GENOTYPING-BY-SEQUENCING USING CUSTOM ION AMPLISEQ™ TECHNOLOGY AS A TOOL FOR GENOMIC SELECTION IN ATLANTIC SALMON

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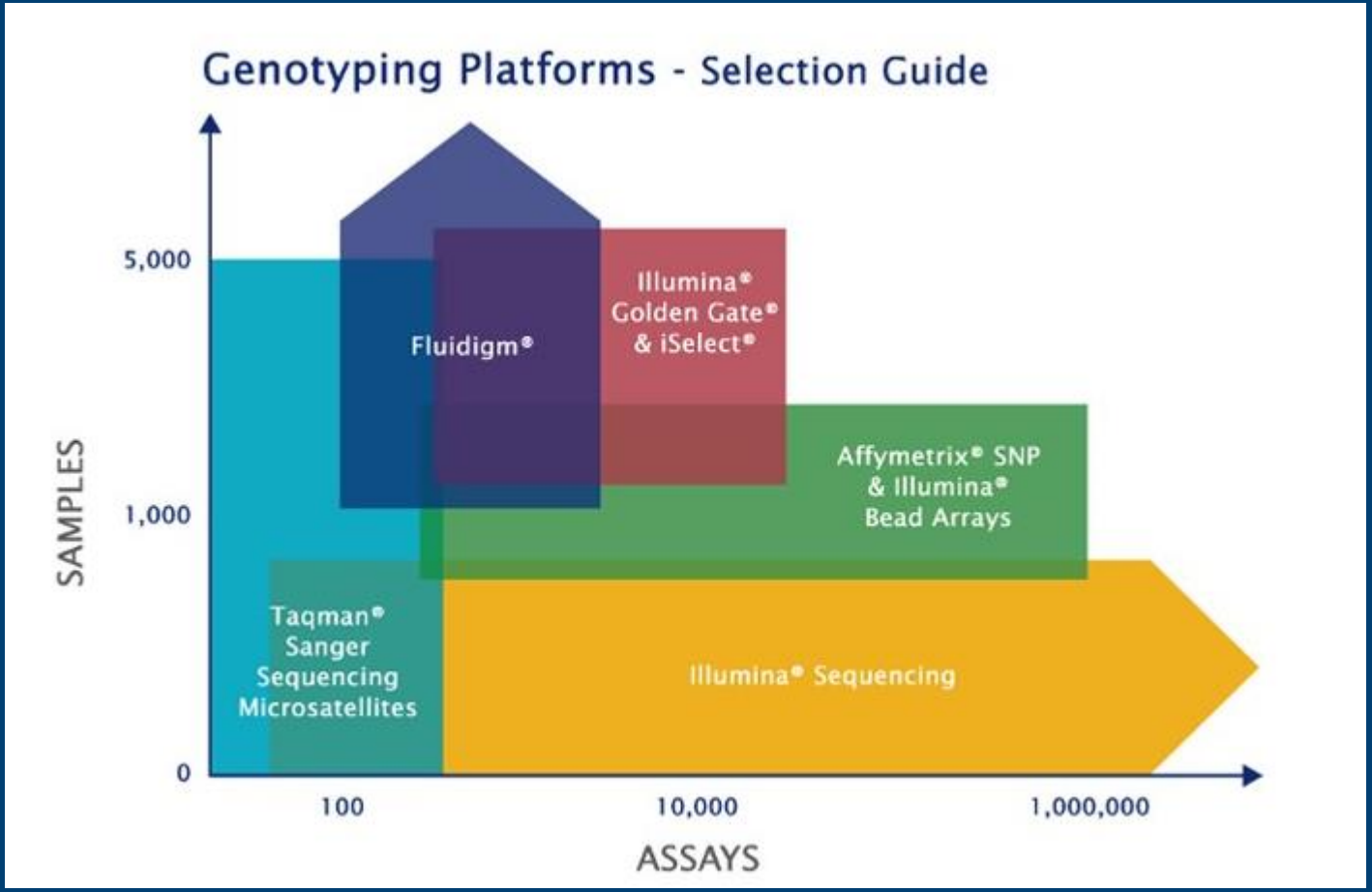
Use of high and low density marker sets in aquaculture

- Genotyping technology development has changed animal breeding
- Within-family genomic selection is a viable alternative to LD based 'population' GS
  - 1000 SNPs should be sufficient for Atlantic salmon
- Low density (1000-3000) sets also very useful for other reasons
  - SNPs tagging QTLs
  - Parentage assignment
  - Traceability
- Genotyping options for 1000-3000 SNPs?

