# Microsatellites development in *Ostrea edulis* and *Mytilus edulis*

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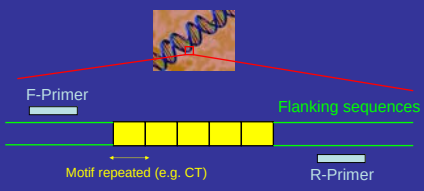
## CONTEXT AND AIM OF THE STUDY


- European flat oyster *Ostrea edulis* and blue mussel *Mytilus edulis*: very valuable commercial species across Europe
- Microsatellite: short fragments of DNA made up of sequences repeated in tandem arrays of 2-6 bp
- Microsatellite features: codominance, abundance, high polymorphism, neutrality, Mendelian inheritance
  → markers of choice for numerous studies
- Only a few microsatellites available for those species (e.g. Launey *et al.* 2002, Presa *et al.* 2002)




**Aim of the study: development of new microsatellites for *O. edulis* and *M. edulis* that could be used for population genetics studies, genetic variability assessment stocks, parentage analysis or genetic and QTL mapping studies**

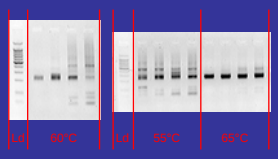
## FLOWCHART

DNA extraction
↓
Construction of genomic library enriched with 2 repeat motifs (GT/AC, CT/AG)
↓
Screening of library with fluorescent probes
↓
Sequencing of positive clone
↓
Primer design (Primer3)
↓
PCR optimisation
↓
Test of polymorphism

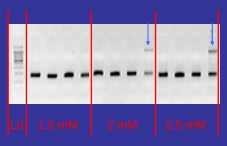

Fluorescently labelled CT probes


## 1st STAGE PCR OPTIMISATION: 2% AGAROSE GEL

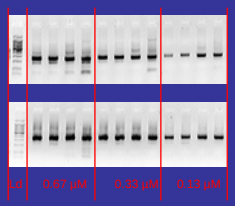
- Optimisation of PCR conditions was first performed on 2% agarose gels, by changing the annealing temperature, the $MgCl_2$ concentration and the primers concentration
- Successful amplification achieved: single band observed for the 4 tested individuals



Effect of annealing temperature



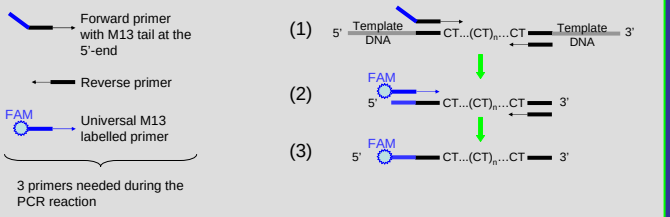
Effect of $MgCl_2$ concentration



Effect of primers concentration

## 2nd STAGE PCR OPTIMISATION: 3130*xl* GENETIC ANALYZER (APPLIED BIOSYSTEMS)

### Economic method for fluorescent labelling of PCR fragments (Schuelke 2000)




**Test panel**
(8 individuals chosen at random)

successful amplification

### Test of polymorphism

- Scoring (30 individuals from wild population)
- Statistical analyses: number of alleles, observed and expected heterozygosity, deviations from Hardy Weinberg equilibrium, estimation of null allele frequency (MICRO-CHECKER)

## RESULTS / DISCUSSION

- Technical problems occurring during the development stage:
  - ❖ occurrence of polymononucleotide repeats in the flanking sequences
  - ❖ visualisation of peaks every one bp (could reflect the non-templated addition of Adenine by *Taq* Polymerase)
  - ❖ amplification of aspecific bands
  - ❖ stuttering
  - ❖ large allele dropout
- General features of newly developed microsatellites:
  - ❖ high polymorphism
  - ❖ significant deviations from Hardy Weinberg equilibrium suggesting occurrence of null alleles

→ Null alleles have commonly been reported for various bivalve species and represent major limitations

**Characterisation of 10 novel microsatellites in *M. edulis* and 28 in *O. edulis***

| | *Mytilus edulis*  | *Ostrea edulis*  |
|---|---|---|
| No. recombinant colonies screened | 750 | 758 |
| Positive signal after hybridization | 157 | 179 |
| No. clones sequenced | 157 | 133 |
| No. primers designed | 62 | 94 |
| Successful amplification (2% agarose gel) | 40 | 76 |
| Test of polymorphism | 11 | 28 |
| **No. polymorphic microsatellites kept** | **10** | **28** |

## REFERENCES

Launey S., Ledu C., Boudry P., Bonhomme F. and Naciri-Graven Y. (2002) Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. The Journal of Heredity 93: 40-47

Presa P., Pérez M. and Diz A. (2002) Polymorphic microsatellite markers for the blue mussels (*Mytilus* spp.). Conservation genetics 3: 441-443

Schuelke M. (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233-235