

SporLaks – Industry-wide tracing of Norwegian farmed Atlantic salmon

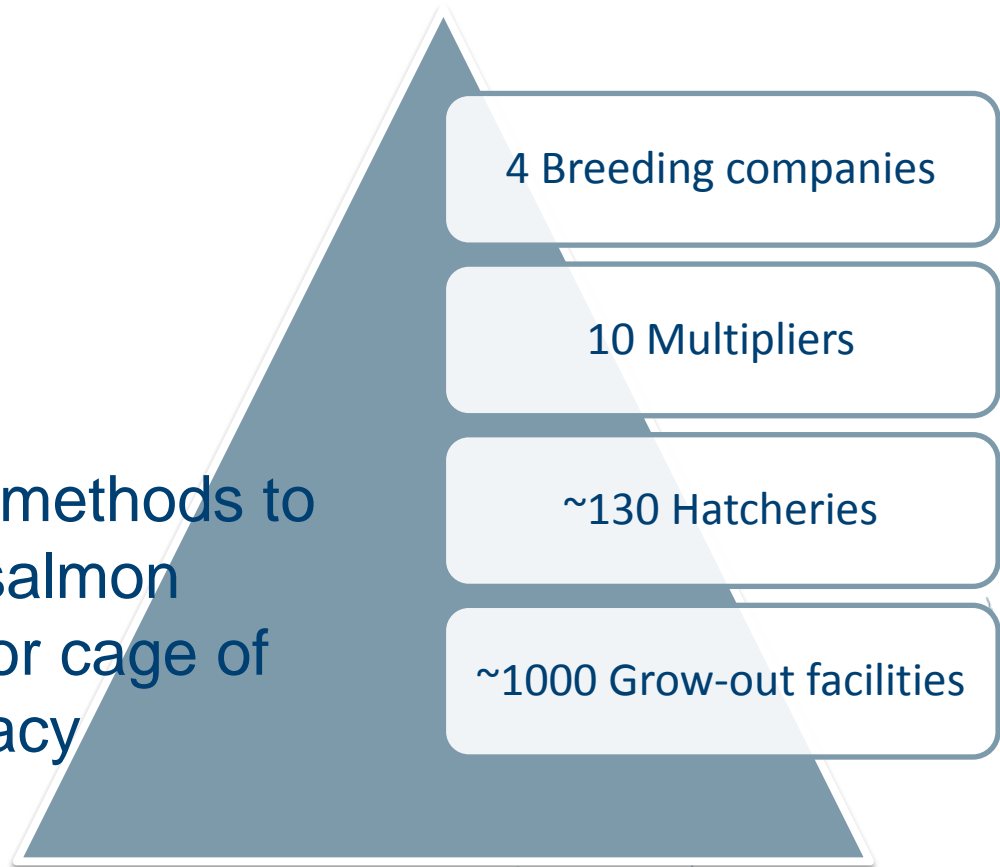
Matthew Baranski & Celeste Jacq* (Nofima)
Sten Karlsson (NINA)

Atlantic salmon production in Norway

Every year 30 000 – 40 000 broodstock used to produce 350 million fish

Project Concept:

To use DNA parentage methods to trace escaped farmed salmon back to egg batch and/or cage of origin with 100% accuracy



Using DNA markers to trace fish in aquaculture



Available online at www.sciencedirect.com



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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

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Simulations of the 'PAR' strategy

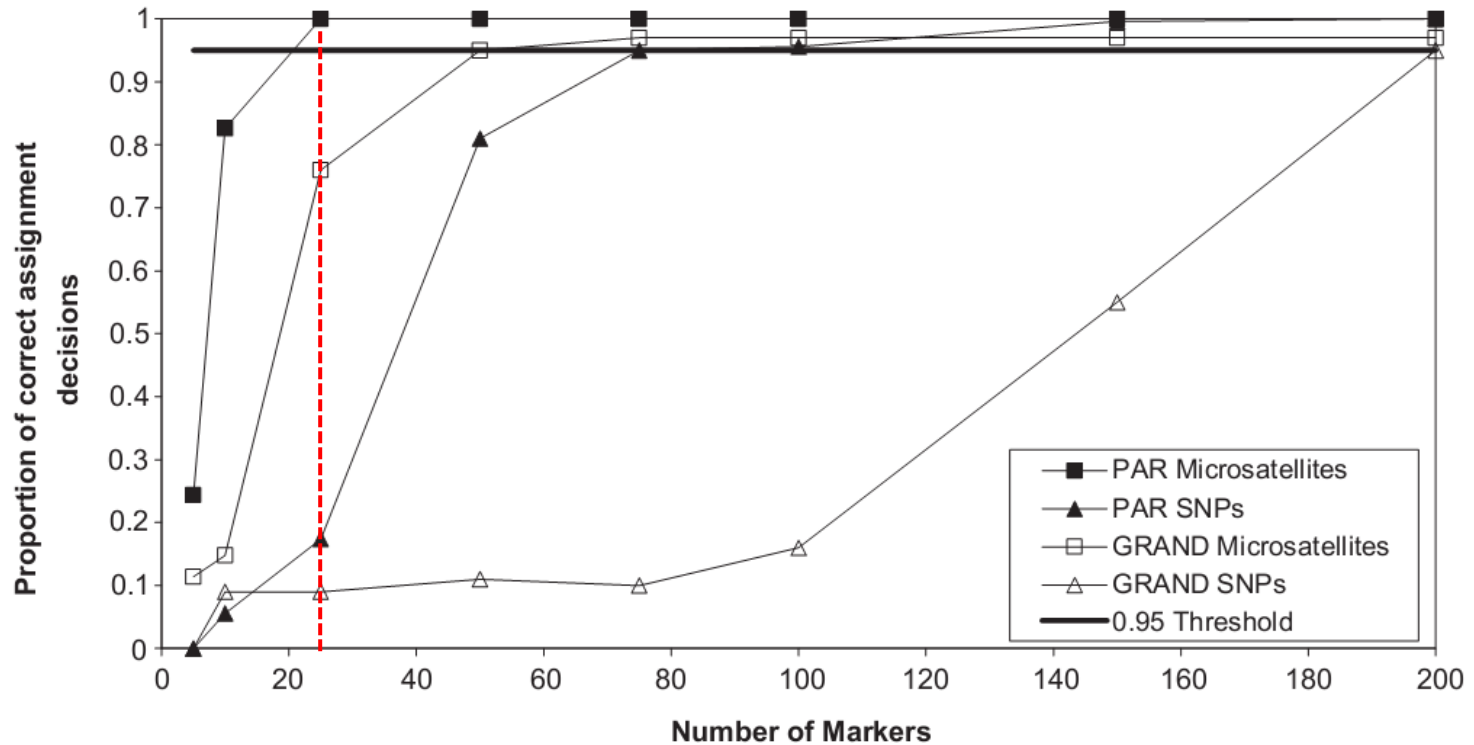
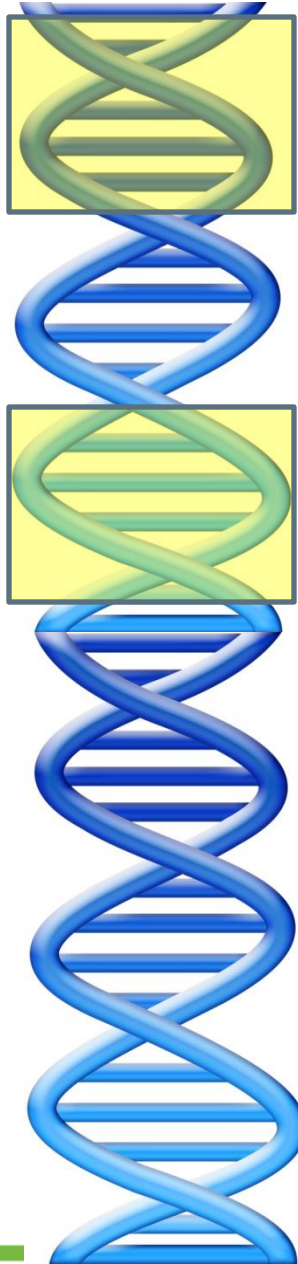


Fig. 5. Proportion of correct assignment decisions from strategies PAR and GRAND with increasing number of microsatellite and SNP markers.

