205) 表达的组成型启动子组成, 所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因 (T-AGRtu.nos-1:1:13, SEQ ID NO: 209) 的3'终止区域。另一个转化对照质粒包含驱动海肾 (*Renilla reniformis*) 荧光素酶编码序列 (RLuc, SEQ ID NO: 206) 表达的组成型启动子组成, 所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因的3'终止区域。

[0189] 使用PEG转化法, 使用植物表达载体pMON80585、pMON109584、pMON118756、pMON124912、pMON140818、pMON140819、pMON140820、pMON140821、pMON140822、pMON140823、

pMON140824、pMON140825、pMON140826、pMON140827、pMON140828、pMON140829、pMON140830、pMON140831、pMON140832、pMON140833、pMON140834、pMON140835、pMON140836、pMON140837、pMON140838和pMON140839转化棉花叶原生质体细胞。用等摩尔量的所述两个转化对照质粒中每一个和测试植物表达载体转化原生质体细胞。测定GUS和荧光素酶活性。通过将等分的如上转化的细胞的裂解制剂放入两个不同的小孔托盘中来进行GUS和荧光素酶的测量。一个托盘用于GUS测量,而另一个托盘用于进行使用双荧光素酶报告基因检测系统(Promega Corp.,Madison,WI;参见例如,Promega Notes Magazine,No:57,1996,p.02)的双荧光素酶检测。每次转化使用4个复制物来进行试样测量。GUS和荧光素酶的平均值显示在下表15中。

[0190] 表15.GUS和荧光素酶表达平均值和GUS/荧光素酶比例

[0191]

构建体	调控元件	SEQ ID NO:	平均 GUS	平均 FLuc	平均 RLuc	GUS/FLuc	GUS/RLuc
pMON109584	EXP-CaMV.35S-enh+Ph.DnaK:1:3	201	5322.8	14842.8	27990.5	0.3586	0.1902
pMON118756	EXP-At.Act7:1:1	202	1006.3	19746.8	25582.3	0.0510	0.0393
pMON124912	无启动子		21	19248.5	25012	0.0011	0.0008
pMON140818	P-CUCme.1-1:1:1rc	155	170.3	17796.8	22026.3	0.0096	0.0077

[0192]

构建体	调控元件	SEQ ID NO:	平均 GUS	平均 FLuc	平均 RLuc	GUS/FLuc	GUS/RLuc
pMON140819	P-CUCme.2-1:1:1	14	34.8	16326.3	21407.5	0.0021	0.0016
pMON140820	P-CUCme.3-1:1:3	15	51.5	17356.8	21523.8	0.0030	0.0024
pMON140821	EXP-CUCme.4:1:1	156	3497.8	18745.3	26065.3	0.1866	0.1342
pMON140823	P-CUCme.6-1:1:1	18	40.8	19533.8	26361.5	0.0021	0.0015
pMON140824	P-CUCme.8-1:1:1	19	22	19701	26278	0.0011	0.0008
pMON140825	P-CUCme.9-1:1:1	20	372.5	21972.3	28755	0.0170	0.0130
pMON140826	P-CUCme.10-1:1:1	21	198	21362.8	28902	0.0093	0.0069
pMON140827	EXP-CUCme.eEFla:1:1	162	725	21589	27635.3	0.0336	0.0262
pMON140828	P-CUCme.15-1:1:2	23	55.3	17706	28846	0.0031	0.0019
pMON140829	P-CUCme.16a-1:1:2	24	14	23289.5	30190	0.0006	0.0005
pMON140830	P-CUCme.17-1:1:2	26	155.5	23178.3	31602.8	0.0067	0.0049
pMON140831	P-CUCme.18-1:1:2	27	86.8	19085.8	22396.5	0.0045	0.0039
pMON140832	P-CUCme.19-1:1:3	167	130	21520.3	27270.5	0.0060	0.0048
pMON140833	P-CUCme.20-1:3	211	88.5	22223.8	30786	0.0040	0.0029
pMON140834	P-CUCme.21-1:1:1	30	98.5	18579	20506.3	0.0053	0.0048
pMON140835	P-CUCme.22-1:1:3	31	363	21780.3	28816.3	0.0167	0.0126
pMON140836	EXP-CUCme.SAMS2:1:1	168	515	17906	23031	0.0288	0.0224
pMON140837	P-CUCme.26-1:1:2	33	125	15529.3	15169.3	0.0080	0.0082
pMON140838	P-CUCme.28-1:1:2	34	115.8	17013.5	22236.5	0.0068	0.0052
pMON140839	EXP-CUCme.29:1:2	212	15.5	16370.3	20409	0.0009	0.0008

[0193] 为了比较棉花叶原生质体中每个启动子的相对活性,将GUS值表示为GUS与荧光素酶活性的比例,并且根据观察到的组成型表达元件组EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3的表达水平进行标准化。下表16显示根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例。下表17显示根据EXP-At.Act7:1:11和EXP-CaMV.317S-enh+Ph.DnaK:1:3标准化的GUS与海肾荧光素酶 (RLuc) 的比例。

[0194] 表16. 根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例

[0195]

构建体	调控元件	SEQ ID NO:	根 据 EXP-At.Act7:1:11 标 准化的 GUS/FLuc	根 据 EXP-CaMV.35S-enh +Ph.DnaK:1:3 标准化 的 GUS/FLuc
pMON109584	EXP-CaMV.35S-enh +Ph.DnaK:1:3	201	7.037	1.000
pMON118756	EXP-At.Act7:1:11	202	1.000	0.142
pMON124912	无启动子		0.021	0.003
pMON140818	P-CUCme.1-1:1:1rc	155	0.188	0.027
pMON140819	P-CUCme.2-1:1:1	14	0.042	0.006
pMON140820	P-CUCme.3-1:1:3	15	0.058	0.008
pMON140821	EXP-CUCme.4:1:1	156	3.662	0.520
pMON140823	P-CUCme.6-1:1:1	18	0.041	0.006
pMON140824	P-CUCme.8-1:1:2	19	0.022	0.003
pMON140825	P-CUCme.9-1:1:2	20	0.333	0.047
pMON140826	P-CUCme.10-1:1:1	21	0.182	0.026
pMON140827	EXP-CUCme.eEF1a:1:1	162	0.659	0.094
pMON140828	P-CUCme.15-1:1:2	23	0.061	0.009
pMON140829	P-CUCme.16a-1:1:2	24	0.012	0.002
pMON140830	P-CUCme.17-1:1:2	26	0.132	0.019
pMON140831	P-CUCme.18-1:1:2	27	0.089	0.013
pMON140832	P-CUCme.19-1:1:3	167	0.119	0.017
pMON140833	P-CUCme.20-1:3	211	0.078	0.011
pMON140834	P-CUCme.21-1:1:1	30	0.104	0.015
pMON140835	P-CUCme.22-1:1:3	31	0.327	0.046
pMON140836	EXP-CUCme.SAMS2:1: 1	168	0.564	0.080
pMON140837	P-CUCme.26-1:1:2	33	0.158	0.022
pMON140838	P-CUCme.28-1:1:2	34	0.134	0.019
pMON140839	EXP-CUCme.29:1:2	212	0.019	0.003

[0196] 表17. 根据EXP-At.Act7:1:11和EXP-CaMV.35S-enh+Ph.DnaK:1:3标准化的GUS与海肾荧光素酶(RLuc)的比例

[0197]

