第 13 卷第 5 期
2006 年 9 月

中 国 水 产 科 学
Journal of Fishery Sciences of China

Vol. 13 No. 5
September 2006

磁珠富集法筛选虾夷扇贝微卫星序列

赵莹莹[1,2]，朱晓琛[1,2]，孙效文[1]，梁利群[1]

(1. 中国水产科学研究院 黑龙江水产研究所，黑龙江 哈尔滨 150070；2. 大连水产学院 生命科学与技术学院，辽宁 大连 116023)

摘要：采用生物素—磁珠吸附微卫星富集法，筛选虾夷扇贝(*Patinopecten yessoensis*)微卫星分子标记，并用同位素法进行二次筛选。结果在筛选的 192 个菌落中获得 136 个阳性克隆，经测序分析，获得微卫星序列 179 个，其中完美型占 50.8%，非完美型 43.0%，混合型占 6.1%。除探针中使用的 CA 重复外，还得到 TC、AG、ACA、CTAT 的重复序列，用引物设计软件 Primer Premier5.0 设计引物 85 对，挑选其中的 40 对合成并进行筛选。[中国水产科学，2006，13(5)：749－755]

关键词：磁珠富集；虾夷扇贝；微卫星

中图分类号：Q959.215　　**文献标识码**：A　　**文章编号**：1005－8737－(2006)05－0749－07

虾夷扇贝(*Patinopecten yessoensis*)为冷水性贝类，是世界最重要的养殖经济贝类之一，原产于俄罗斯千岛群岛的南部水域，日本北海道及本洲北部。中国于 20 世纪 80 年代初从日本引种，并开始养殖，目前已经在黄海北部形成规模化和产业化养殖，其中以大连长海养殖规模最大。当前国产鲜活扇贝主要以国内销售为主，海外市场主要以加工产品为主，扇贝产品易加工、味道鲜美、价格适宜，将会有更为广阔的发展空间。由于经过连续多代的人工养殖，虾夷扇贝养殖群体内已经出现一定的近交现象[1]。

微卫星标记，亦称作简单序列重复(Simple sequence repeats，SSRs)或简单序列长度多态性(Simple sequence length polymophisms，SSLPs)，同其他遗传分子标记相比，微卫星 DNA 标记不仅具有极高的个体特异性、可重复性，而且能够提供丰富的多态位点及基因座位杂合度和纯合度等遗传信息。目前，微卫星 DNA 标记在群体进化研究、核基因组研究、遗传连锁图谱的构建[2]、亲缘关系鉴定、基因定位、品种鉴定等领域，都已得到广泛的应用[3－4]。很多海洋生物，如大西洋鲑(*Salmo salar*)[5]、真鲷(*Pagrus major*)[6]、中国对虾(*Penaeus chinensis*)[7]等已经克隆出很多微卫星标记，并且已经应用于育种以及种群遗传学研究。在扇贝的微卫星研究中，李红蕾等[8]在栉孔扇贝(*Chlamys farreri*)的 6 935 条 ESTs 库中发现了 42 条微卫星序列。但是作为重要经济贝类之一的虾夷扇贝，其微卫星分子标记的筛选和分离至今还未见报道。用生物素包被的磁珠富集法分离微卫星分子标记是一种简单高效的方法[9]，已经应用于一些植物和动物微卫星分子标记的分离[10]，但在贝类的应用还未见报道。本研究采用新型的生物素－磁珠吸附微卫星富集法与放射性同位素杂交法相结合的方式，构建虾夷扇贝部分基因组微卫星文库，计算机软件设计并筛选可作为分子标记的 PCR 引物，以期为虾夷扇贝的种质保护、改良和提高提供技术手段。

1　材料和方法

1.1　虾夷扇贝总 DNA 的提取和酶切、特定片段大小 DNA 片段的回收

样品采自辽宁大连，新鲜的虾夷扇贝闭壳肌，每 0.1 g 加 0.2 mL 裂解液[0.5%十二烷基肌氨酸钠，200 μg/mL Protainase K，0.2 mol/L EDTA(pH 8.0)]50 ℃温育 3～4 h 后用酚、氯仿、异戊醇混合液(体积比为 25:24:1)抽提 2 遍，无水乙醇沉淀，70%

收稿日期：2005－08－24；修订日期：2005－10－31.
基金项目：国家重大基础研究计划项目(2004CB117405).
作者简介：赵莹莹(1980－)，女，硕士研究生，从事水产动物遗传育种研究.
通讯作者：孙效文. E-mail:sunxw2002@163.com，Tel:0451－84842646

乙醇洗涤，自然干燥后加100 μL 0.1×TE溶解。取100 μg虾夷扇贝总DNA，用限制性内切酶 *Sau* 3AI部分酶切，使酶切片段大部分在250～750 bp，有机抽提法终止反应，无水乙醇沉淀回收，溶于200 μL 0.1×TE。参照《分子克隆》进行蔗糖密度梯度离心[11]，分离250～750 bp大小的片段，透析，乙醇沉淀回收备用。

1.2 接头的制备及连接

用单链的寡核苷酸制备接头，每种单链寡核苷酸的浓度均为50 μmol/L。本实验采用Brown接头：等比例混合两组寡聚核苷酸链A（5'－GATCGTCGACGGTACCGAATTCT－3'）和B（5'－GTCAAGAATTCGGTACCGTCGAC－3'），95 ℃变性10 min，然后经过4 h缓慢冷却至10 ℃，最终形成的双链接头为：

5' GATCGTCGACGGTACCGAATTCT A
3' CAGCTGCCATGGCTTAAGAACTG B
Sau 3AI－－*Sal*I－－*Eco*RI

建立20 μL的连接体系，其中包含0.4 mol/L酶切片段，0.5 mol/L接头，0.1 mol/L T_4 DNA连接酶，于16 ℃水浴中过夜连接12～14 h。用旋离柱（centrifugal concentrators，PALL FILTRON公司产品）离心去除多余的接头，并最终浓缩到15 μL左右。

1.3 利用接头连接片段构建"基因组PCR文库"

用连有接头的DNA片段作为模板，寡核苷酸链B作为引物，进行PCR扩增（PE9700型PCR仪），创建基因组PCR文库。程序为94 ℃ 预变性3 min；94 ℃ 1 min，60 ℃ 1 min，72 ℃ 1 min，20个循环；最后72 ℃延伸10 min。反应完毕后，过旋离柱以去除多余的引物和没有参加反应dNTP，并浓缩到15 μL左右。

1.4 微卫星文库的构建

1.4.1 杂交 根据实验材料建立50 μL反应体系：1.5 μL（10 μmol/L）探针（为生物素标记的$(CA)_{15}$），5 μL（50 μmol/L）引物（为合成接头的寡核苷酸链B），15 μL 20×SSC，0.5 μL 10% SDS，16 μL ddH_2O，混合，68 ℃预热。将12 μL（276 ng）DNA 95 ℃变性5 min加入预热的杂交混合液，68 ℃杂交1 h。杂交过程中平衡磁珠。