DNA



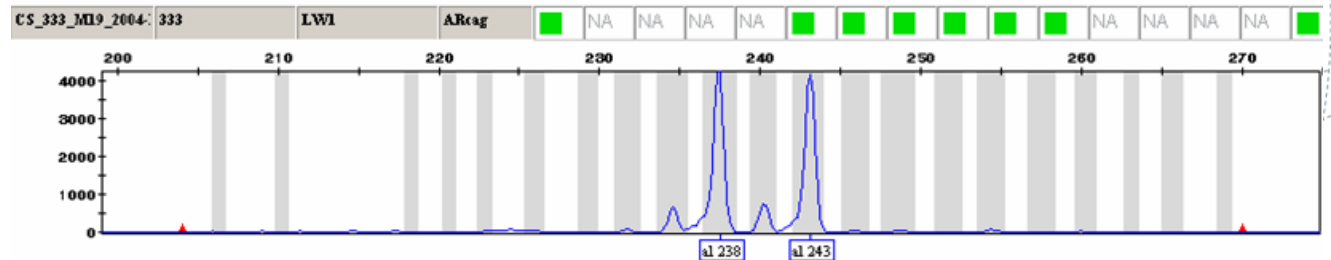
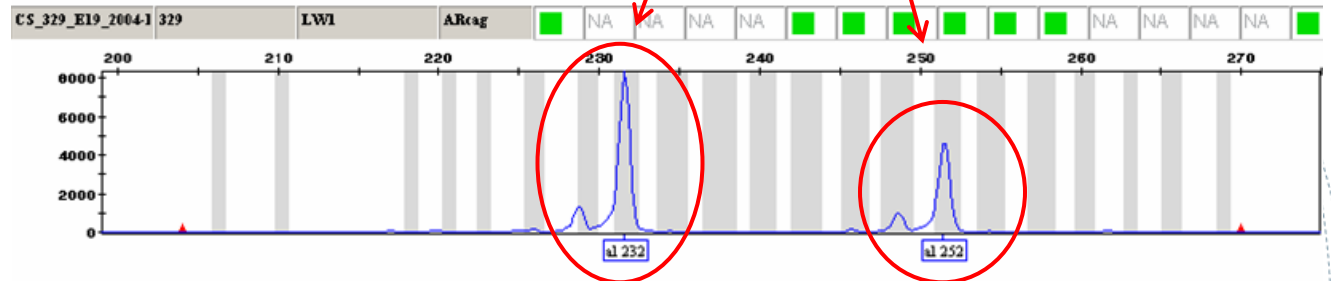
SNP



Molecular markers

ACGTT**T**CGA.....CGTTACAGTGTGTGTGTGTGTGTACGA
ACGT**A**CGA.....CGTTACAGTGTGTGTGTGTGTGTGTACGA
ACGT**A**CGA.....CGTTACAGTGTGTGTGTGTGTGTGTGTGTACGA

Microsatellite



Parentage analysis – exclusion approach

2. Determine multilocus genotypes for each individual potentially used with dams & offspring

Offspring



Candidate parents



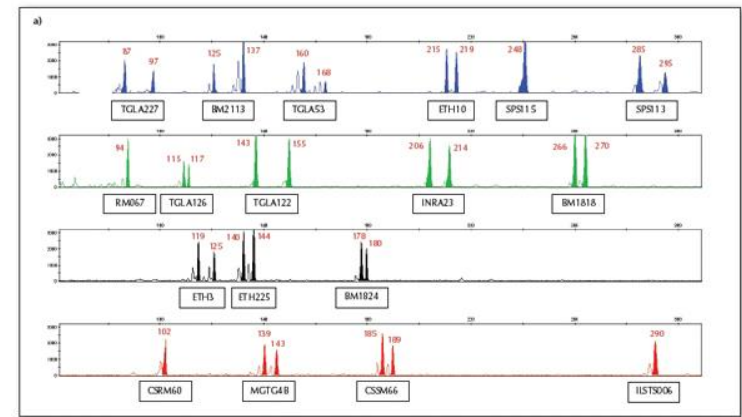
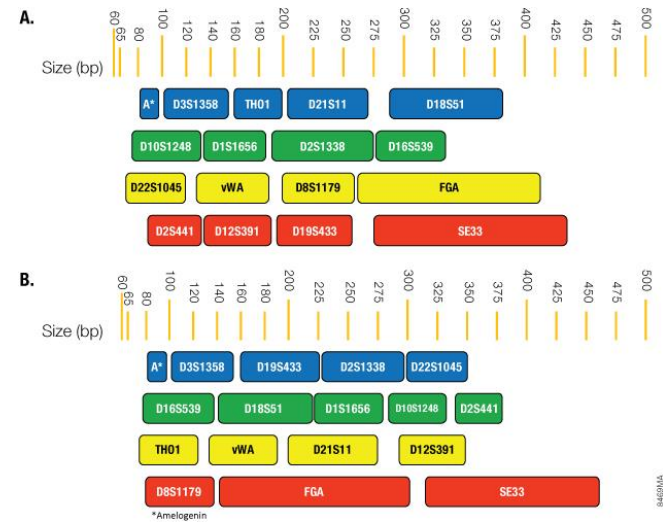


Multiplex PCR

- Amplify a 'panel' of markers together
- Very little DNA required
- Ability to handle mixtures and degraded samples
- Different fluorescent dyes used to distinguish alleles with overlapping size ranges

Established panels of microsatellites

- *Human forensics/paternity*
 - Promega PowerPlex
- *Livestock and terrestrial species*
 - Bovine Genotypes™ Panel 3.1
 - Canine Genotypes™ Panel 1.1
 - Equine Genotypes™ Panel 1.1



Why don't we have such panels in aquaculture species?

1. Not so many top quality markers to choose from
2. Few concerted efforts to make highly optimised multiplexes

How to address this?

1. Use genome sequence data
 - No longer a shortage of markers to choose from
 - Tens to hundreds of thousands of microsatellites present
2. Use the same protocols/reagents as the optimised commercial panels

QDD pipeline using Atlantic salmon genome

BIOINFORMATICS APPLICATIONS NOTE

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Sequence analysis

QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects

Emese Meglécz^{1,*}, Caroline Costedoat¹, Vincent Dubut¹, André Gilles¹,
Thibaut Malausa², Nicolas Pech¹ and Jean-François Martin³

¹Aix-Marseille Université, CNRS, IRD, UMR 6116 – IMEP, Equipe Evolution, Génome et Environnement, Centre Saint-Charles, Case 36, 3 Place Victor Hugo, 13331 Marseille Cedex 3, ²Institut National de la Recherche Agronomique, UMR 1301, INRA/UNSA/CNRS, Equipe BPI, 400, route des Chappes. BP 167. 06903 Sophia-Antipolis Cedex and ³Montpellier SupAgro, INRA, CIRAD, IRD, Centre de Biologie et de Gestion des Populations, Campus International de Baillarguet, CS30016, 34988 Montferrier-sur-Lez, France

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80 markers

Optimise multiplex primer
design

Testing new markers with ‘new generation’ PCR reagents

- Genotyping efficiency critical if thousands of samples are to be genotyped rapidly
- Commercial genotyping kits use ‘advanced’ reagents

