FISKERI- OG HAVBRUKSNÆRINGENS FORSKNINGSFOND

SporLaks – Industry-wide tracing of Norwegian farmed Atlantic salmon

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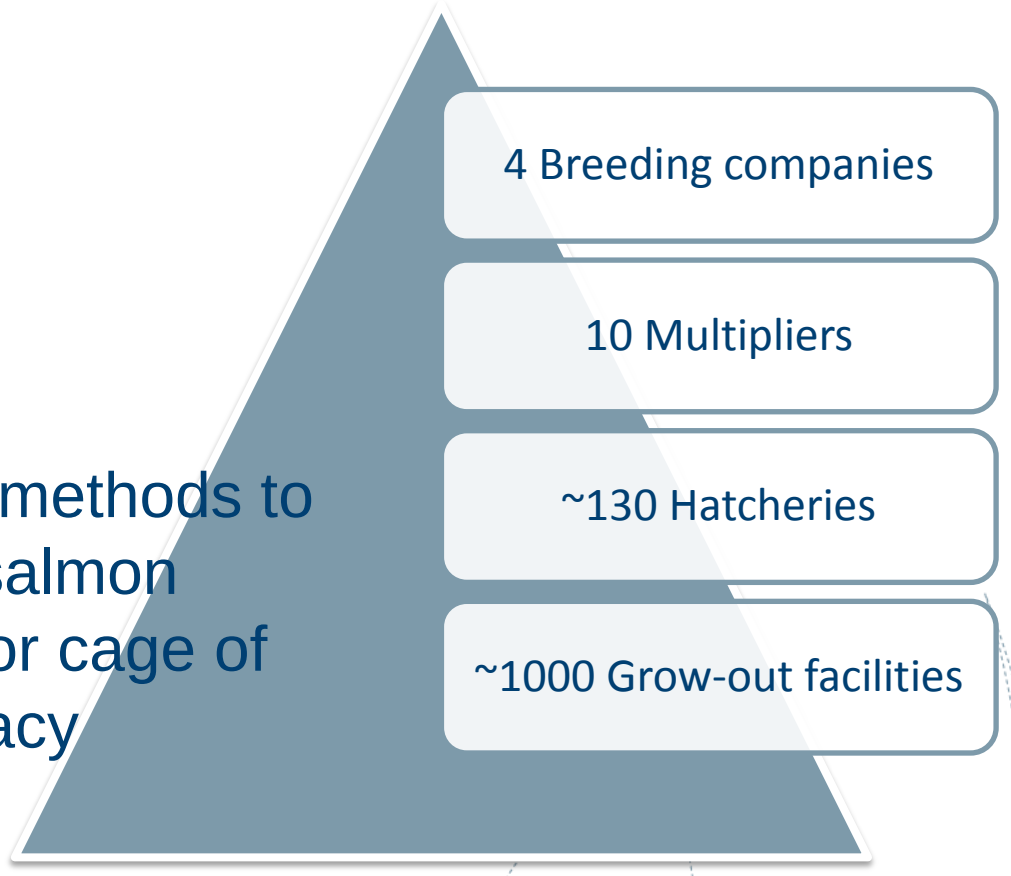
Sten Karlsson (NINA)

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

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Using DNA markers to trace fish in aquaculture

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

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Simulations of the ’PAR’ strategy

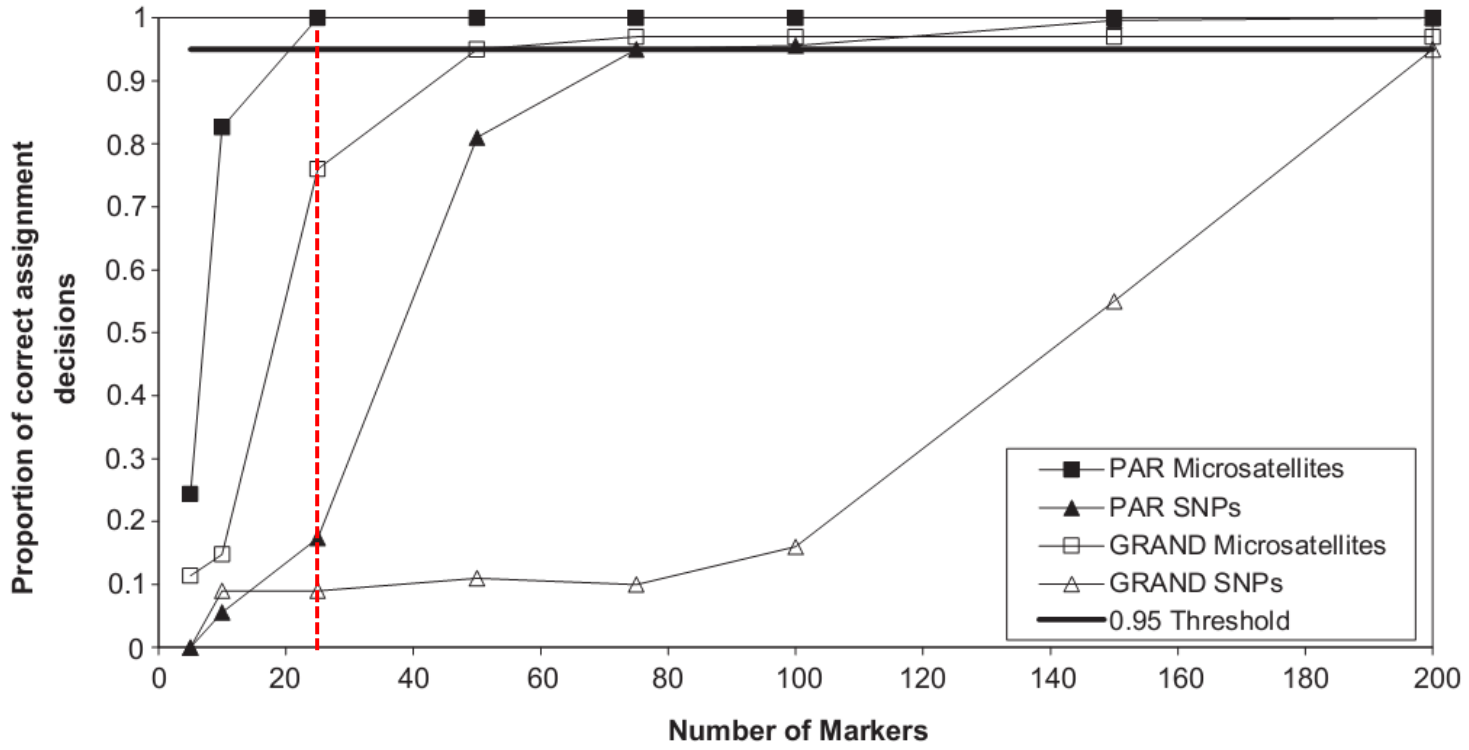


Fig. 5. Proportion of correct assignment decisions from strategies PAR and GRAND with increasing number of microsatellite and SNP markers.

