

## FHF prosjekt 900706:

### “Sporing av laks: SNP-tilnærming”



Matthew Kent, Harald Grove, Teresa Andersstuen, Mariann Arnyasi, Kristil Sundsaasen, Sigbjørn Lien

CIGENE, Dept Animal and Aquaculture Sciences, Norwegian University of Life Sciences UMB, Ås, NORWAY

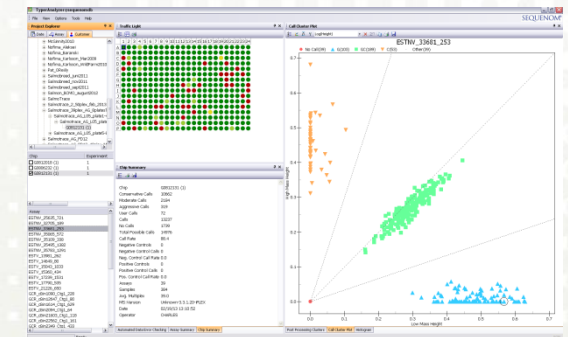


## SNP marker panel development

- ca. 6,000 SNPs genotyped in 756 samples belonging to MOWI, Salmobreed and AquaGen breeding populations was analysed.
- A subset of SNPs (n=114) was identified using the following criteria:
  - SNPs must have high minor allele frequency (MAF >0.45) in all three breeding populations
  - 3-4 SNPs from each chromosome and a wide physical distribution
  - 3 SNPs from mitochondrial genome to provide extra assurance for female assignment

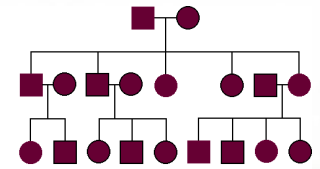
## Genotyping protocol

- A panel of 60 SNPs was genotyped using Sequenom MassArray 4 instrument.
- Genotype assignment is automatic but usually requires manual inspection
- DNA extraction from fin clips has been semi-automated using a BioMek3000 robot. Process includes tissue digestion, protein – nucleotide separation, and DNA precipitation.



## Genotyping protocol - Challenges

- DNA extraction is producing sufficient amounts of high quality material, however contaminant in the DNA extract seems to inhibit genotyping. This can be overcome by dilution.
- Genotyping 60 markers in a single reaction has not been robust, and the set is run as 2 separate reactions.
- “Holes” in the genotyping dataset weakens the power to assign. Missing parental genotypes is the most damaging situation.



## Assignment principles

- Assignment script looks for incorrect Mendelian inheritance (MI) patterns to eliminate impossible offspring-parent pairings. For example:

|                  |                |                    |
|------------------|----------------|--------------------|
| Offspring 1 (AA) |                | → Possible match   |
| Offspring 2 (AB) | + Parent1 (AA) | → Possible match   |
| Offspring 3 (BB) | →              | → Impossible match |

- After identifying *candidate parents*, software considers MI patterns within all possible parent-parent-offspring to eliminate incorrect trios. For example:

|                  |   |                   |                   |
|------------------|---|-------------------|-------------------|
| Offspring 2 (AB) | → | P1 (AA) + P2 (BB) | → Possible trio   |
|                  | → | P1 (AA) + P3 (AA) | → Impossible trio |

## Assignment software - Challenges

- An incomplete set of genotypes (ie not all 60 SNPs) can lead to offspring being (i) unassigned, (ii) assigned to 1 parent only, (iii) assigned to multiple trios
- Additional information can be used to increase certainty and reduce trio combinations. Examples of additional information are:
  - Sex of parents
  - Parental crossings (ie mating scheme)
  - Relationships between parents

## Validation study 1 – many offspring, few parents

- Samples from AquaGen breeding program, selected by NVH and sent with anonymous IDs to CIGENE for assignment. Includes:
  - 230 Parents
  - 520 Offspring
  - 40 unrelated offspring
- Require minimum 40 genotypes for assignment, allow 1 Mendelian mismatch :
  - 16 offspring (2.8%) failed to produce >40 genotypes

## Validation study 1 - assignment

- 230 Parents
- 520 Offspring
- 40 unrelated offspring

| Category                        | Description   | Number |
|---------------------------------|---|--------|
| Unique – full                   | Unique Assignment, valid parent couple                | 496    |
| Uncertain / unrelated / missing | One parent only / no parents / insufficient genotypes | 64     |

Assignment validation rate = 97%



## Validation study 2 – Many parents, few offspring

- Samples selected by NVH and sent with anonymous IDs to CIGENE for assignment. Includes:
  - 496 Parents
  - 279 Offspring
- Require minimum 40 genotypes for assignment, allow 1 Mendelian mismatch:
  - 10 offspring (3.5%) failed to produce >40 genotypes

## Validation study 2 - assignment

- 496 Parents
- 279 Offspring

| Category            | Description   | Number |
|---------------------|---|--------|
| Unique – couple     | Unique Assignment, parent couple                                    | 185    |
| Unique – single     | Only one parent, other unknown                                      | 33     |
| Multiple - couple   | More than one valid couple  | 9      |
| Uncertain / Missing | Multiple parent options and no valid couple/ insufficient genotypes | 52     |