构建体	调控元件	SEQ ID NO:	根 据 EXP-At.Act7:1:1 1 标 准 化 的 GUS/RLuc	根 据 EXP-CaMV.35S-enh h +Ph.DnaK:1:3 标 准化的 GUS/RLuc
pMON109584	EXP-CaMV.35S-enh + Ph.DnaK:1:3	201	4.83	1.00
pMON118756	EXP-At.Act7:1:11	202	1.00	0.21
pMON124912	无启动子		0.02	0.00
pMON140818	P-CUCme.1-1:1:1rc	155	0.20	0.04
pMON140819	P-CUCme.2-1:1:1	14	0.04	0.01
pMON140820	P-CUCme.3-1:1:3	15	0.06	0.01
pMON140821	EXP-CUCme.4:1:1	156	3.41	0.71
pMON140823	P-CUCme.6-1:1:1	18	0.04	0.01
pMON140824	P-CUCme.8-1:1:2	19	0.02	0.00
pMON140825	P-CUCme.9-1:1:2	20	0.33	0.07
pMON140826	P-CUCme.10-1:1:1	21	0.17	0.04
pMON140827	EXP-CUCme.eEF1a:1:1	162	0.67	0.14
pMON140828	P-CUCme.15-1:1:2	23	0.05	0.01
pMON140829	P-CUCme.16a-1:1:2	24	0.01	0.00
pMON140830	P-CUCme.17-1:1:2	26	0.13	0.03
pMON140831	P-CUCme.18-1:1:2	27	0.10	0.02
pMON140832	P-CUCme.19-1:1:3	167	0.12	0.03
pMON140833	P-CUCme.20-1:3	211	0.07	0.02
pMON140834	P-CUCme.21-1:1:1	30	0.12	0.03
pMON140835	P-CUCme.22-1:1:3	31	0.32	0.07
pMON140836	EXP-CUCme.SAMS 2:1:1	168	0.57	0.12
pMON140837	P-CUCme.26-1:1:2	33	0.21	0.04
pMON140838	P-CUCme.28-1:1:2	34	0.13	0.03
pMON140839	EXP-CUCme.29:1:2	212	0.02	0.00

[0198] 从表16和17中可以看出,大部分所测试的表达元件组显示在棉花叶原生质体细胞中驱动转基因表达的能力。在该检测中,一个表达元件组EXP-CUCme.4:1:1 (SEQ ID NO: 156) 显示高于EXP-At.Act7:1:11的转基因表达水平。

[0199] 实施例8:使用转基因盒扩增子在棉花叶原生质体中驱动GUS的调控元件的分析

[0200] 用含有驱动所述 β -葡萄糖醛酸酶 (GUS) 转基因表达的转录的调控表达元件组的转基因盒扩增子转化棉花叶原生质体,并与叶原生质体中的GUS表达进行比较,其中GUS表达由已知的组成型启动子驱动。所述转基因盒扩增子由可操作地连接GUS编码序列 (GUS, SEQ ID NO:206)、可操作地连接3'UTR (T-Gb.FbL2-1:1:1, SEQ ID NO:205) 的EXP序列组成。将GUS平均表达与所述对照EXP元件P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2 (SEQ ID NO:210) 和EXP-At.Atnnt1:1:2 (SEQ ID NO:200) 进行比较。

[0201] 此外,以与如上在实施例2中所描述的相似方式使用用于共-转化和数据标准化的质粒。所述转化对照质粒由驱动萤火虫 (Photinus pyralis) 荧光素酶编码序列 (FLuc, SEQ ID NO:205) 表达的组成型启动子组成,所述组成型启动子可操作地连接5'至来自根癌土壤杆菌胭脂碱合酶基因 (T-AGRtu.nos-1:1:13, SEQ ID NO:209) 的3'终止区域。

[0202] 下表18显示由每个转基因扩增子赋予的GUS表达平均值。下表19显示根据EXP-

At.Atn1:1:2和P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例。

[0203] 表18.GUS和荧光素酶表达平均值和GUS/荧光素酶比例

[0204]

扩增子 ID	调控元件	SEQ ID NO:	平均 GUS	平均 Fluc	GUS/Fluc
空白载体	无 DNA		32.8	14087.5	0.002
pMON124912	无启动子		12	20486.3	0.001
pMON80585	EXP-At.Atn1:1:2	200	55.5	18811	0.003
pMON33449	P-CaMV.35S-enh-1:1:102/ L-CaMV.35S-1:1:2	210	12472.5	19126.3	0.652
56741	CumMe_WSM_SF143981.G5150	36	5.8	17449.5	0.000
56492	CumMe_WSM_SF144839.G5080	37	27.5	16674	0.002
56877	P-CUCme.CumMe_WSM_SF16444. G5140-1:1:1	175	96.3	17237.8	0.006
56485	CumMe_WSM_SF16530.G6000	42	27.3	17858.5	0.002
56844	CumMe_WSM_SF16953.G5180	47	22.3	19398.5	0.001
56500	CumMe_WSM_SF17250.G5910	52	12.3	23980.3	0.001
56754	P-CUCme.WSM_SF17252.G7330-1:1:1	179	16	13848.8	0.001
56740	CumMe_WSM_SF17672.G5610	60	12	16646.8	0.001
56870	CumMe_WSM_SF18287.G5380	66	39.3	13930.5	0.003

[0205]

扩增子 ID	调控元件	SEQ ID NO:	平均 GUS	平均 Fluc	GUS/Fluc
56478	CumMe_WSM_SF18504.G5090	68	11.8	15830.5	0.001
56481	CumMe_WSM_SF18530.G5750	69	6.5	15211.3	0.000
56498	CumMe_WSM_SF18645.G5380	73	36	14569.8	0.002
56746	P-CUCme.CumMe_WSM_SF18716.G5860-1:1:1	184	11	18054.5	0.001
56490	CumMe_WSM_SF18801.G5040	75	21.5	14147.3	0.002
56488	CumMe_WSM_SF19323.G5120	81	15.3	11985.3	0.001
56499	CumMe_WSM_SF19631.G5170	83	12.5	20140.5	0.001
56482	P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1	189	75	18690.5	0.004
56489	CumMe_WSM_SF19850.G5130	86	38.3	19756.5	0.002
56476	CumMe_WSM_SF20355.G5130	91	10.5	27901.8	0.000
56895	CumMe_WSM_SF20431.G6340	95	34.8	16283.8	0.002
56744	CumMe_WSM_SF206458.G5970	98	11	19659	0.001
56480	CumMe_WSM_SF21366.G5980	105	10.8	17367	0.001
56930	CumMe_WSM_SF22070.G5280	109	25.3	14210.5	0.002
56484	CumMe_WSM_SF23181.G5100	117	20.3	13506	0.002
56495	P-CUCme.CumMe_WSM_SF23760.G5200-1:1:1	193	7.8	15138.5	0.001
56971	CumMe_WSM_SF25084.G5580	125	16	16135.3	0.001
56742	P-CUCme.CumMe_WSM_SF25355.G5000-1:1:1	196	18	13782.8	0.001
56494	CumMe_WSM_SF25455.G5370	129	10.5	16089.8	0.001
56751	P-CUCme.CumMe_WSM_SF41124.G5080-1:1:1	199	24.3	17884.3	0.001
56483	CumMe_WSM_SF41644.G6400	143	14.5	13130.5	0.001
56904	CumMe_WSM_SF44933.G5290	147	33	13369	0.002
56743	CumMe_WSM_SF9060.G5120	154	11.3	15230.8	0.001

[0206] 表19. 根据EXP-At.Atn1:1:2和P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2标准化的GUS与萤火虫荧光素酶 (FLuc) 的比例

[0207]

