VERIFICATION OF ISOGENIC CLONAL LINES IN THE ATLANTIC SALMON (*Salmo salar)* THROUGH ddRADseq

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I. Background: Why does it matter to produce clonal lines?

Clonal Lines = **Genetically Identical Individuals**

- Homogenity (decreases variation in experiments)
- Standardisation of the research – refined experimental designs (3Rs)
- Speed of generation (2 consecutive production cycles via Gynogenesis or Androgenesis)
- Reveals genetic variation for many traits
- QTL identification and whole genome sequencing projects

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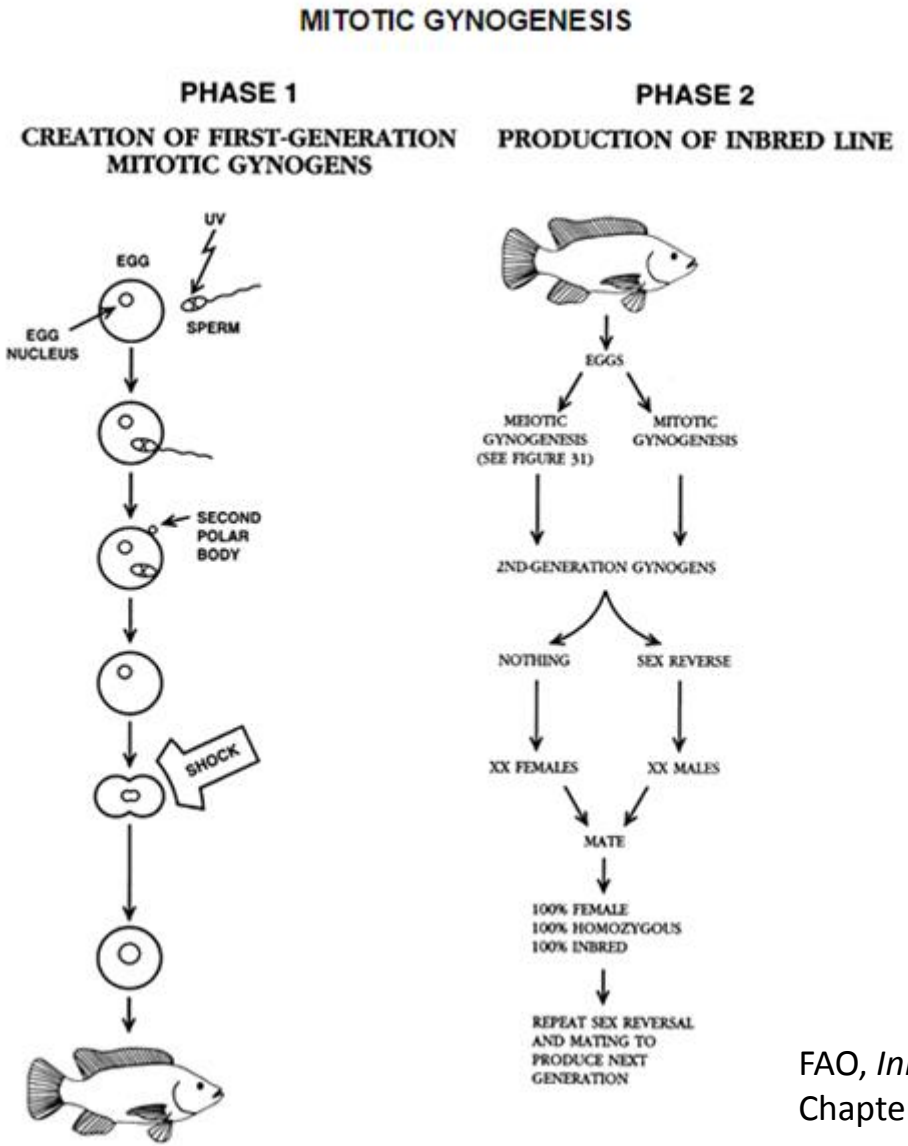
I. Background:
How to produce clonal lines?



