Origins of the Nofima project

ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Aquaculture 250 (2005) 70–81

Aquaculture

www.elsevier.com/locate/aqua-online

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

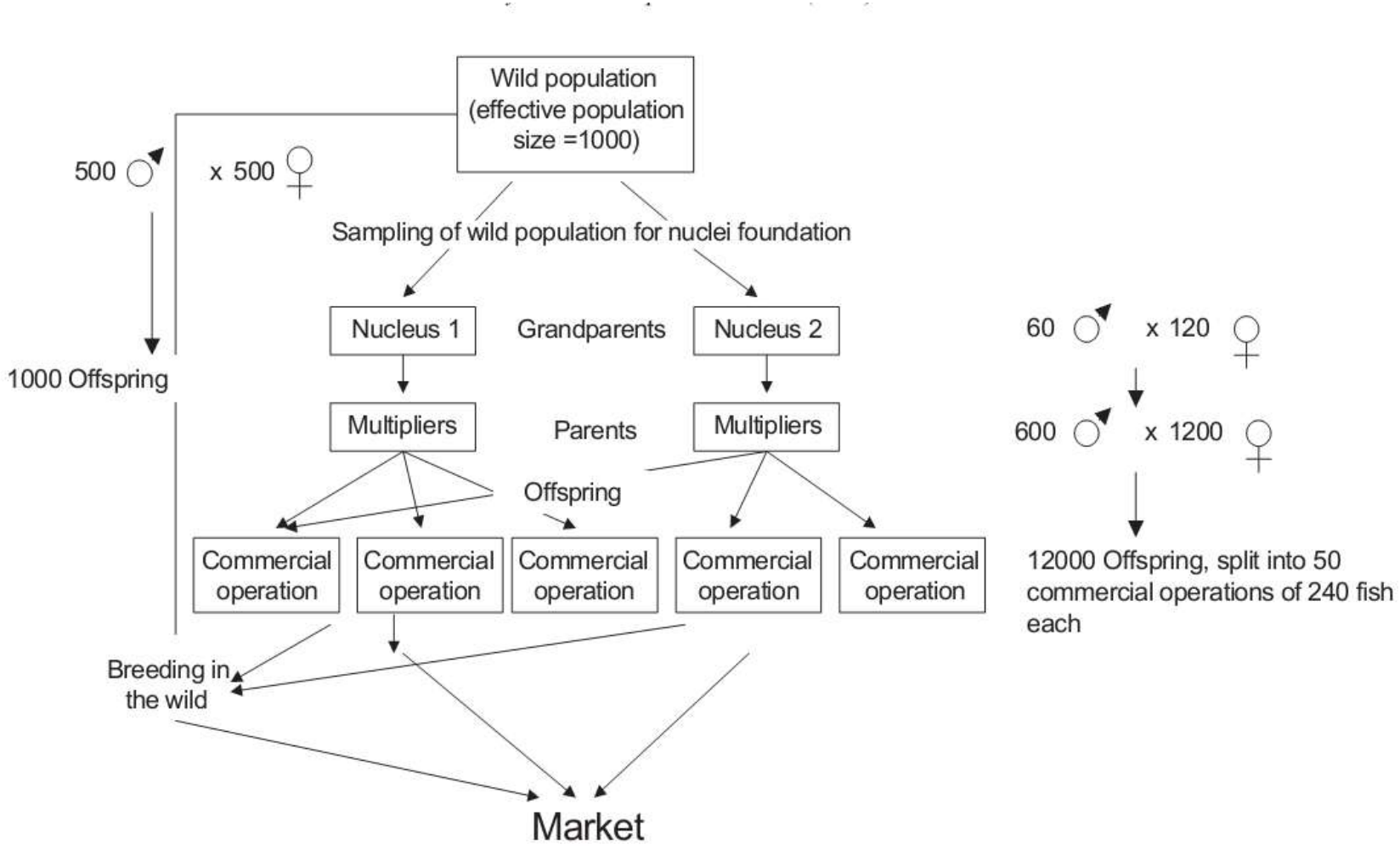
Ben Hayes*, Anna K. Sonesson, Bjarne Gjerde