Q5 polymerase



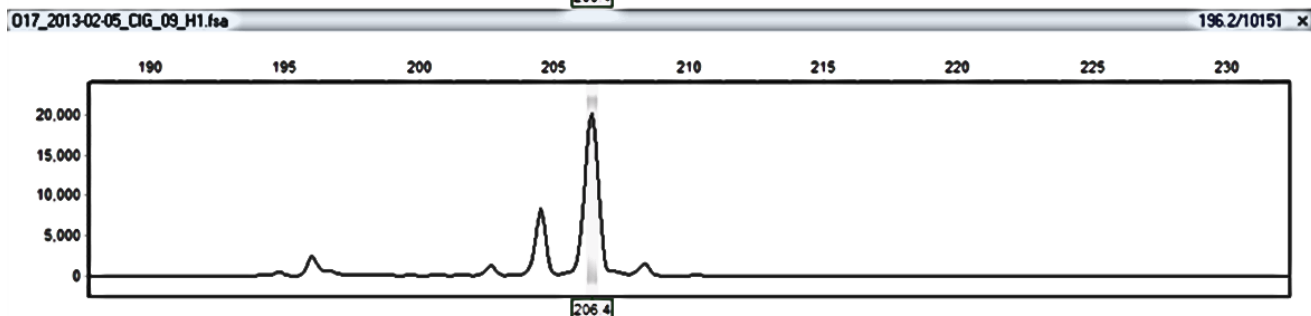
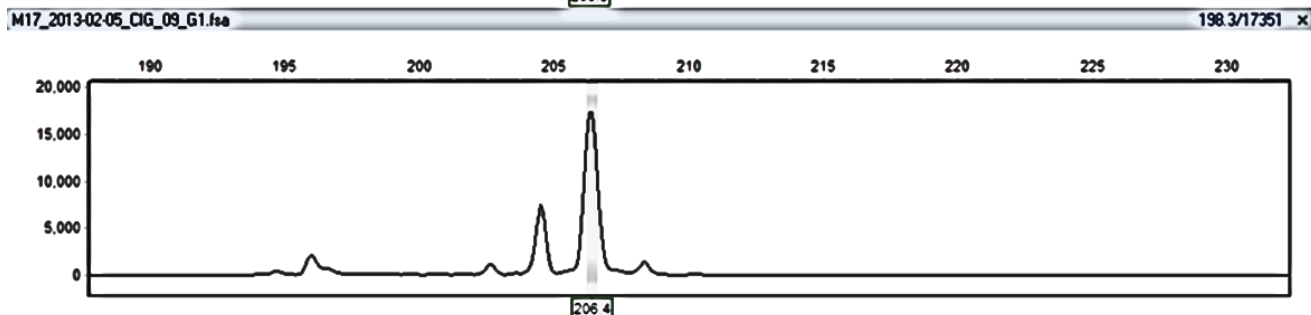
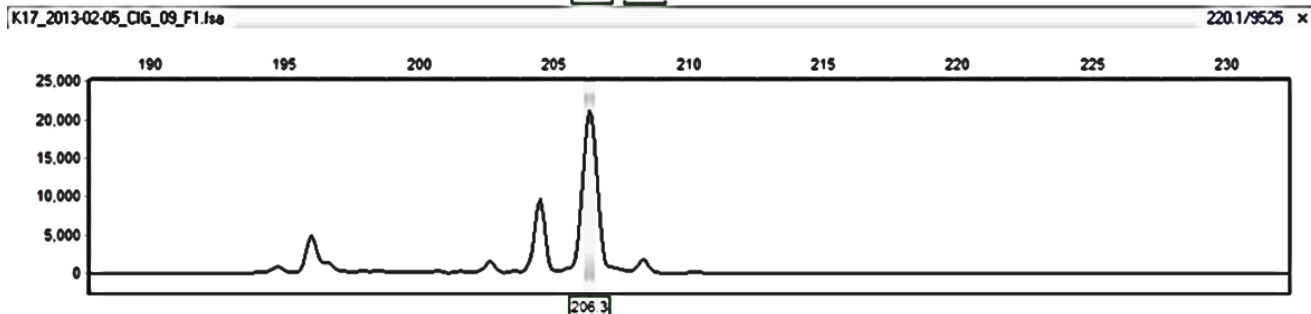
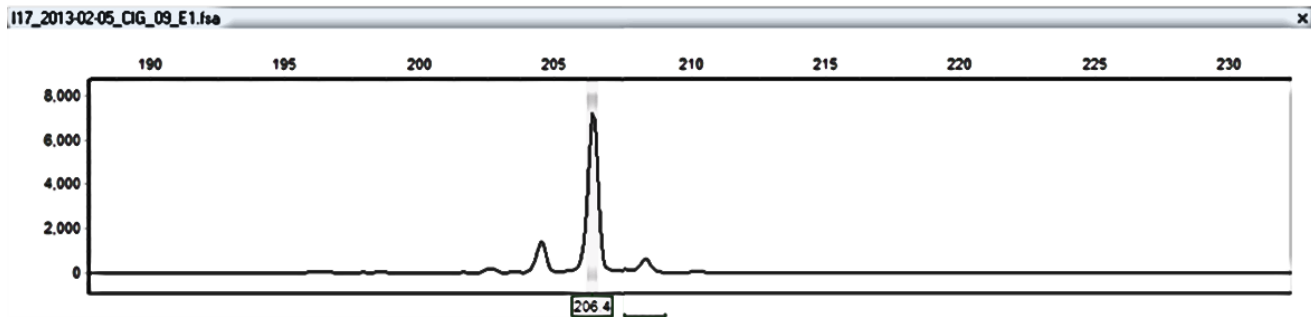
- Extremely high fidelity (>100X higher than Taq)
- Robust – high specificity and yield with minimal optimization
- Very fast (10 s/kb)
- **30-40 minute two-step PCR program**

