

# Origins of the Nofima project



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*Aquaculture* 250 (2005) 70–81

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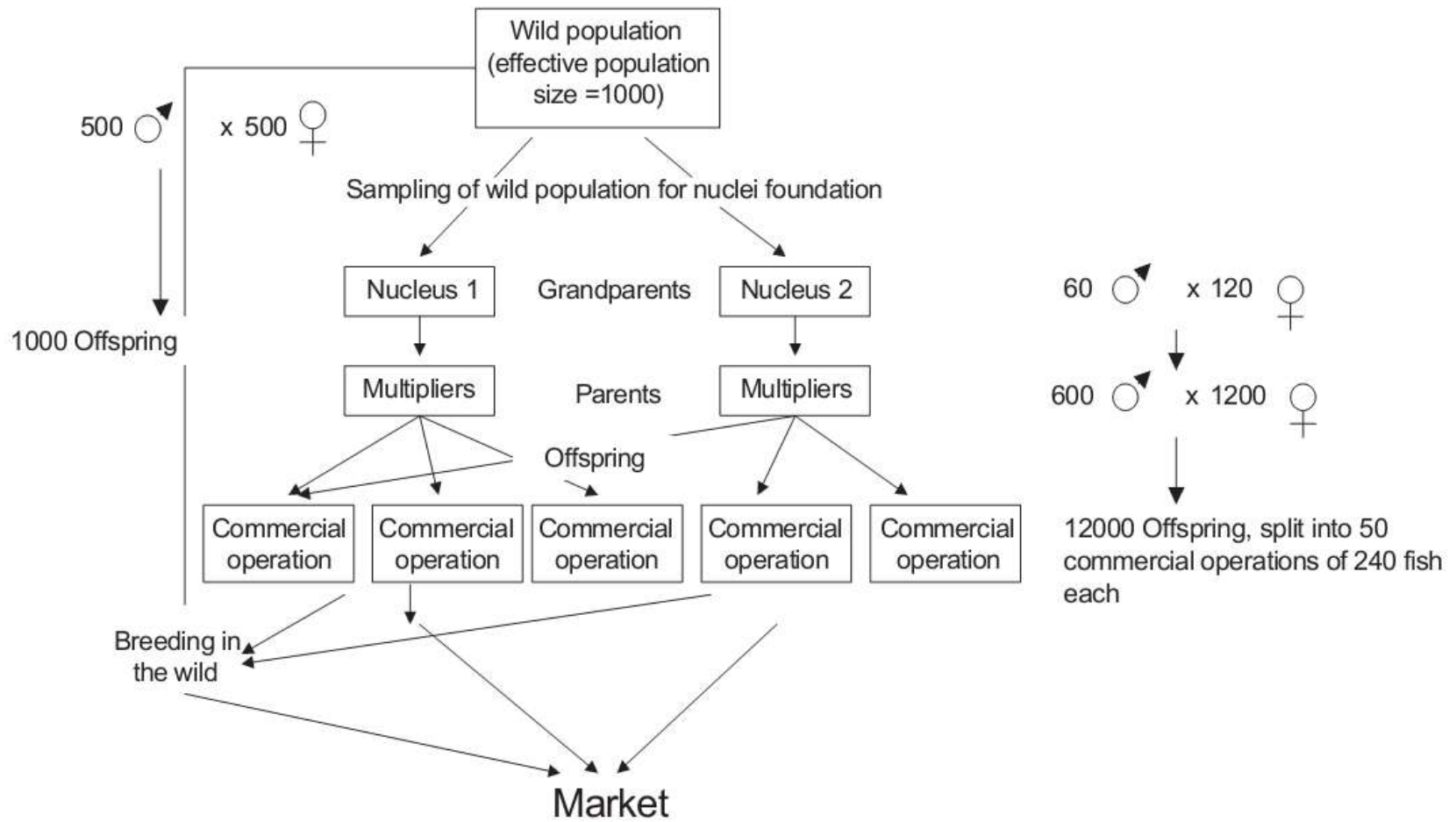
## Evaluation of three strategies using DNA markers for traceability in aquaculture species

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Received 14 May 2004; received in revised form 27 January 2005; accepted 2 March 2005