Various genotyping technologies…



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Genotyping-by-sequencing approaches

Ion AmpliSeq

Restriction Enzyme digestion (RAD)

1. Ideal for *de novo* SNP discovery with no prior sequence
2. Genome complexity is reduced by digesting the DNA with one or two restriction enzymes

Targeted amplicon-based resequencing

1. PCR primers designed to amplify the areas of interest
2. Targets known SNPs

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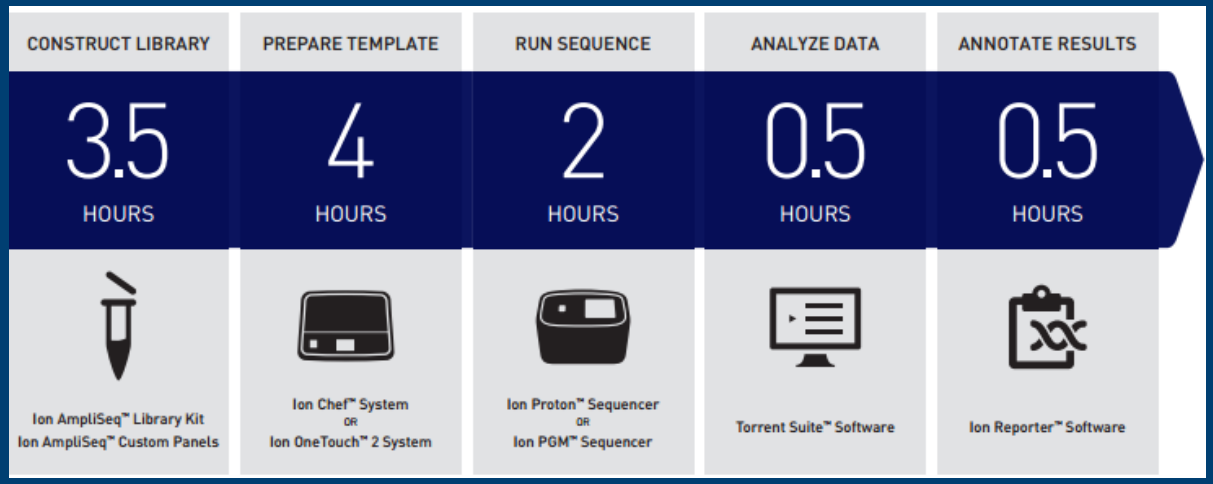
Ion AmpliSeq™ Technology: Multiplex PCR based genotyping by sequencing (GBS)



Up to 24,576 primer pairs per tube

+

10 ng DNA per tube

+

Ion AmpliSeq™ Library Kit

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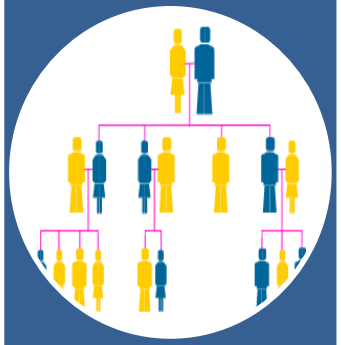
Ion AmpliSeq™ Target Selection Solutions

