

## 波纹唇鱼微卫星分子标记的筛选及适用性分析

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中文摘要: 采用磁珠富集法及利用生物素标记的(CA)<sub>15</sub>寡核苷酸探针从波纹唇鱼(*Cheilinus undulatus*)基因组DNA Bsp143 I 酶切位点的400~1000 bp 片段中筛选CA/GT 微卫星位点。洗脱的杂交片段克隆到PMD18-T 载体上构建富集微卫星基因组文库后, 通过PCR 筛选出阳性克隆进行测序。在120 条序列中有88 条序列包含有重复次数不少于5 次的微卫星位点, 阳性序列比例达到73.33%。其中完美型(perfect)类型微卫星最多的重复次数为26 次。在88 条非冗长序列中, 共有28 条(31.82%)微卫星重复序列两端有侧翼序列能够进行引物设计。用波纹唇鱼3 个个体的混合基因进行引物筛选, 其中的24 对具有清晰的扩增条带。将筛选的24 对引物对波纹唇鱼1 个群体的39 个个体进行遗传多样性分析, 其中4 对引物扩增产物为单态, 20 对扩增产物呈多态性; 20 对扩增多态的引物在39 个个体中扩增出等位基因数为2~12, 平均有效等位基因数为3.5。该波纹唇鱼群体的PIC、H<sub>o</sub>、H<sub>e</sub> 的平均值分别为0.0782、0.8513、0.5667。这20 个微卫星位点适于波纹唇鱼群体遗传结构的研究分析。

中文关键词: [波纹唇鱼\(Cheilinus undulatus\)](#) [微卫星分子标记](#) [筛选](#) [磁珠富集法](#)

## Screening and suitability analysis of microsatellite markers in *Cheilinus undulatus*

**Abstract:** Genomic DNA of *Cheilinus undulatus* was digested by restriction endonuclease Bsp143 I and electrophoresed on agarose gel. The DNA fragments from 400 to 1000 bp were recovered and ligated to Bsp143 I adaptor. Purified and adaptor ligated fragments were hybridized to biotin-labelled (CA)<sub>15</sub> probe and captured by Streptavidin-coated magnetic beads. Target fragments were eluted, and PCR amplified, then the purified PCR products were inserted to PMD18-T vector and transformed into Top 10 component cell. Positive clones in the enriched genomic DNA bank were screened out through PCR method and sequenced. Of 120 positive clones, 88 sequences contained repetition that repeated no less than 5 times, none redundant sequences were found after the multiple sequence alignment analysis, and the 88 non-redundant microsatellite-contained sequences, of which about 73.33% contained positive sequence, and the largest repeat number of perfect type microsatellite was 26. Among the 88 non-redundants, 28 (or 31.82%) had enough flanking region (>150 bp), which is enough to design microsatellite primer pairs. 28 primer pairs were synthesized and tested with the compound DNA of 3 *C. undulatus* individuals. 24 loci were produced with clear bands. A population of 39 individuals were tested with the 24 loci. 20 loci revealed polymorphic, while 4 loci was monomorphic. The primer pairs amplified the loci with relatively high numbers of alleles ranging from 2 to 12 with an average of 3.5 per locus among 20 ployomorphic loci. The polymorphism information content (PIC), observed heterozygosity (H<sub>o</sub>) and expected heterozygosity (H<sub>e</sub>) were 0.0782, 0.8513, and 0.5667, respectively. The 20 ployomorphic loci could be useful for the analysis of population structure from *C. undulatus*.

**keywords:** [Cheilinus undulatus](#) [microsatellite molecular markers](#) [screening](#) [magnetic-bead enrichment method](#)

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