# Simulated scheme



# Simulations of the 'PAR' strategy

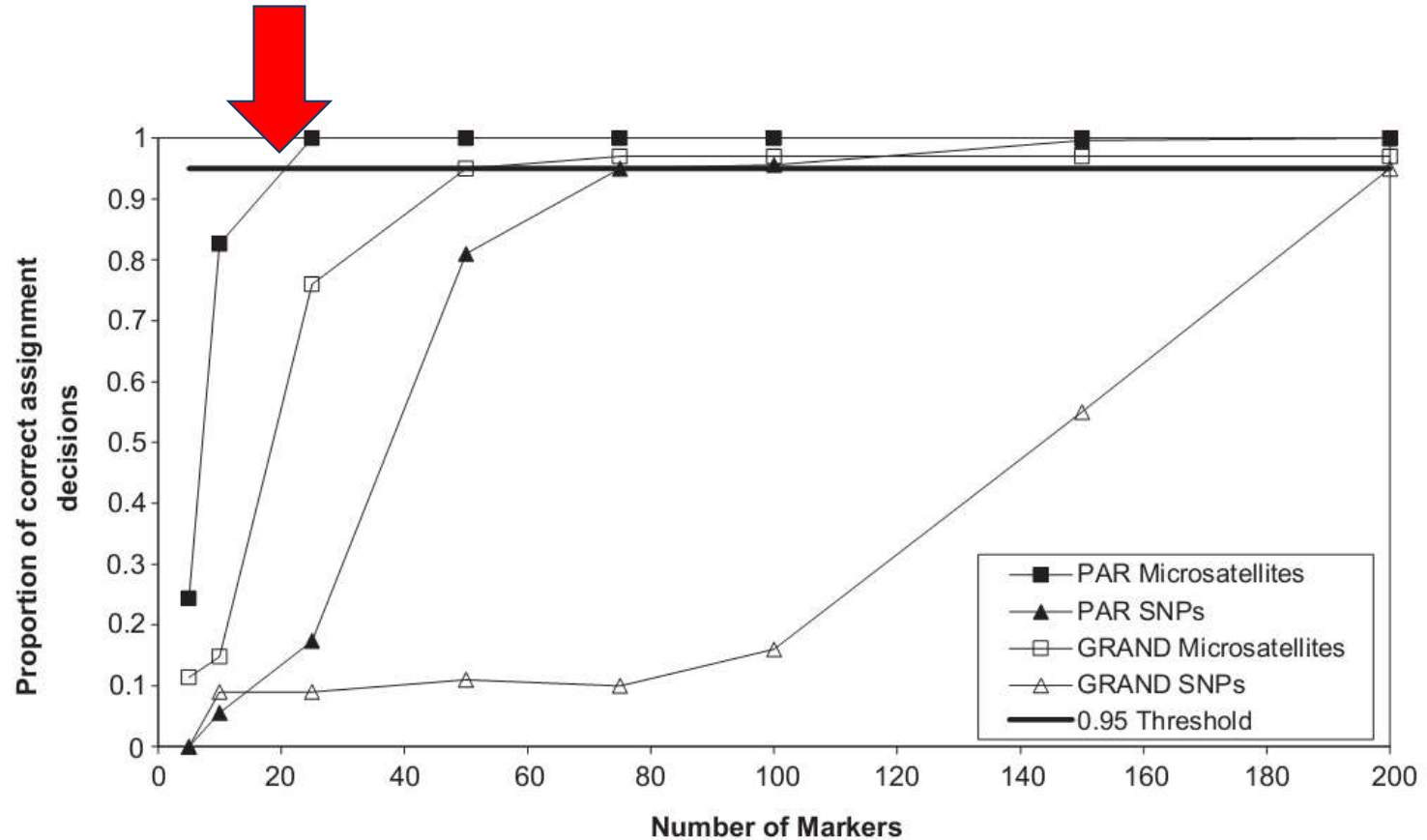
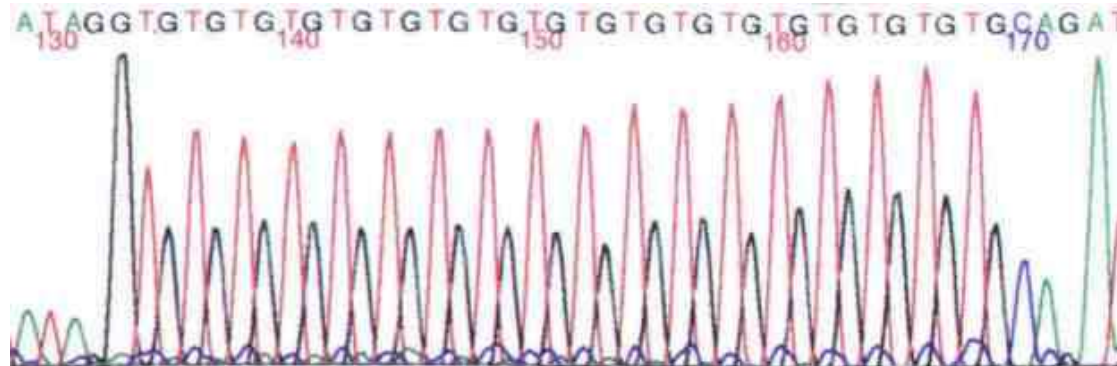


Fig. 5. Proportion of correct assignment decisions from strategies PAR and GRAND with increasing number of microsatellite and SNP markers.

# Microsatellites for parentage assignment

- Microsatellites = STR = SSR = short tandem repeated sequence
- Advantages
  - Highly polymorphic (multi-allelic)
  - Simple protocol
  - Cheap
  - No specialised equipment needed



# Microsatellite marker multiplex

- Aim to produce the equivalent of commercial multiplex identification panels, eg:
  - Bovine Genotypes™ Panel 3.1 (18 loci)
  - Canine Genotypes™ Panel 1.1 (19 loci)
  - Equine Genotypes™ Panel 1.1 (17 loci)