Ion AmpliSeq™ Cancer Panel

46 genes

760 mutations

Ion 314™ Chip



Ion AmpliSeq™ Inherited Disease Panel

~100 diseases

Ion 316™ Chip

Ion AmpliSeq™ Comprehensive Cancer Panel

~400 genes

Ion 318™ Chip

Ion AmpliSeq™ Designer

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12 to 1536 plex per tube

Ion AmpliSeq™ Exome Panel kit

All Exome in 12 tubes

Ready-to-Use Panels & Custom Solutions

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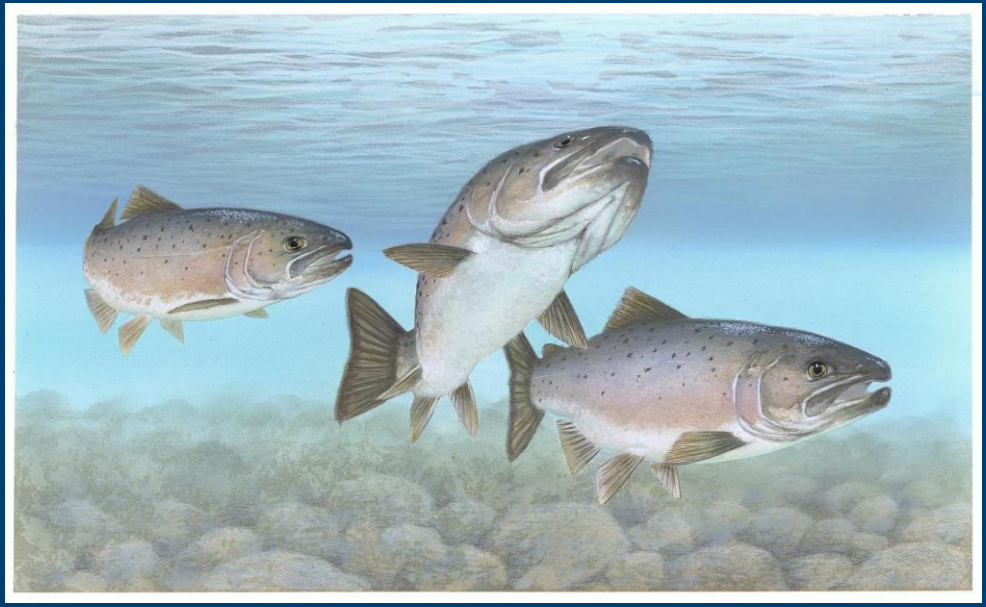
Aim

- To validate Ion Ampliseq technology as a cost-efficient means of genotyping 1000 SNPs in Atlantic salmon



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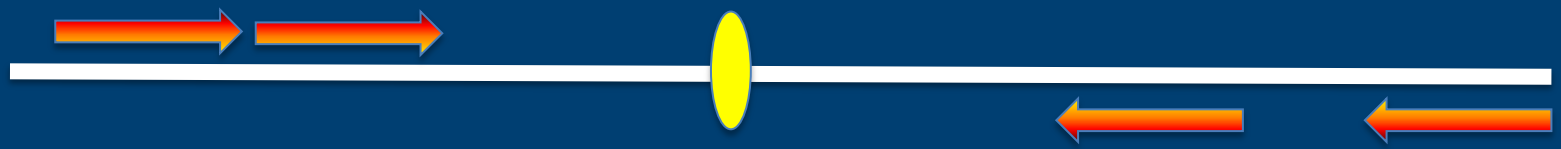
Methods

- 1000 SNP loci were selected from published map
- 40 DNA samples selected for trial
- Primer pool created
- PCR optimised and library prep carried out
- Amplicon sequencing performed on Illumina Nextseq 500 (PE 150bp reads)
- Sequences aligned to amplicon reference sequences with Bowtie2

Primer design for 1000+ amplicons

- Amplicon size of 100-200 bp
- Primers do not need to be right ‘on’ the SNP
- Primers need to be compatible and avoid repetitive sequences etc.
- Target sequences and reference genome submitted to Life Tech
  - Bioinformatics pipeline does its magic



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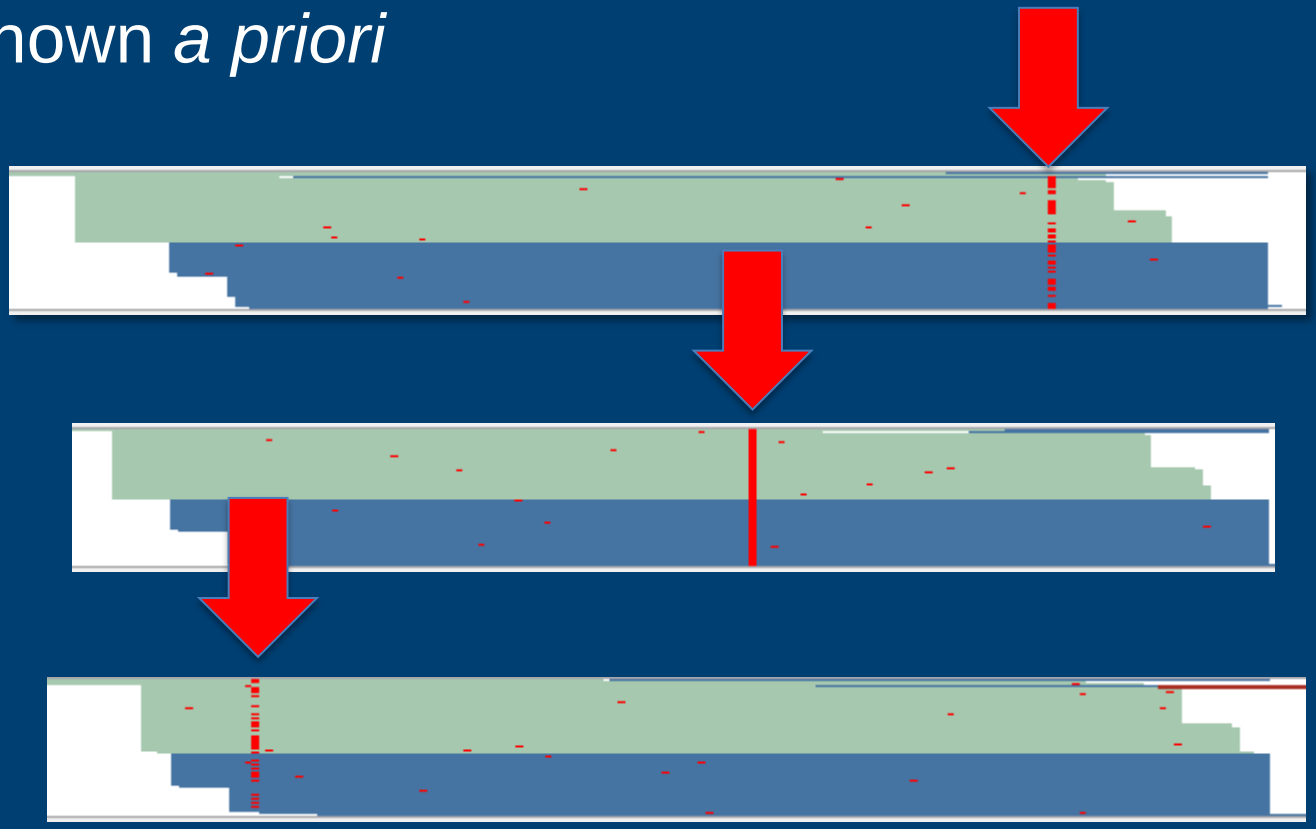
Results

