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柑橘CAPS 标记和AS-PCR 引物的开发

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Development of CAPS Markers and Allele-specific PCR Primers in Citrus

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摘要 以从GenBank中下载的93 955条甜橙[*Citrus sinensis*（L.）Osbeck]EST序列为源序列，利用QualitySNP软件包从中挖掘出1 521个单核苷酸多态性（single nucleotide polymorphism，SNP）候选位点。进一步利用酶切位点查找软件WatCut进行分析，发现其中125个SNP为酶切扩增多态性序列（cleaved amplified polymorphic sequences，CAPS）位点。随机挑选40个CAPS候选位点设计引物，对12个含多种类型的柑橘品种进行分型，以此验证各标记的有效性。同时，还挑选了25个经生物信息学预测可导致其编码蛋白氨基酸序列改变的SNP位点，分别设计出一组等位基因特异PCR（allele specific PCR，AS-PCR）引物，以6个不同类型柑橘品种的DNA为模板进行PCR扩增，扩增产物采用琼脂糖凝胶电泳进行检测，筛选多态性引物。结果显示，40个CAPS候选位点中有26个位点具有酶切扩增多态性，且分型结果稳定，可作为CAPS标记。此外，还筛选获得15组在不同柑橘品种间检测到多态性的AS-PCR引物，重复性好，可有效用于柑橘品种的SNP分型。

关键词： 柑橘 表达序列标签 单核苷酸多态性 酶切扩增多态性序列 等位基因特异PCR

Abstract： The objective of this study was to develop CAPS markers and AS-PCR primers that can be used as molecular genetic markers in cultivar identification and genetic diversity studies. With the QualitySNP software package，1 521 putative single nucleotide polymorphism（SNP）sites were identified among the 93 955 sweet orange [*Citrus sinensis*（L.）Osbeck] expressed sequence tags（ESTs）downloaded from the GenBank. Furthermore，those ESTs containing the putative SNP sites were analysed with WatCut program and 125 putative Cleaved Amplified Polymorphic Sequences（CAPS）sites were obtained. Forty ESTs with CAPS sites were randomly chosen to design primer pairs，with which PCR amplifications were performed and then the amplification products were digested with restriction enzymes. Meanwhile，25 sets of AS-PCR primers were designed according to other 25 putative SNP that change the deduced amino acid type. Afterwards，6 citrus cultivars with different genotypes were used for the validation of each SNP site. As a result，26 CAPS sites with cleaved amplified polymorphism among 12 citrus cultivars were identified，all of them can be used as CAPS markers. Furthermore，fifteen sets of Allele-specific PCR primers with good reproducibility and polymorphism were obtained，which can be applied for further SNP genotyping in citrus. This study indicated that it is feasible to develop CAPS markers and AS-PCR primers based on public citrus EST sequences.

Keywords： citrus， expressed sequence tags（EST）， single nucleotide polymorphism（SNP）， cleaved amplified polymorphic sequences（CAPS）， allele specific PCR

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