## Tetranucleotide microsatellites contribute to a highly discriminating parentage test panel in pig

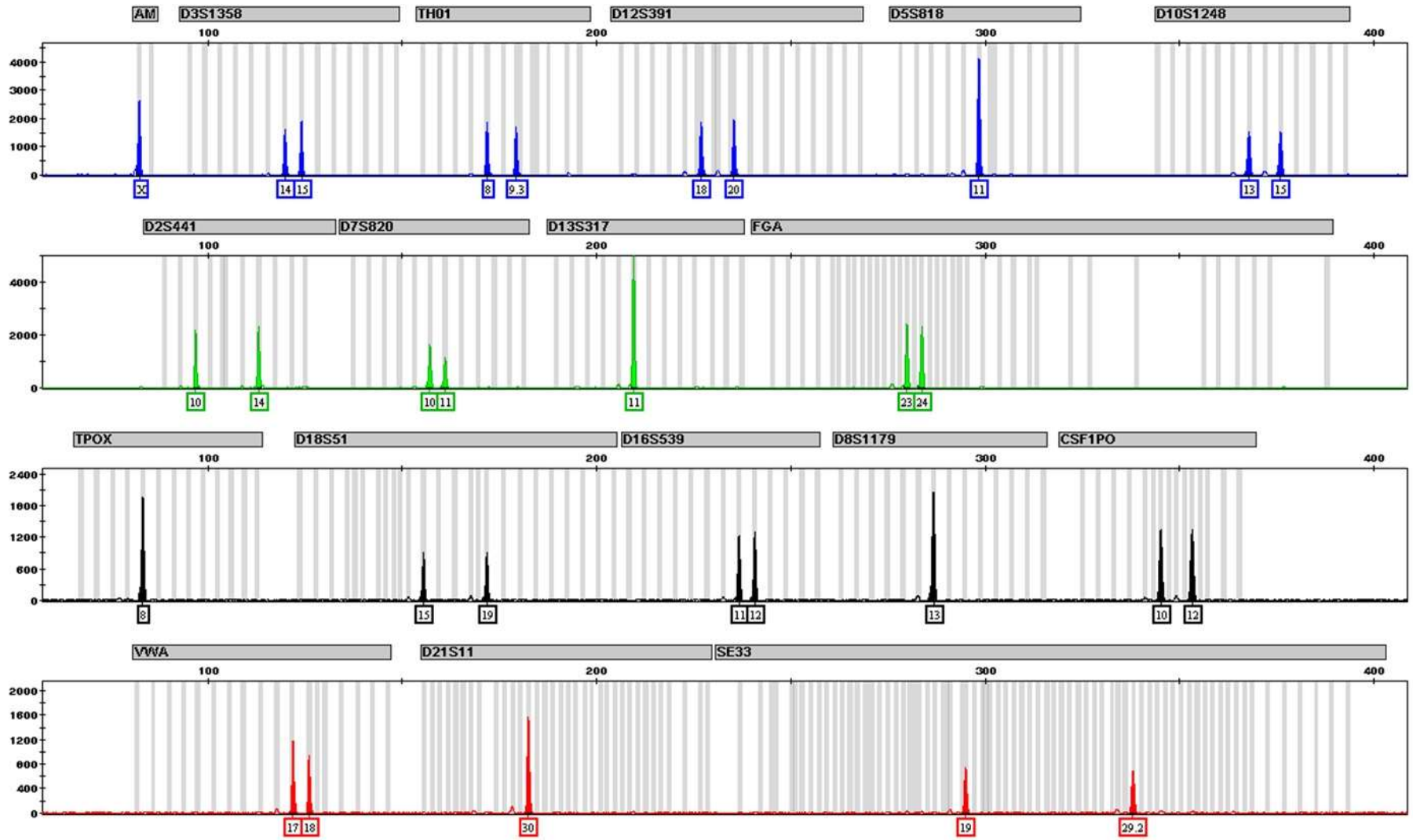
P. Cherel, J. Glénisson and J. Pires

France-Hybrides, 100 Avenue Denis Papin, St Jean de Braye, F-45808 Cedex, France

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### Summary

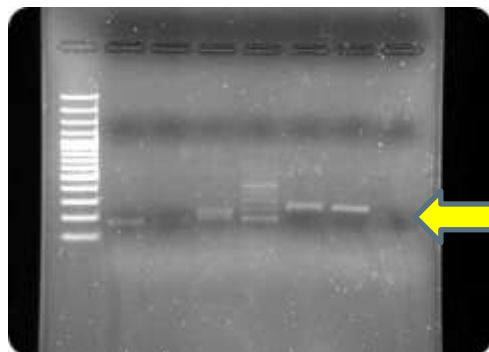
Here, we report genotyping conditions for 434 new polymorphic pig microsatellite markers