DNA

SNP

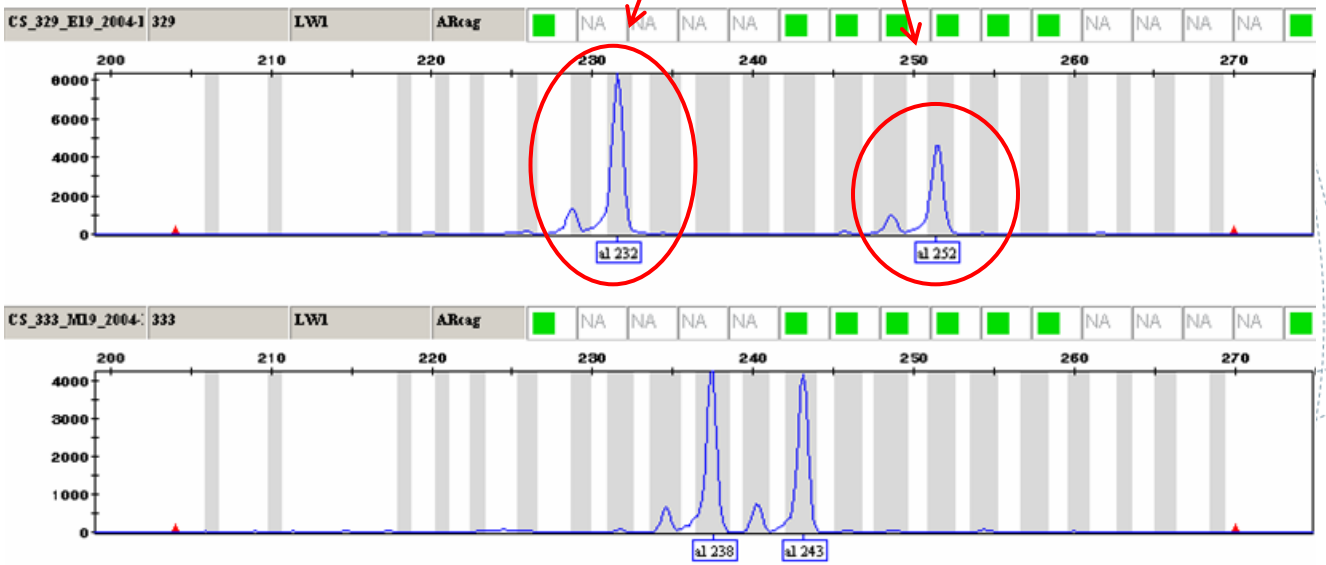
Molecular markers

ACGT**T**CGA……CGTTACAGTGTGTGTGTGTGTACGA

ACGT**A**CGA……CGTTACAGTGTGTGTGTGTGTGTACGA

ACGT**A**CGA……CGTTACAGTGTGTGTGTGTGTGTGTACGA

Microsatellite



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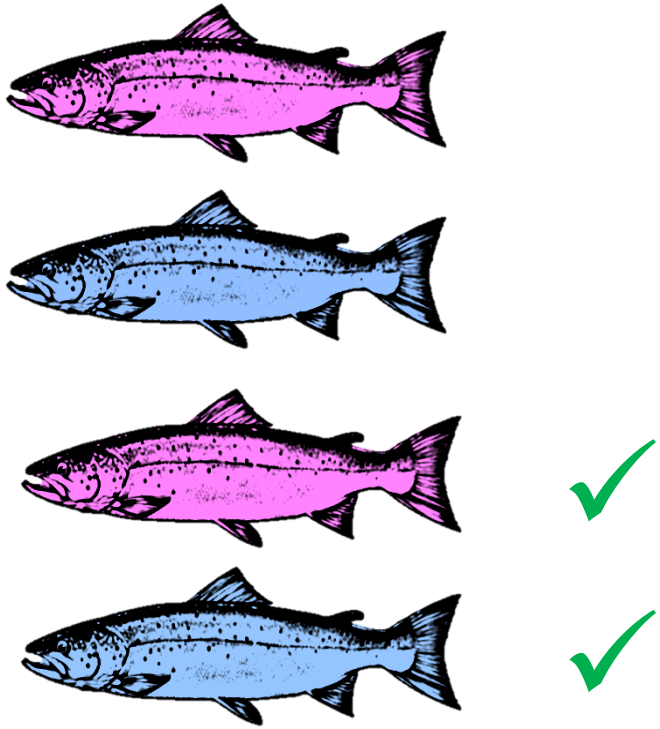
Parentage analysis – exclusion approach