Gynogenesis (G) All maternal inheritance
Androgenesis (A) All paternal inheritance

Spontaneous rise of:

- Haploids (due to failure in shock to deploy diploidy)
- Meiotic Gynogenetics (due to failure in the time of shock – produced by blocking 2nd polar body exclusion therefore enables to 'capture' the results of any crossover events – undesired heterozygosity
- **Essential to verify the isogenic nature of clonal lines**

FAO, *Inbreeding and Broodstock Management* Chapter 6, Chromosome Set Manipulations.

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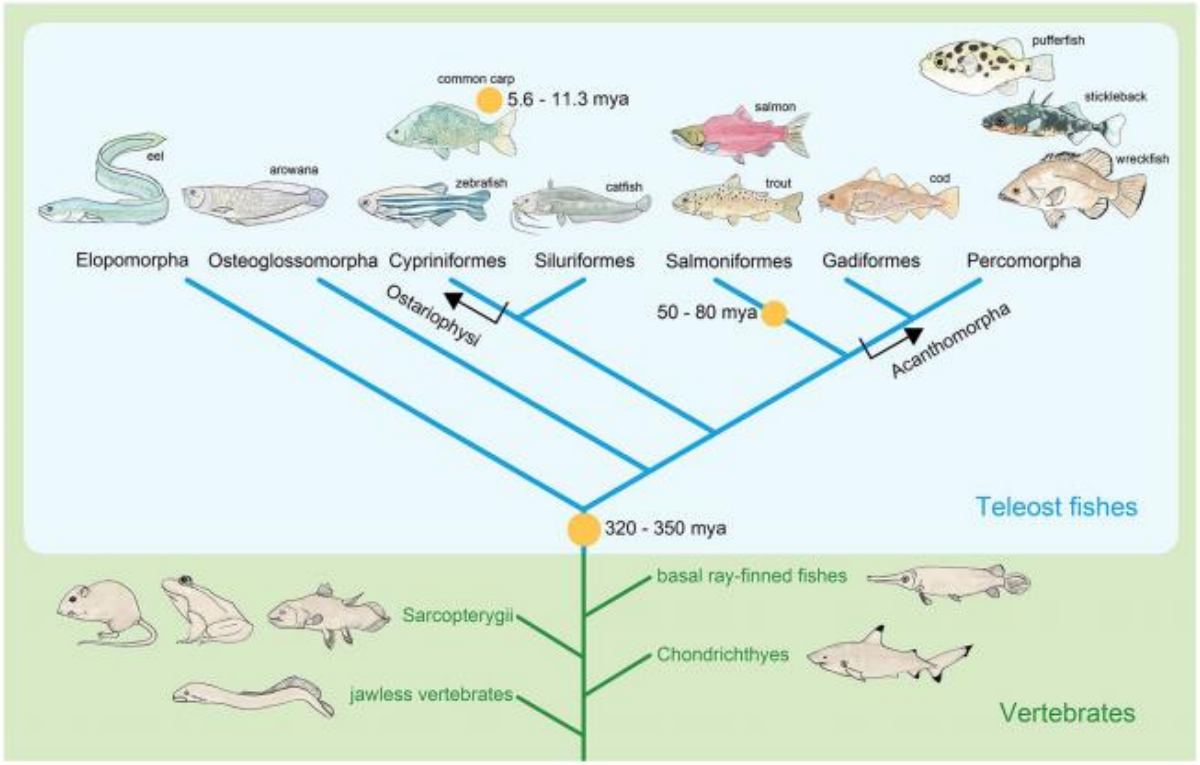
I. Background:
Teleost Specific-WGD



Glasauer & Neuhauss 2014, *Mol Genet Genomics*

WGD results in paralogs loci.!