**18 markers in total**

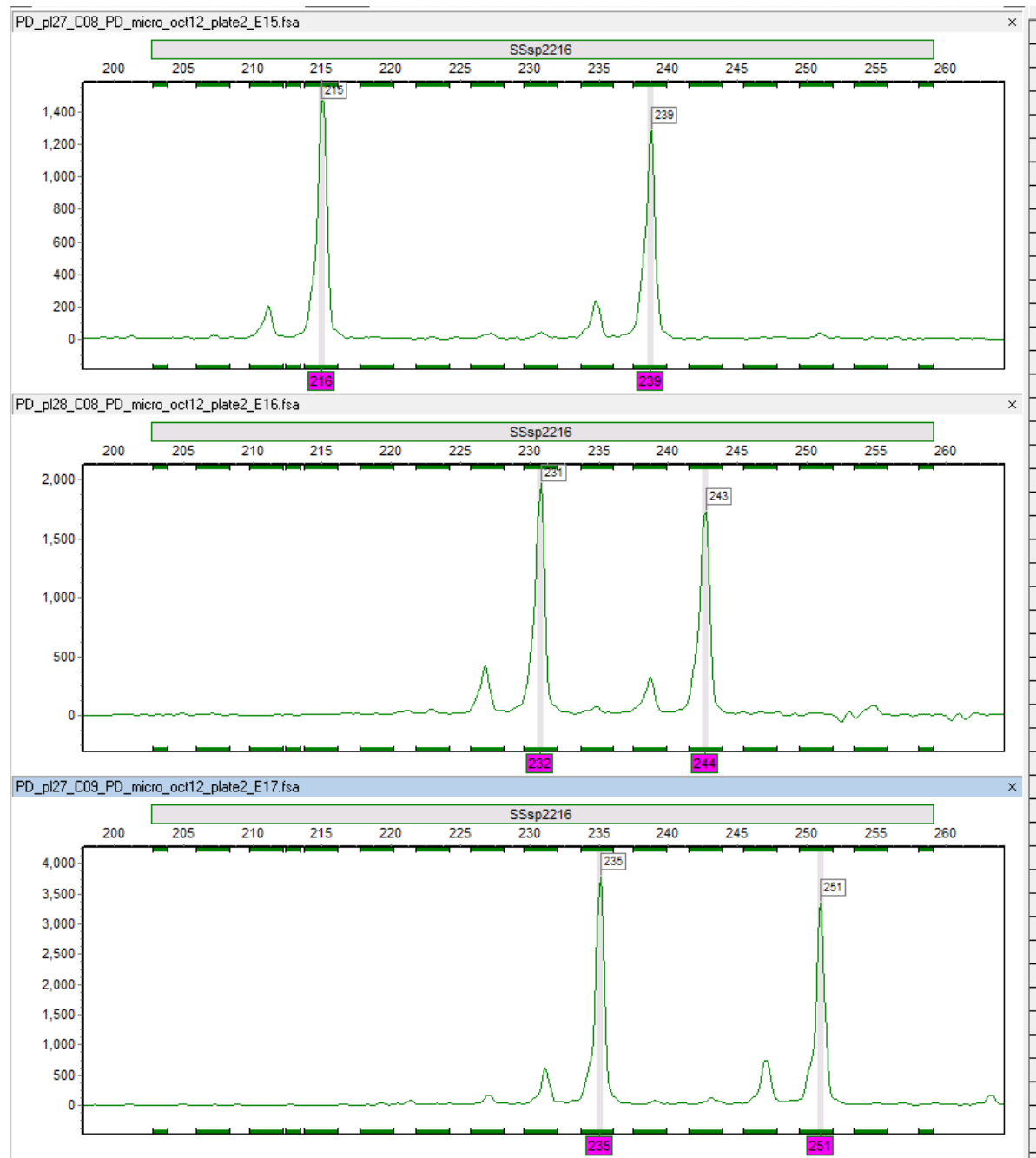
# Development of an efficient microsatellite marker multiplex

- Scan of two Atlantic salmon genome assemblies (public release and Cigene) for new microsatellites performed
  - 26.309 candidate markers discovered in Cigene assembly
  - 22.537 candidate markers discovered in public assembly
- Primers ordered for 80 new markers
  - PCR performed on 96 samples to test initial amplification of 12 markers, high success rate so far