1.4.2 磁珠的平衡 将磁珠轻轻摇匀，吸出100 μL到500 μL的硅化离心管中，放在磁力架上（MPC）1～2 min，轻轻吸出盐溶液。用200 μL B&W洗液（10 mmol/L Tris-Cl，1 mmol/L EDTA，2 mol/L NaCl）洗涤2次，再用200 μL洗液Ⅰ（6×SSC，0.1% SDS）反复洗涤平衡，直到磁珠表面变得顺滑易洗脱。加入200 μL洗液Ⅰ，室温放置直到杂交完毕。

1.4.3 磁珠吸附富集 磁珠上包被有链霉亲和素（Streptavidin），可与探针上的生物素结合，从而通过磁力将探针连同所需的微卫星序列一同分离出来。将杂交完毕的杂交液加入平衡好的磁珠中，25 ℃温育20 min，并轻轻摇动，使生物素和链霉亲和素充分结合。温育后将离心管放置到磁力架上，去除溶液。依次用洗液Ⅰ（6×SSC，0.1% SDS）、洗液Ⅱ（3×SSC，0.1% SDS）、洗液Ⅲ（6×SSC）洗涤磁珠，去除不含微卫星的序列。洗涤方法是：洗液Ⅰ在室温洗2次，每次静置10 min；洗液Ⅱ在68 ℃洗2次，每次静置15 min；洗液Ⅲ在室温快速洗2次。

1.4.4 捕获含有微卫星序列的单链DNA 用200 μL 0.1×TE在室温快速洗2次，加入50 μL 0.1×TE，95 ℃变性10 min，释放出含有微卫星序列的单链DNA，放在磁力架上吸出备用。

1.5 PCR扩增含有微卫星序列的DNA片段

PCR反应体系25 μL，内含4种dNTP的混合PCR缓冲液18 μL，引物（为寡核苷酸链B）0.5 μL，*Taq* DNA聚合酶0.5 μL，根据DNA模板的浓度加入不多于4 μL的模板，加无菌水补足25 μL，PE9700型PCR仪进行扩增。程序为94 ℃预变性3 min；94 ℃ 1 min，60 ℃ 1 min，72 ℃ 1 min，20个循环；最后72 ℃延伸10 min。反应完毕后，过旋离柱以去除多余的引物和没有参加反应的dNTP，浓缩到15 μL左右。电泳检测，并定量。

1.6 连接T－载体与克隆

建立10 μL连接反应体系：2×连接酶缓冲液5 μL，pGEM-T vector 1 μL（购于Promega公司，随载体提供连接酶），插入DNA片段2 μL，T_4 DNA连接酶1 μL（3 U/μL），加无菌去离子水补足10 μL，同时T载体自身连接作为对照。4 ℃连接过夜。用$CaCl_2$制备的感受态大肠杆菌（*Escherichia coli*）DH5α（购于上海博彩生物科技有限公司）进行转化，得到微卫星基因组文库。

1.7 原位杂交，用同位素探针进行二次筛选

通过原位杂交对微卫星文库进行二次筛选。将转化所得的克隆转化到杂交膜上，同时保留完全相同大小的菌板，以待杂交结果出来后挑取阳性克隆。

用同位素标记的$(CA)_{15}$进行杂交，压 X 光片，-70 ℃ 放射自显影 3 d，显影后挑取阳性克隆进行测序分析。

1.8　序列分析与 PCR 引物设计及筛选

经两轮筛选的阳性克隆菌挑到 96 孔培养板中，北京诺塞基因组研究中心有限公司测序，返回序列采用 Premier Primer 5.0 软件包进行引物设计。

2　结果

2.1　克隆及测序结果

挑选 192 个阳性克隆进行测序，成功测序 136 个，其中只有 2 个没有微卫星序列，微卫星含量达到 98.53%。共得到 179 个重复次数在 5 次以上的微卫星序列，除探针中使用的 CA 重复外，还得到 TC、AG、ACA、CTAT 的重复序列。虾夷扇贝微卫星的重复次数主要集中在 20～60，最高达到 133 次重复。根据 Weber 提出的标准[12]，按照微卫星核心序列排列方式的差异，将虾夷扇贝的微卫星序列分为完美型(perfect)、非完美型(imperfect)和混合型(compound)等 3 种类型。其中完美型 91 个，占 50.8%；不完美型 77 个，占 43.0%；混合型 11 个，占 6.1%。

2.2　引物设计与扩增结果

采用 Premier Primer 5.0 软件包进行引物设计，其中有 47 个序列由于侧翼序列不完全无法设计，共设计引物 85 对，采用常规 PCR[13]对合成的 40 对引物进行筛选，有 34 对引物可扩增出目的片段，可以用于虾夷扇贝的遗传学分析，图 1 为 HLJX-007 引物对虾夷扇贝基因组 DNA PCR 扩增电泳图，序列合成及筛选结果见表 1。

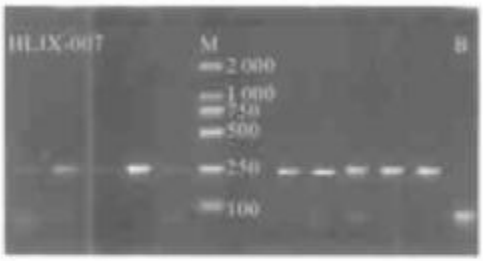

图 1　微卫星引物 HLJX－007 对虾夷扇贝基因组 DNA PCR 扩增电泳图

M：为分子量标准；B：对照

Fig.1　Genomic DNA of Japanese scallop by primer HLJX-007

M: DL2000 molecular marker; B: Blank

3　讨论

微卫星序列的获得主要有两种途径：(1)从已知的核酸序列中进行检索，或者根据其他物种已知微卫星的侧翼序列设计引物，但是可检索的资源相对有限，而且工作量大、不能筛选出该物种特有的微卫星；(2)用经典的分子生物学方法构建富含有微卫星位点的基因组文库，通过杂交筛选出含有微卫星序列的阳性克隆。

本研究采用生物素—磁珠吸附微卫星富集与放射性同位素相结合的方法，构建了虾夷扇贝微卫星富集文库。微卫星富集文库法，就是用链霉亲和素的磁珠亲和捕捉生物素标记的微卫星探针上结合的基因组 DNA 片段，获得高度富集了微卫星小插入片段的基因组文库。在此基础上又增加了用 CA 重复序列的同位素探针进行 2 次筛选，这种结合了两种方法的优点是，灵敏度高、周期短而且目的性强、阳性克隆率及效率高，一次可获得大量的微卫星序列。富集文库的阳性克隆率一般都较高，Kandpal 等[14]为 90%，李齐发等[15]为 77%，Edwards 等[16]为 70%。本方法采用生物素—磁珠吸附微卫星富集与同位素杂交的两步筛选，所测 136 个克隆中有 134 个序列含有微卫星，达到 98.53%，说明两步筛选法具有明显的优势，节约经费，所构建的虾夷扇贝微卫星富集文库是一个高质量的文库，可以进行大规模测序筛选。