Sequence variants found in duplicated genomes:

1. Paralogues Sequence Variants (PSVs) - fixed sites, no polymorphism
2. SNPs – allelic polymorphism
3. Multisite Variants (MSVs) – polymorphism found across paralogs

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Aim of the study

- Verification of optimised genome irradiation protocol in Salmon
- Verification of successful production of isogenic clonal lines

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II. Materials and Methods

Production of Clonal fish

- Sperm was diluted to 5 x 10^8 ml^{-1} and irradiated at 170 $\mu W.cm^{-2}$ with 254nm UV light
- Pressure shocks used 4400-4800 min°C post-fertilization

See Online

AquaExcel_deliverables_optimsation of G1 fish production in salmon

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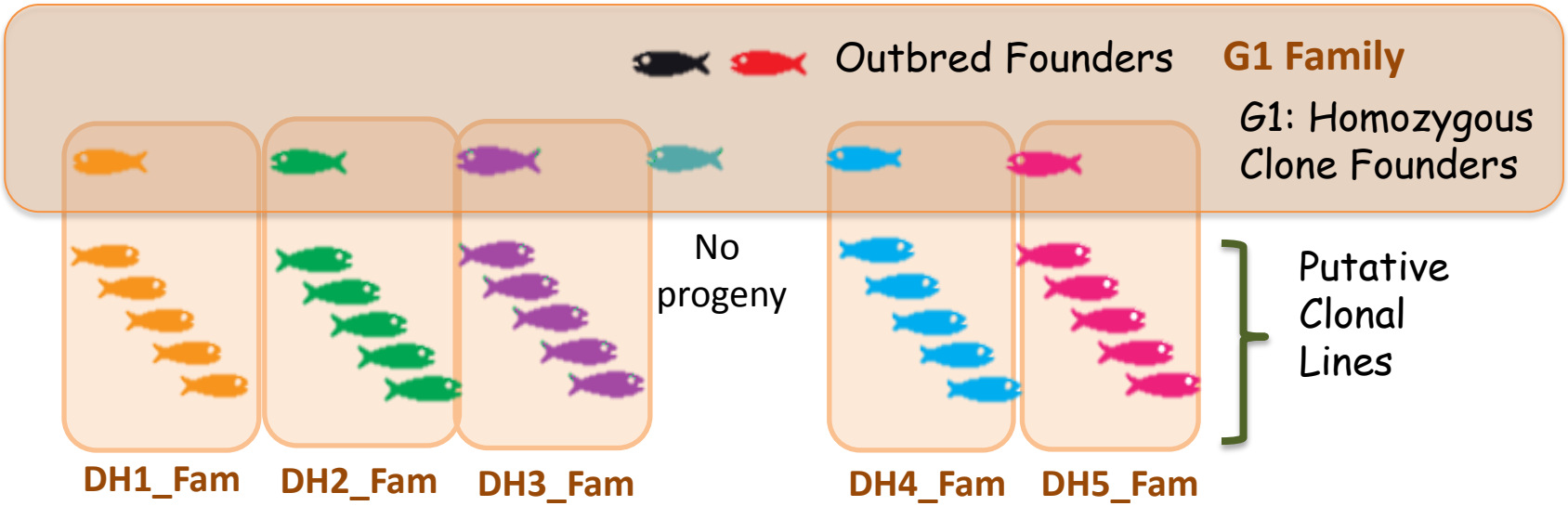
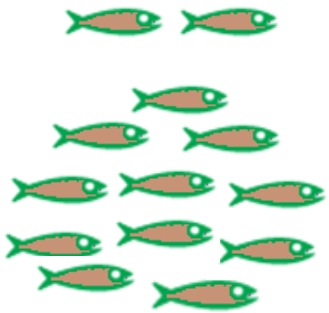
Experimental Design
Putative Clonal Lines