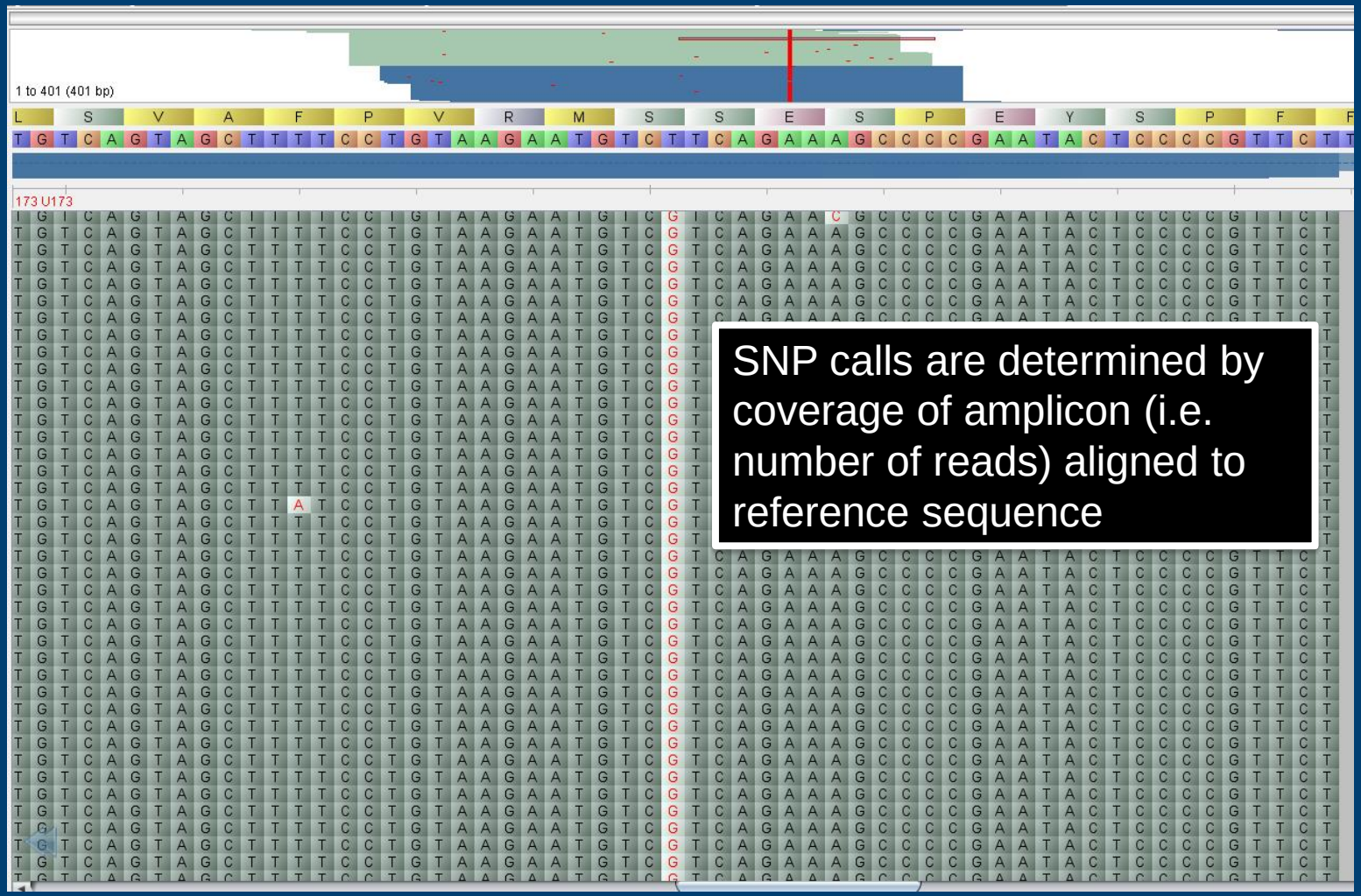
- We obtained sequence for 941 targeted SNPs with a minimum coverage of 100x in all samples
- Alignment pipeline took less than a minute
- Average coverage per locus ranged from 551x to 8925x
- Genotype concordance between replicate samples was 100%
- Some issues with sensitivity to template to be resolved