Example marker 1

Polymorphic



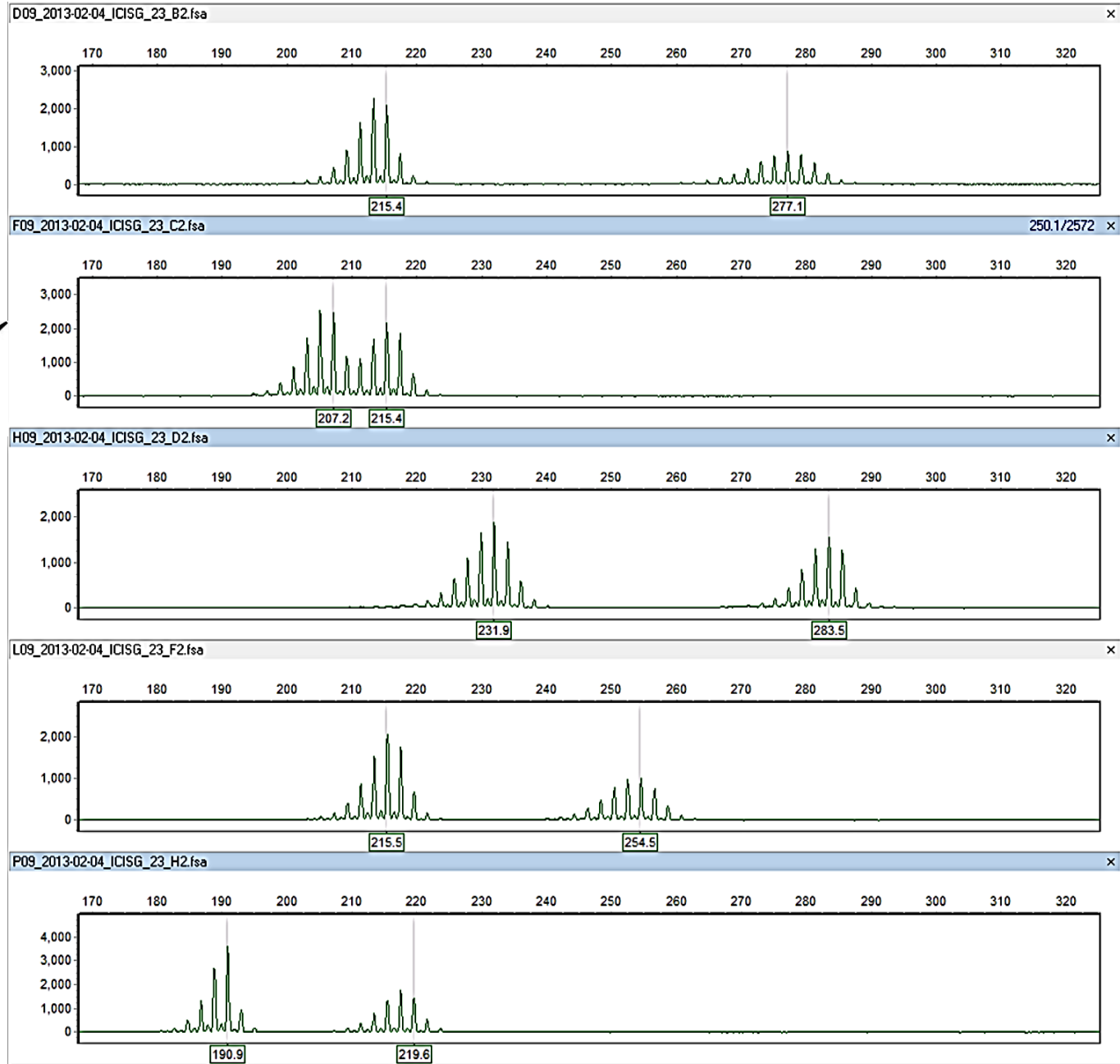
Quality



Example marker 2

Polymorphic ✓

Quality ✗

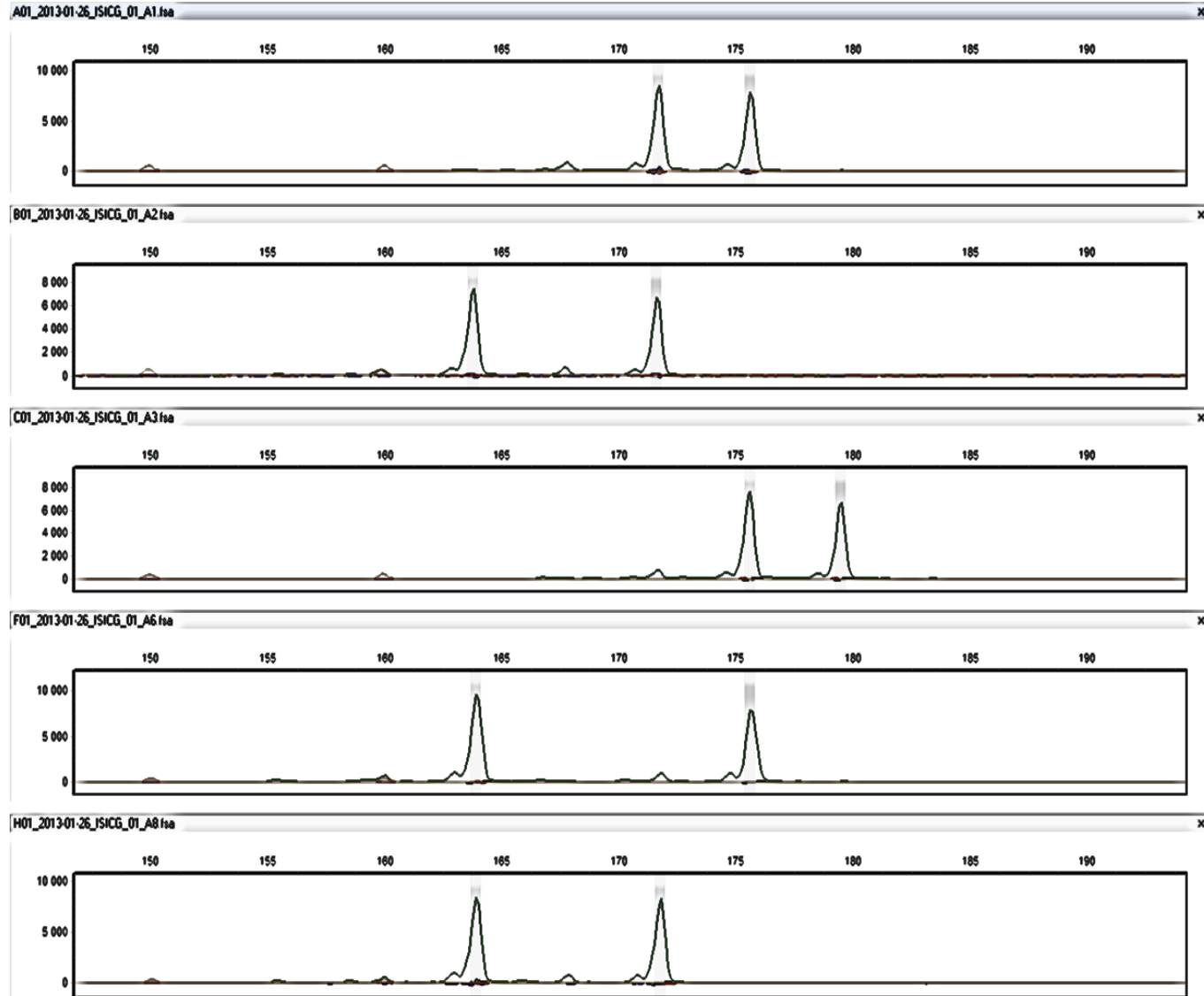


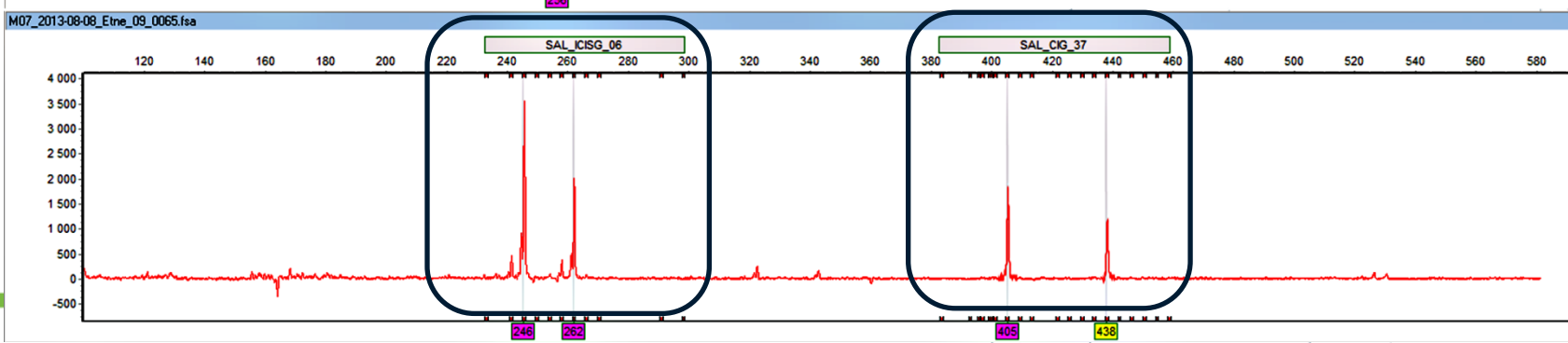
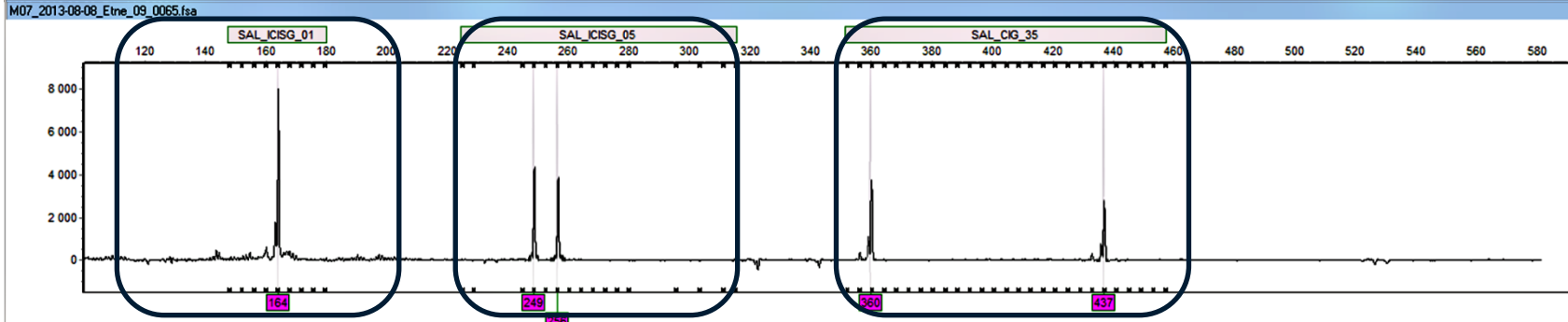