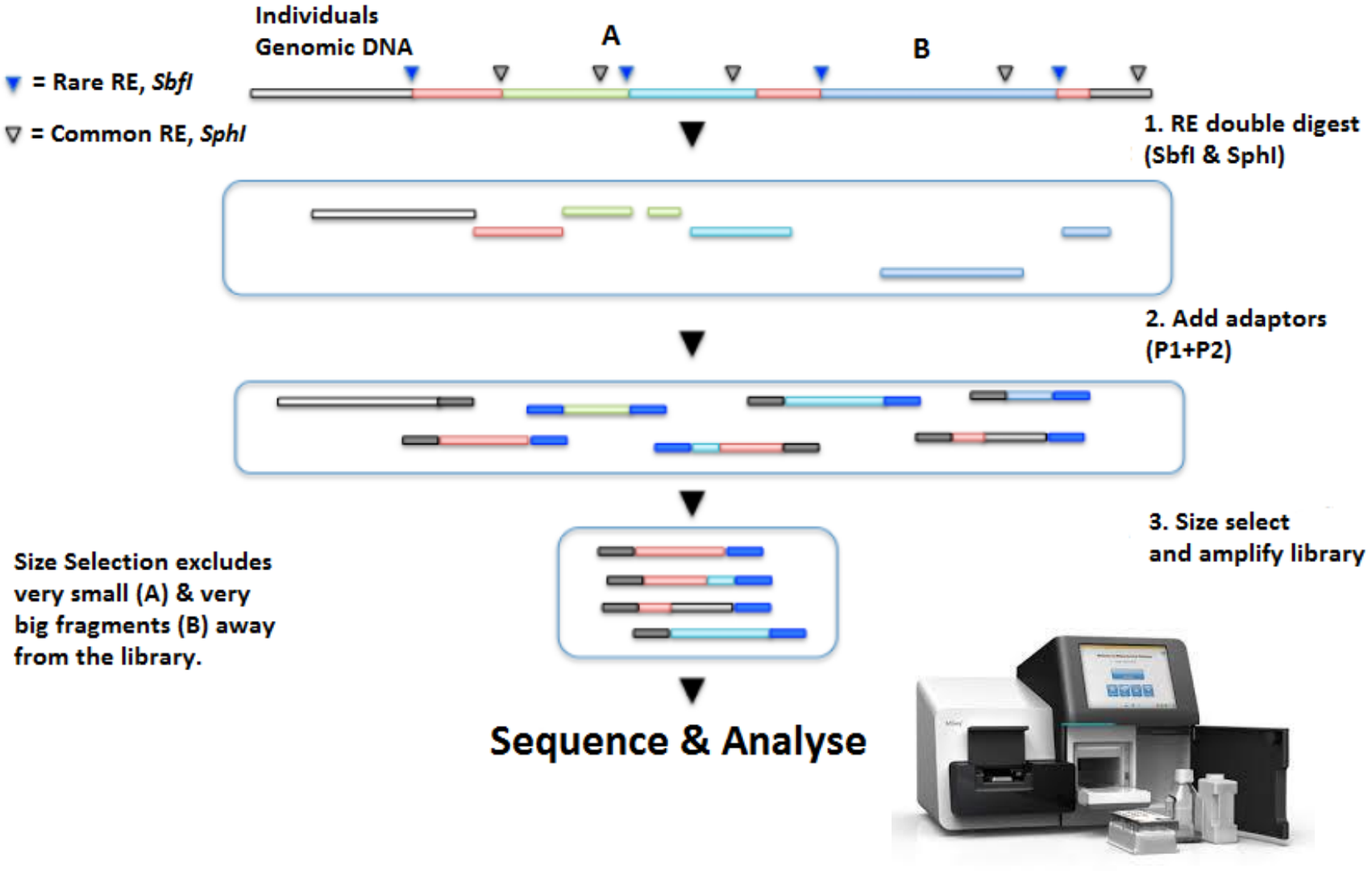


Haploid Family
Parents+Progeny
(PSVs/MSVs)

DH_Fams: G2 fish (putative clonal lines)

II. Material and Methods

Double Digest RADseq (ddRADseq)



