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# 虫草属EST-SSR标记系统的建立研究

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中文摘要:目的:通过球孢虫草、蛹虫草EST设计EST-SSR引物,建立虫草属EST-SSR标记系统。 方法 :从NCBI公共数据库下载获得虫草EST,利用Sequece Seiners 1.2软件去除冗余序列并设计引物,进行PAGE电泳。 结果 :通过去除EST总序列中低质量的和冗余的序列后,得到全长为2 953 173 bp的4 556条无冗余球孢虫草EST。从中发掘出718个EST-SSR,分布于616条EST中,出现频率是15.8%。平均分布频率是每4 096 bp出现1个,三核苷酸重复序列有419个,是出现最多的重复类型。蛹虫草EST去冗余后得到1 363条无冗余EST,共含有1 117个EST-SSR,出现频率为81.95%,出现最多的重复类型是A核苷酸重复。根据球孢虫草EST-SSR序列,设计合成50对引物,有扩增产物的引物为34对,占总设计引物数的68%。根据蛹虫草EST-SSR,设计合成40对引物,有扩增产物的引物为39对,占总设计引物数的97.5%。基于SSR标记进行聚类分析,7种虫草无性型均能分开,且分为4支。 结论 :虫草属EST-SSR出现频率较高、类型较丰富、多态性潜能较高,具有较高的利用价值。球孢虫草和蛹虫草EST开发的SSR标记在虫草属有较好的转移性与通用性,可以很好的应用于虫草种间遗传关系的研究。应用虫草物种EST建立分子标记是一条简便而又有效的途径。

中文关键词:虫草属 球孢虫草 蛹虫草 EST-SSR 引物设计

## Study of EST-SSR marker system of *Cordyceps*

**Abstract:**Objective : To establish the EST-SSR marker system for *Cordyceps* by using ESTs of *C. bassiana* and *C. militaris*. Method : The ESTs of *Cordyceps* were downloaded from the public database of NCBI, and the redundant ESTs with low quality were removed. The EST-SSR primers were designed by Sequece Seiner 1.2. And the primers were screened through PAGE-Electrophoresis. Result : The 4 556 non-redundant ESTs which from *C. bassiana* with total length of 2 953 173 bp were selected. 718 EST-SSRs distributed in 616 ESTs were totally screened out, accounting for 15.8%of the non-redundant ESTs. It was discovered that the average distance of EST-SSSR was 1/4 096 bp in EST-SSRs distribution of *C. bassiana*. Trinucleotide repeats were the most abundant types with 419 repeated sequences. Regarding to *C. militaris*, totally 1 363 non-redundant ESTs were acquired, from which 1 117 EST-SSRs were screened, and rate of SSR sites in ESTs was 81.95%. The leading motif of SSR was nucleotide A. The 50 pairs of EST-SSR primers were designed according to the ESTs of *C. bassiana*,and preliminary test showed the 34 pairs of primers amplified clear fragments,accounting for 68% of all primers. Furthermore, the 39 of the 40 pairs of primers from the ESTs of *C. militaris* were found to be amplified as the clear fragments, accounting for 97.5%. The phylogenetic analysis revealed that different anamorph of *Cordyceps* spieces were divided into four branches. Conclusion : The EST-SSR of *Cordyceps* had comparably higher utility value. The EST-SSR markers developed from ESTs of *C. bassiana* and *C.militaris* had well transferability in *Cordyceps*. And it was suggested that the EST-SSR markers should be an easy and effective way to assay molecular genetic structure of *Cordyceps*.

**keywords:***Cordyceps* *Cordyceps bassiana* *Cordyceps militaris* EST-SSR primer design

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