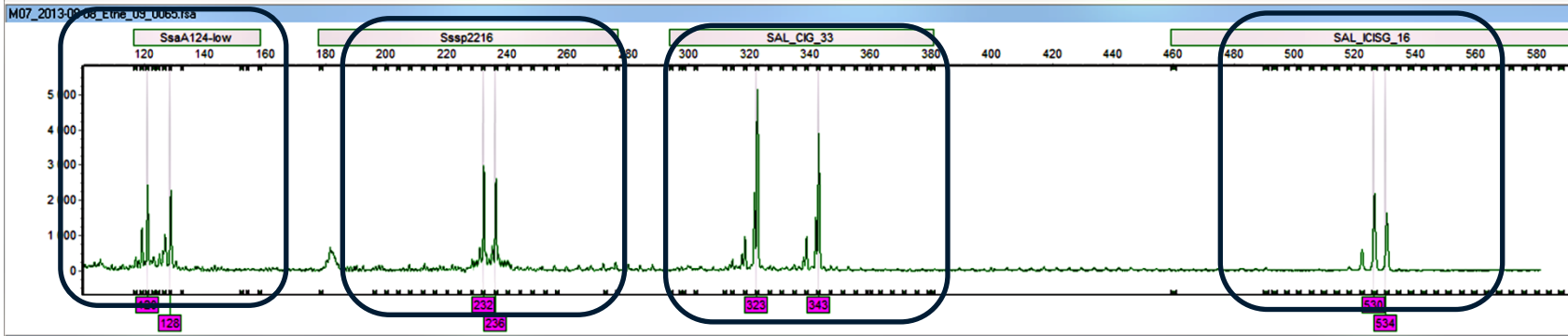
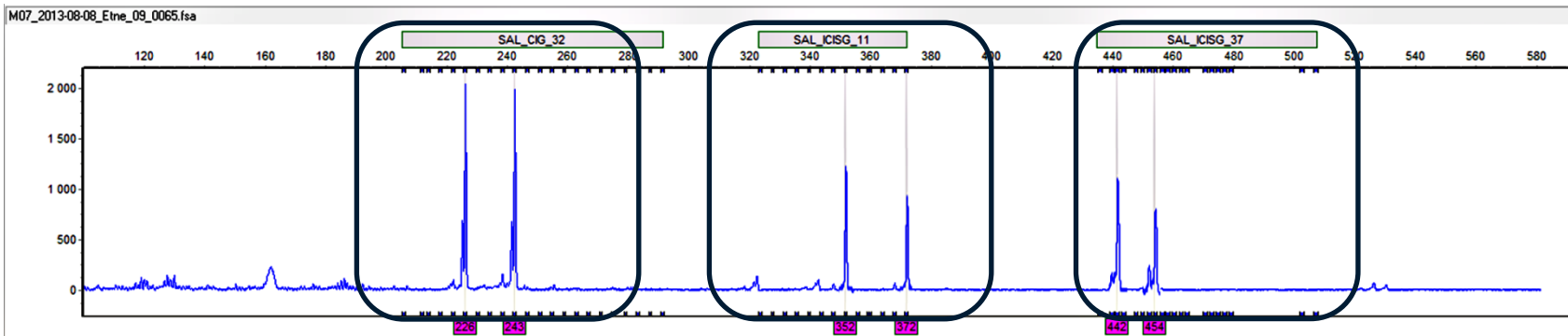
Example marker 3

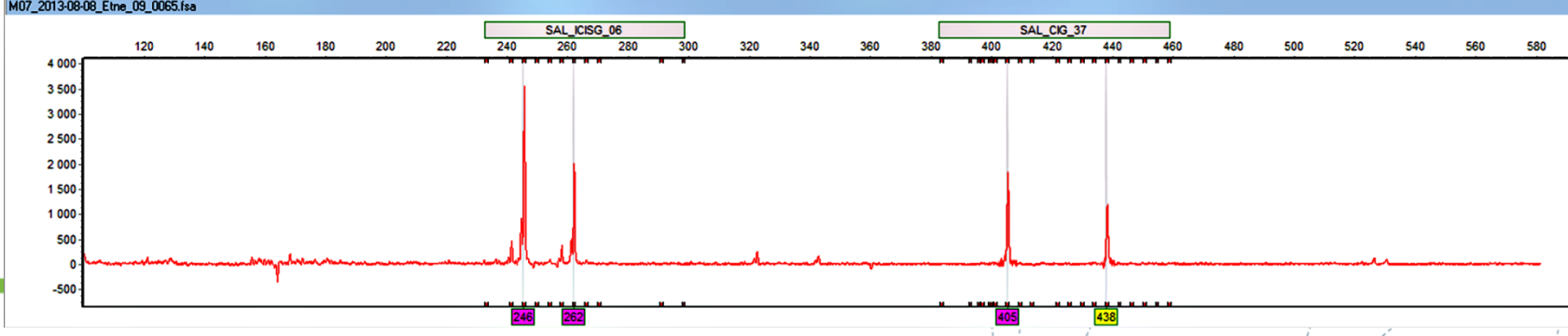
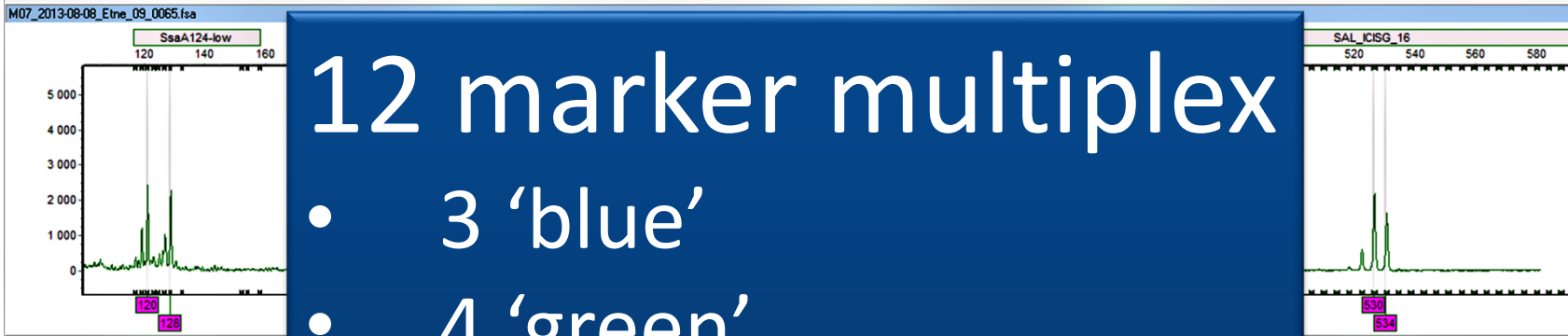
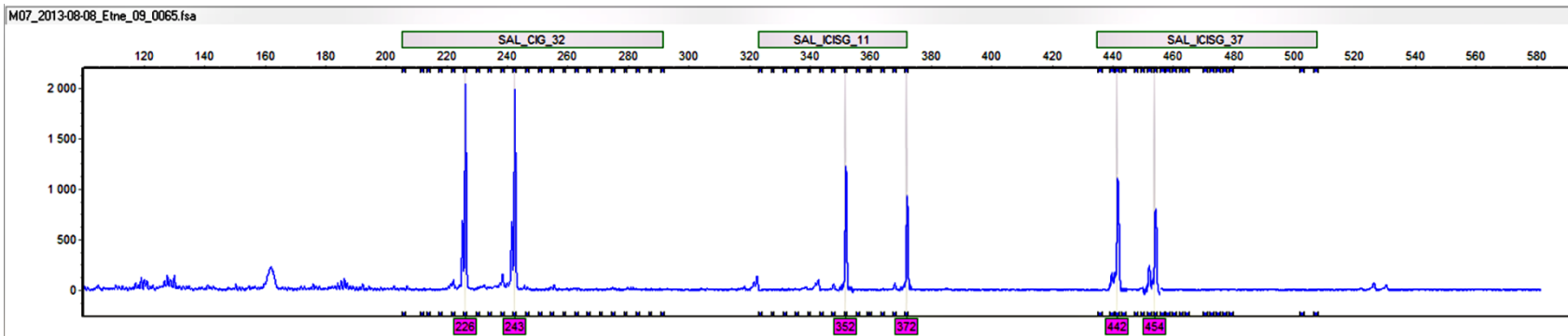
Polymorphic