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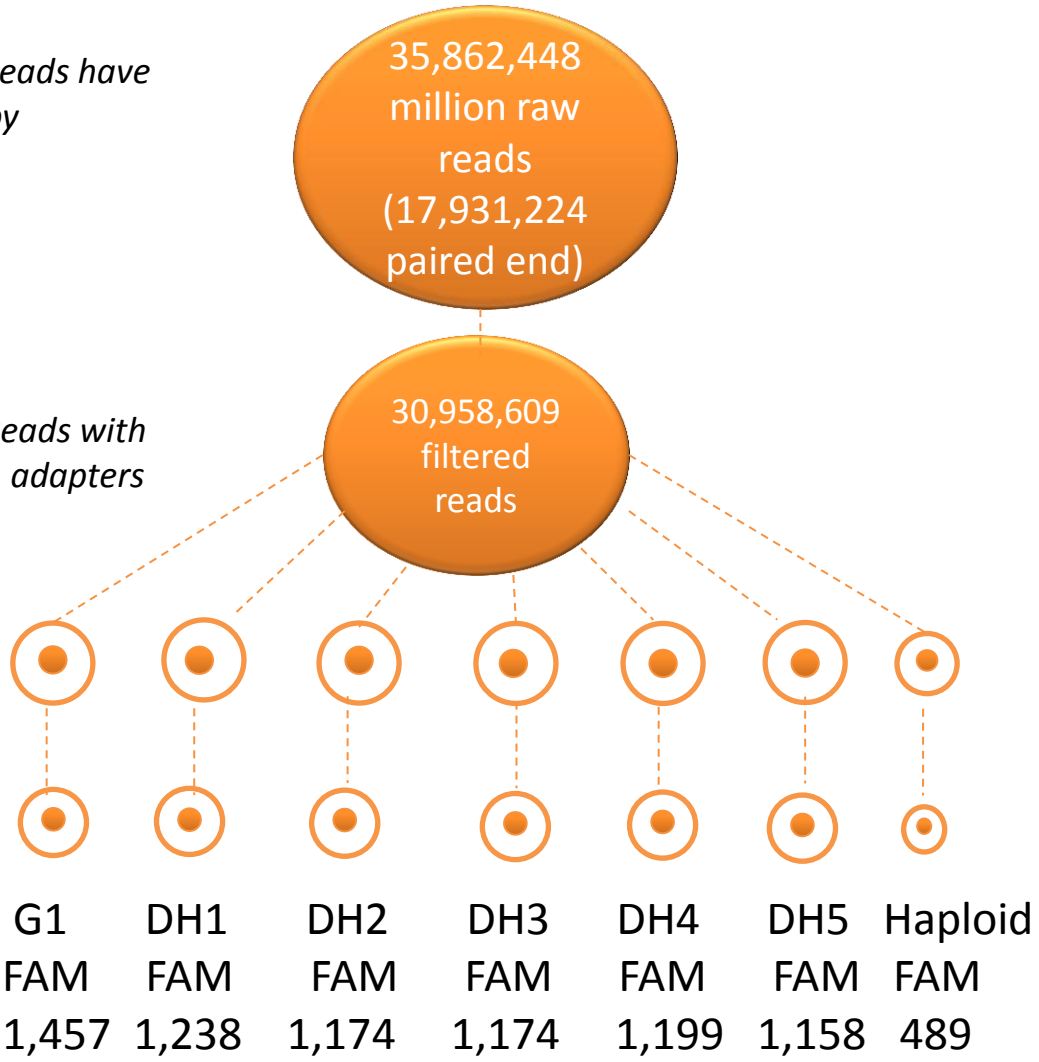
III. Results: Sequencing & RAD tag summary

***Raw Reads**: All reads have been produced by sequencer*

***Filtered reads**: Reads with right barcodes & adapters combination*



Reads used by Stacks to create individual paired-end markers

Total RAD markers identified in each FAM

Loci retrieved in 70% of the samples

Stacks package (Catchen et al., 2011).