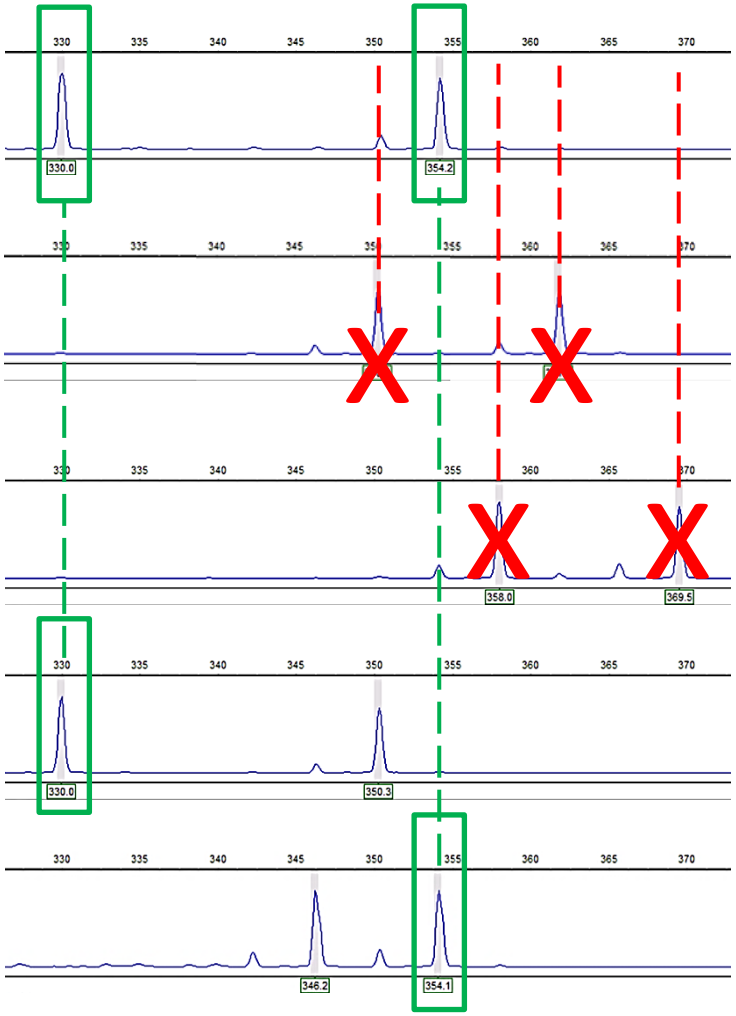
Offspring

Candidate parents



2. Determine multi-locus genotype for all potential sires, dams & offspring

1. Determine multi-locus genotype for all potential sires, dams & offspring



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

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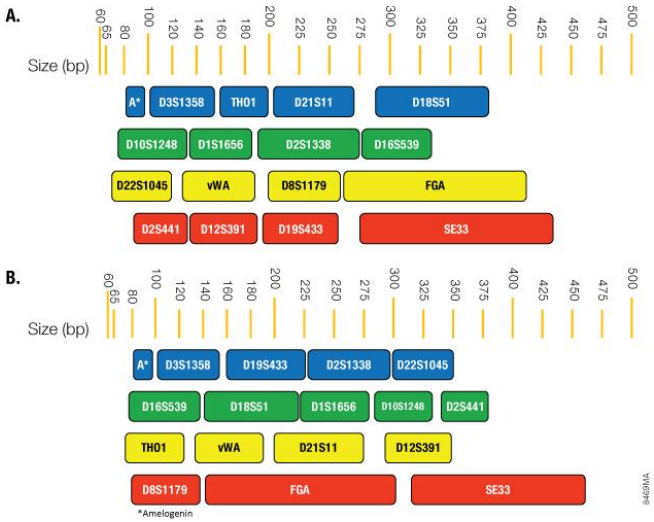
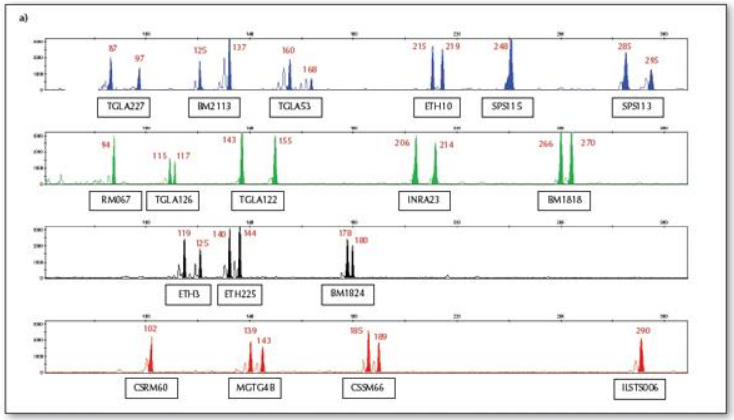
Established panels of microsatellites

- *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

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QDD pipeline using Atlantic salmon genome

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Sequence analysis

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

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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**

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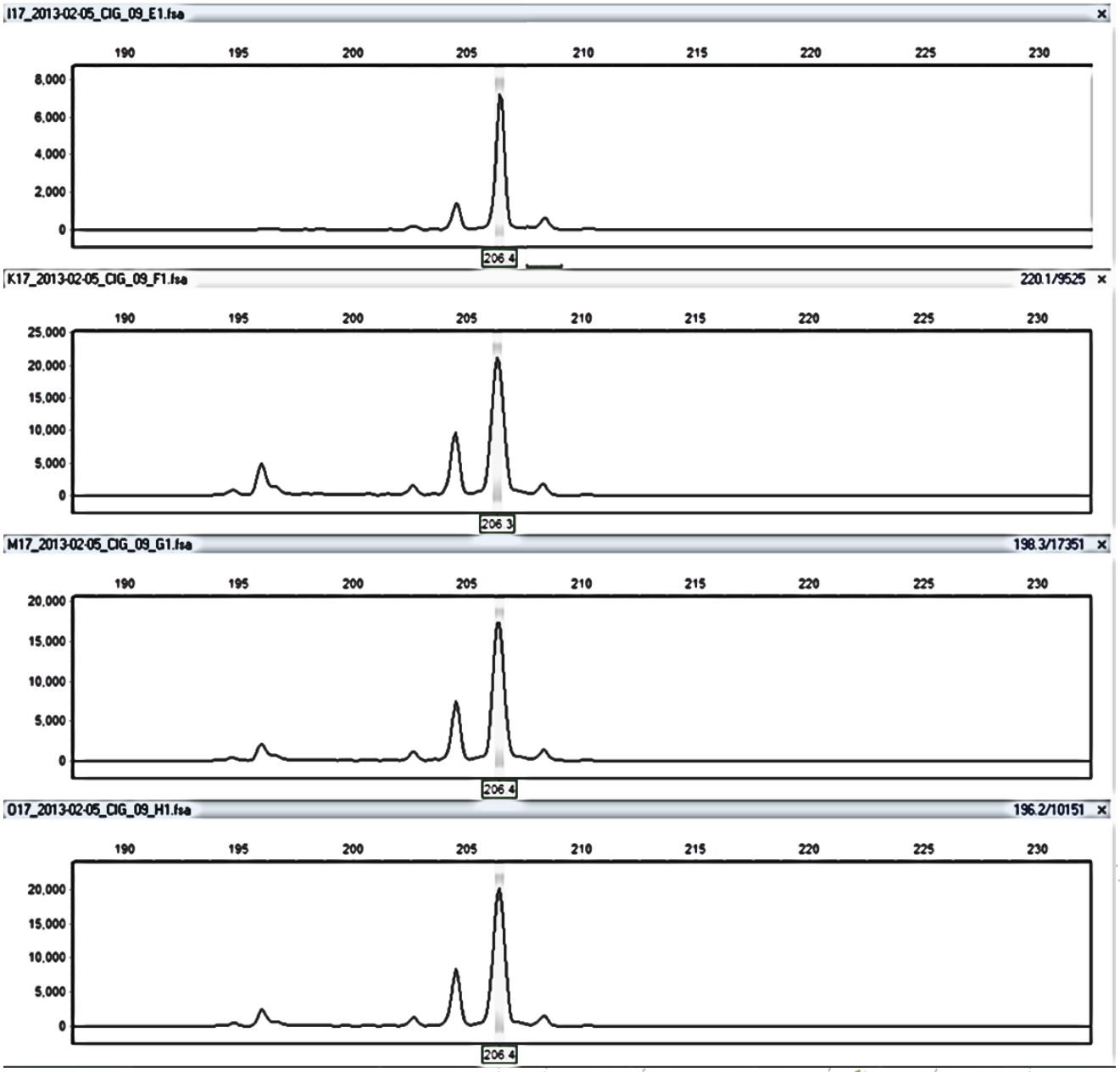
Example marker 1