与人和其他动物相比，虾夷扇贝微卫星序列中完美型较低，而非完美型较高，这可能是由于所抽样测序的克隆较少，不能完全反映其微卫星的分布情况，也可能和虾夷扇贝所处生态环境条件相关，基因组中发生的碱基替换、错配和不等交换等增加了遗传多样性，以适应复杂的自然环境[15]。

微卫星核心序列突变率相对较高(10^{-5}～10^{-3})，微卫星长度的变化是微卫星多态性的基础[17]。微卫星寡核苷酸的重复数在同一物种的不同基因型间差别很大，具有十分丰富的多态性。Weber[12]认为，只有在双碱基重复序列重复次数大于 12 次时，微卫星标记才有可能表现出较高 PIC (polymorphic information content)值，当 n≥16 时，可提供的 PIC 在 0.5 以上[18]才可以进行相应的多态性分析。在富集的过程中，磁珠经过多个洗涤的过程，可以将一些因重复数目较少而附着不牢固的微卫星序列洗去，使所获得的微卫星序列较长，重复

表1 虾夷扇贝微卫星分子标记及其引物

Tab.1 Microsatellite markers and their primers in Japanese scallop(*Patinopecten yessoensis*)

微卫星标记 Microsatellite marker	引物序列 5'－3' Primer sequence	序列大小/bp Sequence size	重复序列 Repeat sequence	退火温度/℃ Annealing temperature
HLJX-002	F:CCAGGGTCAAACCAAACA R:ACAGCCCAATCCCAAAGA	130	$(AC)_{23}$	60
HLJX-003	F:TACCGTCGATGATCAAAC R:TTTTACTGTGGAGGGTTG	264	$(CA)_{93}$	59
HLJX-005	F:ATAGTAGCCACTGAGGTAGA R:GGTAAGATTTGGTGTTCG	263	$(TG)_4(ATTTT)_2$ $(AC)_{60}$	57
HLJX-006	F:AGCGGAGTCACCCTACAAG R:CAGGACGGCACATAAAGC	171	$(TG)_{23}$	58
HLJX-007	F:TGCAGGTGCAGATAAAGC R:TCAGTGCTGCTACGAGGA	218	$(TG)_{34}$	60
HLJX-008	F:TCTGGACAACTGCTGGAA R:ATCAGAAACGAGGACAGA	201	$(AC)_{80}$	58
HLJX-010	F:GTCTGACCACCAGCCAACT R:GTGCCATGATGACGAGATAA	314	$(CA)_{126}$	60
HLJX-011	F:CTGTGCATAGTGGGCTCT R:CAAGGTTGAAGTCCGTGT	261	$(TC)_{10}(AC)_{87}$	55
HLJX-012	F:TGAACGAGGACACGAAGA R:AAGCTGCTCTGGATAAGG	169	$(CA)_{54}$	55
HLJX-013	F:AGAGCGAGTCTGGAAACC R:AGGAAGTCTGTCTTAGCG	285	$(AC)_{51}$	60
HLJX-014	F:AGCTGGACGACTGAGTAC R:TGAGTGACTGAAGGTGGC	212	$(AC)_{34}$	56
HLJX-015	F:CCCCTTGATTTCGTTTGT R:TGGTTCTGGGTAGTGAGC	113	$(TG)_{20}$	52
HLJX-016	F:AGCTGGACGACTGAGTAC R:TGAGTGACTGAAGGTGGC	212	$(AC)_{34}$	58
HLJX-017	F:AGCGTGAAAGTAGATAAGG R:TTCTGTTTGGTCGGTTTG	288	$(AC)_{67}$	55
HLJX-018	F:GATCAATGAAACGTCTGC R:ATGGTGGAGTCAAAGTCG	237	$(AC)_{64}$	59
HLJX-019	F:AGAGTCCCTCCACTTCAT R:TCTTGGCTGAGATGGTAA	148	$(AC)_{43}$	58
HLJX-020	F:GAAAGCATCTGGCTACAC R:ACTAAACCCTCCAACTCG	166	$(AC)_{27}$	54
HLJX-021	F:TGTGCTTGCTTCTCCCTG R:TCCTGTAATCGTGGTGGC	290	$(AC)_{41}$	60
HLJX-022	F:CCCATATCGGTAACTGCA R:TTCCTCCATCTGTCTTCA	124	$(CA)_{40}$	50
HLJX-023	F:AGCTCCGACTTGCTGTAC R:TGGCTGGCTGCTTAGTGT	216	$(AC)_{78}$	50
HLJX-024	F:GAAATCCCATTCTCCTGC R:CTGAGATGCCGACTGACT	111	$(AC)_{20}$	52
HLJX-025	F:ACACTGTCTGCTGTTTCTC R:GTGTATGTTCTTGTGCTGA	258	$(CA)_{93}$	50

续表

微卫星标记 Microsatellite marker	引物序列 5'－3' Primer sequence	序列大小/bp Sequence size	重复序列 Repeat sequence	退火温度/℃ Annealing temperature
HLJX-026	F:TTTCACTAGCTGTAGATTT R:TACACTGAGTTTGTCCTG	290	$(AC)_{101}$	60
HLJX-027	F:TAAGAGCATAAGGGCAGGTG R:ACCGTCAAAGCTCACCCA	287	$(CA)_{100}$	55
HLJX-028	F:CGGTTTTGTGCCTGTGGT R:AGAGGAGCTTGTGCGTGT	217	$(AC)_{67}$	59
HLJX-029	F:CATTTAGCCTGCTCCATC R:CCATACACCGAACCCATA	155	$(GT)_{19}$	58
HLJX-030	F:AGAATGAGTCTGGATGGT R:AAAGGCTCTGAACAAACA	206	$(AC)_{59}$	58
HLJX-031	F:TACGTACCCACTCACCCA R:GAGCCGTTAGGAAGGTGT	278	$(AC)_{115}$	58
HLJX-034	F:TCAGCCGAATCTCCTTAC R:GCACGTTTCATATCACCC	217	$(CA)_{31}$	58
HLJX-035	F:TATGCCCATCCTCAACAA R:TCCAGCCCAGCTCTAATG	163	$(AC)_{23}$	52
HLJX-036	F:GAGACCAAAGCCCAATAA R:AACGATCAGAGGGAAAGA	174	$(TG)_{22}$	57
HLJX-037	F:ATCTGGACTGCCATCTAC R:AATTCGACCTGAACAAGC	191	$(TG)_{26}$	60
HLJX-038	F:TGTTTTCCCTGTTGAGCC R:TTTTGTCTGCCTGGTTAT	279	$(AC)_{75}$	50
HLJX-039	F:CACAATGGAAAGGGAATC R:AAGCGGTAGAAGCACAGG	228	$(TG)_{33}$	52
HLJX-040	F:AAGATTTTCAGCCGAGAC R:TACCCCTACACCCCTATT	224	$(CA)_{62}$	53

注：F.正向引物；R.反向引物.
Note: F, Forward primer; R, Reverse primer.