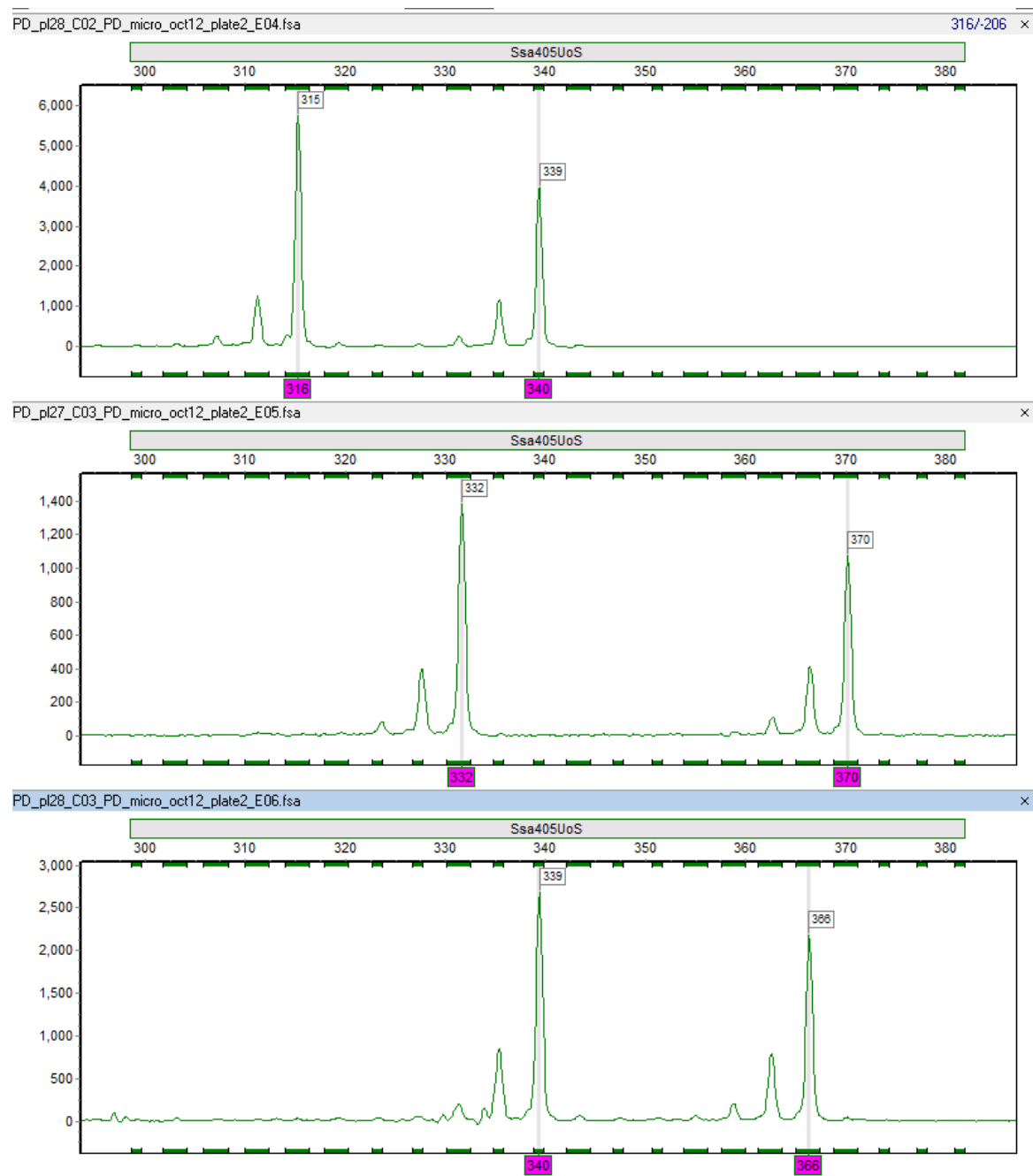
Successfully amplified markers

# Existing high quality markers