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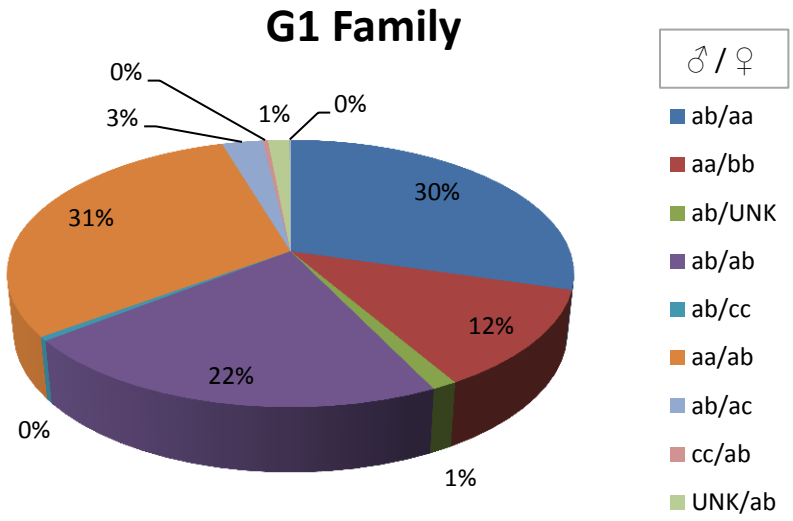
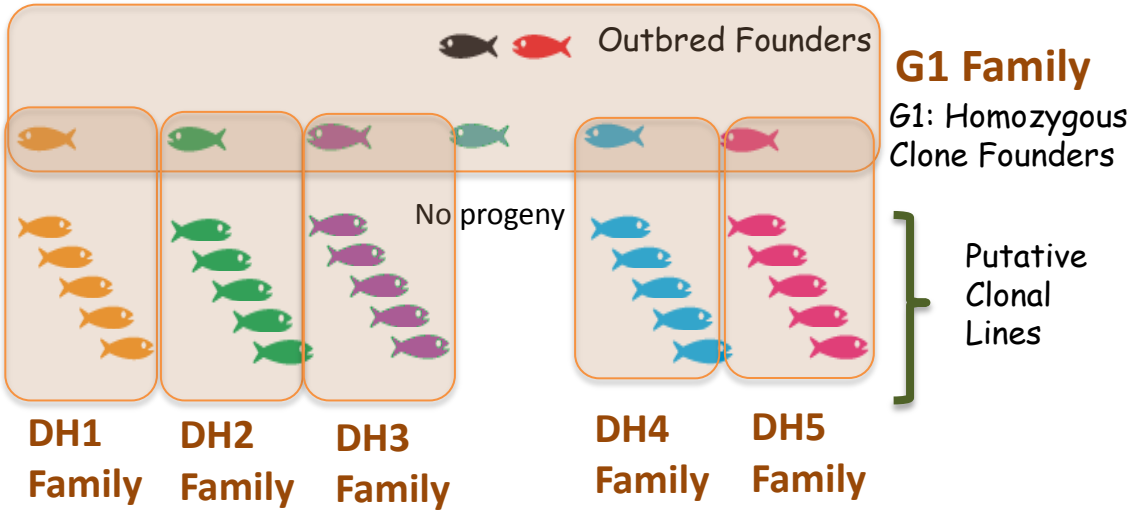
III. Results

Distribution of RAD alleles in G1 FAM

♂ / ♀

G1 Family with 6DHs Progeny			
Map types available	RAD alleles (total loci)	Potentail Paternal contributor loci	% of Potential Contributor Loci
ab/aa	431	13	3.0
aa/bb	175	1	0.6
ab/UNK	18	0	0.0
ab/ab	314	93	29.6
ab/cc	7	1	14.3
aa/ab	445	11	2.5
ab/ac	40	8	20.0
cc/ab	5	0	0.0
UNK/ab	21	0	0.0
ab/cd	1	0	0.0
TOTAL	1457	127	8.7





G1 Family

G1: Homozygous Clone Founders

Putative Clonal Lines

- 8.7% potential paternal contributor loci, but WGD.??!