次数较多。Smulders 等[19]认为重复次数多的微卫星既能在种间又能在种内产生多态性，但重复次数少的微卫星，仅能在种间产生多态性。因此，研究所选用的微卫星序列其核心序列重复次数应当在较高的水平上，避免由于微卫星核心序列过短，造成微卫星标记筛选中多态性引物比例过低。本研究所得到的微卫星序列重复数在 10 次以上的有 157 个，占总数的 87.71%，这对筛选多态信息含量高的分子标记，进行种群的遗传多样性研究是具有一定的参考价值。

虾夷扇贝为中国引进的品种，国内学者对于遗传多样性研究很少。高悦勉等[1]用同工酶研究了大连沿海诸岛的养殖群体的遗传结构，同工酶是基因的表达产物，受环境和发育阶段影响较大，有时不能代表物种遗传本质上的差异[20]；日本学者 Nagashima 等应用 mtDNA 研究了日本沿海虾夷扇贝的遗传结构[21]，而 mtDNA 反应的是其母系遗传特性，还不能真正代表虾夷扇贝基因组水平的多态性状况[22]。因此，利用微卫星等重复性高和共显性比例高的分子标记对于虾夷扇贝种群的遗传多态性研究，可能会得到更准确和更精细的结论。研究得到的微卫星标记可以用于虾夷扇贝种群遗传学分析和多样性鉴定，从分子水平对中国虾夷扇贝养殖群体的遗传变异水平、近交衰退和性状退化进行有效分析，建立分子遗传选育技术方法，为遗传育种、种质鉴定、种苗放流等提供理论依据，实现虾夷扇贝的种质资源科学管理及持续利用。另外，微卫星标记也是用于基因作图的一种重要的分子标记，如果开发出更多的虾夷扇贝标记的话，则可以用来构建虾夷扇贝遗传连锁图谱，这对于品种选育、种系评估都具有重要意义。

致谢：感谢大连水产学院孙洋、刘丹同学提供虾夷扇贝DNA样品。

参考文献：

[1] 高悦勉，李国喜，赵银娟. 大连沿海虾夷扇贝养殖群体遗传结构的研究[J]. 大连水产学报，2004，19(2)：142－145.

[2] 孙效文，梁利群. 鲤鱼的遗传连锁图谱(初报)[J]. 中国水产科学，2000，7(1)：1－5.

[3] 周 莉，刘静霞，桂建芳. 应用微卫星标记对鲢核发育银鲫的遗传多样性初探[J]. 动物学研究，2001，22(4)：257－264.

[4] Naish K-A, Skibinski O. Tetranucleiotide microsatellites loci for Indian major carp (*Catlacatla*) [J]. Journal of Fish Biology, 1998, (53)：886－889.

[5] Ashie T N, Daniel G B, Edward P C, et al. Parentage and relatedness determination in farmed Atlantic Salmon (*Samo salar*) using mincrosatellite markers[J]. Aquaculture, 2000, 182：73－83.

[6] Ricardo P E, Motohiro T, Nobuhiko Taniguchi, et al. Genetic variability and pedigree tracing of hatchery-reared stock of red sea bream(*Pagrus major*) used for stock enhance ment, based on microsatellite DNA markers[J]. Aquaculture, 1999, 173：411－421.

[7] 徐 鹏，周岐华，相建海. 中国对虾微卫星DNA的筛选[J]. 海洋与湖沼，2001，32(3)：255－259.

[8] 李红霞，宋林生，王玲玲，等. 栉孔扇贝EST中微卫星标记的筛选[J]. 高技术通讯，2003，13(12)：72－75.

[9] Brown J, Hardwick L J, Wright A F. A simple method for rapid isolation of microsatellites from yeast artificial chromosomes[J]. Molecular and Cellular Probes, 1995, 9：53－58.

[10] Carol A R, Arcangeli L, Block A B. Development of 11 microsatellite loci for population studies in the swordfish, *Xiphias gladius* (Teleostei：Scombridae)[J]. Molecular Ecology Notes, 2003, 3：147－149.

[11] 萨姆布鲁克 J，拉塞尔 D W. 分子克隆实验指南(第三版)[M]. 北京：科学出版社，2002.

[12] Weber J L. Informativeness of human $(dC\text{-}dA)_n\text{-}(dG\text{-}dT)_n$ polymorphisms[J]. Genomics, 1990, 7：524－530.

[13] 梁利群，常玉梅，董浚智，等. 微卫星DNA标记对乌苏里红普罗鱼遗传多样性的分析[J]. 水产学报，2004，28(3)：241－244.

[14] Kandpal R P, Kandpal G, Weissman S M. Construction of libraries enriched for sequence repeats and jumping Ys, and hybridization selection for region-specific markers[J]. Proceding of the National Academy of the USA, 1994, 91：88－92.

[15] 李齐发，赵兴波，罗晓林，等. 牦牛基因组微卫星富集文库的构建与分析[J]. 遗传学报，2004，31(5)：489－494.

[16] Edwards K J, Barker J H, Daly A, et al. *Microsatellite* libraries enriched for several microsatellite sequences in plants[J]. Biotechniques, 1996, 20：758－760.

[17] 刘志毅，相建海. 微卫星在海洋动物遗传分析中的应用[J]. 海洋科学，2001，25(6)：11－13.

[18] Orit G, Pearse D E, Aviae J. Phylogenetic assesment of legth variation at a microsatellite locus. Proceedings of the National Academy of Sciences[J]. Proceedings of the National Academy of Sciences, 1997, 94(10)：745－749.

[19] Smulders M J M, Bredemeijer G, Rus-kortekaas W, et al. Use of short microsatellites from database sequences to generate polymorphisms among Lycopersicon esc Lent mol/L c Ltivas and accessions of other Lycopersicon species[J]. Theor Appl Genet, 1997, 97：264－272.

[20] R$_i$˙os C, Sanz S, Saavedra C, et al. Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia：Pectinidae), across the Almeria Oran front[J]. Journal of Experimental Marine Biology and Ecology, 2002, 267：223－244.

[21] Nagashima K, Sato M, Kawamata K, et al. Genetic structure of Japanese scallops population in Hokkaido, analyzed by mitochondrial haplotype distribution[J]. Marine Biotechnology. 2003, 7(1)：1－10.

[22] 杨 光，任文华，钮明华，等. 条纹原海豚mtDNA控制区序列变异分析[J]. 动物学报，2002，48(1)：131－134.