扩增子 ID	调控元件	SEQ ID NO:	根据 EXP-At.Atn1:1:2 标准化的 GUS/Fluc	根据 P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2 标准化的 GUS/Fluc
空白载体	无 DNA			
pMON12491 2	无启动子			
pMON80585	EXP-At.Atn1:1:2	200	1.000	0.005
pMON33449	P-CaMV.35S-enh-1:1:102/ L-CaMV.35S-1:1:2	210	221.025	1.000
56741	CumMe_WSM_SF143981.G5150	36	0.113	0.001
56492	CumMe_WSM_SF144839.G5080	37	0.559	0.003
56877	P-CUCme.CumMe_WSM_SF16444 .G5140-1:1:1	175	1.893	0.009
56485	CumMe_WSM_SF16530.G6000	42	0.518	0.002
56844	CumMe_WSM_SF16953.G5180	47	0.390	0.002
56500	CumMe_WSM_SF17250.G5910	52	0.174	0.001
56754	P-CUCme.WSM_SF17252. G7330-1:1:1	179	0.392	0.002
56740	CumMe_WSM_SF17672.G5610	60	0.244	0.001
56870	CumMe_WSM_SF18287.G5380	66	0.956	0.004
56478	CumMe_WSM_SF18504.G5090	68	0.253	0.001
56481	CumMe_WSM_SF18530.G5750	69	0.145	0.001
56498	CumMe_WSM_SF18645.G5380	73	0.837	0.004
56746	P-CUCme.CumMe_WSM_SF18716 .G5860-1:1:1	184	0.207	0.001
56490	CumMe_WSM_SF18801.G5040	75	0.515	0.002

[0208]

扩增子 ID	调控元件	SEQ ID NO:	根据 EXP-At.Atn1:1:2 标准化的 GUS/Fluc	根据 P-CaMV.35S-enh-1:1:102/L-CaMV.35S-1:1:2 标准化的 GUS/Fluc
56488	CumMe_WSM_SF19323.G5120	81	0.433	0.002
56499	CumMe_WSM_SF19631.G5170	83	0.210	0.001
56482	P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1	189	1.360	0.006
56489	CumMe_WSM_SF19850.G5130	86	0.657	0.003
56476	CumMe_WSM_SF20355.G5130	91	0.128	0.001
56895	CumMe_WSM_SF20431.G6340	95	0.724	0.003
56744	CumMe_WSM_SF206458.G5970	98	0.190	0.001
56480	CumMe_WSM_SF21366.G5980	105	0.211	0.001
56930	CumMe_WSM_SF22070.G5280	109	0.603	0.003
56484	CumMe_WSM_SF23181.G5100	117	0.509	0.002
56495	P-CUCme.CumMe_WSM_SF23760.G5200-1:1:1	193	0.175	0.001
56971	CumMe_WSM_SF25084.G5580	125	0.336	0.002
56742	P-CUCme.CumMe_WSM_SF25355.G5000-1:1:1	196	0.443	0.002
56494	CumMe_WSM_SF25455.G5370	129	0.221	0.001
56751	P-CUCme.CumMe_WSM_SF41124.G5080-1:1:1	199	0.461	0.002
56483	CumMe_WSM_SF41644.G6400	143	0.374	0.002
56904	CumMe_WSM_SF44933.G5290	147	0.837	0.004
56743	CumMe_WSM_SF9060.G5120	154	0.251	0.001

[0209] 从上表18中可以看出,当与所述无启动子的对照相比时,不是所有的EXP序列都显示驱动转基因表达的能力。然而,EXP序列P-CUCme.CumMe_WSM_SF16444.G5140-1:1:1 (SEQ ID NO:175) 和P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1 (SEQ ID NO:189) 显示以与EXP-At.Atn1:1:2相似或更高的水平驱动大豆子叶原生质体中转基因表达的能力。如上表19所示,在该检测中,EXP序列P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1 (SEQ ID NO:189) 显示以高于EXP-At.Atn1:1:2的水平驱动转基因表达的能力。

[0210] 实施例9:在稳定转化的大豆中驱动GUS的调控元件的分析

[0211] 用含有驱动所述 β -葡萄糖醛酸酶(GUS)转基因表达的EXP序列的植物表达载体转化大豆植物。

[0212] 通过对染色的组织切片的检查定性地和定量地检测由EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1)、EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7)、P-CUCme.1-1:1:1rc (SEQ ID NO:155)、P-CUCme.2-1:1:1 (SEQ ID NO:14)、P-CUCme.3-1:1:3 (SEQ ID NO:15)、EXP-CUCme.4:1:1 (SEQ ID NO:156)、EXP-CUCme.5:1:1 (SEQ ID NO:159)、P-CUCme.6-1:1:1 (SEQ ID NO:18)、P-CUCme.8-1:1:2 (SEQ ID NO:19)、P-CUCme.9-1:1:2 (SEQ ID NO:20)、P-CUCme.10-1:1:1 (SEQ ID NO:21)、EXP-CUCme.eEF 1a:1:1 (SEQ ID NO:162)、P-CUCme.15-1:1:2 (SEQ ID NO:23)、P-CUCme.17-1:1:2 (SEQ ID NO:26)、P-CUCme.18-1:1:2 (SEQ ID NO:27)、P-CUCme.19-1:1:3 (SEQ ID NO:167)、P-CUCme.20-1:3 (SEQ ID NO:211)、P-CUCme.21-1:1:1 (SEQ ID NO:30)、EXP-CUCme.SAMS2:1:1 (SEQ ID NO:168)、P-CUCme.26-1:1:2 (SEQ ID NO:33)、EXP-CUCme.29:1:2 (SEQ ID NO:212)、P-CUCme.CumMe_WSM_SF25355.G5000-1:1:1 (SEQ ID NO:196)、P-CUCme.CumMe_WSM_SF17111.G5790-1:1:1 (SEQ ID NO:177)、P-CUCme.CumMe_WSM_SF22531.G5120-1:1:1 (SEQ ID NO:192)、P-CUCme.CumMe_WSM_SF18488.G5340-1:1:1 (SEQ ID NO:181)、P-CUCme.CumMe_WSM_SF23760.G5200-1:1:1 (SEQ ID NO:193)、EXP-CUCme.WSM_SF19064.G5690:1:1 (SEQ ID NO:185)、P-CUCme.WSM_SF17252.G7330-1:1:1 (SEQ ID NO:179)、P-CUCme.CumMe_WSM_SF18634.G5190-1:1:1 (SEQ ID NO:183)、P-CUCme.CumMe_WSM_SF19647.G5760-1:1:1 (SEQ ID NO:188)、P-CUCme.CumMe_WSM_SF25936.G5450-1:1:1 (SEQ ID NO:197)、P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1 (SEQ ID NO:189)、CumMe_WSM_SF206458.G5970 (SEQ ID NO:98) 和P-CUCme.CumMe_WSM_SF18716.G5860-1:1:1 (SEQ ID NO:184) 驱动的GUS转基因的表达。每种植物表达载体由来自根癌土壤杆菌的右边界区域、第一转基因盒、第二转基因选择盒和来自根癌土壤杆菌的左边界区域组成,其中所述第一转基因盒由可操作地连接5'至含有衍生自土豆光诱导组织特异性ST-LS1基因(基因库登记号:X04753)的可加工内含子的 β -葡萄糖醛酸酶(GUS, SEQ ID NO:206)用编码序列的、可操作地连接5'至来自海岛棉FbLate-2基因(T-Gb.FbL2-1:1:1, SEQ ID NO:205)的3'终止区域的EXP序列组成;所述第二转基因选择盒用于选择赋予除草剂草甘膦(由拟南芥肌动蛋白7启动子驱动)抗性的转化的植物细胞。

[0213] 将上述EXP序列克隆成如下表20-23所示的植物表达构建体,并利用土壤杆菌介导转化法用于转化大豆植物。使用所选择组织的组织切片定性地和定量地检测GUS的表达。