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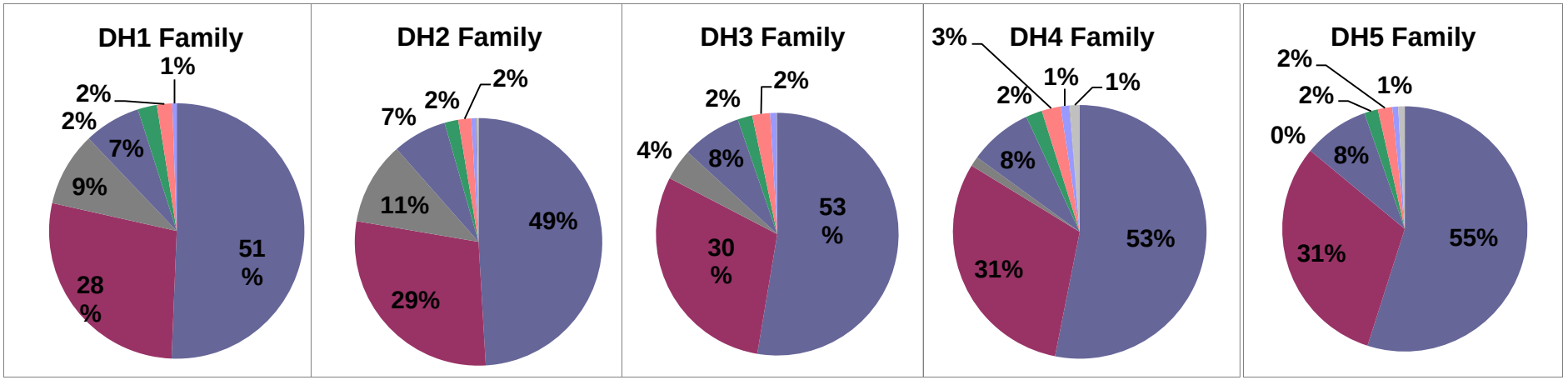
III.Results:

Distribution of RAD alleles in DH Fams



79%	78%	83%	84%	86%

♂ / ♀

ab/aa aa/bb ab/ab aa/ab ab/cc

UNK/ab ab/ac ab/UNK ab/cd cc/ab

III.Results:
Investigation of putative sire contribution

Potential Putative Paternal Contributor Loci ??

BLAST_NCBI_WGS of Salmon

- **NO convincing sign of any paternal contribution to offspring**

- Repetitive elements (transposons)
- PSVs / MSVs
- Noise of salmon genome

BLAST_NCBI_RefSEQ

- It was used to prove the existence of repetitive elements

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III.Results:

Control test to identify true SNPs

	G1_FAM	DH1_FAM	DH2_FAM	DH3_FAM	DH4_FAM	DH5_FAM
Total RAD loci	1457	1238	1174	1174	1199	1158
Potential sire cont loci	127	325	270	320	336	262
All female cont loci	1330	913	904	854	863	896
Further investigated	30	10	10	10	10	10

G1_FAM Frequencies

57 %	True SNPs
20 %	Paralogous loci
23 %	Repetitive elements

DH_FAM Mean Frequencies

34 %	True SNPs
44 %	Paralogous loci
22 %	Repetitive elements

Estimated true SNP markers in G1_Fam: **758**

Estimated true SNP markers in each DH_Fams: **301**

Frequency of haploid derived heterozygous putative SNPs were 30%

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IV. Conclusion

- ✓ Verification of optimised genome irradiation procedure for the Atlantic salmon
- ✓ Verification of isogenic nature of 5 clonal lines in the Atlantic salmon

- ddRADseq is a cost-effective and quick method, generating hundreds of diagnostic markers

Thanks, any questions??