Microsatellite enrichment by magnetic beads in japanese scallop, *Patinopecten yessoensis*

ZHAO Ying-ying[1,2], ZHU Xiao-chen[1,2], SUN Xiao-wen[1], LIANG Li-qun[1]

(1. Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Heilongjiang Harbin 150070, China; 2. Life Science and Technology Institute, Dalian Fisheries University, Liaoning Dalian 116023, China)

Abstract: Japanese scallop (*Patinopecten yessoensis*) is one of the most important cultured seashells in the world. It is distributed widely over the cold seas along the coastline of the northern islands of Japan, the northern part of the Korean Peninsula, Sakhalin, and the Kuril Islands, and was introduced to China from Japan more than 20 years ago. Along with nearly 20 years' artificial culture, available DNA markers are needed for its genetic research. Microsatellites recently have become an extremely popular marker in a wide variety of genetic investigations. In this research, genomic DNA was extracted from muscle of Japanese scallop by normal methods and digested with restriction enzyme *Sau*3AI. Fragments of 400 – 900 bp were isolated and then ligated short linkers (20 bp). They were to create a "whole genome PCR library". Microsatellite isolated from genomic DNA was hybridized with a biotin-labeled SRS (simple repeat sequence) probe $(CA)_{15}$, and the hybrid mixture was incubated with magnetic beads coated with streptavidin. After washing to remove the non-SRS fragments, the eluted single-stranded DNA contains the selected microsatellite DNA. The selected DNA are then amplified using primers designed complementary to the linkers, cloned into the pGEM-T vector and transform into competent *E. coli* DH5α. Then the genomic library was second-screened for microsatellite DNA using $(CA)_{15}$ probes labeled with γ^{32}P-ATP at 5' end the probes hybridized with bacterial colonies. As a result, 136 positive clones were identified from 192 clones, and 179 microsatellites were found. Among the 179 microsatellites, 50.8% were perfect, 43.0% were imperfect and the rest were compound type (6.1%). Except the biotin-labeled SRS probes CA, there were TC, AG, ACA and CATA in the microsatellites. Finally, 85 microsatellite primers were designed by software Premier Primer 5.0, and 40 pairs were screened, 34 pairs were amplified. They made positive contribution to exploring genomes, and developing genetic linkage maps, as well as made possible the systematic search for quantitative trait loci (QTL) of the Japanese scallop. [Journal of Fishery Sciences of China, 2006, 13(5): 749 – 755]

Key words: enrichment by magnetic beads; Japanese scallop; microsatellite

第13卷第5期
2006年9月

中国水产科学
Journal of Fishery Sciences of China

Vol. 13 No. 5
September 2006

Development of microsatellite DNA markers of silver carp (*Hypophthalmichthys molitrix*) and their application in the determination of genetic diversities of silver carp and bighead carp (*Aristichthys nobilis*)

LIAO Mei-jie[1], YANG Guan-pin[1], ZOU Gui-wei[2], WEI Qi-wei[2], WANG Deng-qiang[2]

(1. College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China; 2. Changjiang Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jingzhou 434000, China)

Abstract: In order to evaluate the genetic diversity of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichys nobilis*) and determine their genetic differentiation, a GT microsatellite containing short fragment genomic DNA library of silver carp was constructed. Of 97 randomly selected and sequenced clones, 87 contained a GT repeat motif. From 21 of them, 22 pairs of primers were designed and used to investigate the polymorphism of 32 silver carp individuals and 7 bighead carp individuals collected from the Yangtze River basin, the most important spawning and hatching ground of these two fish species. All the markers developed amplify both silver carp and bighead carp DNAs. A total of 129 alleles were detected at 22 loci in the total sample. The number of alleles per locus ranges from 3 to 10 with an average of 5.9. All marker loci revealed high polymorphisms, with values ranging from 0.33 to 2.00 (Shannon's diversity index) with the mean of 1.22. [Journal of Fishery Sciences of China, 2006, 13 (5): 756 - 761]

Key words: microsatellite DNA marker; simple sequence repeat marker; *Hypophthalmichthys molitrix*; *Aristichys nobilis*; genetic diversity; cross-species amplification

CLC number: Q959.4 **Document code**: A **Article ID**: 1005 - 8737 - (2006)05 - 0756 - 06

Silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) are two of the four most important pond-cultured fish species which inhabit in the major river basins of China, including Heilongjiang River, the Yangtze River and the Pearl River. Their cultivation could be dated back more than 1 000 years ago in the Tang Dynasty[3]. They prey on different organisms and live in different layers of water, utilizing effectively the water body and natural feeds when polycultured. They have been introduced into more than 20 countries and regions in the world in order to improve water quality or harvest for human consumption, although some ecological problems have been associated with their introduction. Of the four fish species, silver carp is the most intensively cultured one in China due to its characteristics of fast growth, strong tolerance of stress conditions and low cost of cultivation.

In the traditional culture operation, fry of silver carp were caught from nature and cultured in ponds or lakes. With the increase of human population and the intensification of economical activity, natural spawning and hatching grounds were either polluted or altered, accelerating the loss of genetic resource and the decrease of natural fry production accordingly[4]. Artificial propagation techniques for silver carp and

Received date: 2006 - 01 - 22; **Revised date**: 2006 - 03 - 29.

Foundation: The Cooperative Project of The Key Lab of Freshwater Germplasm and Biotechnology of the Chinese Ministry of Agriculture (LFB20040503); The National Natural Science Foundation of China (30471318).

Author biography: LIAO Mei-jie (1981 -), female, a Ph. D student, is engaged in study on the molecular genetics of marine organisms. E-mail: changablecat@yahoo.com.cn

Corresponding author: YANG Guan-pin. E-mail: yguanpin@mail.ouc.edu.cn

bighead carp were developed in China in 1958. Unfortunately, these techniques evolved quickly to become the major and currently almost sole approach for obtaining fry. Due to inappropriate broodstock management, continuous artificial propagation over a long period of time has caused tremendous decrease of genetic diversity and deterioration of disease resistance and growth performance. In addition, escape of cultured silver carp in frequent large-scale flooding has disturbed the genetic diversity of natural populations. In recent years, large-scale fry release has been proposed and practiced, which will interfere further with the wild populations. However, it is infeasible to manage and exploit silver carp and bighead carp genetic resources without the development of a set of appropriate genetic markers. Moreover, silver carp and bighead carp can be artificially hybridized[1], even in commercial aquaculture hatcheries[6]. These observations imply their close phylogenetic relationship, although the fertility of their filial generation has not yet been assessed. A set of appropriate markers will facilitate the determination of the affinity between the two species.