Amplicon sequences and SNPs

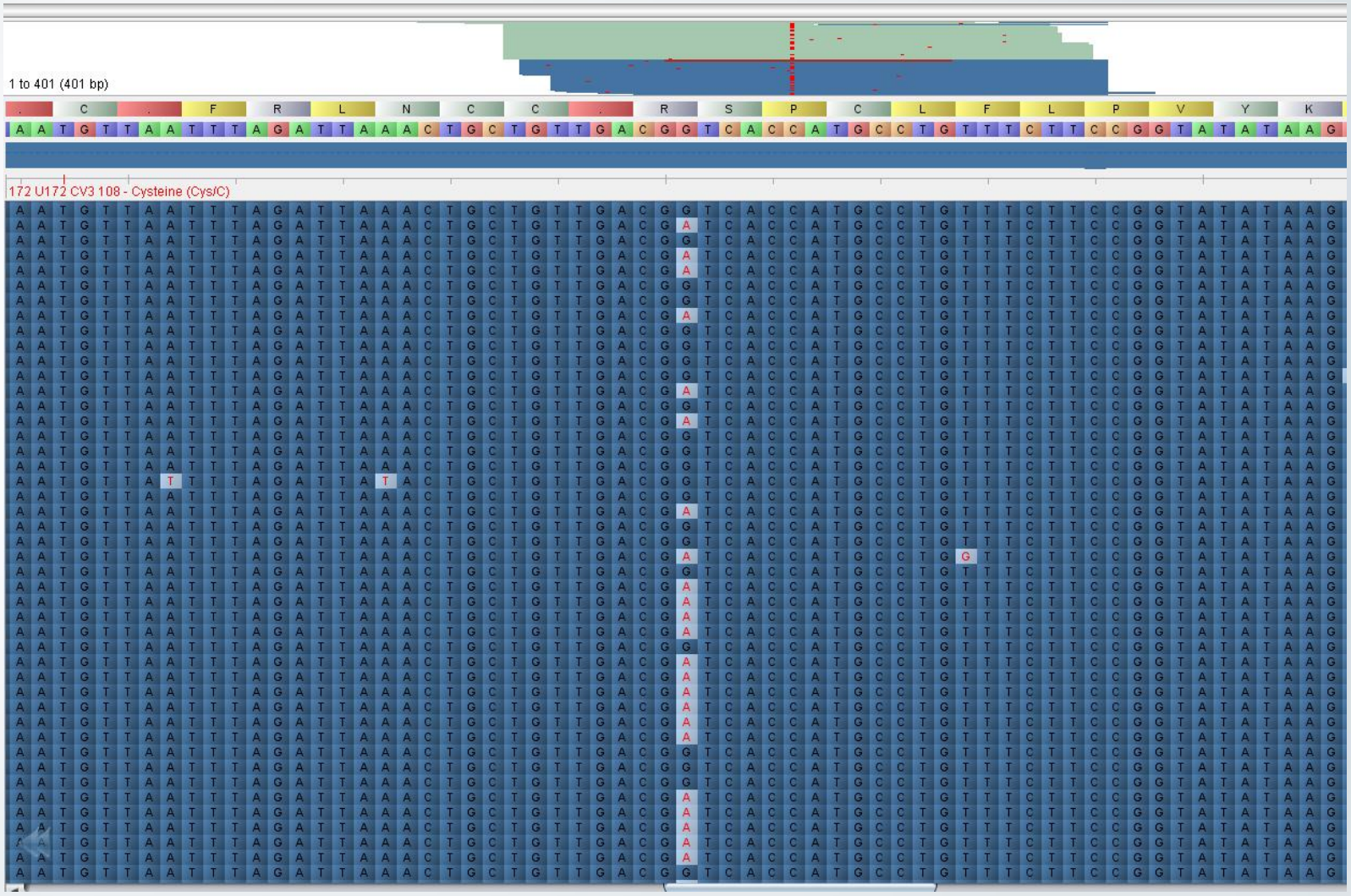
- SNP can be anywhere in the amplicon, position is known *a priori*