Quality







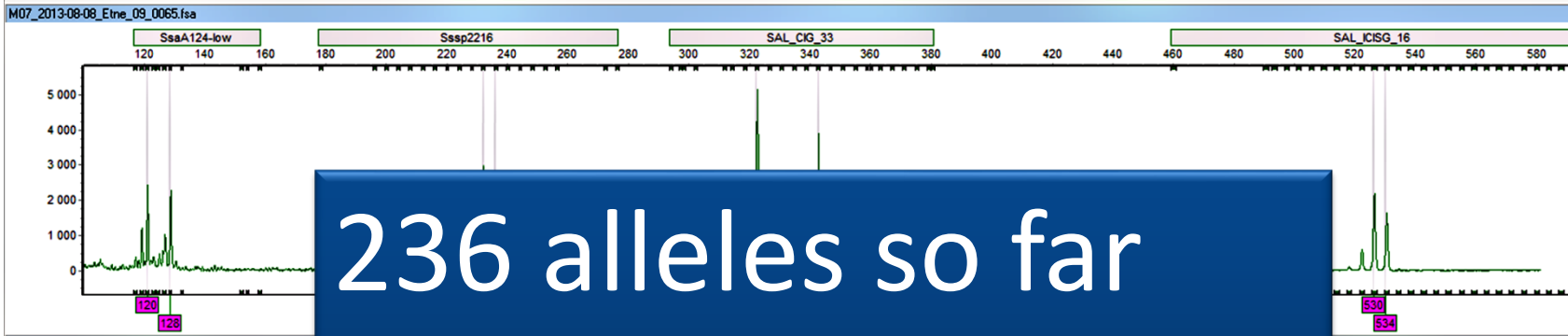
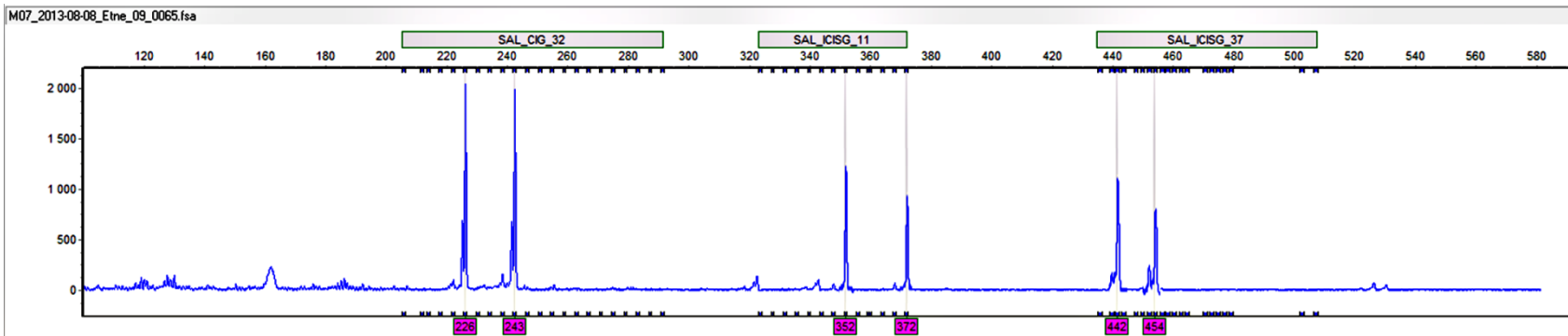
12 marker multiplex

- 3 'blue'
- 4 'green'
- 3 'yellow'
- 2 'red'

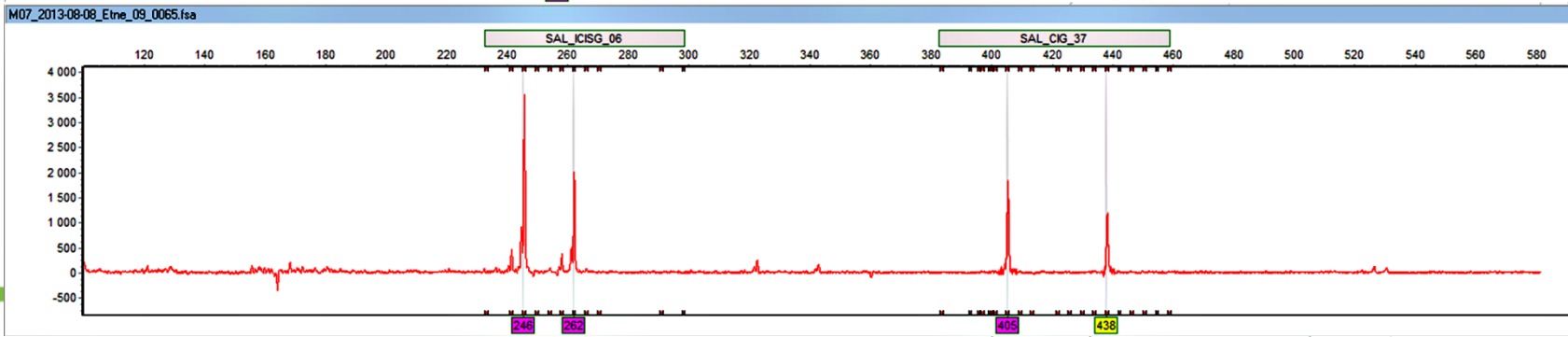
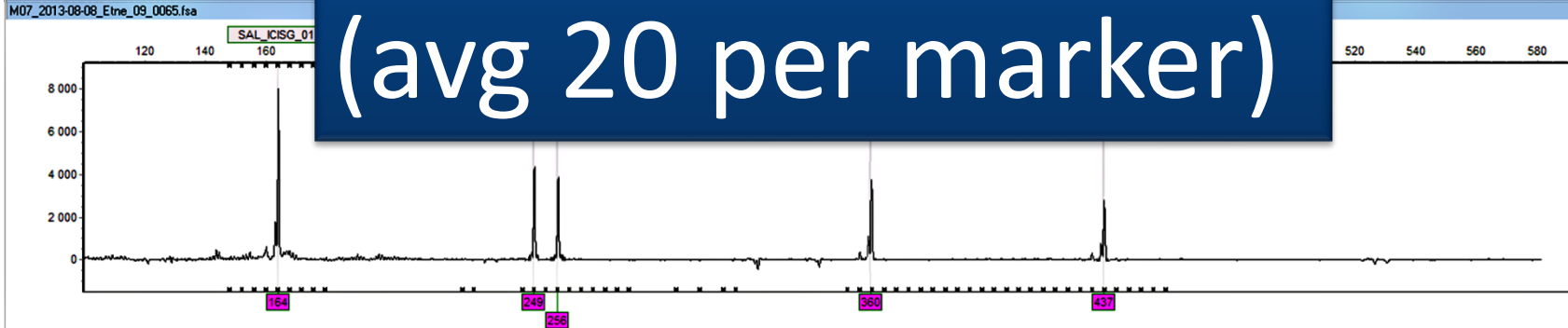
Wild fish sampling