To date, genetic marker systems including allozyme[2,11], RAPD[13,15], and RFLP of mtDNA[7,14] have been used in studies of the genetic diversity of silver carp or bighead carp. However, these marker systems have disadvantages. The number of allozyme markers is limited, and the information provided by RAPD does not correspond to specifically amplified loci and comparison among experiments and laboratories is inaccurate. In contrast, microsatellite DNA or simple sequence repeat(SSR) markers have proven to be an extremely valuable tool for genome mapping, genetic studies and conservation and management of genetic resources[5]. Genomic microsatellite DNA markers have been used in silver carp and bighead carp, but the number is very limited[6,9]. In the present study, cloning and characterization of 22 new microsatellite loci of silver carp are reported.

1 Materials and methods

1.1 DNA Extraction

Thirty-two individuals of silver carp and 7 individuals of bighead carp were collected from the Yangtze River of China. Genomic DNA was isolated from alcohol-preserved individual muscle tissues using the phenol chloroform method as modified by Taggart et al in 1992[8].

1.2 Isolation of microsatellite DNA containing fragments

Fragments containing microsatellite DNA were isolated from the genomic DNA of silver carp using the FIASCO (Fast Isolation by AFLP of Sequences Containing Repeats) method described in detail by Zane et al. in 2002[12]. Genomic DNA was simultaneously digested with *Mse* I and ligated with a *Mse* I AFLP adaptor(5'-TAC TCA GGA CTC AT-3' /5'-GAC GAT GAG TCC TGA G-3') in a 25 μL mixture containing 250 ng genomic DNA, 1 × buffer Y$^+$, 1 × T4 DNA ligase buffer, 1 μmol/L adaptor, 200 μmol/L ATP, 5 U *Mse* I(Sangon, Shanghai, China) and 5 U T4 DNA Ligase(Sangon, Shanghai, China) at 37 ℃ for 3 h and diluted to 250 μL(1∶10). The digestion-ligation mixture was amplified in a total volume of 20 μL containing 1 × *Taq* DNA polymerase buffer(Promega), 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTP (each), 0.4 U *Taq* DNA polymerase (Promega), 5 μL diluted digestion-ligation mixture and 120 ng AFLP adaptor-specific primer(5'-GAT GAG TCC TGA GTA A-3'). The PCR reaction was cycled 20 times at 94 ℃ for 30 s, 53 ℃ for 30 s, and 72 ℃ for 1 min[10].

Following Travis Glenn's protocol (http://www.uga.edu/srel/DNA_Lab/protocols.htm), the hybridization of biotinylated $(GT)_{12}$ probe and SSR motif was carried out in a 100 μL volume containing 250 − 500 ng PCR product, 80 pmol biotinylated $(GT)_{12}$ probe, 4.2 × SSC and 0.07% SDS. The mixture was denatured at 95 ℃ for 3 min, annealed at room temperature for 15 min and diluted with 300 μL of TEN_{100}. Single-stranded DNA fragments with biotinylated probe were captured with 100 μL streptavidin-coated beads (MagenSphere Megnedic Separation Products, Promega) extensively washed with TEN_{100} (10 mmol/L TrisHCl, 1 mmol/L EDTA,

100 mmol/L NaCl, pH 7.5) and resuspended in 40 μL TEN_{100} at room temperature for 30 min with occasional agitation. To minimize nonspecific binding of DNA, 1 μg genomic DNA(of any unrelated species) was mixed with the beads ahead of adding the hybridization mixture. The bead-probe-DNA complex was separated in a magnetic field from hybridization buffer and washed extensively. DNA fragments with microsatellites were recovered by denaturing at 95 ℃ or using NaOH as described.

DNA recovered was precipitated together with yeast tRNA carrier with isopropanol and sodium acetate and resuspended in 10 μL water. Of these, 2 μL were used to amplify DNA fragments containing microsatellites. The PCR was carried out as described above.

1.3 Isolation of SSR-containing DNA fragments and SSR primer design

DNA fragments containing microsatellites was ligated with the pMD18-T vector (TaKaRa, Dalian, China), and transferred into *E. coli* JM109 by electroporation. Each recombinant was subjected to three PCR screenings using two universal sequencing primers, a forward one with $(GT)_{12}$ oligonucleotide and a reverse one with $(GT)_{12}$ oligonucleotide, respectively, in order to ensure that the sequenced fragments contain the SSR in the middle region. Selected clones were sequenced, trimmed and used to design SSR primers with the help of Primer Premier 5 Software (http://www.premierbiosoft.com/primerdesign/).

1.4 Polymorphism analysis

SSR primers designed were used to amplify genomic DNA of silver carp and bighead carp individuals. The PCR mixture contained 1 × buffer, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTP(each), 200 μmol/L primers(each direction) and about 50 ng DNA. The reaction was carried out by denaturing at 94 ℃ for 1 min, annealing at the temperature optimized for each primer pair for 1 min and extending at 72 ℃ for 1 min for 30 cycles, followed by an extra extension step at 72 ℃ for 10 min. The optimized annealing temperatures of different primer pairs were listed in T_A column of Table 1. The PCR product was separated on a 6% denaturing polyacrylamide gel and visualized by silver staining.

1.5 Statistical analysis

Allele frequencies were obtained by direct counting. Genetic diversity (G) of a population was expressed as the Shannon's diversity index, $-\Sigma f_i * \ln f_i$, where f_i is the frequency of the i^{th} allele at a locus in a population. "G" can be partitioned into that within subpopulations and that among subpopulations, or that within silver carp population and bighead carp population (G_{sb}) and that between them. $G_{sb} = (G_s \times N_s + G_b \times N_b)/N_{sb}$, where G_s and N_s are genetic diversity and number of individuals of silver carp, respectively, and G_b and N_b, genetic diversity and the number of individuals of bighead carp, respectively. $D_{sb} = (G - G_{sb})/G_{sb}$, where, D_{sb} is the differentiation of silver carp and bighead carp. The t-test was used to determine the significance of differences of both averages of the genetic diversities between silver carp and bighead carp and the frequencies of an allele in the two populations.

2 Results and discussion

In order to evaluate the genetic diversity of silver carp and bighead carp and determine the genetic differentiation of these two species, a GT SSR-containing short genomic DNA fragment library of silver carp was constructed. The enrichment was highly effective. About 30% recombinants were found to contain inserts with a SSR motif in the middle position. Of 97 clones surviving PCR screening, 87 contained a SSR motif in middle position after being sequenced. As the first batch, 22 pairs of primers were designed according to 21 sequences and used to investigate polymorphism of 32 silver carp individuals and 7 bighead carp individuals collected from Jingzhou of the Yangtze River (Tab. 1). All the markers developed amplified both silver carp and bighead carp DNAs.