AKVAFORSK, Institute for Aquaculture Research, P.O. 5010, 1432 Ås, Norway

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

Nofima

Simulated scheme



Simulations of the 'PAR' strategy

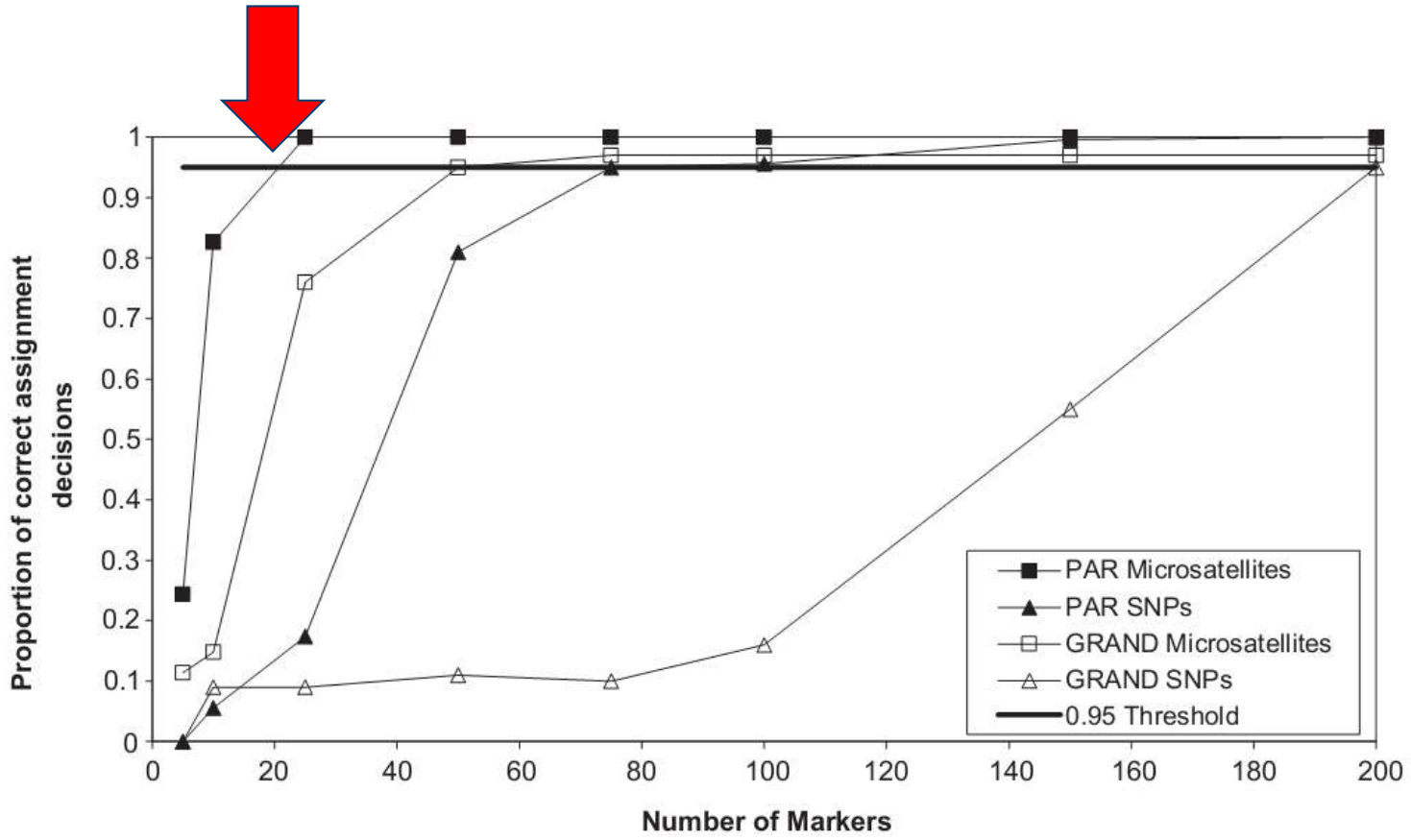
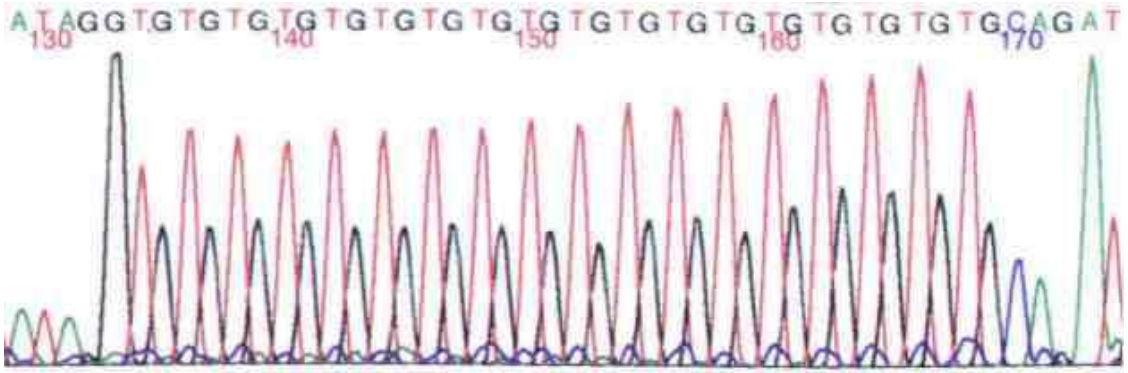


Fig. 5. Proportion of correct assignment decisions from strategies PAR and GRAND with increasing number of microsatellite and SNP markers.