[0214] 组织化学的GUS分析用于转化植物的定性的表达分析。用GUS染色溶液X-Gluc (5-溴-4-氯-3-吲哚基-b-葡萄糖苷酸) (1毫克/毫升) 培育整个组织切片持续适当长度的时间,冲洗并目测检查蓝色染色。通过使用所选择的植物器官和组织直接目测检查或在显微镜下检查,定性地确定GUS活性。检查R₀代植物在Vn5根、R1根、Vn5库叶、Vn5源叶、R1源叶、R1叶柄、黄色豆荚胚、黄色豆荚子叶、R3未成熟种子、R3豆荚、R5子叶和R1花中的表达。

[0215] 对于定量分析,从所选择的转化的玉米植物组织提取总蛋白质。1微克总蛋白质与50微升总反应体积中的荧光底物4-甲基伞形酮基(methyleumbelliferyl)- β -D-葡萄糖苷酸(MUG)一起使用。所述反应产物4-甲基伞形酮基(4-MU) 在高pH时荧光最大,其中所述羟基被离子化。加入碳酸钠的碱性溶液同时停止所述检测,并调节pH以定量荧光产物。使用具有Micromax读数器的Fluoromax-3 (Horiba; Kyoto, 日本),将狭缝宽度设置为在2nm激发和在

3nm发射,测定在365nm激发、在445nm发射的荧光。

[0216] 下表20和21显示在R₀代植物组织中测量的定量表达平均水平。在两个表中,未检测的那些组织显示为空白单元。

[0217]

表 20.在 R₀ 代转化的大豆植物的 Vn5 根、R1 根、Vn5 库叶、Vn5 源叶、R1 源叶和 R1 叶柄中的平均 GUS 表达

构建体	调控元件	SEQ ID NO:	Vn5 根	R1 根	Vn5 库叶	Vn5 源叶	R1 源叶	R1 叶柄
pMON138776	EXP-CUCme.Ubq1:1:1	1	4				4	4
pMON138778	EXP-CUCme.Ubq1:1:3	7	16		1	2	13	23
pMON140818	P-CUCme.1-1:1:1rc	155	48.21		22.35	20.24	33.01	78.17
pMON140819	P-CUCme.2-1:1:1	14						
pMON140820	P-CUCme.3-1:1:3	15						
pMON140821	EXP-CUCme.4:1:1	156	96.82		28.32	39.17	322.98	280.03
pMON140822	EXP-CUCme.5:1:1	159	28.88				41.11	
pMON140823	P-CUCme.6-1:1:1	18	23.94				32.14	30.22
pMON140824	P-CUCme.8-1:1:2	19						
pMON140825	P-CUCme.9-1:1:2	20	22.06				21.22	23.08
pMON140826	P-CUCme.10-1:1:1	21						
pMON140827	EXP-CUCme.eEF1a:1:1	162	189.24	153.52	59.6	37.44	103.01	130.6
pMON140828	P-CUCme.15-1:1:2	23	30.53					
pMON140830	P-CUCme.17-1:1:2	26	51.62		30.07	31.08	30.49	60.14
pMON140831	P-CUCme.18-1:1:2	27	57.38					30.03
pMON140832	P-CUCme.19-1:1:3	167	23.07		50.21	59.73	65.58	137.42
pMON140833	P-CUCme.20-1:3	211	23.15		61.6	118.76	502.55	119.46
pMON140834	P-CUCme.21-1:1:1	30					25.49	
pMON140836	EXP-CUCme.SAMS2:1:1	168	230.89	184.88	65.44	53.36	118.82	351.49
pMON140837	P-CUCme.26-1:1:2	33	56.21		26.81	45.07	51.61	47.42
pMON140839	EXP-CUCme.29:1:2	212	82.17		45.2	28.27	64.96	109.9
pMON144926	P-CUCme.CumMe_WSM_SF25355.G 5000-1:1:1	196	28.53					

[0218]

构建体	调控元件	SEQ ID NO:	Vn5 根	R1 根	Vn5 库叶	Vn5 源叶	R1 源叶	R1 叶柄
pMON144927	P-CUCme.CumMe_WSM_SF17111.G 5790-1:1:1	177	23.62					
pMON144928	P-CUCme.CumMe_WSM_SF22531.G 5120-1:1:1	192	75.62		23	20.46	21.78	39.77
pMON144931	P-CUCme.CumMe_WSM_SF18488.G 5340-1:1:1	181	43.2					52.55
pMON144933	P-CUCme.CumMe_WSM_SF23760.G 5200-1:1:1	193	25.61		20.45	0	0	28.69
pMON146941	EXP-CUCme.WSM_SF19064.G5690: 1:1	185	33.5		0	0	24.27	47.82
pMON144932	P-CUCme.WSM_SF17252.G7330-1:1 :1	179	32.54		23.76	21.5	0	22.21
pMON146940	P-CUCme.CumMe_WSM_SF18634.G 5190-1:1:1	183	0		0	0	0	0
pMON147340	P-CUCme.CumMe_WSM_SF19647.G 5760-1:1:1	188	28.9		0	0	29.77	25.82
pMON147342	P-CUCme.CumMe_WSM_SF25936.G 5450-1:1:1	197	50.15		24.26	0	29.38	29.91
pMON147343	P-CUCme.CumMe_WSM_SF19839.G 5090-1:1:1	189	36.05		25.7	27.54	22.85	37.15
pMON144929	CumMe_WSM_SF206458.G5970	98						
pMON147304	P-CUCme.CumMe_WSM_SF18716.G 5860-1:1:1	184	35.01		21.17	21.23	22	44.57

表 21.在 R₀ 代转化的大豆植物的黄色豆荚胚、黄色豆荚子叶、R3 未成熟种子、R3 豆荚、R5 子叶和 R1 花中的平均 GUS 表达

[0219]

构建体	调控元件	SEQ ID NO:	黄色豆荚胚	黄色豆荚子叶	R3 未成熟种子	R3 豆荚	R5 子叶	R1 花
pMON138776	EXP-CUCme.Ubql:1:1	1	12	9	13	11	10	7
pMON138778	EXP-CUCme.Ubql:1:3	7	3	1	13	9	13	27
pMON140818	P-CUCme.1-1:1:1rc	155	100.79	117.5	38.31	84.72	132.27	66.8
pMON140819	P-CUCme.2-1:1:1	14					20.35	36.18
pMON140820	P-CUCme.3-1:1:3	15						
pMON140821	EXP-CUCme.4:1:1	156	86.68	225.53	105.62	342.07	119.08	184.92
pMON140822	EXP-CUCme.5:1:1	159	21.48	32.27	21.47	21.66		36.88
pMON140823	P-CUCme.6-1:1:1	18	38.75		23.03		25.32	58.7
pMON140824	P-CUCme.8-1:1:2	19					90.33	25.77
pMON140825	P-CUCme.9-1:1:2	20	132.04			20.56	34.78	
pMON140826	P-CUCme.10-1:1:1	21					22.34	
pMON140827	EXP-CUCme.eF1a:1:1	162	200.28	291.26	58.21	131.17	114.29	130.38
pMON140828	P-CUCme.15-1:1:2	23			142.24	26.2		
pMON140830	P-CUCme.17-1:1:2	26	343.34	302.94	65.55	80.94	137.02	62.7
pMON140831	P-CUCme.18-1:1:2	27	103.17	135.97	30	34.62	88.14	23.73
pMON140832	P-CUCme.19-1:1:3	167	30.96	64.46		316.66		53.46
pMON140833	P-CUCme.20-1:3	211	174.62	524.88		222.04	59.43	124.68
pMON140834	P-CUCme.21-1:1:1	30			28.15	20.52	23.89	
pMON140836	EXP-CUCme.SAMS2:1:1	168	110.23	159.43	61.99	248.96	49.17	224.24
pMON140837	P-CUCme.26-1:1:2	33	56.73	50.06	70	143.05	25.06	49.92
pMON140839	EXP-CUCme.29:1:2	212	251.76	237.2	49.16	89.28	114.92	57.84
pMON144926	P-CUCme.CumMe_WSM_SF25355.G5000-1:1:1	196			21.41		22.23	
pMON144927	P-CUCme.CumMe_WSM_SF17111.G5790-1:1:1	177	58.84	28.94			20.97	
pMON144928	P-CUCme.CumMe_WSM	192	135.62	152.48	30.45	51.71	129.72	42.2