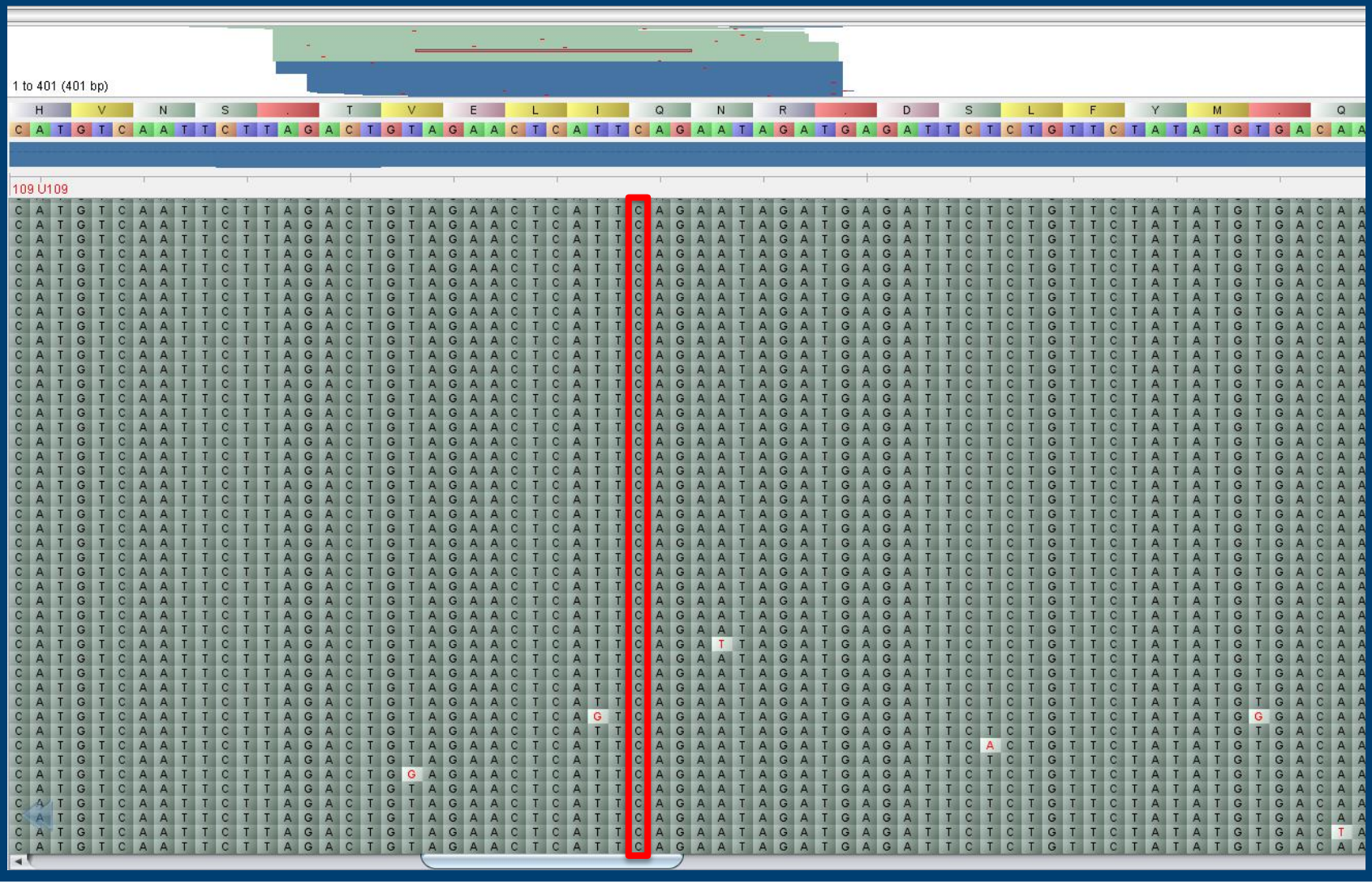
Homozygote G/G genotype



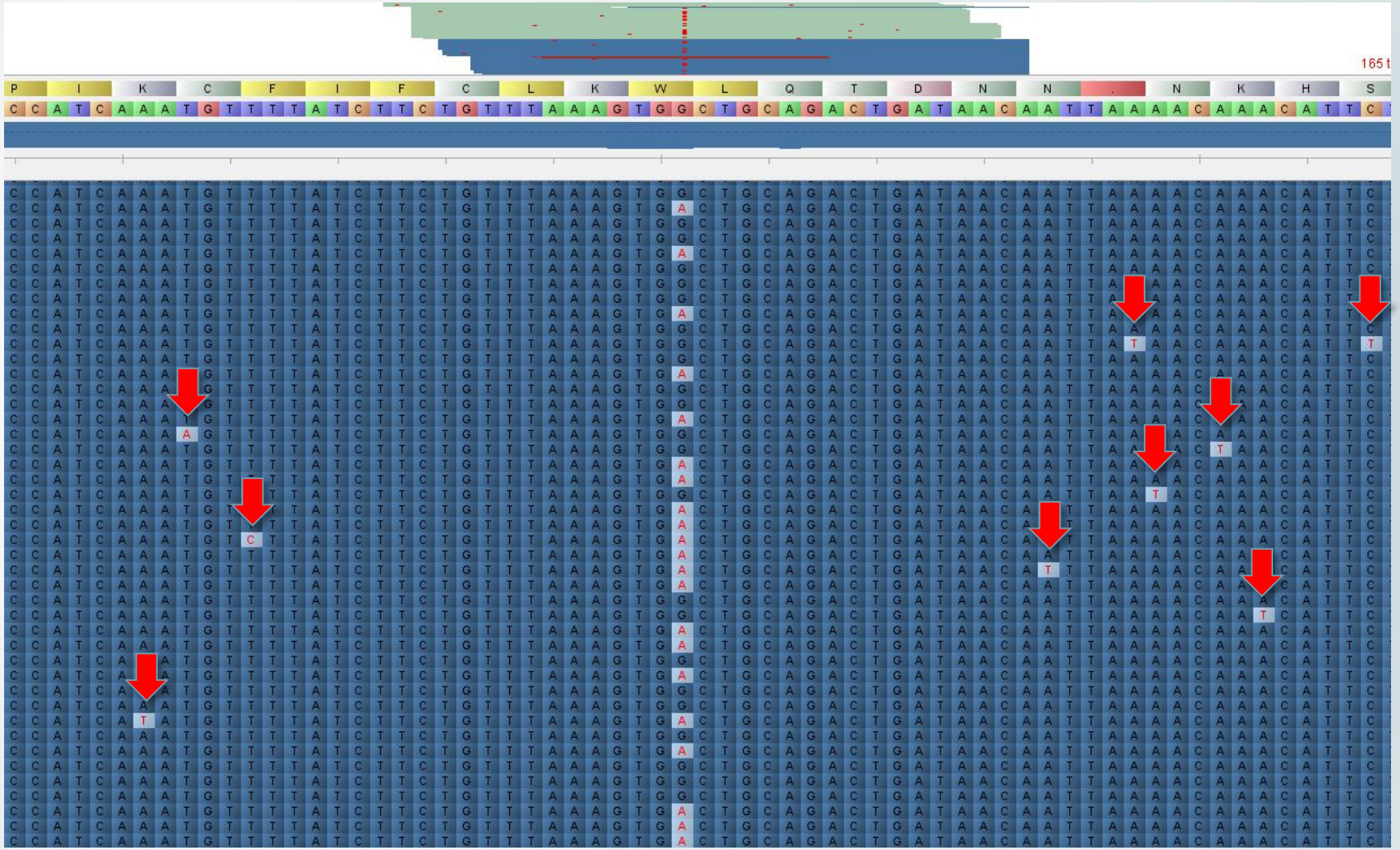
Heterozygote A/G genotype



Homozygote C/C genotype



Seq errors not a problem with sufficient coverage



Conclusions and future development

- Ion AmpliSeq was validated as an efficient GBS tool in Atlantic salmon
- 'Ideal' SNP number for within-family genomic selection, parentage assignment, targeted MAS, traceability…..
- Phase 2 trial underway
  - 2000 new genomewide distributed SNPs selected
  - Will be sequenced on the 'native' Ion Proton platform
  - Multiplex level of 384 samples per PI chip, target 100x coverage
  - Cost target **10 euros per sample** (optimise target no.+ multiplex level)
- Future PII and PIII chips promise further efficiency improvements
  - Competition from alternative systems, eg. Illumina Truseq Amplicon, Affymetrix