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)

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

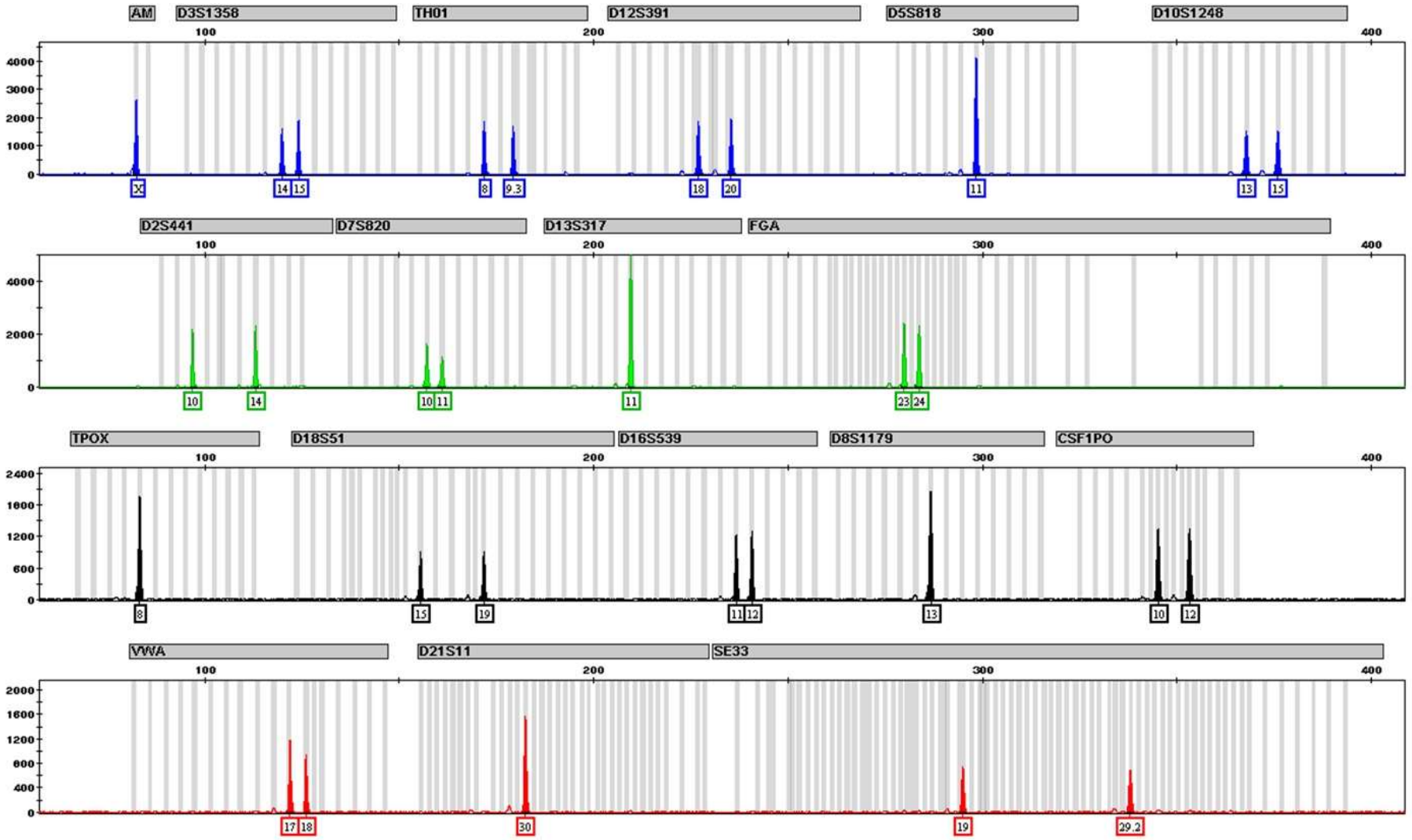
SHORT COMMUNICATION doi:10.1111/j.1365-2052.2011.02187.x