[0220]

构建体	调控元件	SEQ ID NO:	黄色豆荚胚	黄色豆荚子叶	R3 未成熟种子	R3 豆荚	R5 子叶	R1 花
	SF22531.G5120-1:1:1							
pMON144931	P-CUCme.CumMe_WSM_SF18488.G5340-1:1:1	181	866.94		23.26	21.49		
pMON144933	P-CUCme.CumMe_WSM_SF23760.G5200-1:1:1	193			29.03	34.9	69.63	24.42
pMON146941	EXP-CUCme.WSM_SF19064.G5690:1:1	185			36.69	83.08	89.81	33.99
pMON144932	P-CUCme.WSM_SF17252.G7330-1:1:1	179			34.29	39.89	113.83	0
pMON146940	P-CUCme.CumMe_WSM_SF18634.G5190-1:1:1	183			30.25	0	0	0
pMON147340	P-CUCme.CumMe_WSM_SF19647.G5760-1:1:1	188			25.73	28.28	24.04	23.35
pMON147342	P-CUCme.CumMe_WSM_SF25936.G5450-1:1:1	197			104.02	80.27	31.06	26.8
pMON147343	P-CUCme.CumMe_WSM_SF19839.G5090-1:1:1	189						29.09
pMON144929	CumMe_WSM_SF206458.G5970	98			24.42	25.33		
pMON147304	P-CUCme.CumMe_WSM_SF18716.G5860-1:1:1	184				283.49		61.43

[0221] 从表20和21可以看出,取决于用于驱动表达的EXP序列,EXP序列EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1)、EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7)、P-CUCme.1-1:1:1rc (SEQ ID NO:155)、P-CUCme.2-1:1:1 (SEQ ID NO:14)、EXP-CUCme.4:1:1 (SEQ ID NO:156)、EXP-CUCme.5:1:1 (SEQ ID NO:159)、P-CUCme.6-1:1:1 (SEQ ID NO:18)、P-CUCme.8-1:1:2 (SEQ ID NO:

19)、P-CUCme.9-1:1:2 (SEQ ID NO:20)、P-CUCme.10-1:1:1 (SEQ ID NO:21)、EXP-CUCme.eEF1a:1:1 (SEQ ID NO:162)、P-CUCme.15-1:1:2 (SEQ ID NO:23)、P-CUCme.17-1:1:2 (SEQ ID NO:26)、P-CUCme.18-1:1:2 (SEQ ID NO:27)、P-CUCme.19-1:1:3 (SEQ ID NO:167)、P-CUCme.20-1:3 (SEQ ID NO:211)、P-CUCme.21-1:1:1 (SEQ ID NO:30)、EXP-CUCme.SAMS2:1:1 (SEQ ID NO:168)、P-CUCme.26-1:1:2 (SEQ ID NO:33)、EXP-CUCme.29:1:2 (SEQ ID NO:212)、P-CUCme.CumMe_WSM_SF25355.G5000-1:1:1 (SEQ ID NO:196)、P-CUCme.CumMe_WSM_SF17111.G5790-1:1:1 (SEQ ID NO:177)、P-CUCme.CumMe_WSM_SF22531.G5120-1:1:1 (SEQ ID NO:192)、P-CUCme.CumMe_WSM_SF18488.G5340-1:1:1 (SEQ ID NO:181)、P-CUCme.CumMe_WSM_SF23760.G5200-1:1:1 (SEQ ID NO:193)、EXP-CUCme.WSM_SF19064.G5690:1:1 (SEQ ID NO:185)、P-CUCme.WSM_SF17252.G7330-1:1:1 (SEQ ID NO:179)、P-CUCme.CumMe_WSM_SF18634.G5190-1:1:1 (SEQ ID NO:183)、P-CUCme.CumMe_WSM_SF19647.G5760-1:1:1 (SEQ ID NO:188)、P-CUCme.CumMe_WSM_SF25936.G5450-1:1:1 (SEQ ID NO:197)、P-CUCme.CumMe_WSM_SF 19839.G5090-1:1:1 (SEQ ID NO:189)、CumMe_WSM_SF206458.G5970 (SEQ ID NO:98) 和 P-CUCme.CumMe_WSM_SF18716.G5860-1:1:1 (SEQ ID NO:184) 定量地显示在一些或全部检测组织中驱动转基因表达的能力。

[0222] 所选择的组织切片的组织学分析进一步提供了许多EXP序列表达的证据。虽然定量分析显示相当低的表达水平,但EXP-CUCme.Ubq1:1:1 (SEQ ID NO:1) 和EXP-CUCme.Ubq1:1:3 (SEQ ID NO:7) 显示在所有组织中观察到染色的组成型表达模式。这类驱动要求组成型低表达水平的转基因表达的表达式可能是最偶然的。由P-CUCme.1-1:1:1rc (SEQ ID NO:155) 驱动的表达显示在库叶和源叶维管束和木质部以及在根皮、韧皮部、木质部、内皮、中柱和尖端中表达。在所有组织中观察到由EXP-CUCme.4:1:1 (SEQ ID NO:156) 驱动的表达,其中在所述植物的繁殖期中观察到最高的表达。仅在V5库叶和R1花花药中观察到由P-CUCme.10-1:1:1 (SEQ ID NO:21) 驱动的表达。由EXP-CUCme.eEF1a:1:1 (SEQ ID NO:162) 驱动的表达显示在黄色豆荚胚和子叶中观察到最高表达的组成型表达模式。在R1代中的黄色豆荚胚活性比R0代中高5倍(参见下表23)。由P-CUCme.15-1:1:2 (SEQ ID NO:23)、P-CUCme.17-1:1:2 (SEQ ID NO:26) 和P-CUCme.18-1:1:2 (SEQ ID NO:27) 驱动的表达显示组织学组成型表达水平。除所述V5根和R1叶柄之外,由P-CUCme.19-1:1:3 (SEQ ID NO:167) 驱动的表达显示组织学组成型表达模式。R3豆荚显示最高的表达。

[0223] 除在V5根的表达之外,由P-CUCme.20-1:3 (SEQ ID NO:211) 驱动的表达显示组织学组成型表达模式。R8阶段子叶中的表达最高。由EXP-CUCme.SAMS2:1:1 (SEQ ID NO:168) 驱动的表达显示在所有组织中具有组织学观察到的表达的组成型表达模式。在R1代中观察到GUS表达增加(参见下表22和23)。R1阶段花和叶柄显示在大豆中的最高表达水平。由P-CUCme.CumMe_WSM_SF22531.G5120-1:1:1 (SEQ ID NO:192) 驱动的表达显示在R8阶段子叶和胚中具有最高表达的组织学组成型表达模式。由P-CUCme.CumMe_WSM_SF18488.G5340-1:1:1 (SEQ ID NO:181) 驱动的表达显示组成型表达水平,而在黄色豆荚胚中观察到定量的高表达。