Assignment validation rate = 98%

## Validation study 3 – Wild fish

- 95 wild fish (5 fish x 19 rivers) provided by NINA

| Category            | Description  | Number |
|---------------------|--|--------|
| Mismatch – A        | Mismatches $\geq 3$  | 87     |
| Mismatch - B        | 2 Mismatches   | 6      |
| Uncertain / Missing | Multiple parent options and no valid couple/insufficient genotypes | 2      |

- No wild fish assigned to known parents

## Extraction protocols

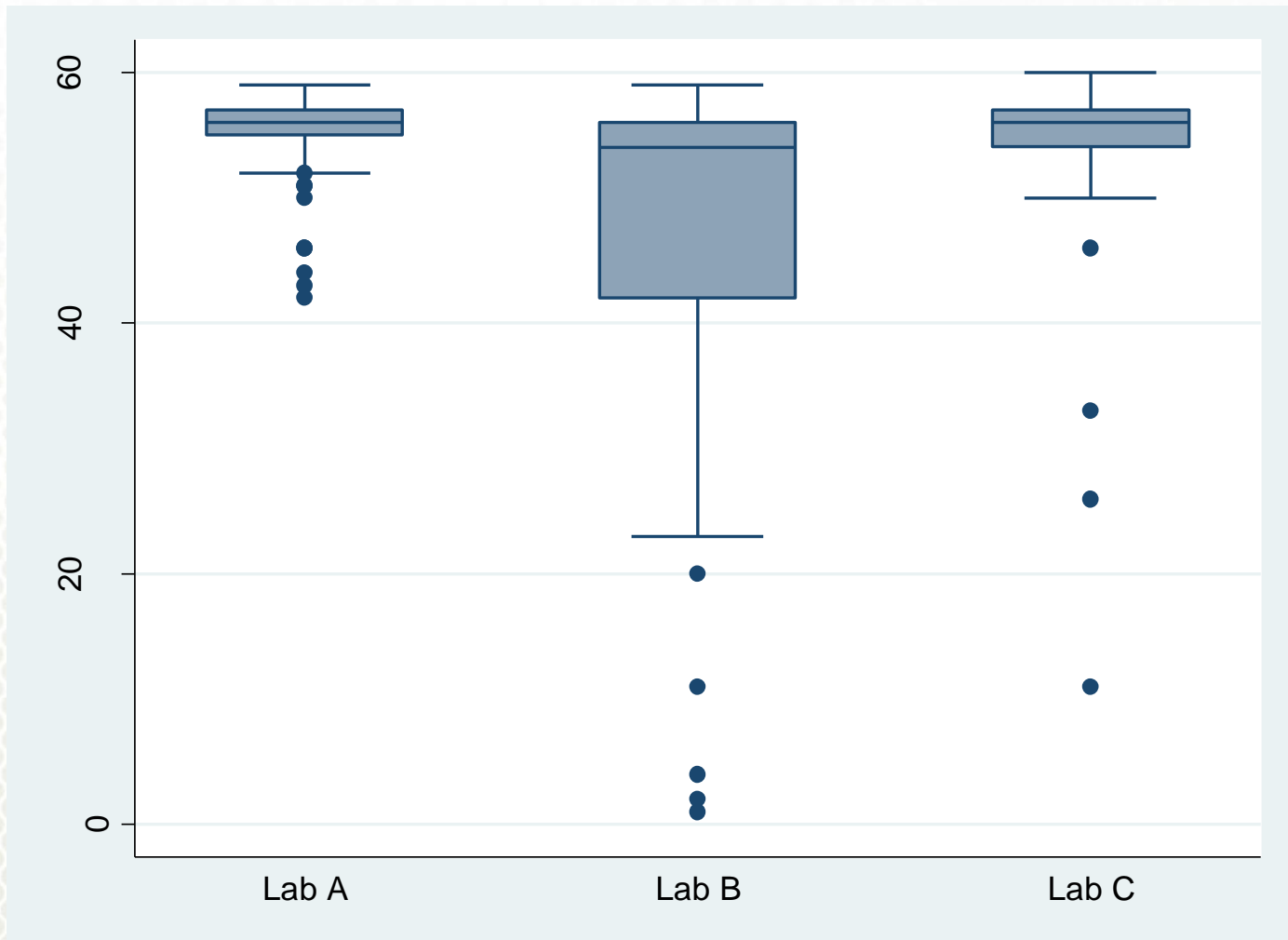
- To assess issues related to practical implementation of genetic tracing, samples were stored, extracted and genotyped under different conditions.
- Variables included:
  - Tissue type (bukfinne, fettfinne, skjell)
  - Preservation Method (ethanol, red-spirit, frozen)
  - Extraction (chelex, precipitation)
  - Operator (3 sites)
- Samples genotyped with SNPs, “number of genotypes” used to represent DNA quality.