Polymorphic

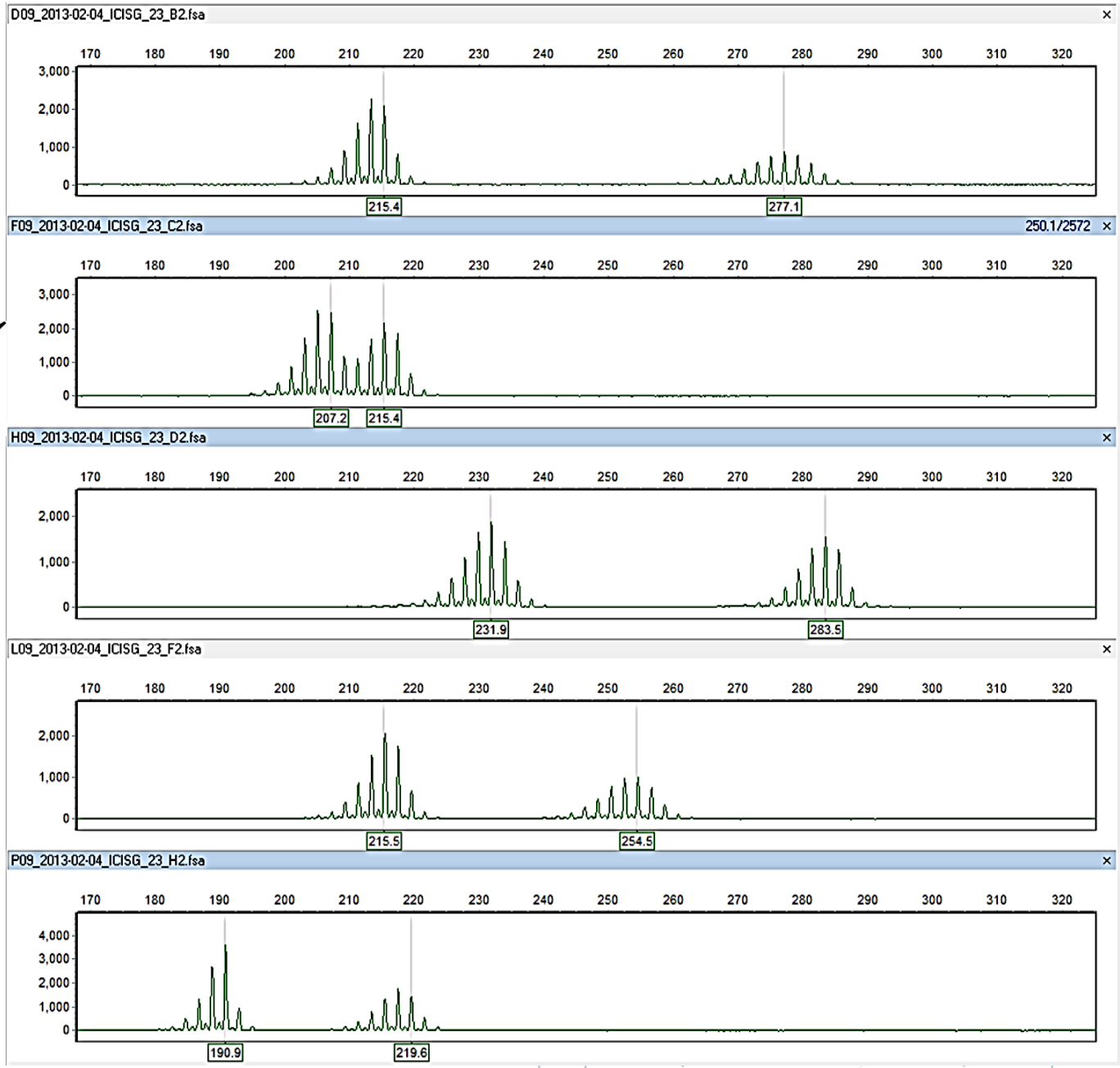
Quality



Example marker 2

Polymorphic

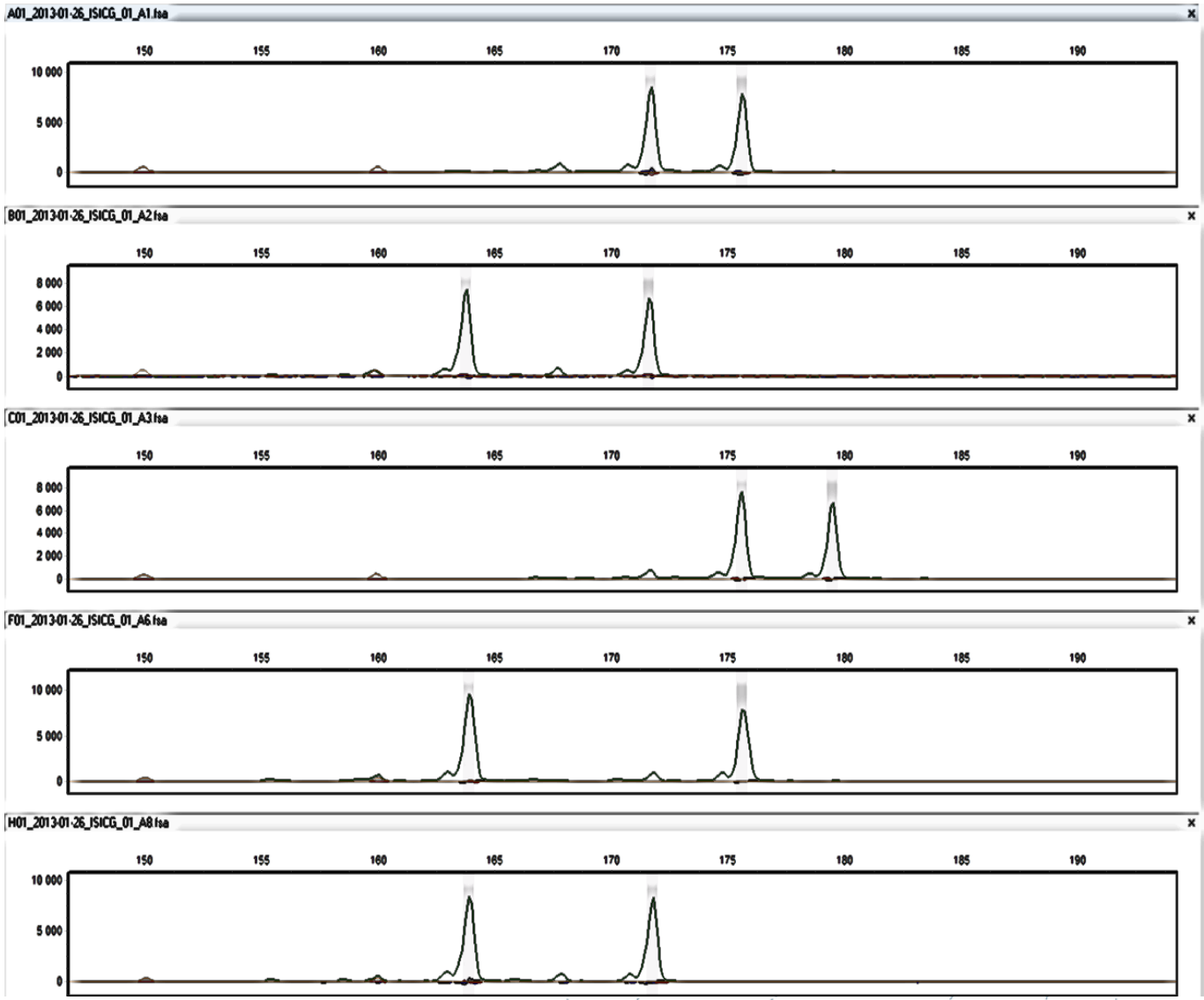
Quality



Example marker 3

Polymorphic ✓

Quality ✓



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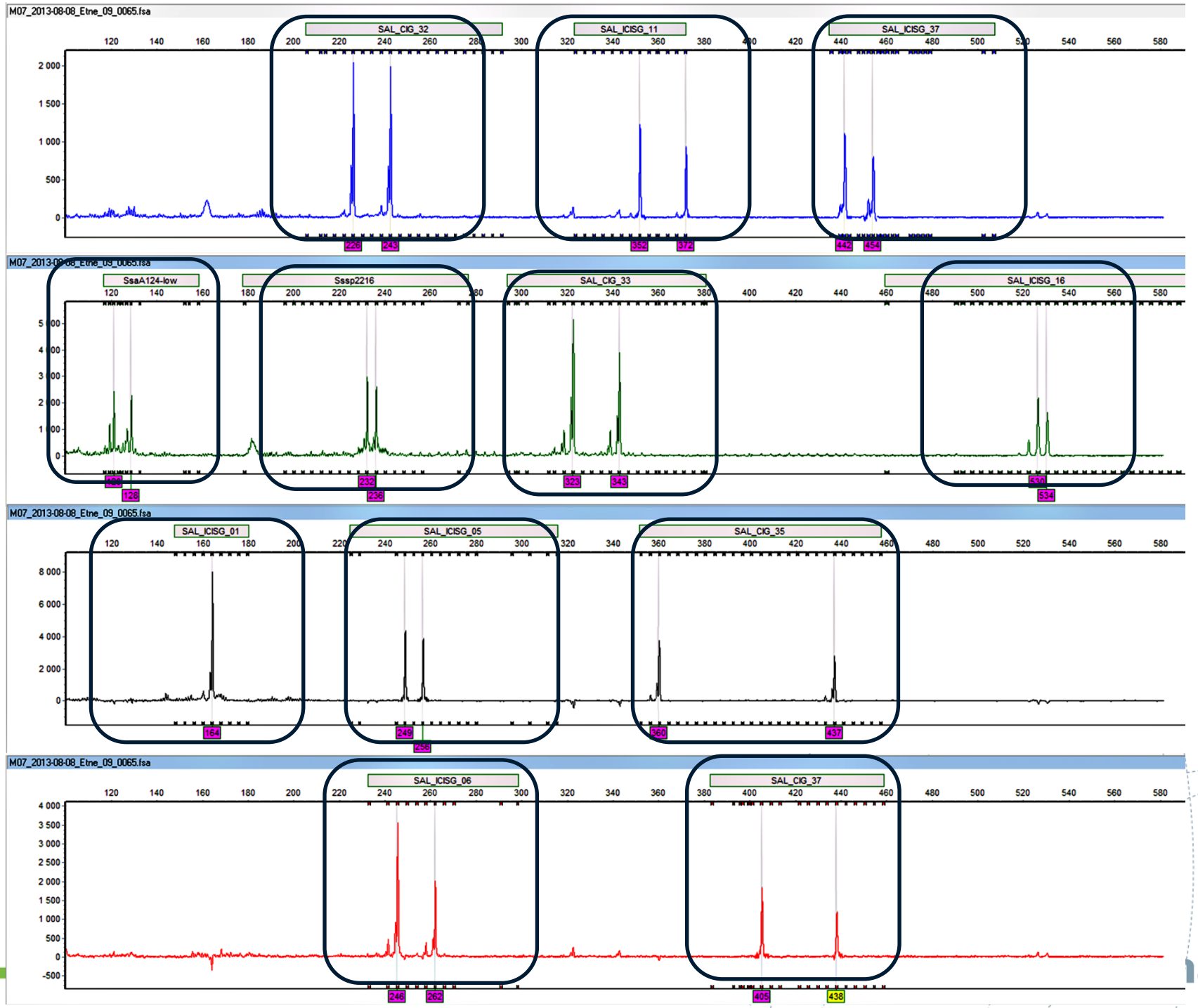
M07_2013-08-08_Etne_09_0065.fsa
SAL_CIG_32
SAL_ICISG_11
SAL_ICISG_37
226
243
352
372
442
454
M07_2013-08-08_Etne_09_0065.fsa
SsaA124-low