[0224] 将用包含EXP-CUCme.eEF 1a:1:1 (SEQ ID NO:162) 和EXP-CUCme.SAMS2:1:1 (SEQ ID NO:168) 的质粒构建体转化的R₀代植物结籽,并分析R₁代植物的GUS表达。分析R₁代植物在Vn5根、Vn5库叶、Vn5源叶、R1源叶、R1叶柄、黄色豆荚胚、黄色豆荚子叶、R3未成熟种子、R3

豆荚、R5子叶和R1花中的表达。表22和23显示在R₁代转化植物的每个组织中测量的平均GUS表达。

[0225]

表 22.在 R₁ 代转化的大豆植物的 Vn5 根、 Vn5 源叶、 R1 源叶、 R1 叶柄中的平均 GUS 表达

构建体	调控元件	SEQ ID NO:	Vn5 根	Vn5 库叶	Vn5 源叶	R1 源叶	R1 叶柄
pMON140827	EXP-CUCme.eEF1a:1:1	162	145.84	50.24	43.73	107.98	357.67
pMON140836	EXP-CUCme.SAMS2:1:1	168	260.41	65.52	51.12	129.86	623.42

表 23.在 R₁ 代转化的大豆植物的黄色豆荚胚、黄色豆荚子叶、 R3 未成熟种子、 R3 豆荚、 R5 子叶、 R1 花中的平均 GUS 表达

构建体	调控元件	SEQ ID NO:	黄色豆荚胚	黄色豆荚子叶	R3 未成熟种子	R3 豆荚	R5 子叶	R1 花
pMON140827	EXP-CUCme.eEF1a:1:1	162	1098.51	764.83	288.77	214.6	459.62	394.77
pMON140836	EXP-CUCme.SAMS2:1:1	168	219.04	291.58	241.48	382.73	397.91	653.23

[0226] 从上表22和23可以看出,由EXP-CUCme.eEF1a:1:1 (SEQ ID NO:162) 和EXP-CUCme.SAMS2:1:1 (SEQ ID NO:168) 在 R_1 代中驱动的表达显示,在 R_1 代许多组织中观察到相对于 R_0 代表达增加的组成型表达水平。

[0227] *****

[0228] 已经阐明并描述了本发明的原则,在不背离这种原则的情况下可以改变本发明的安排和细节,这对本领域技术人员应是显而易见的。在权利要求书的精神和范围内的所有变型均意图包括在本发明范围内。本文引用的所有出版物和公开的专利文件在此以引用方式引入,如同具体地和单独地表示每个单独的出版物或专利申请均以引用方式引入。

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[0004]	M.奥法托勒
[0005]	R.W.舒尔茨
[0006]	X.韦
[0007]	W.吴
[0008]	S-P.杨
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[7134]	ctcgactggg cagatgaaca tggcatcgtg gtgattgatg aaactgctgc tgtcggcttt 1260
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 [7152] <220>
 [7153] <221> misc_feature
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[7182] tacgtcgcca gtcaagtaac aaccgcgaaa aagttgcgcg gaggagtgtgt gtttgtggac 1560
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 [7197] aggcacgtcg tgcctcacat cgagcccgtg gctagatgca tcatccctga tctgatcgga 240
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P-CUCme.Ubq1-1:1:15 (SEQ ID NO: 2)	ATCTGAAAGGAACACCTAGCAAGGGGCTACTCTACAAGCATACTAAGTCTACAAAAGCTAG
P-CUCme.Ubq1-1:1:16 (SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17 (SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15 (SEQ ID NO: 2)	AGTTGTATGGTTATGCAGAAGACCTGGACAAAAAGAAGATCACTCGCTGCTTTTACTTTTA
P-CUCme.Ubq1-1:1:16 (SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17 (SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15 (SEQ ID NO: 2)	TCCTAAGAGGAAATGTGATTTTATGGAAGTTTAACCTATAGCCTGTAGTGGCACTATTCA
P-CUCme.Ubq1-1:1:16 (SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17 (SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15 (SEQ ID NO: 2)	CAACAAAAGTAAAGTTTATAGCCATGACTGAAGTTGTTAAAGAAGTCGCTCTGGCTAAAG
P-CUCme.Ubq1-1:1:16 (SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17 (SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15 (SEQ ID NO: 2)	GACTACTTGAAGAACTTGGCTTCTTTTAACAGTCAGTAAACATCATGTGTAGTATAAA
P-CUCme.Ubq1-1:1:16 (SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17 (SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15 (SEQ ID NO: 2)	GTGCAATACACTTGTCTAAAAATCTGCAATATCACGAAAGAAGTAAAGCATATTGATGTGA
P-CUCme.Ubq1-1:1:16 (SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17 (SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19 (SEQ ID NO: 12)	-----

图1a

P-CUCme.Ubq1-1:1:15	(SEQ ID NO: 2)	AGCTATATGTCATTAGAGAAAGTCATAGCAAAGAGAAAAAGTAACAGTATCAAAGGTTTCAGA
P-CUCme.Ubq1-1:1:16	(SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15	(SEQ ID NO: 2)	CAAAAGAAAAATGCAGCAGATATGTTGACTAAAAATAGTTACTAATGCTAAACTCGAGCACT
P-CUCme.Ubq1-1:1:16	(SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15	(SEQ ID NO: 2)	GCCTACAGTTGCTCAAGGTAATAGACTACTTTAAAGAATAGAATCAGAAGAAATAGTCAT
P-CUCme.Ubq1-1:1:16	(SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15	(SEQ ID NO: 2)	TGGTAGCAATAAAATTCAGGTGGAGGATTGTTAAAAAGAAGAGTGAATTTTATTACTTA
P-CUCme.Ubq1-1:1:16	(SEQ ID NO: 6)	-----
P-CUCme.Ubq1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15	(SEQ ID NO: 2)	AAGAAAAATCTCGGTGAAACTCGAAAGATCTCGATTGAACTCTATTGCTTAAGAACCTG
P-CUCme.Ubq1-1:1:16	(SEQ ID NO: 6)	-----TCGGTGAAACTCGAAAGATCTCGATTGAACTCTATTGCTTAAGAACCTG
P-CUCme.Ubq1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme.Ubq1-1:1:15	(SEQ ID NO: 2)	GTGAAGCTCGAGAGATCTTGATACAATCCCAGTGCCCTAACTCTTCAACAAGCTAAGCAA
P-CUCme.Ubq1-1:1:16	(SEQ ID NO: 6)	GTGAAGCTCGAGAGATCTTGATACAATCCCAGTGCCCTAACTCTTCAACAAGCTAAGCAA
P-CUCme.Ubq1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme.Ubq1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme.Ubq1-1:1:19	(SEQ ID NO: 12)	-----