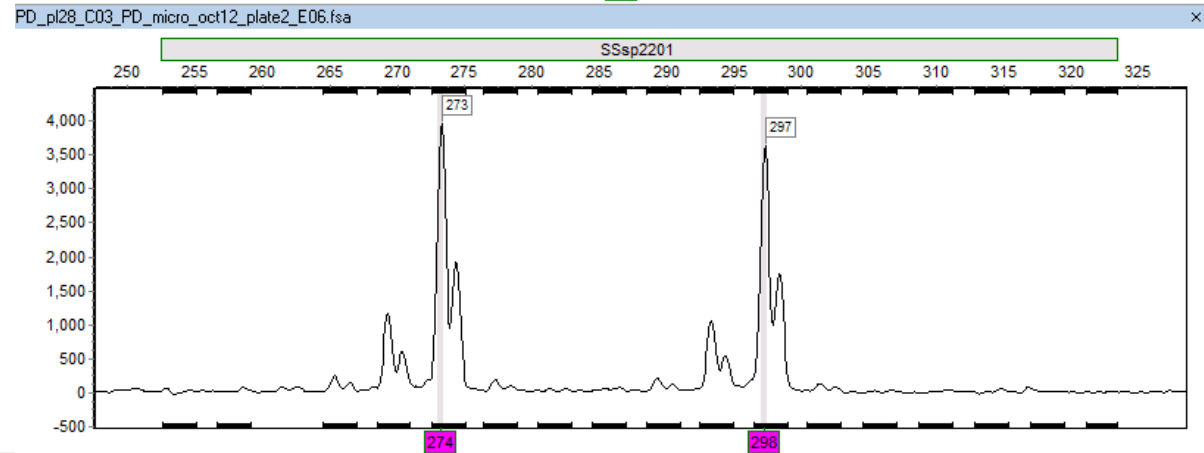
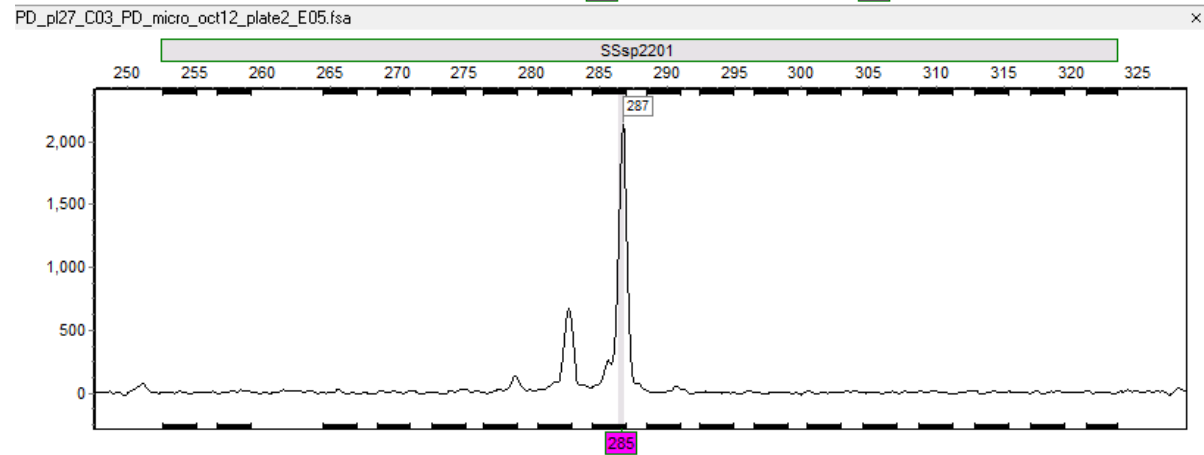
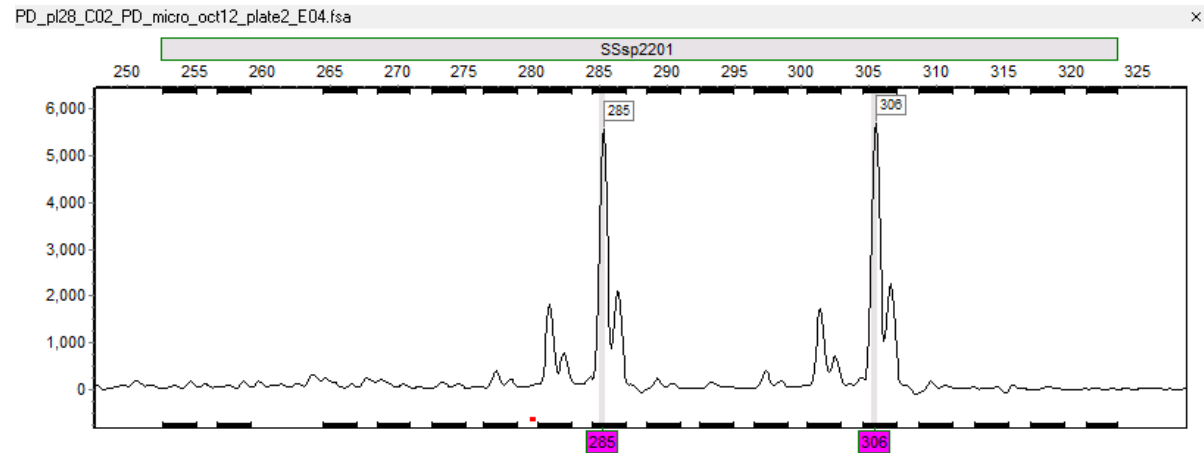




