AFLP analysis as a tool to investigate genetic diversity in microalgae

Cecilia Rad-Menéndez
Scottish Association for Marine Science (SAMS), Oban, UK

PREAMBLE. AFLP analyses have proven able to resolve genetic variations when other genetic markers have reached their limits (Werner et al. 2001, Müller et al. 2005) and so have the potential to reveal genetic diversity and differentiate between populations. The method described below is based on Müller et al. (2005), with slight modifications.

1. To ensure reproducibility of this technique, DNA should be extracted twice on two separate occasions, using fresh reagents etc. Then the protocol outlined below should be conducted three times: twice using aliquots from one extraction and the third time using the other extraction.
2. Restriction and ligation of total genomic DNA should be performed in a single reaction as described by Mannschreck et al. (2002). Incubate aliquots (5.5µl) of high molecular genomic DNA with 1U *Mse*I, 5U *Eco*RI and 1 Weiss Unit T4 DNA ligase (New England Biolabs, Frankfurt, Germany) in T4 Ligase buffer with 55 mM NaCl, 0.55 µg BSA, EcoR1 and Mse1 adapters [5 and 50 pmol, sequences as in Vos et al. (1995)] in a total volume of 11µl for 3h at 37°C.
3. The pre-selective amplification is modified from (Müller et al. 2005) as follows: use 4µl of the reactions with 2.5 pmol of the primers: *Mse*I+0 (GATGAGTCCTGAGTAA) and *Eco*RI+0 (GACTGCGTACCAATTC) (Vos et al. 1995), 10µl of Taq PCR Mastermix (Qiagen, Hilden, Germany) and 5µl double distilled water, in a total volume of 20µl. The parameters for the amplification are: 5 min at 94°C, followed by 20 cycles of 20 sec at 94°C, 30 sec at 56°C and 120 sec at 72°C.
4. Check the quality of the pre-selective amplification on a 1.5% (w/v) agarose gel, and depending on the amount of PCR products generated, dilute them (typically between 0 to 1/10) prior to selective amplification.
5. In the next amplification, use 4µl of the diluted pre-selective amplification product in a total reaction volume of 20µl with the following primer combinations: *Eco*RI+A (GACTGCGTACCAATTCA, 10 pmol), *Eco*RI+C (GACTGCGTACCAATTCC, 5 pmol) and *Mse*I+C (GATGAGTCCTGAGTAAC, 10 pmol). Label *Eco*RI+A with the fluorochrome 6-FAM and EcoRI+C with VIC (Applied Biosystems, Foster City, CA, USA). Use the following selective amplification conditions: 5 min at 94°C, followed by 10 cycles of 20 sec at 94°C, 30 sec at 65°C with 1°C decrease after each cycle down to 56°C and 120 sec at 72°C, followed by 20 cycles of 20 sec at 94°C, 30 sec at 56°C and 120 sec at 72°C.

Algae: Method has been successfully employed for a variety of unicellular algae including unicellular greens and diatoms.

Apparatus: SIGMA 1-14 microcentrifuge (Sigma-Aldrich Ltd., Dorset, UK), electrophoresis chamber, ABI 3730 capillary sequencer (Applied Biosystems), ABI GeneMapper (GenePool Sequencing Facility, Edinburgh, UK)

Solutions: Plant DNAZOL Reagent (Gibco BRL, Grand Island, NY, USA), Taq PCR Mastermix Kit (Qiagen, Hilden, Germany), QIAquick PCR Purification Kit(Qiagen, Hilden, Germany),

Additional information:

Vos et al. 1995 *AFLP: a new technique for DNA fingerprinting. Nucl Acids Res **23**: 4407-4414.*

Mannschreck et al. 2002 *Evaluation of an emission inventory by comparison of modeled and measured emission ratios of individual HCs, CO and NOx. Atmospheric Environment **36**: S81-S94.*

Müller et al. 2005 *Distinction between multiple isolates of Chlorella vulgaris (Chlorophyta, Trebouxiophyceae) and testing for conspecificity using amplified fragment length polymorphism and ITS rDNA sequences. J Phycol **41**: 1236-1247*

culturecollection
of algae and protozoa

6. AFLP analysis of the samples should be conducted using an ABI 3730 capillary sequencer, or equivalent. Combine a small volume (1µl) of the ten times diluted selective amplification with 1µl of the fifty times diluted GeneScan 1200 Liz Size Standard (20-1200bp range; Applied Biosystems, Foster City, CA, USA) and analyse using ABI GeneMapper, or equivalent (e.g. GenePool Sequencing Facility, Edinburgh, UK).
7. On receipt of the sequence data, import into GelQuest software (SequentiX - Digital DNA processing, Klein Raden, Germany) or equivalent, and analyse as required.