Tab.1 Motifs, primer sequences and primer annealing temperatures for microsatellite DNA (or simple sequence repeat, SSR) markers of silver carp developed in this study

表 1 白鲢各微卫星 DNA(或简单序列重复,SSR)标记的核心序列、引物序列和扩增的复性温度

Locus 座位	Accession no. 序列获取号	SSR motif 核心序列	Forward primer(5'－3') 正向引物	Reverse primer(5'－3') 反向引物	T_A*/℃ 复性温度
BL2 **	DQ136002	$(TG)_{16}$	TAAGCGTGCATGGCCCTAAAT	TGAACGAAGGTCTTACGGGTG	49
BL5	DQ136003	$(TG)_{27}$	CCTGTGCCTTTGAACTCTGA	CCCTCCACCATACTGACAAG	52
BL6	DQ136004	$(TG)_8$	TTCTATAGCAGTCCTGCTGATTTAC	CACTAGCGTGACGGGAAATA	57
BL8-1 ***	DQ136005	$(TCCA)_6$	TATTGACTGCATCTGGGTCTT	AGGTTATGTTTAGCCCAGTCG	58
BL8-2	DQ136005	$(GT)_9$	CCCGACTGGGCTAAACATA	TCATTTGGGAGGCAGACAC	52
BL11	DQ136006	$(TG)_{11}$	GTCATCAAACTAAGCCATCAG	GCATTTCACCTGTAGCATCTC	54
BL12	DQ136007	$(TG)_9$	AATGAGCAATCAGGCACAGAG	GGGTGTAATGAGGCTATGTTT	54
BL14	DQ136008	$(TG)_{13}$	CGGCACTCAGAAATGATGGGG	CATGGAGAGCAGGAAGAGTTG	54
BL15	DQ136009	$(TG)_8$	TACTGATACTCCGTCCCCT	GCACCTGTAATCCCAAAT	54
BL18	DQ136010	$(TG)_{18}$	CGAGACAAATAAGGTTGGATA	CACAAAGAAACTGGAACAAAGAG	52
BL23	DQ136011	$(GT)_5T(TG)_4$	CCTTCGTTTGACGGACAG	GATGTGGTGATTTCAGCAG	53
BL40	DQ136012	$(GA)_8$	TGACATAAAGAGCCACCATTG	CATAGTCACCCGATCAATACTG	54
BL42	DQ136013	$(GT)_{14}$	TGCCGATGTTATGTTTGCT	TGCTTGTGGGTGAGTTTCT	52
BL46	DQ136014	$(GT)_9$	AGTCCTGCTGTTGCTGTATG	CTCCTGCTCCACCTTCCT	55
BL52	DQ136015	$(TG)_{12}$	CAGAATCCAGAGCCGTCAG	CACCGAACAGGGAACCAA	54
BL55	DQ136016	$(GT)_{14}$	AAGGAAAGTTGGCTGCTC	GGCTCTGAGGGAGATACCAC	52
BL56	DQ136017	$(GT)_{16}$	TTAGGTGAACCCAGCAGC	AAGAAGCATTAGTGCAGATGAGTAC	54
BL58	DQ136018	$(GT)_9$	TTCCTGCCTGTGCTCCAT	TTGCATTGATGCTGTCCC	52
BL62	DQ136019	$(TG)_{13}$	ATATTAACATCTGCCGAAGC	ACAACCAGCAGTCTGAAGC	52
BL64	DQ136020	$(TG)_{23}$	GCCAGGCTAGAAGAACCACC	TTGCAGCACAGTTACCAAGACA	55
BL65	DQ136021	$(GT)_{14}$	TTAGAGCCATTAGAGGAAAA	ACACGGAAGCCATTGTTG	51
BL66	DQ136022	$(TG)_9$	TTTGTTTCCGCCGTGGTG	GGTTCAGGGTTCAATGTCC	54

Note: * Annealing temperature optimized for the amplification of the DNA region flanked by the primer pair; ** Named using the initial of the Chinese name of silver carp; *** Two loci are located in the same DNA fragment.

注: * 针对引物界定区域扩增优化的复性温度; ** 根据鲢中文名命名; *** 2 个遗传座位在同一 DNA 片段上.

A total of 129 alleles were detected at 22 loci in the total sample. The number of allele at a locus ranged from 3 to 10, with an average of 5.9. All marker loci revealed high polymorphisms, with values ranging from 0.33 to 2.00 (Shannon's diversity index) with the mean of 1.22 in the total sample (Tab. 2). The genetic diversity indexes of silver carp at 22 loci ranged from 0.19 (*BL66*) to 1.95 (*BL64*) with an average of 1.16, and those of bighead carp from 0.00 (*BL8-1* and *BL8-2*) to 1.61 (*BL58*) with an average of 0.80. On the average of all 22 loci, the genetic diversity of silver carp is significantly higher than that of bighead carp (1.16 vs 0.80, $P<0.01$). The genetic differentiation of silver carp and bighead carp varied from locus to locus, ranging from 2.1% (*BL46*) to 61.7% (*BL66*) with an average of 12.8% (Tab.2).

These microsatellite DNA markers will certainly facilitate the genetic monitoring of farmed stocks, characterization of genetic structure and clarification of the species status of the two species and in particular the possibility of gene flow between them. These markers are just in the first batch, and more markers will be developed based on the library constructed in this study. We will use these newly developed markers to evaluate the diversities of two species by using samples representing the communities appropriately. We may achieve this ultimate goal by this issue ourselves or more effectively by collaborating with the investigators and institutes interested in this issue. SSR markers will make such trials easy; the data from different sources can be integrated and compared with each other if SSR alleles are sequenced.

Tab.2 Genetic diversity and number of allele(in parentheses) of silver carp and bighead carp at each locus and the genetic differentiation between them detected by using different microsatellite DNA markers developed

表2 白鲢和鳙鱼各微卫星标记座位上的遗传多样性和等位基因数及两种鱼之间的遗传分化

Locus 遗传座位	Silver carp 鲢	Bighead carp 鳙	Total sample 总样品	Differentiation/% 遗传分化
BL2	0.69(2)	0.68(2)	0.90(3)	23.6
BL5	1.05(6)	0.69(3)	1.02(6)	2.9
BL6	1.10(5)	0.69(2)	1.07(5)	10.3
BL8-1	0.70(5)	0.00(1)	0.60(5)	5.3
BL8-2	0.52(3)	0.00(1)	0.45(3)	7.1
BL11	1.51(6)	0.51(3)	1.43(6)	7.7
BL12	0.28(3)	0.51(3)	0.33(3)	3.0
BL14	1.65(8)	0.99(4)	1.73(8)	12.1
BL15	0.82(4)	1.00(3)	0.97(4)	12.4
BL18	1.88(8)	1.08(3)	1.83(8)	5.5
BL23	0.45(2)	0.82(3)	0.72(3)	28.9
BL40	1.05(3)	0.69(2)	1.02(3)	2.9
BL42	1.25(7)	0.41(2)	1.21(7)	18.5
BL46	1.22(5)	0.83(3)	1.17(5)	2.1
BL52	0.56(4)	0.68(2)	0.66(4)	12.1
BL55	1.94(9)	1.20(4)	2.00(9)	10.0
BL56	1.76(8)	1.47(5)	1.96(10)	12.8
BL58	1.60(10)	1.61(6)	1.72(10)	7.0
BL62	1.62(8)	1.57(6)	1.88(9)	14.4
BL64	1.95(9)	1.49(5)	2.00(9)	7.5
BL65	1.68(6)	0.27(2)	1.63(6)	12.9
BL66	0.19(2)	0.41(2)	0.60(3)	61.7
Average	1.16 **	0.80 **	1.22	12.8

Note: ** Difference significant at $P<0.01$.