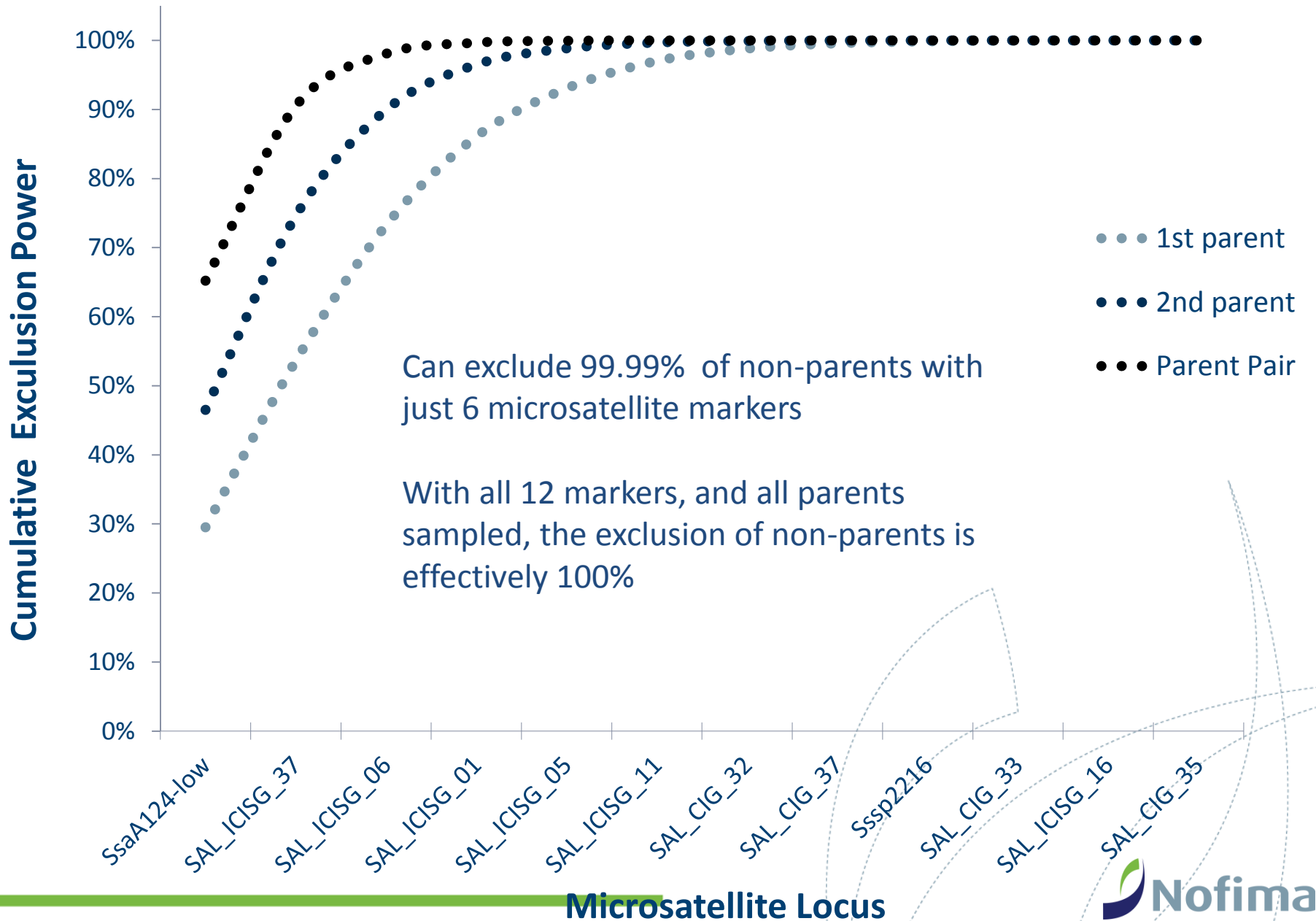
1. Skallelv
2. Repparfjord
3. Skibotn
4. Målselvvassdraget
5. Beiarelva
6. Røssåga
7. Fusta
8. Vefsna
9. Namsen
10. Skauga
11. Bævra
12. Driva
13. Eira
14. Flekke
15. Flåmselva
16. Eio
17. Etne
18. Audna
19. Glomma



236 alleles so far
(avg 20 per marker)



Parentage assignment accuracy



Validation study

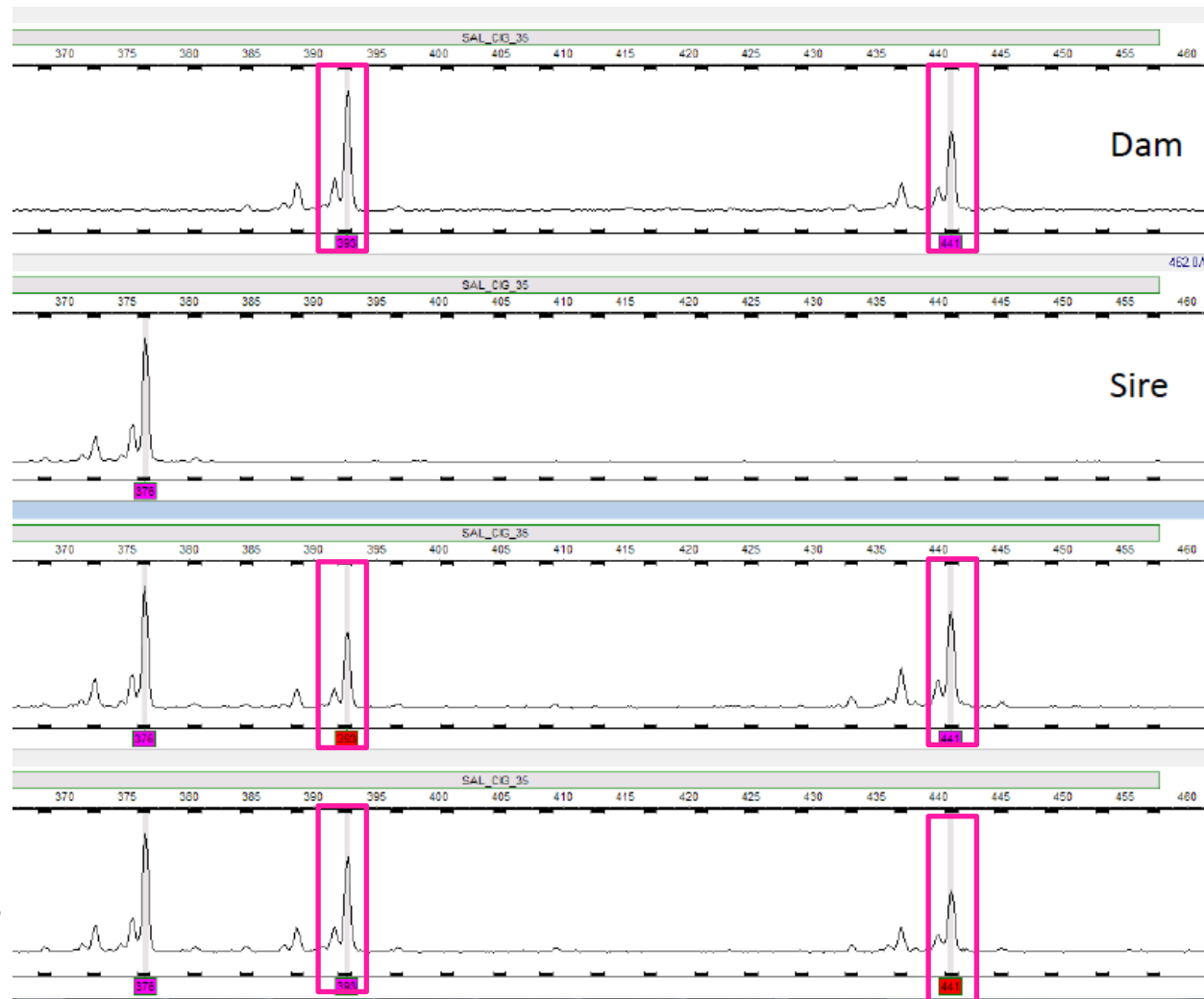
- “Blind test” of parentage assignment using offspring from 230 Aqua Gen families (112 dams & 118 sires) + unrelated fish