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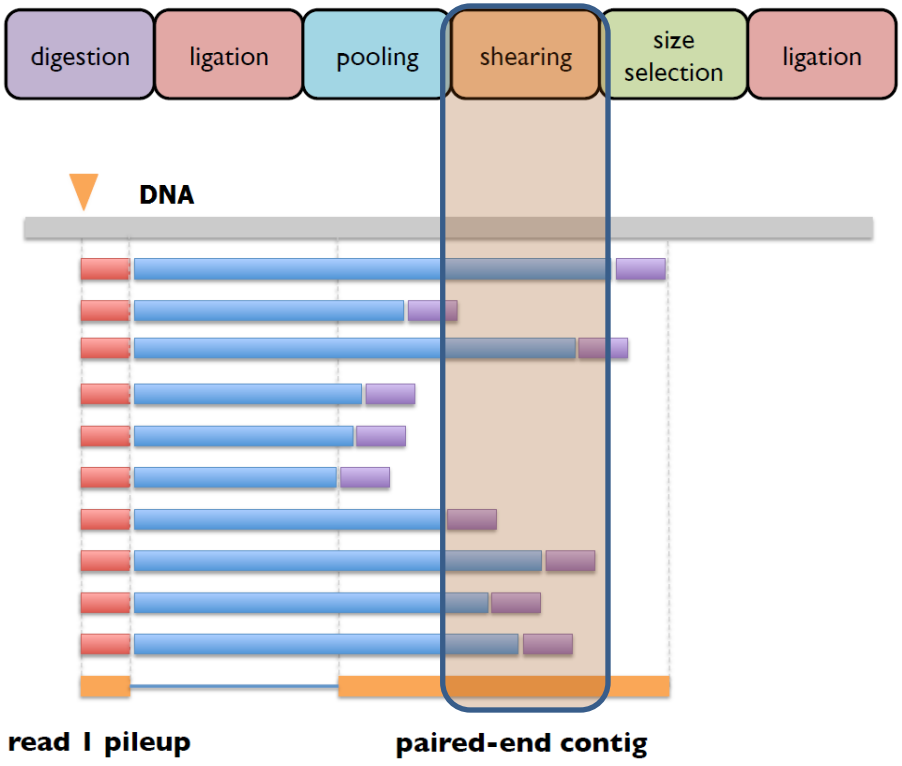
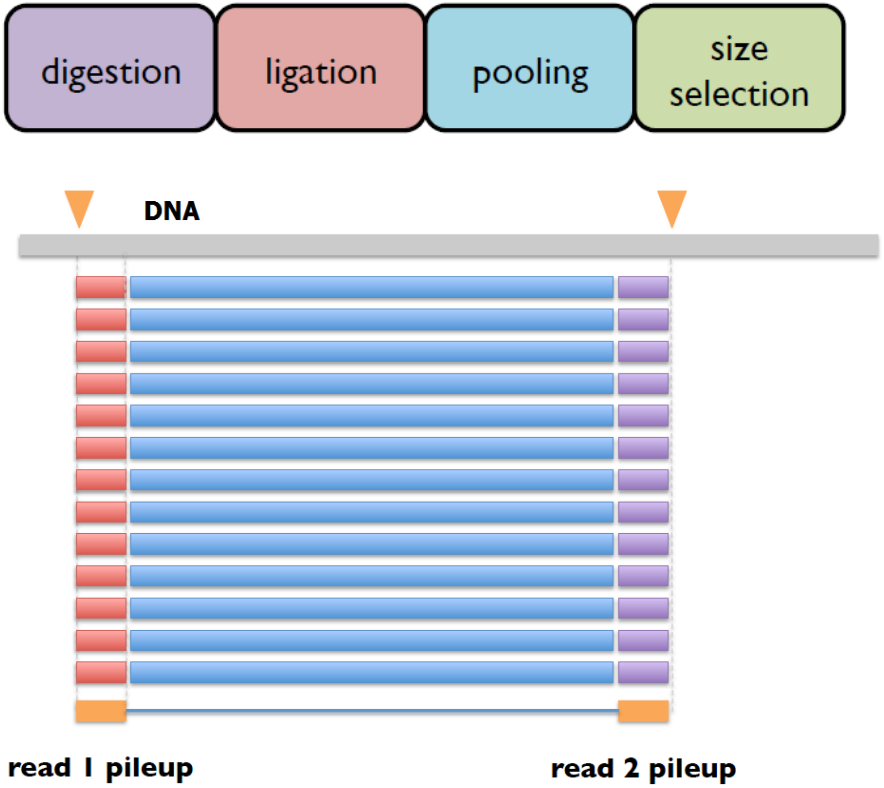
II.Material Methods:

Why PCR duplicates cannot be removed from ddRADseq paired end reads?

RADseq



ddRADseq



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II.Material Methods:

Difference between meiotic and mitotic gynogenesis