Sssp2216
SAL_CIG_33
SAL_ICISG_16
128
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343
534
M07_2013-08-08_Etne_09_0065.fsa
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SAL_ICISG_05
SAL_CIG_35
164
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360
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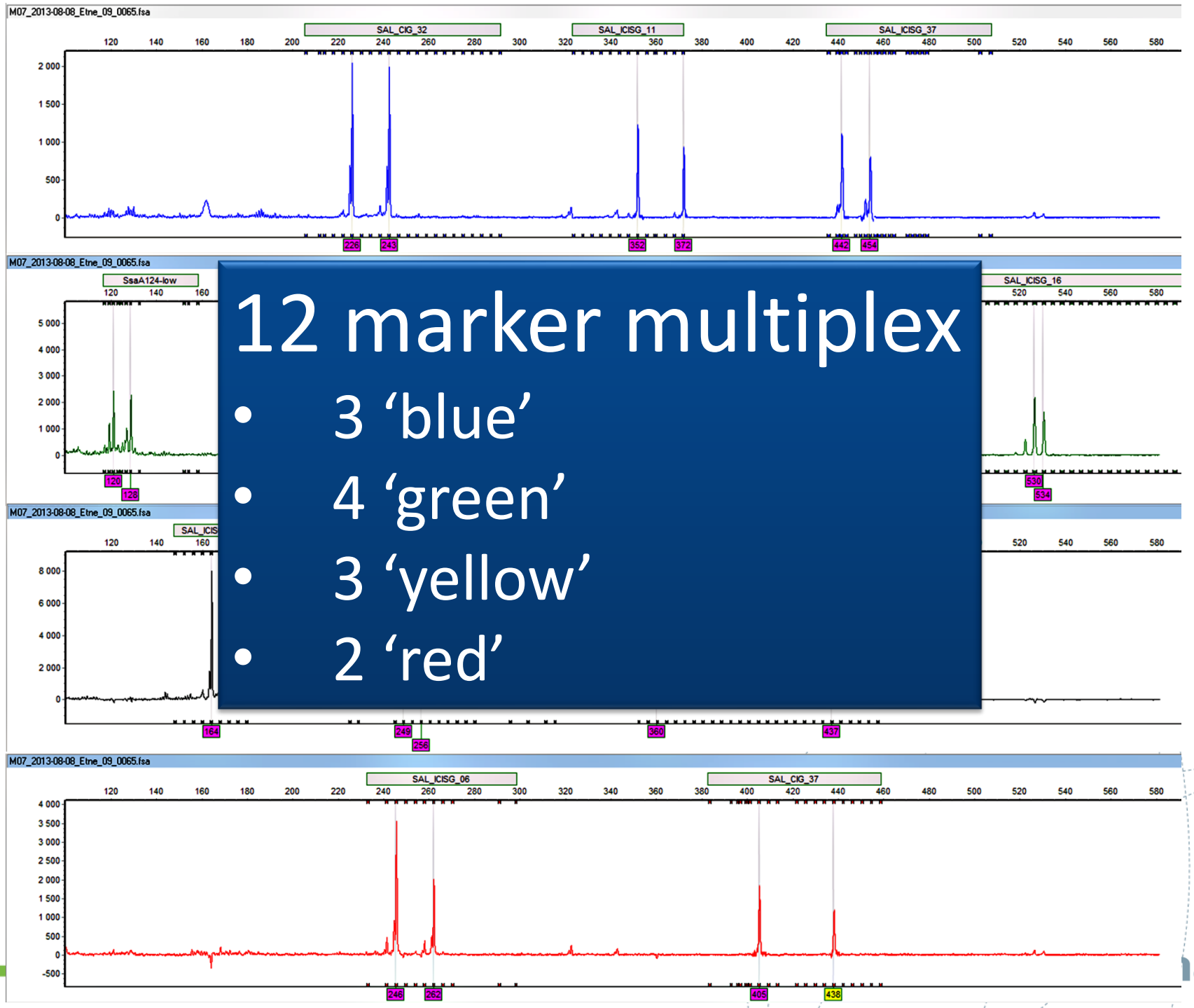
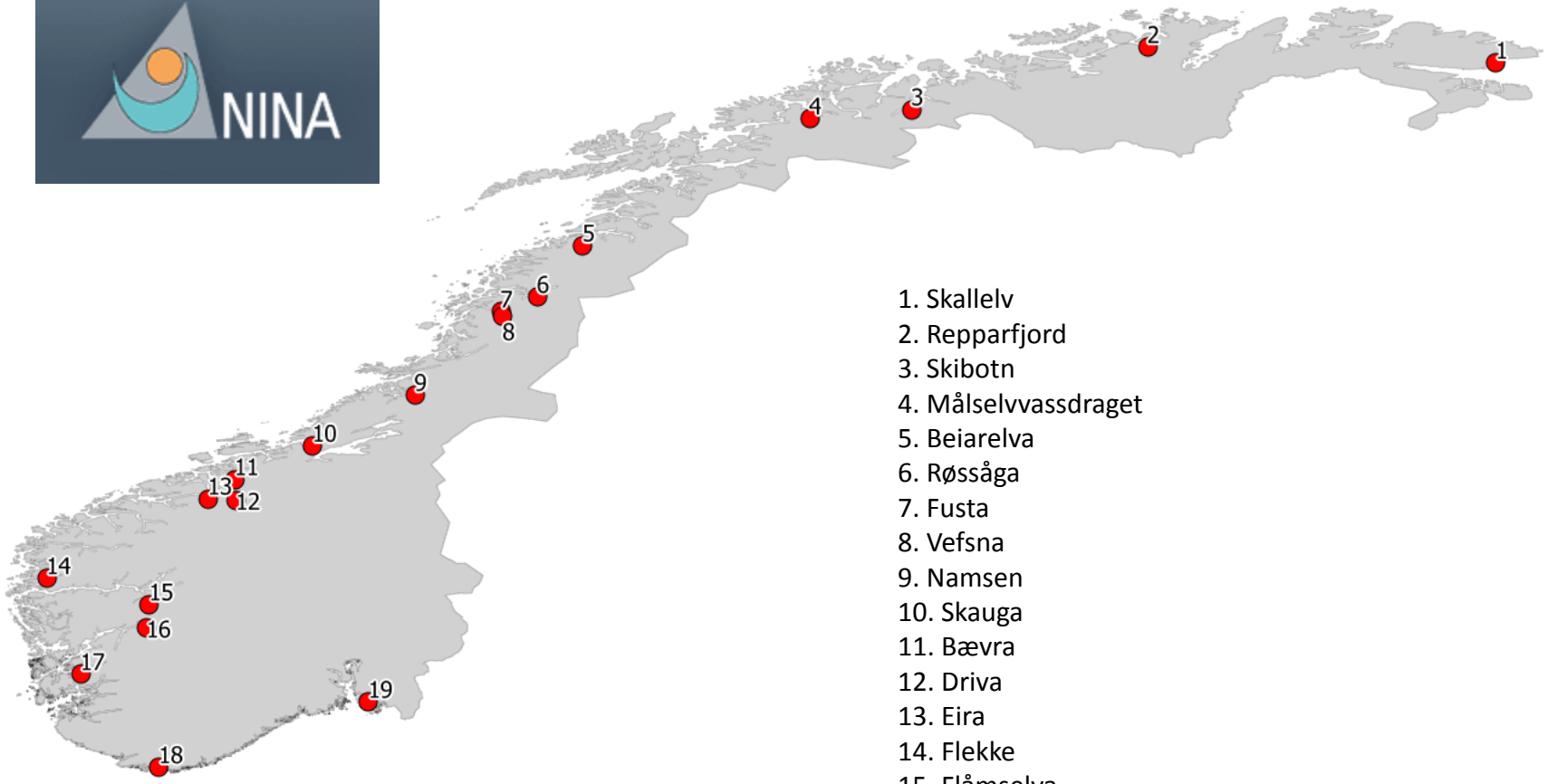
SAL_CIG_37
248
262
405
438