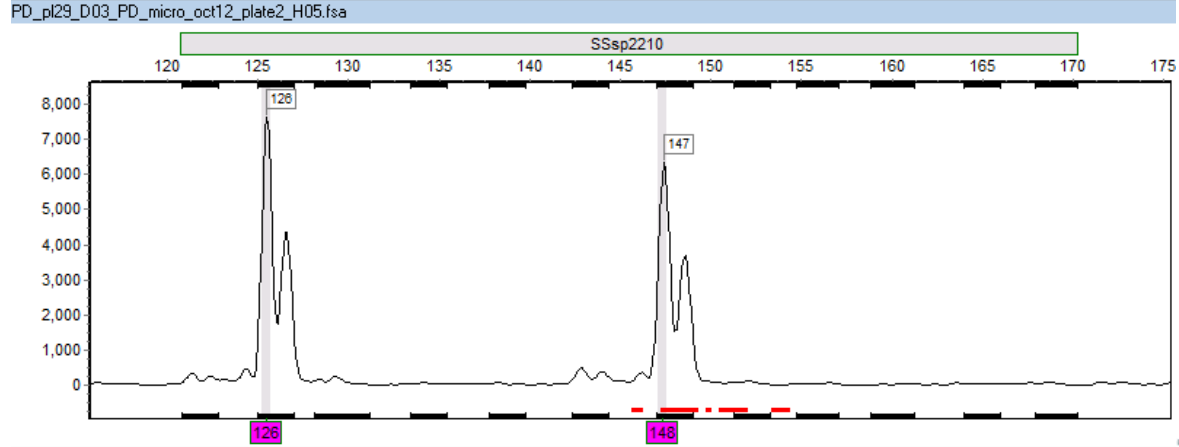
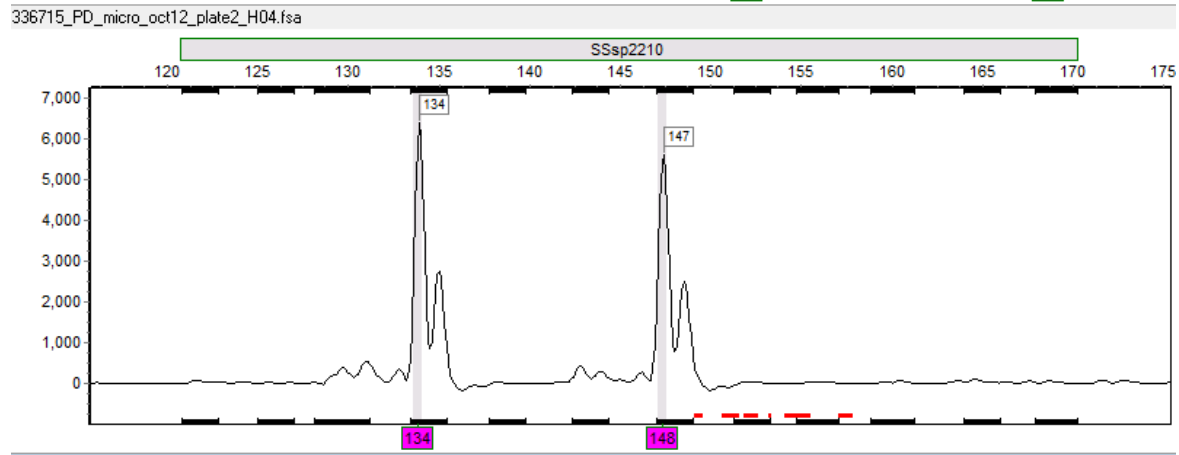
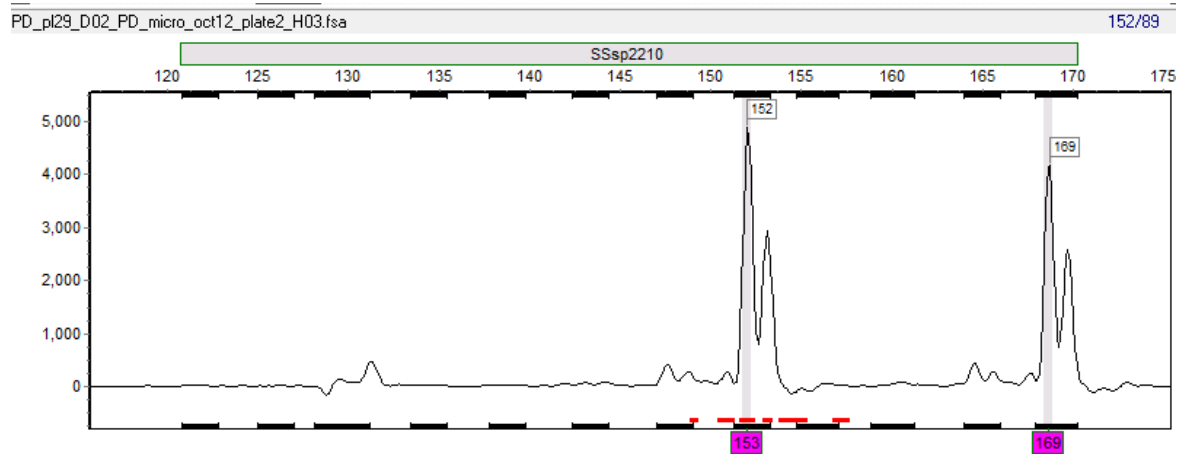
# Existing high quality markers