图1b

P-CUCme, Ubq1-1-1:1:15	(SEQ ID NO: 2)	GTTGTACTGTGGGGCTCAATCTCGGTTCAATCTCGACGCACCTGATGCTTTGTTCCCTGT
P-CUCme, Ubq1-1-1:1:16	(SEQ ID NO: 6)	GTTGTACTGTGGGGCTCAATCTCGGTTCAATCTCGACGCACCTGATGCTTTGTTCCCTGT
P-CUCme, Ubq1-1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme, Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15	(SEQ ID NO: 2)	CTACTCGATGAAGAAGCAATTACTTCTCAGGACAACTCGGTACCCCTAAATACAGATTTT
P-CUCme, Ubq1-1-1:1:16	(SEQ ID NO: 6)	CTACTCGATGAAGAAGCAATTACTTCTCAGGACAACTCGGTACCCCTAAATACAGATTTT
P-CUCme, Ubq1-1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme, Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15	(SEQ ID NO: 2)	GAGCTTCGTGATCCTACAACTGAAATCAAAATAGAAAAACTAATAAGTTAGTTAGATTTG
P-CUCme, Ubq1-1-1:1:16	(SEQ ID NO: 6)	GAGCTTCGTGATCCTACAACTGAAATCAAAATAGAAAAACTAATAAGTTAGTTAGATTTG
P-CUCme, Ubq1-1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme, Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15	(SEQ ID NO: 2)	TTATATTTACTGCCATTAAATAAATCTGTAAATGTAATAATAAACCATTAACTCAATAT
P-CUCme, Ubq1-1-1:1:16	(SEQ ID NO: 6)	TTATATTTACTGCCATTAAATAAATCTGTAAATGTAATAATAAACCATTAACTCAATAT
P-CUCme, Ubq1-1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme, Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15	(SEQ ID NO: 2)	GAAATATAGAAATGAGAAAAAGAAAAAGTTAAAGAGAGAGAGGAGAAAACTCAT
P-CUCme, Ubq1-1-1:1:16	(SEQ ID NO: 6)	GAAATATAGAAATGAGAAAAAGAAAAAGTTAAAGAGAGAGAGGAGAAAACTCAT
P-CUCme, Ubq1-1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme, Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15	(SEQ ID NO: 2)	TTTCAAATTCTCTATACTTGTGTTGATCCTTGAATAAGTTGAATAAAGCTCTATGGCGGC
P-CUCme, Ubq1-1-1:1:16	(SEQ ID NO: 6)	TTTCAAATTCTCTATACTTGTGTTGATCCTTGAATAAGTTGAATAAAGCTCTATGGCGGC
P-CUCme, Ubq1-1-1:1:17	(SEQ ID NO: 8)	-----
P-CUCme, Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----

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P-CUCme, Ubq1-1-1:1:15 (SEQ ID NO: 2)	TTCAAAGTGGATGTAGGCACATATTAGTCGAACCAACAATAAATTTGTTATGTTCTTTTGCT
P-CUCme, Ubq1-1-1:1:16 (SEQ ID NO: 6)	TTCAAAGTGGATGTAGGCACATATTAGTCGAACCAACAATAAATTTGTTATGTTCTTTTGCT
P-CUCme, Ubq1-1-1:1:17 (SEQ ID NO: 8)	-----AGTCGAACCAACAATAAATTTGTTATGTTCTTTTGCT
P-CUCme, Ubq1-1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15 (SEQ ID NO: 2)	ATTCCTTGTAATCTCCATAAATAATTTTCTTACTAAGCTCTAGAAAATCTGCTTGTCAAGAG
P-CUCme, Ubq1-1-1:1:16 (SEQ ID NO: 6)	ATTCCTTGTAATCTCCATAAATAATTTTCTTACTAAGCTCTAGAAAATCTGCTTGTCAAGAG
P-CUCme, Ubq1-1-1:1:17 (SEQ ID NO: 8)	ATTCCTTGTAATCTCCATAAATAATTTTCTTACTAAGCTCTAGAAAATCTGCTTGTCAAGAG
P-CUCme, Ubq1-1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15 (SEQ ID NO: 2)	ATTAGGTATCATTTATGCTTTTATATTTTCCCTTCGGTTGCATAATCTTGAGCTAGTTAAG
P-CUCme, Ubq1-1-1:1:16 (SEQ ID NO: 6)	ATTAGGTATCATTTATGCTTTTATATTTTCCCTTCGGTTGCATAATCTTGAGCTAGTTAAG
P-CUCme, Ubq1-1-1:1:17 (SEQ ID NO: 8)	ATTAGGTATCATTTATGCTTTTATATTTTCCCTTCGGTTGCATAATCTTGAGCTAGTTAAG
P-CUCme, Ubq1-1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15 (SEQ ID NO: 2)	ATCGAGAGGTTACTGTTGTTGAAACCGAGATTAGTATCTTTGGATTAAACACGTCCTACC
P-CUCme, Ubq1-1-1:1:16 (SEQ ID NO: 6)	ATCGAGAGGTTACTGTTGTTGAAACCGAGATTAGTATCTTTGGATTAAACACGTCCTACC
P-CUCme, Ubq1-1-1:1:17 (SEQ ID NO: 8)	ATCGAGAGGTTACTGTTGTTGAAACCGAGATTAGTATCTTTGGATTAAACACGTCCTACC
P-CUCme, Ubq1-1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15 (SEQ ID NO: 2)	AAAATTTGAAATTTTGTTATTTACCCCATTCATTGGATAATAAGCAATTCCTTATAGTGTTA
P-CUCme, Ubq1-1-1:1:16 (SEQ ID NO: 6)	AAAATTTGAAATTTTGTTATTTACCCCATTCATTGGATAATAAGCAATTCCTTATAGTGTTA
P-CUCme, Ubq1-1-1:1:17 (SEQ ID NO: 8)	AAAATTTGAAATTTTGTTATTTACCCCATTCATTGGATAATAAGCAATTCCTTATAGTGTTA
P-CUCme, Ubq1-1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19 (SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:1:15 (SEQ ID NO: 2)	TCAATTAAACTCCTATAAAGTGTAATAATTGAATCCATGAACTATTTTCATATGTAATCT
P-CUCme, Ubq1-1-1:1:16 (SEQ ID NO: 6)	TCAATTAAACTCCTATAAAGTGTAATAATTGAATCCATGAACTATTTTCATATGTAATCT
P-CUCme, Ubq1-1-1:1:17 (SEQ ID NO: 8)	TCAATTAAACTCCTATAAAGTGTAATAATTGAATCCATGAACTATTTTCATATGTAATCT
P-CUCme, Ubq1-1-1:1:18 (SEQ ID NO: 10)	-----
P-CUCme, Ubq1-1-1:1:19 (SEQ ID NO: 12)	-----