注: ** 差异显著($P<0.01$).

References:

[1] de Almeida-Toledo L F, Bigoni A P V, Bernardino G, et al. Chromosomal location of NORs and C bands in F_1 hybrids of bighead carp and silver carp reared in Brazil[J]. Aquaculture, 1995, 135: 277 - 284.

[2] Jiang J, Xiong Q, Yao R. Comparative studies on isozyme of black carp, grass carp, silver carp and bighead carp[J]. Hereditas(Beijing), 1998, 20(2): 19 - 22.

[3] Li S, Fang F. On the geographical distribution of the four kinds of pond-cultured carps in China[J]. Acta Zoologica Sinica, 1990, 36: 244 - 250.

[4] Liu Z J, Cordes J F. DNA marker technologies and their applications in aquaculture genetics[J]. Aquaculture, 2004, 238: 1 - 37.

[5] Liu S, Chen D, Duan X, et al. Monitoring of the four famous Chinese carps resources in the middle and upper reaches of the Yangtze River[J]. Resources and Environment in the Yangtze Basin, 2004, 13(2): 183 - 186.

[6] Mia M Y, Taggart J B, Gilmour A E, et al. Detection of hybridization between Chinese carp species (*Hypophthalmichthys molitrix* and *Aristichthys nobilis*) in hatchery broodstock in Bangladesh, using DNA microsatellite loci [J]. Aquaculture, 2005, 247: 267 - 273.

[7] Song P, Li X, Xiong Q. Comparative study on the maps of nine restriction endonucleases of mtDNA of silver carp (*Hypophthalmichthys molitrix*) and bighead (*Aristichthys nobilis*)[J]. J Fish China, 1994, 18(3): 221 - 230.

[8] Taggart J B, Hynes R A, Prodohl P A, et al. A simplified protocol for routine total DNA isolation from salmonid fishes[J]. J Fish Biol, 1992, 40: 963 - 965.

[9] Tong T, Wang Z, Yu X, et al. Cross-species amplification in silver carp and bighead carp with microsatellite primers of common carp[J]. Molecul Ecol Not, 2002, 2: 245 - 247.

[10] Vos P, Hogers R, Bleeker M, et al. AFLP: a new technique for DNA fingerprinting[J]. Nucl Acids Res, 1996, 23: 4 407 - 4 414.

[11] Wu L, Wang Z. Biochemical genetic structure and variation in a

natural population of silver carp from the middle reaches of the Yangtze River[J]. Acta Hydrobiol Sin, 1997, 21(2): 157 - 162.

[12] Zane L, Bargelloni L, Patarnello T. Strategies for microsatellite isolation: a review[J]. Mol Ecol, 2002, 11: 1 - 16.

[13] Zhang D, Zhang X, Yang D, et al. Comparative study on the genetic diversities of silver carp artificial propagation populations in different geographical regions[J]. Freshw Fish, 2000, 30(3): 38 - 41.

[14] Zhang S, Wang D, Deng H, et al. Mitochondrial DNA variation of silver carp and grass carp in populations of the middle reaches of the Yangtze River revealed by using RFLP-PCR[J]. Acta Hydrobiol Sin, 2002, 26(2): 142 - 147.

[15] Zhang S, Deng H, Wang D, et al. Population structure and genetic diversity of silver carp and grass carp from populations of Yangtze River system revealed by RAPD[J]. Acta Hydrobiol Sin, 2001, 25(4): 324 - 330.

鲢微卫星标记研制及其在鲢和鳙遗传多样性研究中的应用

廖梅杰[1]，杨官品[1]，邹桂伟[2]，危起伟[2]，汪登强[2]
(1. 中国海洋大学 海洋生命学院，山东 青岛 266003；2. 中国水产科学研究院 长江水产研究所，湖北 荆州 434000)

摘要：鲢(*Hypophthalmichthys molitrix*)和鳙(*Aristichys nobilis*)是中国特有的四大家鱼中 2 个重要成员，主要分布于黑龙江、长江和珠江水系。传统人工养殖依靠天然鱼苗。但是，人口增长和人类经济活动加剧使其天然产卵和孵化场消失或遭到破坏。人工育苗技术虽然解决了鱼苗供应问题，但由于遗传资源管理和使用方法的不完善，使两种鱼的生长表现、抗病抗逆性和遗传多样性等都有明显的降低。另外，因洪水导致的养殖个体逃逸也使天然群体的遗传多样性受到干扰。近年来，比较大规模的人工鱼苗放流实践也加剧了对天然群体遗传多样性的扰动。对这些问题的深入研究迫切需要一套适用的分子标记。为评价鲢和鳙的遗传多样性、确定它们的遗传分化和地理分化、科学合理地管理和开发利用遗传资源，本研究构建了富集 GT 微卫星序列的基因组短片段文库。随机选择并测序的 97 个克隆中有 87 个含有微卫星序列。根据其中的 21 条序列，设计了 22 对微卫星标记引物并用来分析了在长江荆州段捕获的 32 尾野生鲢和 7 尾野生鳙的遗传多样性。所有标记引物在两种鱼中通用。在全部样品中共发现 129 个等位基因。每位点等位基因数在 3~10 个，平均 5.9 个。不同标记揭示的遗传多样性指数在 0.33~2.00，平均 1.22。由于使用的鱼个体数少，如鳙，只有 7 个个体，样品也只来源于长江荆州江段。本研究无法基于两种鱼的天然分布，对两种鱼的遗传分化、地理种群多样性比较、养殖群体和天然群体差异等问题进行深入分析。但是，这组标记的研制将有助于这 2 个中国特有经济鱼种的遗传多样性分析、遗传资源的管理及开发利用等相关研究。本研究中，微卫星 DNA 标记的研制使用了固定有微卫星核心序列的磁珠。这样的磁珠与两端接有已知序列的 DNA 片段杂交能富集出含有微卫星核心序列的片段。通过扩增和连接转化，可方便地获得大量含微卫星核心序列的片段。与已有的方法不同的是，本研究用 AFLP 方法的某些步骤使片段两端加上已知引物序列，方便易行。迄今，这两种鱼还没有微卫星标记连锁图谱。构建这样的图谱是本项目研究的长远目标。[中国水产科学，2006, 13(5): 756 - 761]

关键词：微卫星 DNA 标记；简单重复序列标记；鲢；鳙；遗传多样性；种间通用性