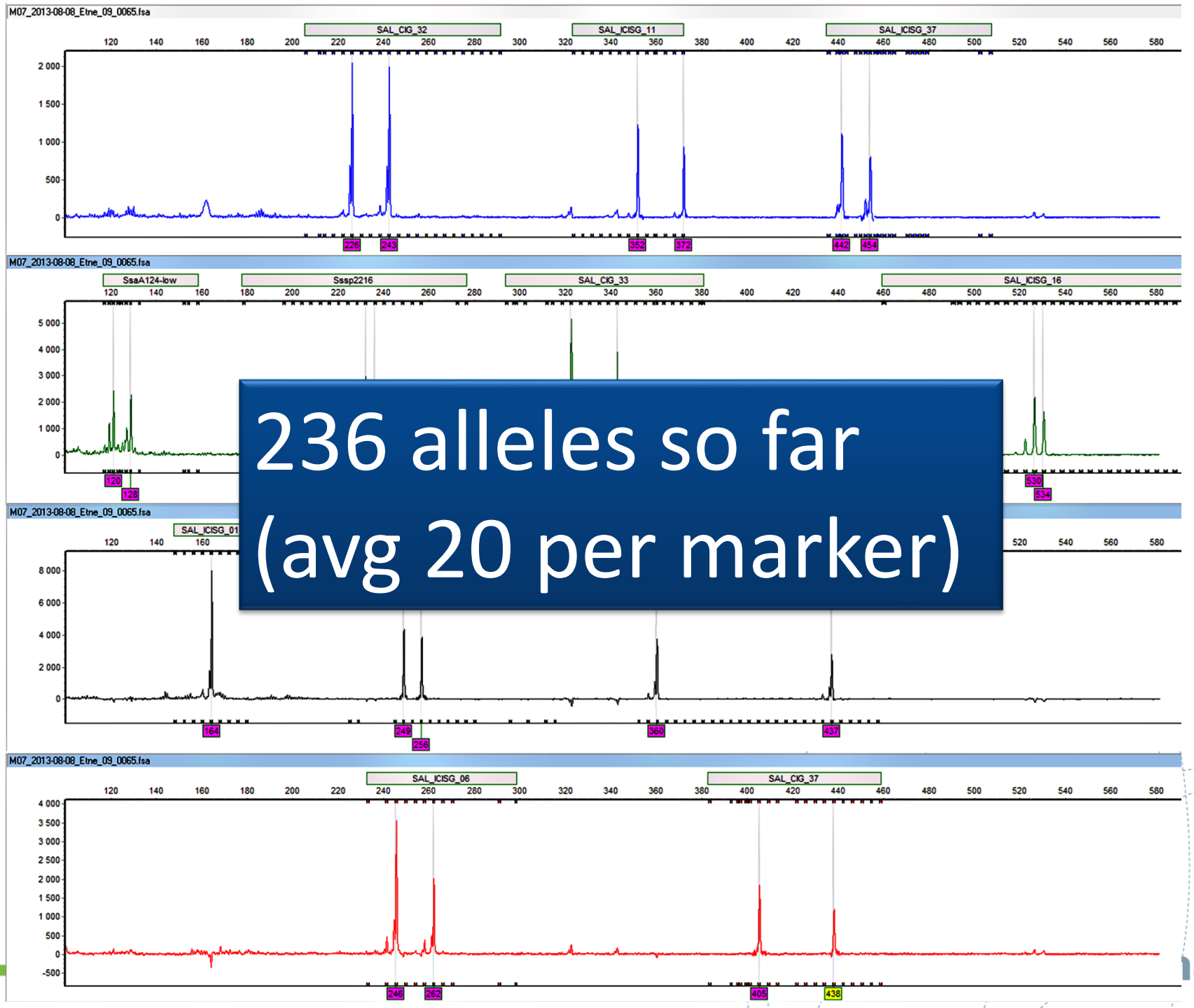
12 marker multiplex

- 3 ‘blue’
- 4 ‘green’
- 3 ‘yellow’
- 2 ‘red’