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P-CUCme. Ubq1-1-1:1:15	(SEQ ID NO: 2)	TAATAAAATGAATTTAGAAGTTTAAATTAATAATAATATATTTTGTATGCTATTTTTCAAAG
P-CUCme. Ubq1-1-1:1:16	(SEQ ID NO: 6)	TAATAAAATGAATTTAGAAGTTTAAATTAATAATAATATATTTTGTATGCTATTTTTCAAAG
P-CUCme. Ubq1-1-1:1:17	(SEQ ID NO: 8)	TAATAAAATGAATTTAGAAGTTTAAATTAATAATAATATATTTTGTATGCTATTTTTCAAAG
P-CUCme. Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme. Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme. Ubq1-1-1:1:15	(SEQ ID NO: 2)	TTTGAAGAAATGTGTTAAATTGATACACATACAAAAATCTAGGTTTTCATGAAAAACTAT
P-CUCme. Ubq1-1-1:1:16	(SEQ ID NO: 6)	TTTGAAGAAATGTGTTAAATTGATACACATACAAAAATCTAGGTTTTCATGAAAAACTAT
P-CUCme. Ubq1-1-1:1:17	(SEQ ID NO: 8)	TTTGAAGAAATGTGTTAAATTGATACACATACAAAAATCTAGGTTTTCATGAAAAACTAT
P-CUCme. Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----
P-CUCme. Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme. Ubq1-1-1:1:15	(SEQ ID NO: 2)	GGAAGTGAAGATAGCATCTAATATTTTATGACACAAAAATGCAAACTAATATATAAAGGA
P-CUCme. Ubq1-1-1:1:16	(SEQ ID NO: 6)	GGAAGTGAAGATAGCATCTAATATTTTATGACACAAAAATGCAAACTAATATATAAAGGA
P-CUCme. Ubq1-1-1:1:17	(SEQ ID NO: 8)	GGAAGTGAAGATAGCATCTAATATTTTATGACACAAAAATGCAAACTAATATATAAAGGA
P-CUCme. Ubq1-1-1:1:18	(SEQ ID NO: 10)	-----TGACACAAAAATGCAAACTAATATATAAAGGA
P-CUCme. Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme. Ubq1-1-1:1:15	(SEQ ID NO: 2)	TTTAATTAATTTTATAGGTTTCAAATTTGTTAGACTTGTCAAATACAAAATTTTATTGA
P-CUCme. Ubq1-1-1:1:16	(SEQ ID NO: 6)	TTTAATTAATTTTATAGGTTTCAAATTTGTTAGACTTGTCAAATACAAAATTTTATTGA
P-CUCme. Ubq1-1-1:1:17	(SEQ ID NO: 8)	TTTAATTAATTTTATAGGTTTCAAATTTGTTAGACTTGTCAAATACAAAATTTTATTGA
P-CUCme. Ubq1-1-1:1:18	(SEQ ID NO: 10)	TTTAATTAATTTTATAGGTTTCAAATTTGTTAGACTTGTCAAATACAAAATTTTATTGA
P-CUCme. Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme. Ubq1-1-1:1:15	(SEQ ID NO: 2)	ACCAAATACATACAAACATCAAAATTAAGAACAGAAAACTAAATTCAAATGAAATTTAT
P-CUCme. Ubq1-1-1:1:16	(SEQ ID NO: 6)	ACCAAATACATACAAACATCAAAATTAAGAACAGAAAACTAAATTCAAATGAAATTTAT
P-CUCme. Ubq1-1-1:1:17	(SEQ ID NO: 8)	ACCAAATACATACAAACATCAAAATTAAGAACAGAAAACTAAATTCAAATGAAATTTAT
P-CUCme. Ubq1-1-1:1:18	(SEQ ID NO: 10)	ACCAAATACATACAAACATCAAAATTAAGAACAGAAAACTAAATTCAAATGAAATTTAT
P-CUCme. Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----
P-CUCme. Ubq1-1-1:1:15	(SEQ ID NO: 2)	TAA TAGAAAAATTAGAAAAAAGAAAAAGAAAAATAAAGGAATCGTATTGTTTTTCCTTC
P-CUCme. Ubq1-1-1:1:16	(SEQ ID NO: 6)	TAA TAGAAAAATTAGAAAAAAGAAAAAGAAAAATAAAGGAATCGTATTGTTTTTCCTTC
P-CUCme. Ubq1-1-1:1:17	(SEQ ID NO: 8)	TAA TAGAAAAATTAGAAAAAAGAAAAAGAAAAATAAAGGAATCGTATTGTTTTTCCTTC
P-CUCme. Ubq1-1-1:1:18	(SEQ ID NO: 10)	TAA TAGAAAAATTAGAAAAAAGAAAAAGAAAAATAAAGGAATCGTATTGTTTTTCCTTC
P-CUCme. Ubq1-1-1:1:19	(SEQ ID NO: 12)	-----

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P-CUCme, Ubq1-1-1:15	(SEQ ID NO: 2)	CTTTTCCCAATTGAGAGGTGAATAAAGCTAATTGAGCTGCTCTAACTTCCTAAATCTTTA
P-CUCme, Ubq1-1-1:16	(SEQ ID NO: 6)	CTTTTCCCAATTGAGAGGTGAATAAAGCTAATTGAGCTGCTCTAACTTCCTAAATCTTTA
P-CUCme, Ubq1-1-1:17	(SEQ ID NO: 8)	CTTTTCCCAATTGAGAGGTGAATAAAGCTAATTGAGCTGCTCTAACTTCCTAAATCTTTA
P-CUCme, Ubq1-1-1:18	(SEQ ID NO: 10)	CTTTTCCCAATTGAGAGGTGAATAAAGCTAATTGAGCTGCTCTAACTTCCTAAATCTTTA
P-CUCme, Ubq1-1-1:19	(SEQ ID NO: 12)	-----
P-CUCme, Ubq1-1-1:15	(SEQ ID NO: 2)	TGCTTTCCCAATAAAGCTTTCCCAACTGCGCGTAATCGTATAAAATGGAATAATGACCTTT
P-CUCme, Ubq1-1-1:16	(SEQ ID NO: 6)	TGCTTTCCCAATAAAGCTTTCCCAACTGCGCGTAATCGTATAAAATGGAATAATGACCTTT
P-CUCme, Ubq1-1-1:17	(SEQ ID NO: 8)	TGCTTTCCCAATAAAGCTTTCCCAACTGCGCGTAATCGTATAAAATGGAATAATGACCTTT
P-CUCme, Ubq1-1-1:18	(SEQ ID NO: 10)	TGCTTTCCCAATAAAGCTTTCCCAACTGCGCGTAATCGTATAAAATGGAATAATGACCTTT
P-CUCme, Ubq1-1-1:19	(SEQ ID NO: 12)	-----TCGTATAAAATGGAATAATGACCTTT
P-CUCme, Ubq1-1-1:15	(SEQ ID NO: 2)	CCAACTAGATTCTTCCAGAACTAAACAAATACGTAAACACGCAAGTAATCAAAGACACGTTT
P-CUCme, Ubq1-1-1:16	(SEQ ID NO: 6)	CCAACTAGATTCTTCCAGAACTAAACAAATACGTAAACACGCAAGTAATCAAAGACACGTTT
P-CUCme, Ubq1-1-1:17	(SEQ ID NO: 8)	CCAACTAGATTCTTCCAGAACTAAACAAATACGTAAACACGCAAGTAATCAAAGACACGTTT
P-CUCme, Ubq1-1-1:18	(SEQ ID NO: 10)	CCAACTAGATTCTTCCAGAACTAAACAAATACGTAAACACGCAAGTAATCAAAGACACGTTT
P-CUCme, Ubq1-1-1:19	(SEQ ID NO: 12)	CCAACTAGATTCTTCCAGAACTAAACAAATACGTAAACACGCAAGTAATCAAAGACACGTTT
P-CUCme, Ubq1-1-1:15	(SEQ ID NO: 2)	CATTTTCCTATAGAATATTATAGTTATTTCGTGATTAAACGGAAGTCGGCAATTTTAGGTAT
P-CUCme, Ubq1-1-1:16	(SEQ ID NO: 6)	CATTTTCCTATAGAATATTATAGTTATTTCGTGATTAAACGGAAGTCGGCAATTTTAGGTAT
P-CUCme, Ubq1-1-1:17	(SEQ ID NO: 8)	CATTTTCCTATAGAATATTATAGTTATTTCGTGATTAAACGGAAGTCGGCAATTTTAGGTAT
P-CUCme, Ubq1-1-1:18	(SEQ ID NO: 10)	CATTTTCCTATAGAATATTATAGTTATTTCGTGATTAAACGGAAGTCGGCAATTTTAGGTAT
P-CUCme, Ubq1-1-1:19	(SEQ ID NO: 12)	CATTTTCCTATAGAATATTATAGTTATTTCGTGATTAAACGGAAGTCGGCAATTTTAGGTAT
P-CUCme, Ubq1-1-1:15	(SEQ ID NO: 2)	AAATACGTGAATTCTCGAGCGCTAATTT
P-CUCme, Ubq1-1-1:16	(SEQ ID NO: 6)	AAATACGTGAATTCTCGAGCGCTAATTT
P-CUCme, Ubq1-1-1:17	(SEQ ID NO: 8)	AAATACGTGAATTCTCGAGCGCTAATTT
P-CUCme, Ubq1-1-1:18	(SEQ ID NO: 10)	AAATACGTGAATTCTCGAGCGCTAATTT
P-CUCme, Ubq1-1-1:19	(SEQ ID NO: 12)	AAATACGTGAATTCTCGAGCGCTAATTT

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