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

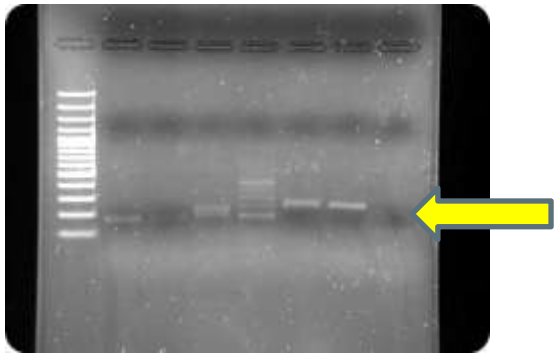
Summary Here, we report genotyping conditions for 434 new polymorphic pig microsatellite markers



18 markers in total

Development of an efficient microsatellite marker multiplex

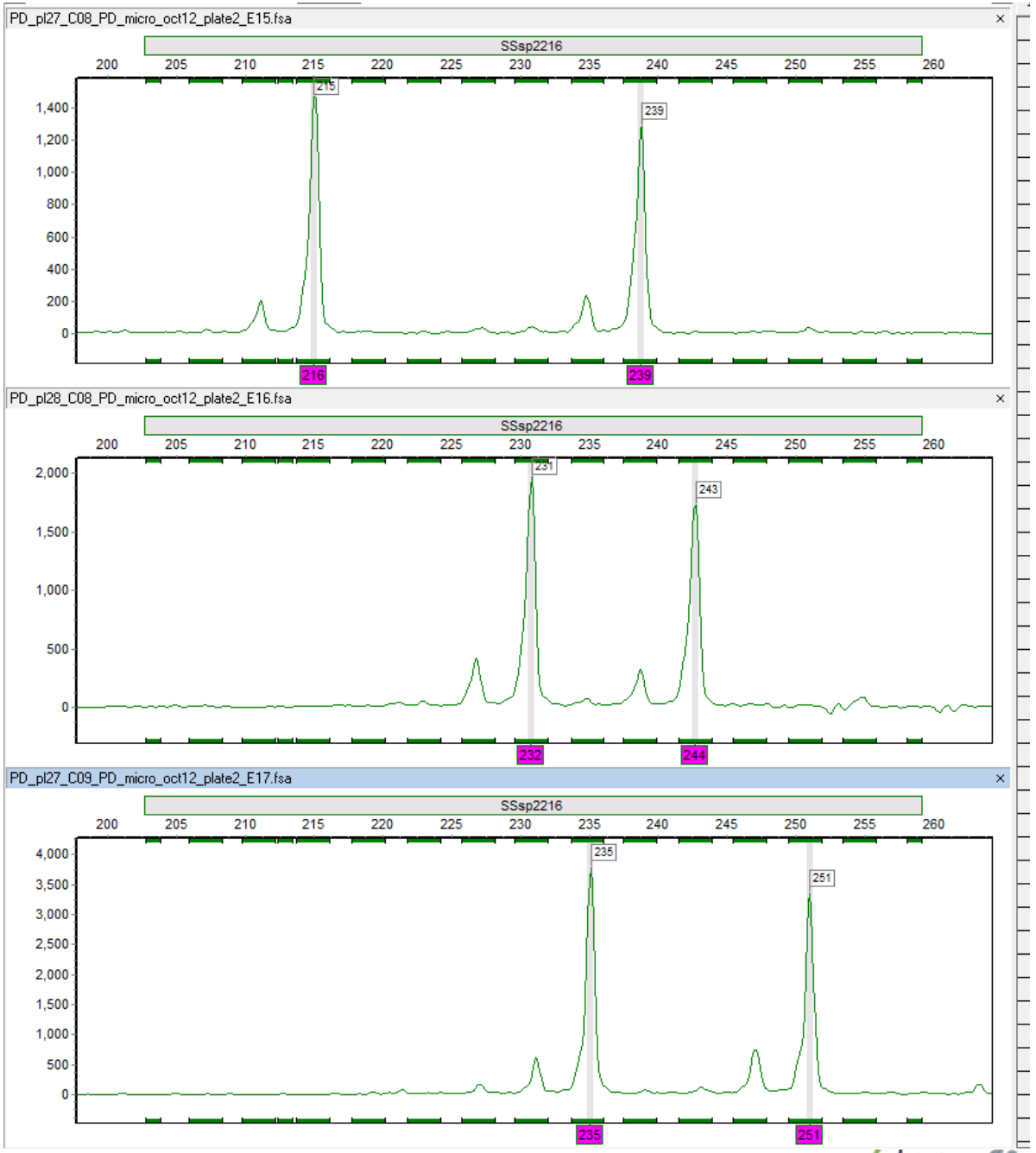
- Scan of two Atlantic salmon genome assemblies (public release and Cigene) for new microsatellites performed
  - 26.309 candidate markers discovered in Cigene assembly
  - 22.537 candidate markers discovered in public assembly
- Primers ordered for 80 new markers
  - PCR performed on 96 samples to test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



