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野生大豆未成熟种子总mRNA的分离及其cDNA的分子克隆[1)]

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摘要 用氯化锂沉淀法从野生大豆(G.soja)未成熟种子中制备总RNA，经oligo(dT)-纤维素柱亲和 层析，获得总mRNA，在兔网织红细胞体系中表现出一定翻译活性。以总mRNA为模板，oligo(dT)12 18 为引物，反转录酶催化合成第一链cDNA，RNaseH-DNA聚合酶I协同合成第二链cDN A。双链cDNA的长度大约为200-5000bp，且不存在发夹结构。将双链cDNA修补后钝端连接到p UC19质粒的SmαI位点，转化E.coli JM107，获得800多个白色重组子克隆。快速电泳检测 及酶切分析表明，多数重组子带有插入片段，其中3个重组子的插入片段长度大致为1700bp 、2600bp和1400bp。

关键词 野生大豆,mRNA,cDNA,克隆

分类号

Isolation of Total mRNA from the Immature Beijing Wild Soybean Seeds and Molecul ar Cloning of Its Complementary DNA

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Abstract

Total RNA of the immature Beijing wild soybean seeds was isolated by LiCl precip itation method.Total mRNA was purified by oligo (dT)-cellulose affinity chromato graphy.The mRNA thus prepared showed enough translation activity when assayed in the rabbit reticulocyte cell-free system in vitro.Using the total mRNA as templ ate and oligo(dT)12-18 as primer,the first strand and second strand cDNA were sy nthesized respectively by AMV reverse transcriptase and RNase H-DNA polymerase I .The length of the double stranded cDNA synthesized was about 200-5000bp .the ds -cDNA with perfect plunt ends was inserted into the smal site of pUC19 by blunt end ligation. The resultant recombinant molecules sere used to transform E.coli strain JM107,and more than 800 white clones were obtained.Repid electrophoresis and EcoRI-HindIII digestion analysis showed that most of the recombinant plasmi nds from the white clones contained insertions with various lengths and 3 out of 4 tested were about 1700,2600 and 1400bp in length respectively.

Key words Wild soybean mRNA cDNA Cloning

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