Development of genome-wide microsatellite resources in a commercially important mussel species (Mytilus chilensis)

Article *in* Animal Genetics · December 2015

DOI: 10.1111/age.12398

7 authors, including:

Cristian Araneda
University of Chile
49 PUBLICATIONS **266** CITATIONS
SEE PROFILE

Fabiola Lafarga
Ensenada Center for Scientific Research an…
31 PUBLICATIONS **70** CITATIONS
SEE PROFILE

María Angélica Larraín
University of Chile
27 PUBLICATIONS **169** CITATIONS
SEE PROFILE

doi: 10.1111/age.12398

Development of genome-wide microsatellite resources in a commercially important mussel species (*Mytilus chilensis*)

Cristián Araneda*, Ximena Noriega†, Felipe Jilberto*, Jorge Ruíz†, Coral Jara†, Fabiola Lafarga‡ and María Angélica Larraín†

*Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, Avda. Santa Rosa 11315, La Pintana, Santiago 8820808, Chile; †Departamento de Ciencias de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Sergio Livingstone 1007, Independencia, 8380492 Santiago, Chile; ‡Departamento de Acuicultura, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Carretera Ensenada-Tijuana 3918, Zona Playitas, 22860 Ensenada, Baja California, México

Accepted for publication 28 October 2015

Source description: The Chilean blue mussel (*Mytilus chilensis*) is one of the most cultivated and marketed marine bivalves of the *Mytilus* genus. It is one of the top five mollusc species produced worldwide[1] and is widely appreciated for its nutritional value and use in prepared food.[1,2] In spite of its commercial importance, genetic resources for this species are limited. Here, we describe 16 polymorphic microsatellite markers. Pyrosequencing using a 454 GS Junior instrumentation yielded 9976 sequences with an average read length of 384 bp (Accession No. SAMN04158627) and allowed for the design of primers for 115 *in silico* microsatellite loci (Table S1). Eighty-nine microsatellite loci were tested for amplification in *M. chilensis* ($n = 15$), 17 of which were validated.

PCR conditions and genotyping: Microsatellite genotyping was performed using the M13-tailed primer method.[3] PCR amplifications were carried out in 15-μl reactions containing 1× manufacturer's PCR buffer, 1.5–3.2 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.16 μM of primer reverse, 0.16 μM of fluorescent-labelled universal M13 primer (6-FAM, VIC, NED or PET), 0.04 μM of primer forward with M13 tail, 0.1 U of Taq polymerase (Thermo Scientific®) and 40–60 ng of genomic DNA. Thermal cycling parameters were initial denaturation at 95 °C for 2 min followed by 30 cycles of denaturation at 95 °C for 30 s, primer-specific annealing temperature for 30 s (Table S2), extension at 72 °C for 30 s, then eight additional cycles at 94 °C (30 s), 53 °C (45 s) to bind M13-labelled primer and 72 °C (45 s) with a final extension at 72 °C for 12 min. Amplicons labelled with different fluorescent dyes were pooled in one reaction tube and run on an ABI-3130XL genetic analyser with LIZ GS-500 as an internal size standard (Applied Biosystems®). Alleles were scored with PEAK SCANNER v1.0 (Applied Biosystems®) and checked for possible genotyping errors with MICRO-CHECKER v2.2.3.[4] Genetic diversity was assessed using CERVUS v3.0.[5] Excluding the monomorphic *Mch07-Uch* marker, observed (H_O) and expected (H_E) heterozygosities ranged from 0 to 0.60 and from 0.26 to 0.95 respectively (Table S2). The number of alleles by locus (N_A) and polymorphism information content (PIC) ranged from 2 to 17 and from 0.062 to 0.915 respectively (Table S2).

We also tested the amplification of these markers in another eight mussel species: *Mytilus edulis* ($n = 16$), *Mytilus galloprovincialis* ($n = 16$), *Mytilus californianus* ($n = 7$), *Mytilus trossulus* ($n = 8$), *Choromytilus chorus* ($n = 8$), *Perumytilus purpuratus* ($n = 8$), *Semimytilus algosus* ($n = 8$) and *Aulacomya atra* ($n = 8$). The success rate ranged from 13 markers (*M. edulis*) to zero microsatellites (*S. algosus*) (Table S3).

We conclude that the markers we have developed show sufficient polymorphism for application in population genetic studies and paternity/maternity exclusion analysis.

Acknowledgement: Conicyt-Fondecyt Grant 11303302.

References

1 Astorga M. (2014) *Frontiers in Genetics* **5**, 435.
2 Larraín M.A. *et al.* (2014) *Food Research International* **62**, 104–10.
3 Schuelke M. (2000) *Nature Biotechnology* **18**, 233–4.
4 Van Oosterhout C. *et al.* (2004) *Molecular Ecology Notes* **4**, 535–8.
5 Kalinowski S.T. *et al.* (2007) *Molecular Ecology* **16**, 1099–106.

Correspondence: M. A. Larraín (mlarrain@uchile.cl)

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Microsatellite loci identified *in silico* within the *Mytilus chilensis* 454 sequencing.

Table S2 Primer sequences and characteristics for 17 microsatellite loci validated for *Mytilus chilensis*.

Table S3 Number of alleles detected in the cross-amplification of microsatellite markers derived from the Chilean blue mussel in other mussel species. No amplification was observed for *Semimytilus algosus*.

A G E | Journal Code | 12398 | Manuscript No. | WILEY | Dispatch: 30.11.15 | No. of pages: 1 | CE: Ashok Raj | PE: Prasanna