## Significant effects

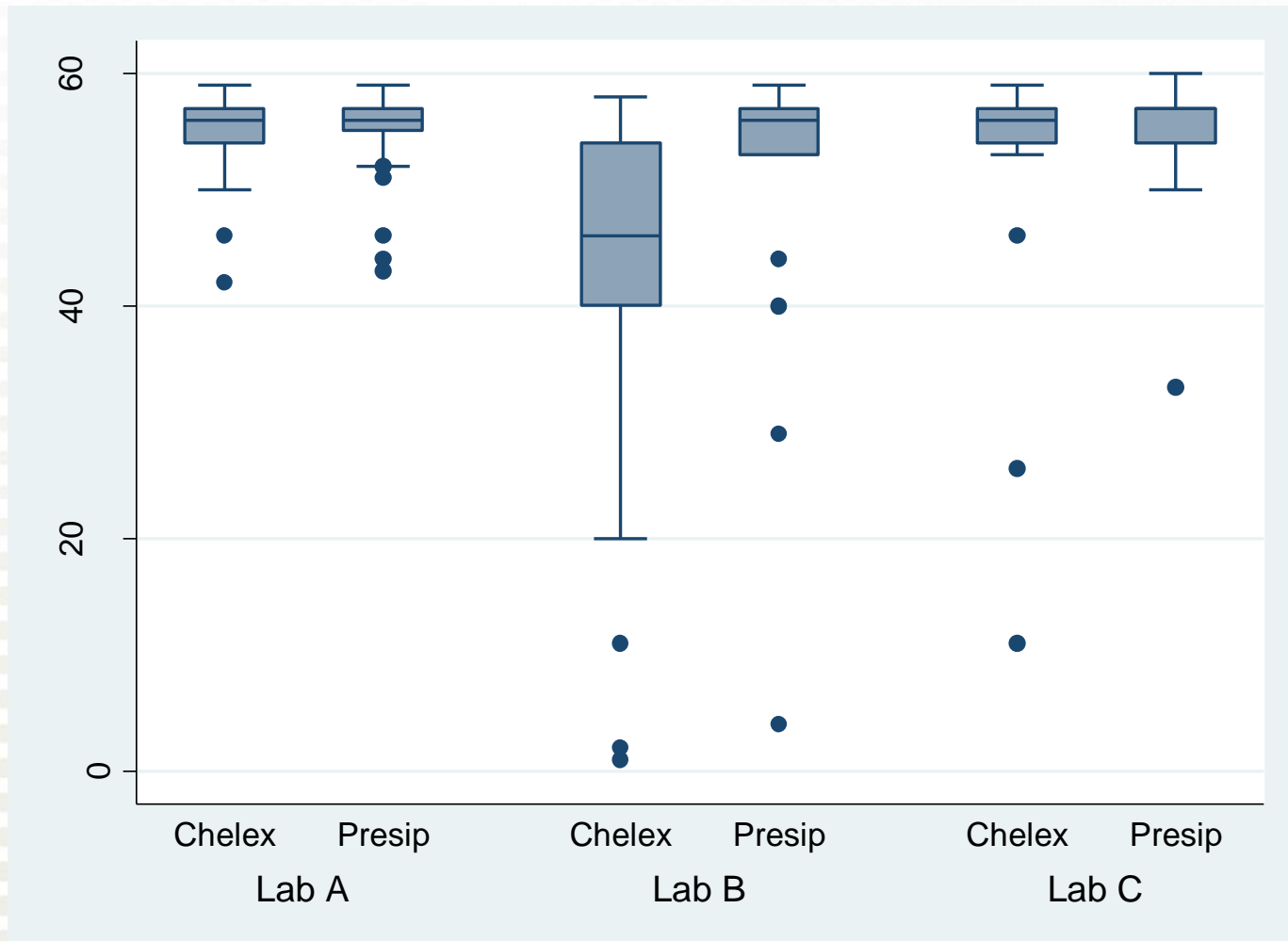
- Statistical analysis
  - effect of tissue, preservation, extraction, and site on # genotypes reported
  - generalized linear models with gamma distribution and identity link function
- # genotypes ranges from 1 – 60, mean 52, median 56

| Effect of ... | ... on number of genotypes                                 | P value |
|---------------|--|---------|
| Tissue        | Not significant  | 0,7     |
| Preservation  | Not significant  | 0,8     |
| Extraction    | Significant (precip gave 3.7 more genotypes than chelex)   | 0,018   |
| Site          | Significant (Sites A and C gave 7-8 more genotypes than B) | 0,001   |

## Site vs. #genotypes



## Site and Extraction vs. #genotypes



## Conclusion

- SNP based technology demonstrates good ability to assign farmed fish to their parents.
- The current SNP set has the power to differentiate wild and farmed.
- Standardization (and optimization) of the DNA extraction methodology is important.
- Further implementation of SNP-tracing would benefit from a redesign of the SNP set to include more markers and/or achieve single assay throughput.



## Synergies

- Routine testing of salmon DNA using SNP technology is performed large scale today, including:
  - Sample collection,
  - sample storage,
  - DNA extraction,
  - genotyping and reporting of data
- Thoughtful design of SNP panels can create added value, ie markers can be included that not only allow for tracing, but can provide broodstock information to producers.