Wild fish sampling

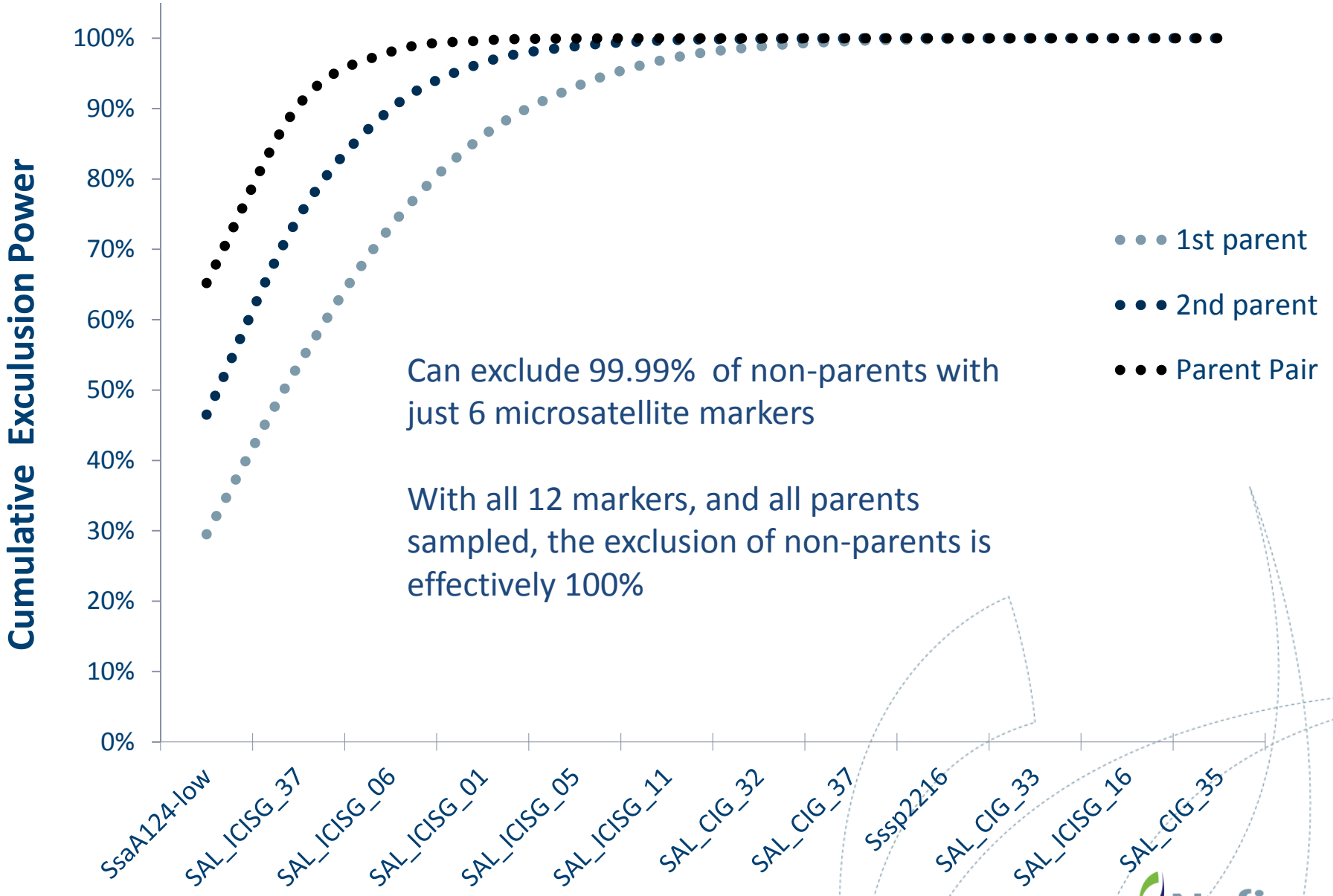


1. Skallelv
2. Repparfjord
3. Skibotn
4. Målselvvassdraget
5. Beiarelva
6. Røssåga
7. Fusta
8. Vefsna
9. Namsen
10. Skauga
11. Bævra
12. Driva
13. Eira
14. Flekke
15. Flåmselva
16. Eio
17. Etne
18. Audna
19. Glomma



236 alleles so far (avg 20 per marker)

Parentage assignment accuracy



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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

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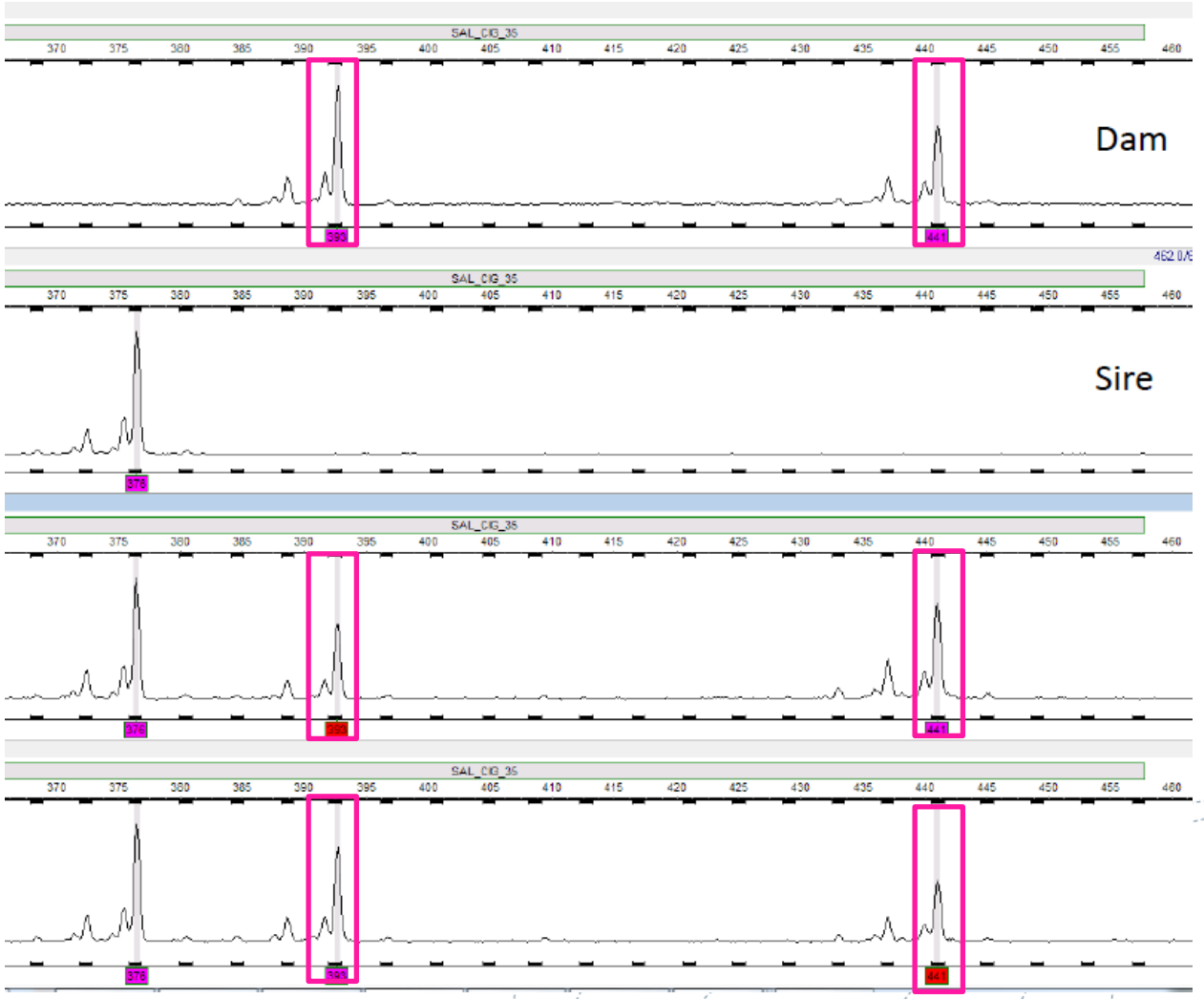
«Bonus» validation study results