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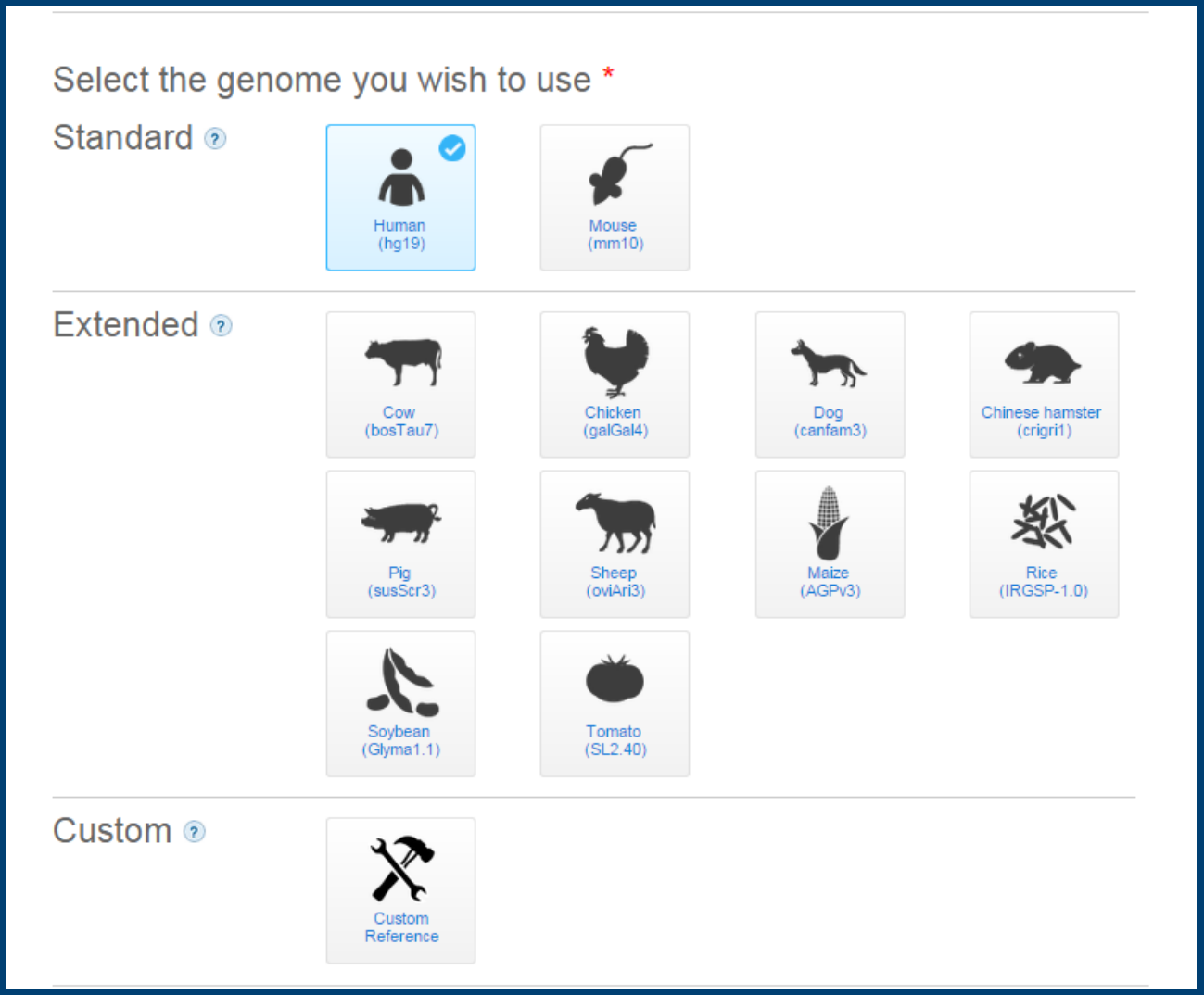
Customised panels – Aquaculture species soon?



Thanks for your attention

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