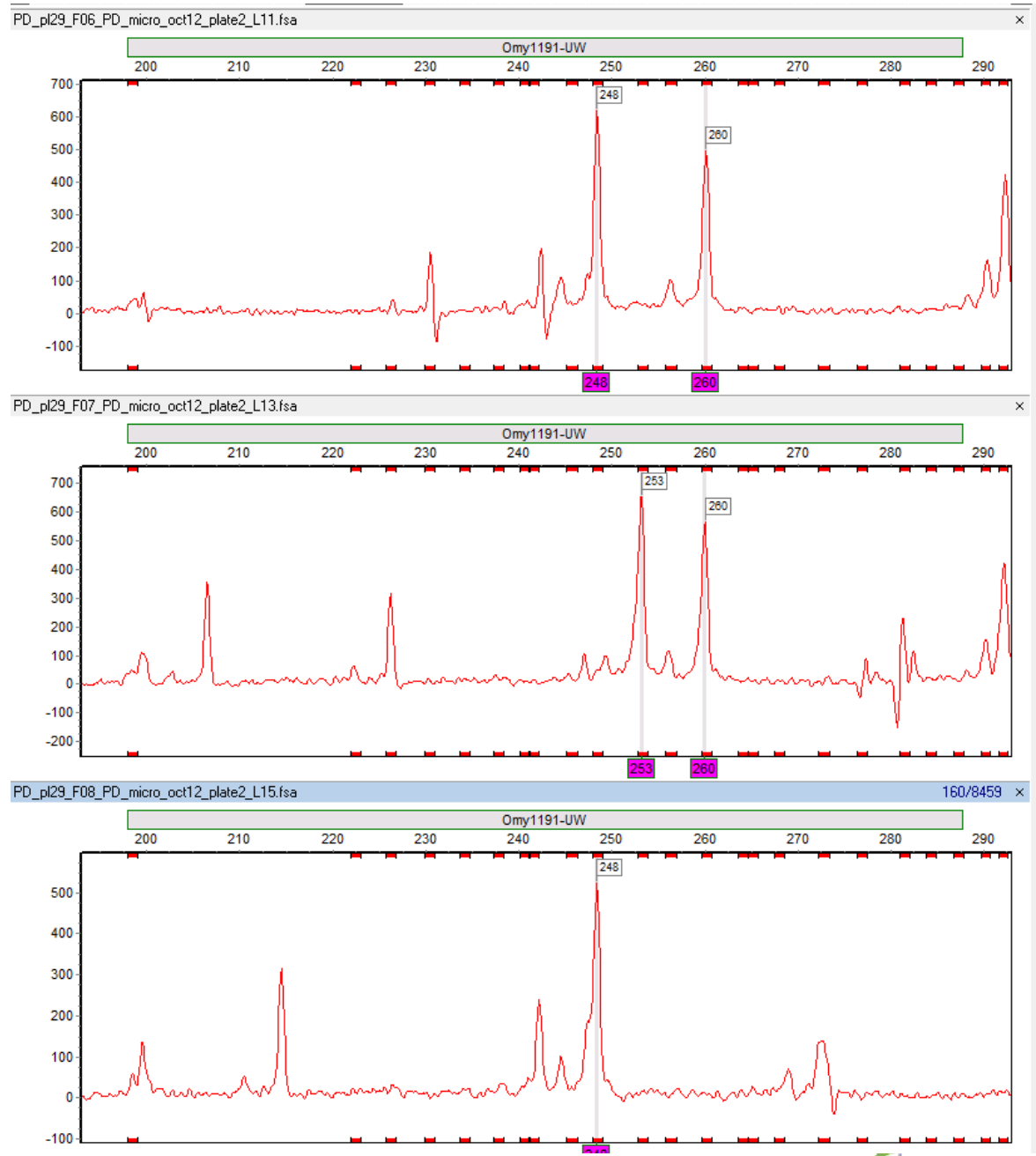
# Existing high quality markers



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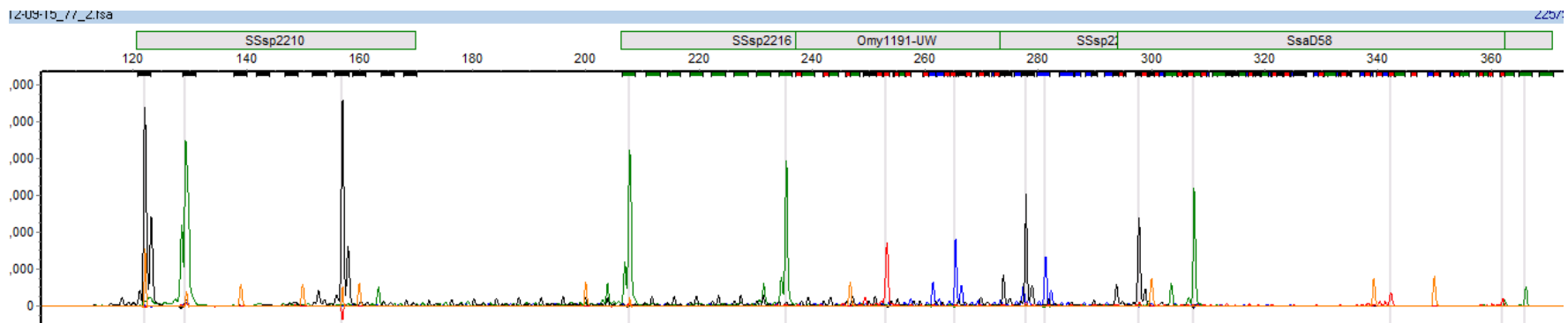


# Not so good marker....



# Preliminary results from a 'halfway' multiplex

- Seven highly polymorphic markers
  - Five very high quality and two lower quality
- Tested with a dataset from Aqua Gen
  - 362 offspring assigned parental crosses out of 384
  - Shows that even seven highly polymorphic markers have high power
- Final multiplex of 12-16 markers should dramatically better
  - Important for crosses of closer relatives

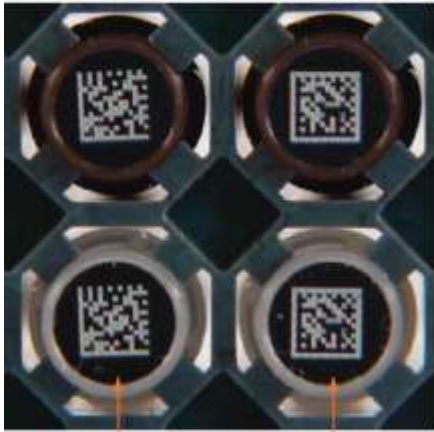


# Optimisation of sampling, transport, DNA extraction and storage methods

- Proposed tracing scheme will depend of sampling and genotyping of 50.000+ samples per year
- Huge logistical challenge
- Methods and protocols are needed to ensure:
  - Efficient sampling of thousands of fish by workers with a range of skill levels
  - Secure tracking, handling and transport of samples
  - Adequate preservation of tissue for downstream analysis
  - High throughput DNA extraction and genotyping
  - Efficient data analysis and data storage

# Sampling and sample preservation

- A pre-requisite for downstream lab processing is the '96-well' format
- Room temperature sample preservation is preferred
- No downstream manual handling of samples
  - 3mm tissue sample appears to be a good compromise between field practicality and lab processability
- Different sampling equipment being evaluated
- Need to ensure simple and effective protocols that can be used by whole industry



2D-coded tubes  
(white on black)

2D-coded tubes  
(black on white)





# Efficient lab processing – DNA

- Many different methods available for DNA extraction
- Range in speed, cost, throughput and final product quality
- Three methods are being compared:
  - Chelex
  - High-salt precipitation
  - Silica column kit
  - Methods will be evaluated and compared for:
    - Speed, cost, throughput, and DNA quality
- **Most importantly, do they produce DNA that produces consistent amplification quality in the SNP and microsatellite assays**
- Do we want archival DNA or 'one time' DNA?