Successfully amplified markers

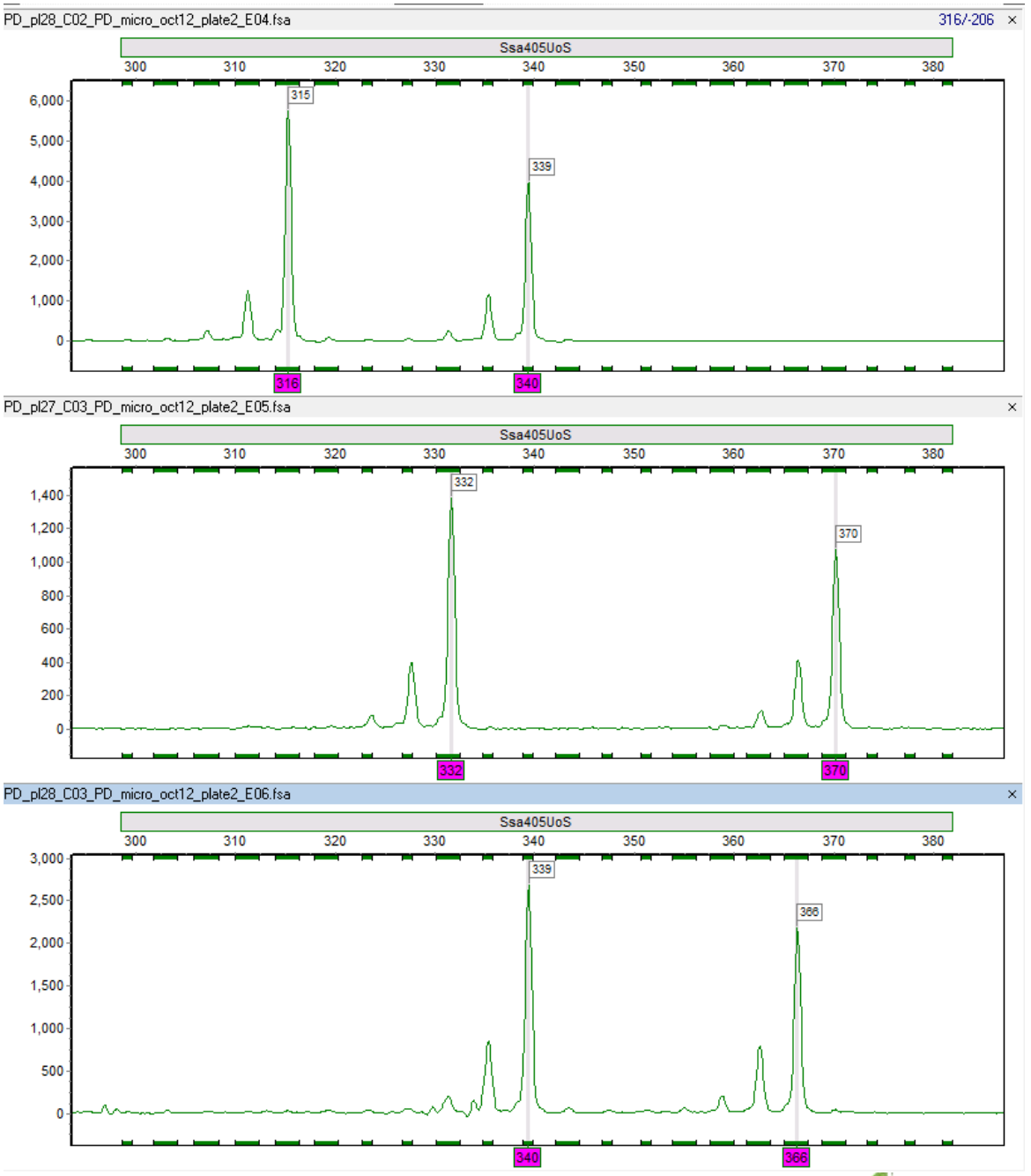
Nofima

Existing high quality markers

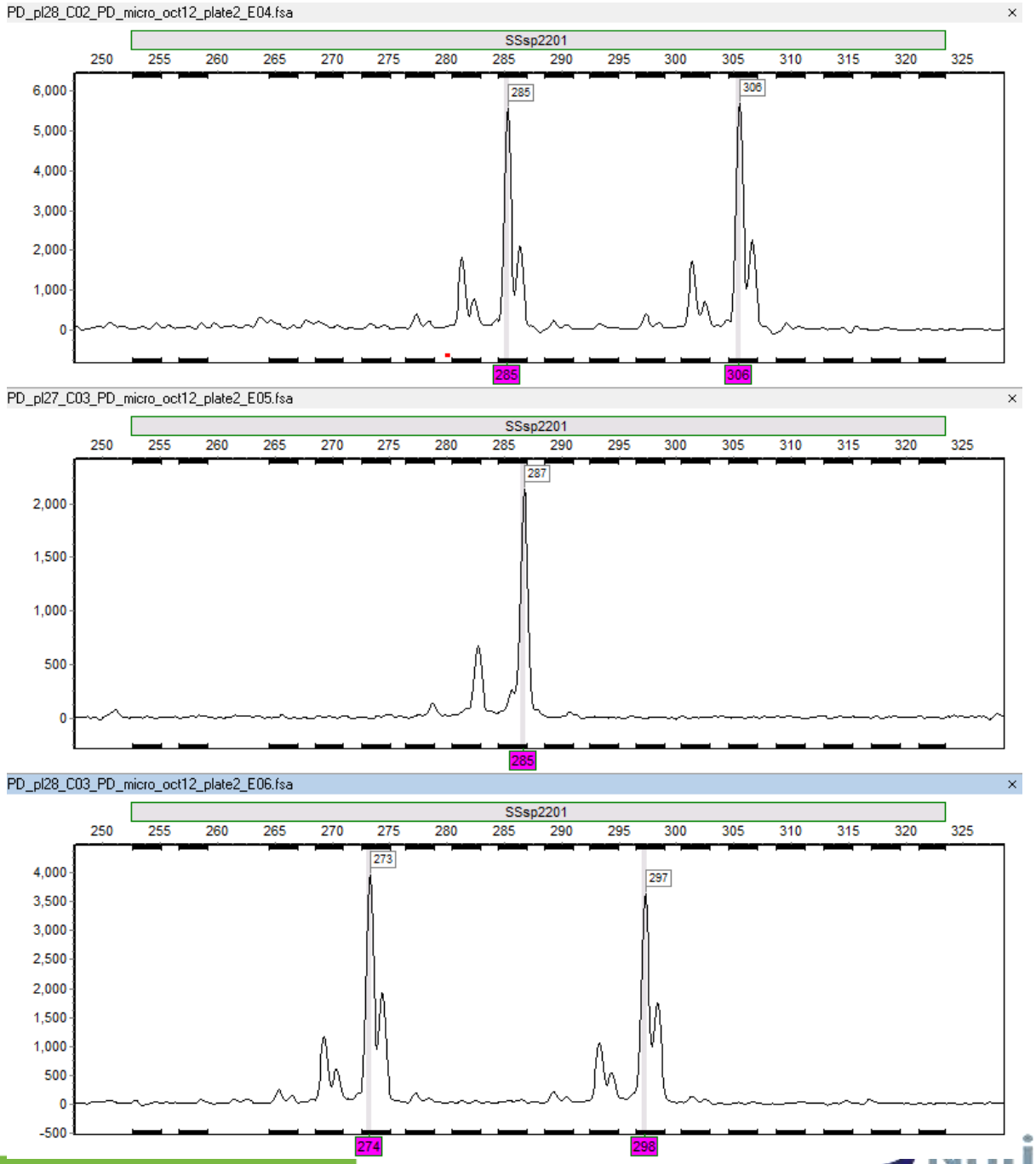


Nofima

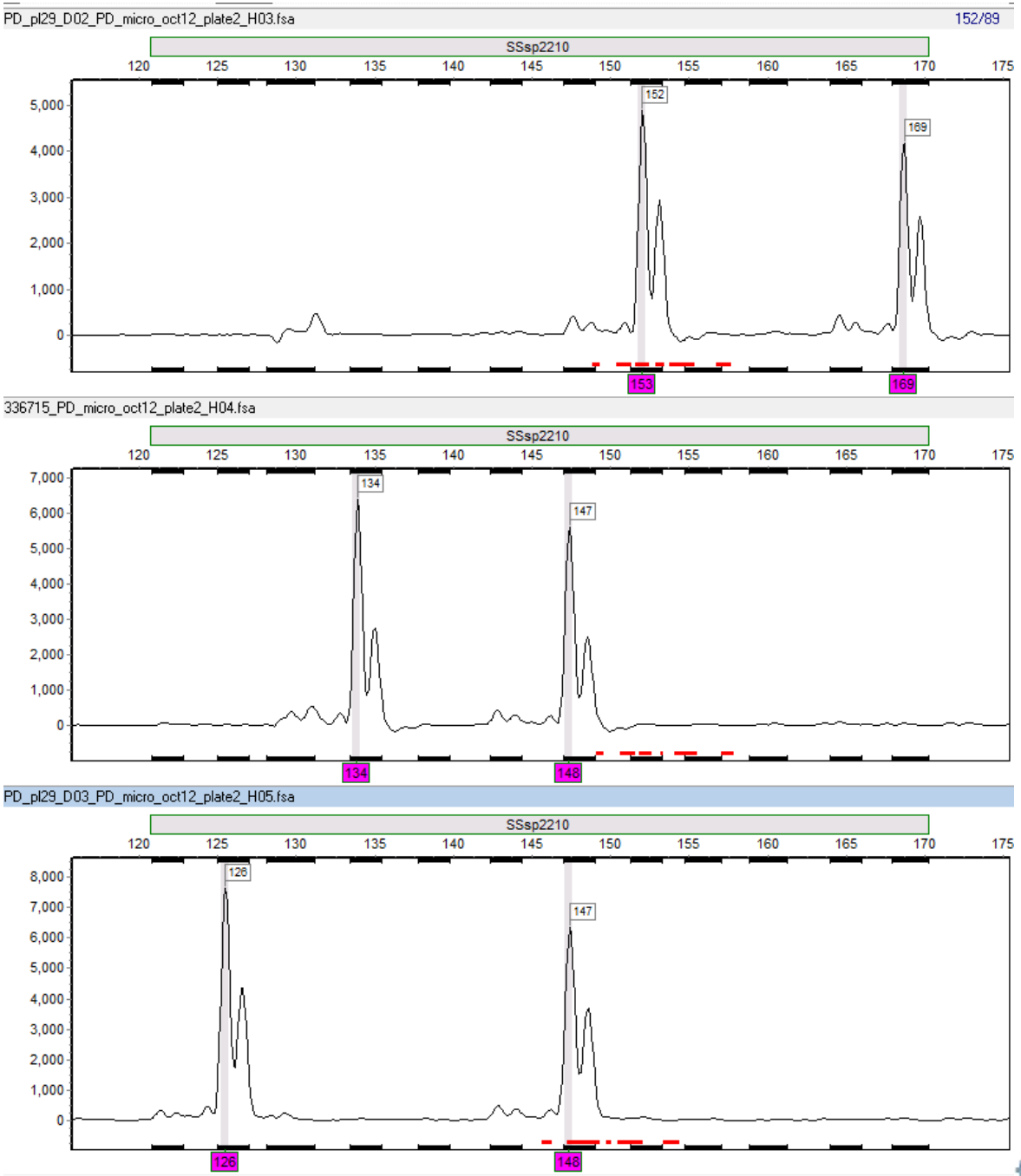