Validation set	Assigned to 1 or more parent	% Correct according to pedigree
520 offspring from 230 AquaGen families	519 (99.8%)	97%
40 unrelated AquaGen fish	0	100%
88 wild salmon	0	100%

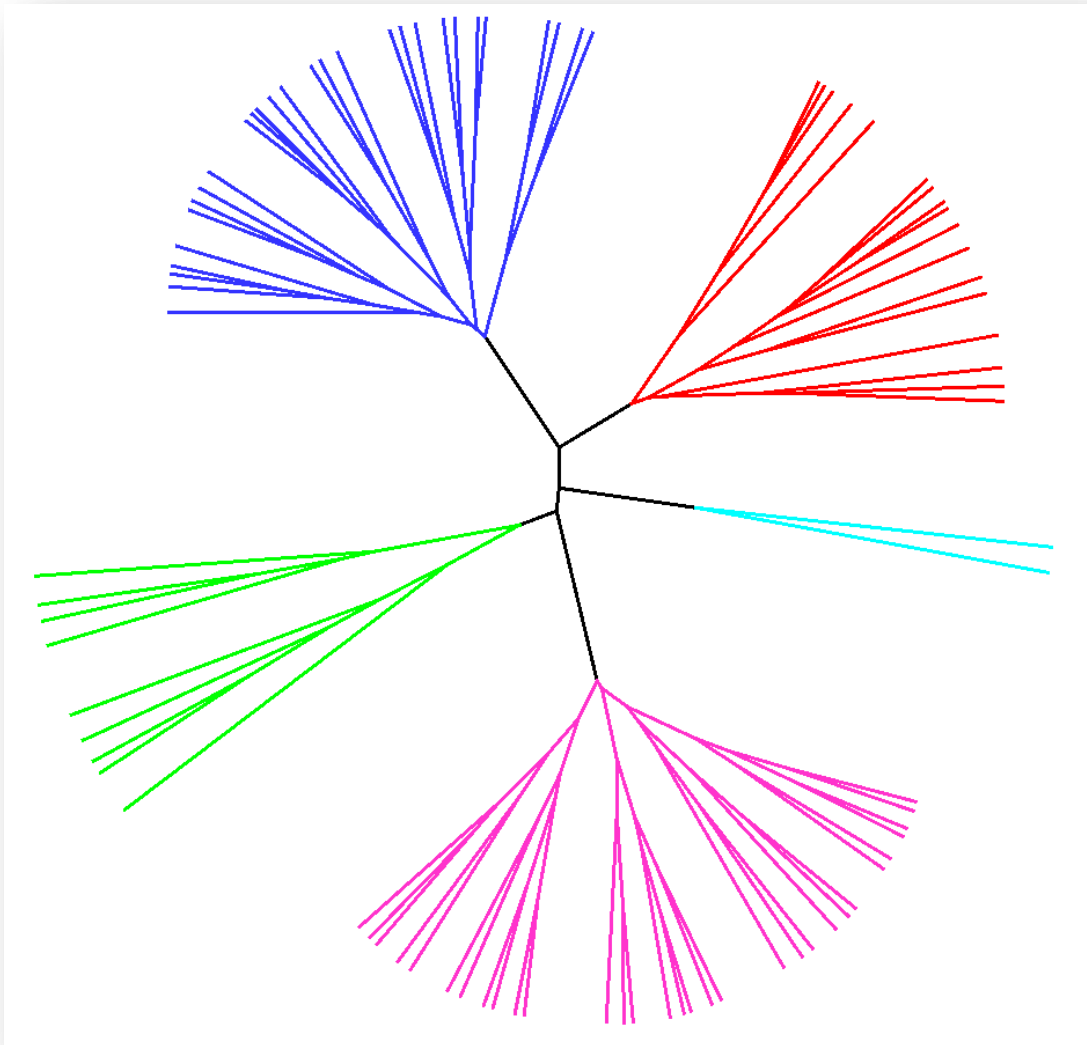
- 1 dam couldn't be genotyped
- 504 offspring assigned to 2 parents, 15 assigned to a single parent
- 1 fish couldn't be assigned
- No un-related farmed fish & wild fish could be assigned to parents

«Bonus» validation study results

- 4 of the wild fish had unusual genotype patterns
 - Further testing showed these were Salmon X Trout hybrids
- 2 of the Aqua Gen offspring had 3 alleles at most microsatellites
 - Assigned to parents
 - Offspring inherited both copies of mother's genes + 1 copy of father's



Relatedness clustering with multiplex



- Wild hatchery-reared smolt
- Progeny from single pair crosses of 5 males and 5 females
- Average relatedness within each cluster (family) = 50%

High-throughput

Example for 384 samples

DNA extraction

Crude method eg. Chelex

2 hours

PCR

Q5 polymerase + robotics

1 hour

Genotyping

ABI 3730xl capillary sequencer

4 hours

Logistical challenges

- Proposed tracing scheme will depend on 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

Sampling and sample storage

- Individual barcoded tubes in rack format
 - Read ID directly into database, no human error
- Room temperature storage in ethanol
- Compatible with lab robotics
- 'Biopsy' sampling ensures consistent sample size and good yield



Genotyping effective with 'poor quality' DNA

- Tested three commonly used methods
 - Microsatellite genotyping performed well with Chelex[®]
 - cheapest, quickest, “roughest” method

Time

DNA Quality

Cost

Chelex

Salt precipitation method

Chelex

Commercial Kits

Commercial Kits

Salt precipitation method

Salt precipitation method

Chelex

Commercial kits

Ongoing work

- Validate multiplex performance in different laboratories
- Marker data (allele number and frequencies) being fed into simulations
 - Test power of marker set at industry wide level



Conclusions

- Thousands of new markers identified from the Atlantic salmon genome
- Efficient multiplex of 12 high quality markers developed
- Protocol optimised for high-throughput genotyping at low cost
- Very high assignment power and very good assignment rates achieved for breeding companies
- Challenge lies in scaling up to whole-industry level and the logistics of sampling, genotyping, egg tracking
 - Rigorous database needed