- 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



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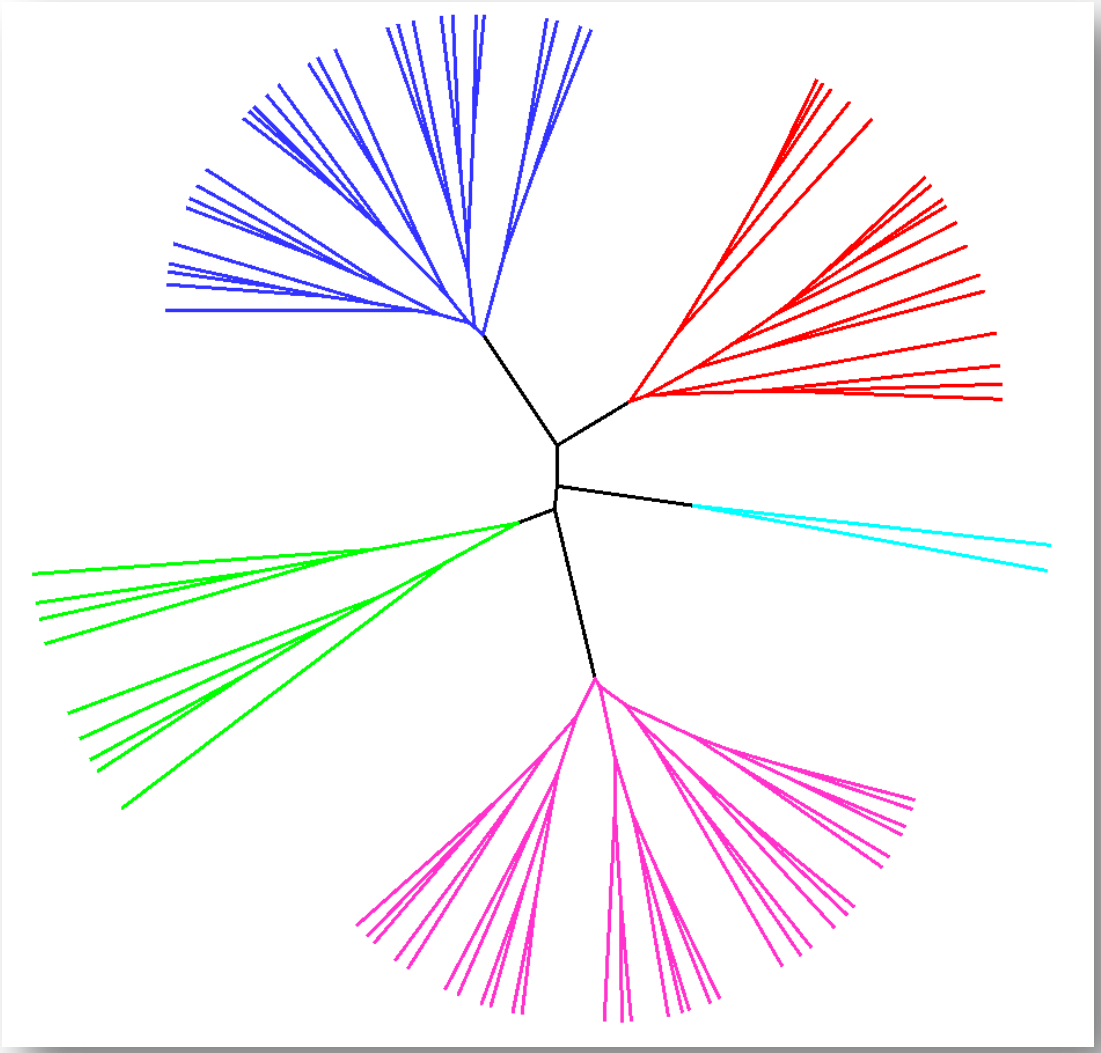
Relatedness clustering with multiplex



- Wild hatchery-reared smolt
- Progeny from single pair crosses of 5 males and 5 females
- Average relateness 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

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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

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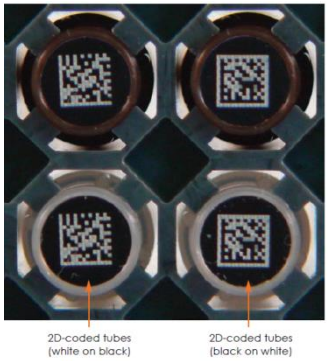
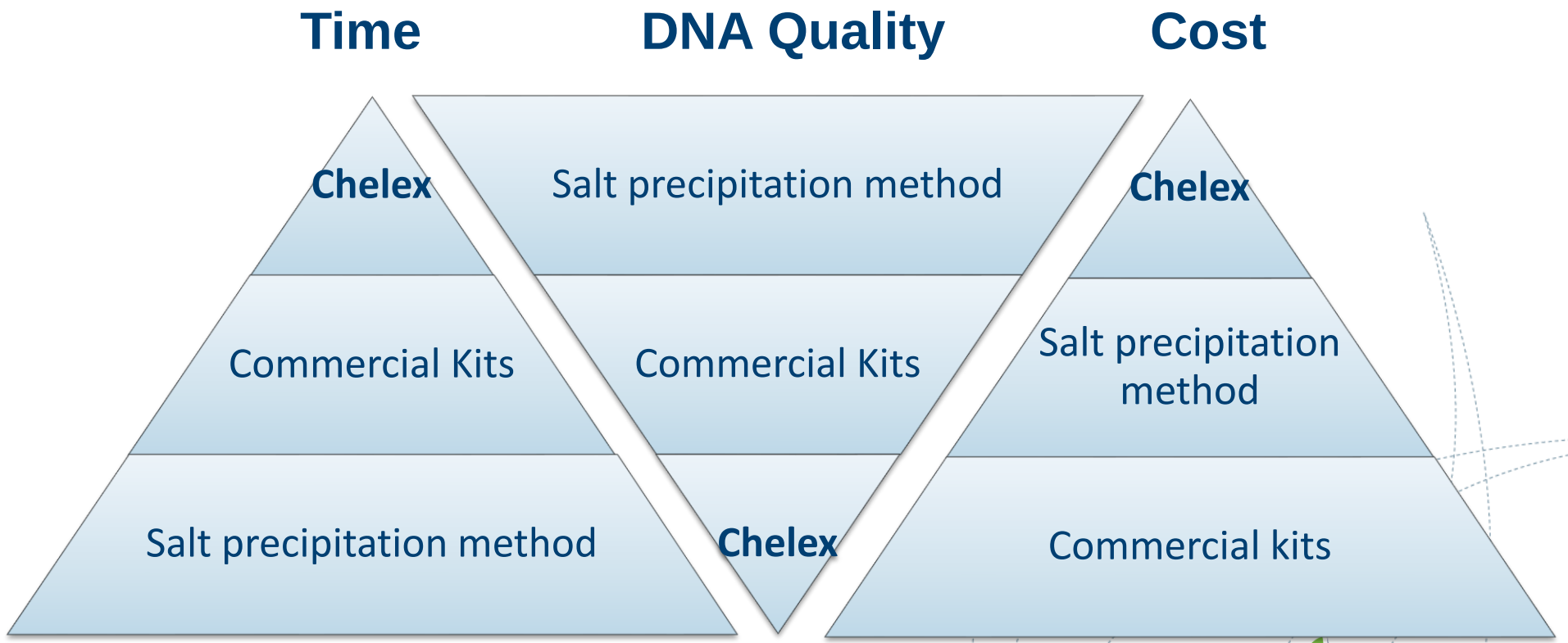
Sampling and sample storage

- 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



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

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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

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