Existing high quality markers



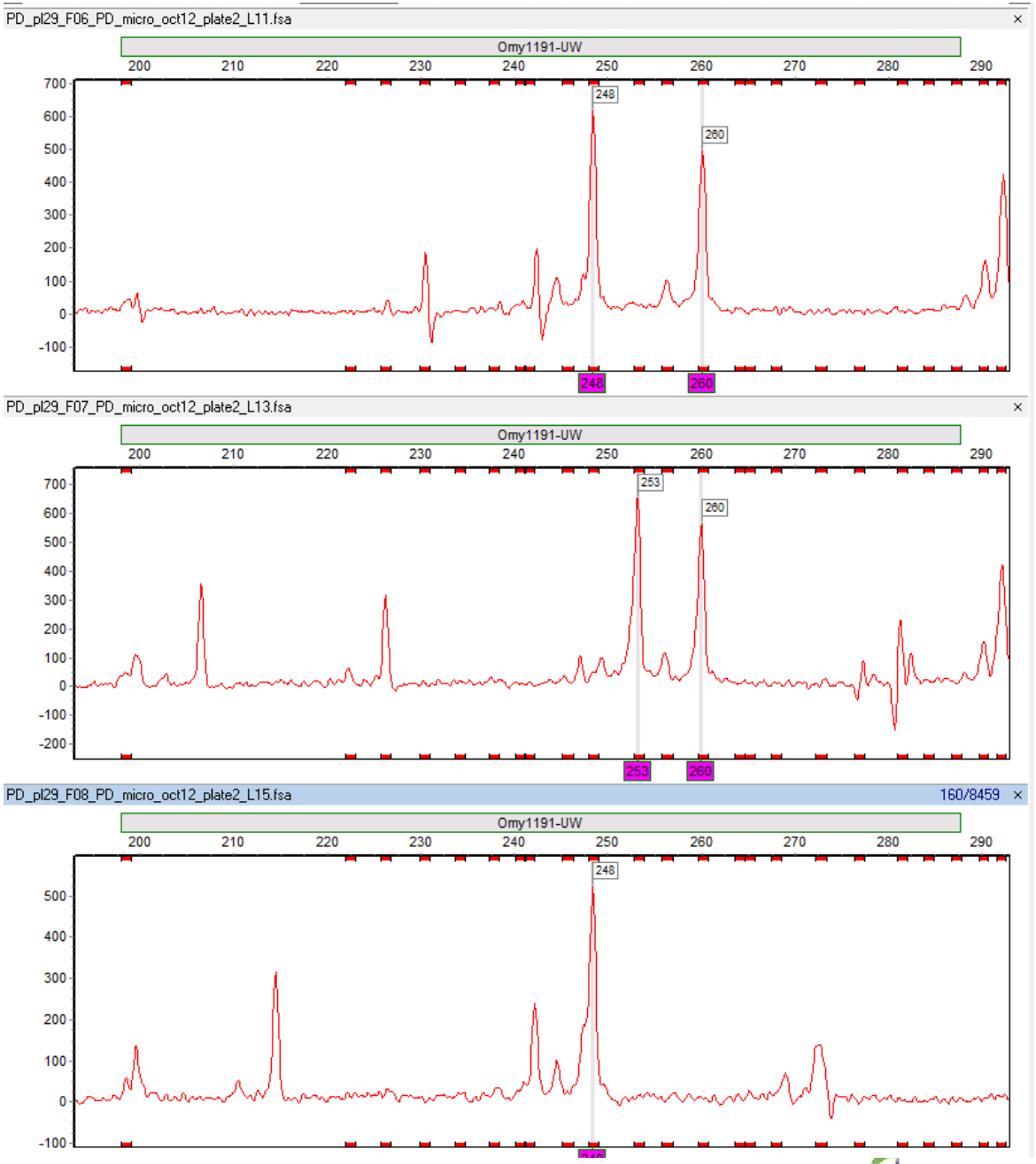
Existing high quality markers



Existing high quality markers

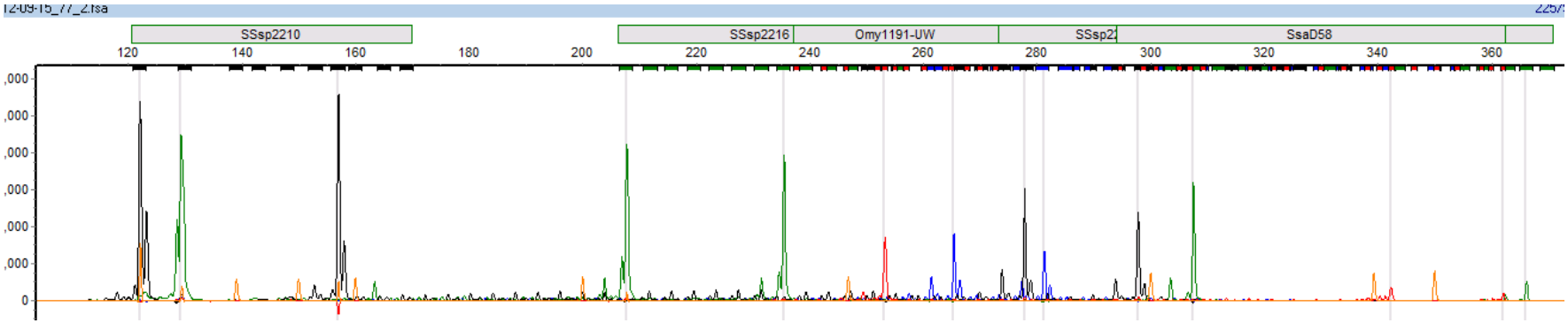


Not so good marker….



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



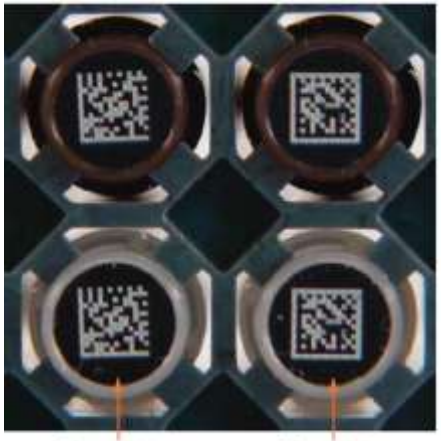
Optimisation of sampling, transport, DNA extraction and storage methods

- 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

Nofima

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)


Thermo
Thermo
Thermo

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?

Nofima