



# Gene expression profiles defining host resistance to Infectious Pancreatic Necrosis in Atlantic salmon fry



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ISGA XII 21st-26th June 2015

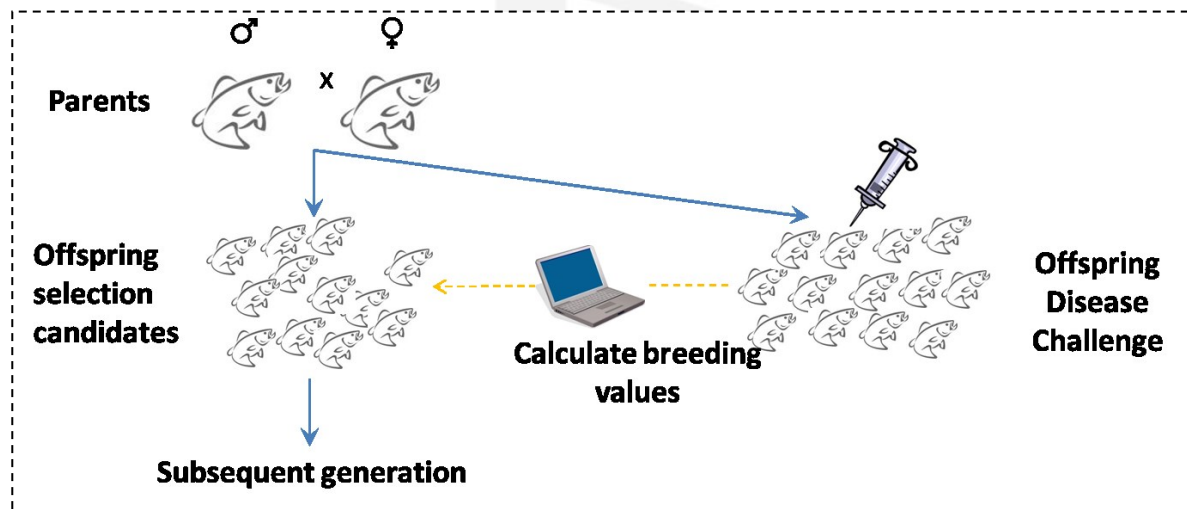
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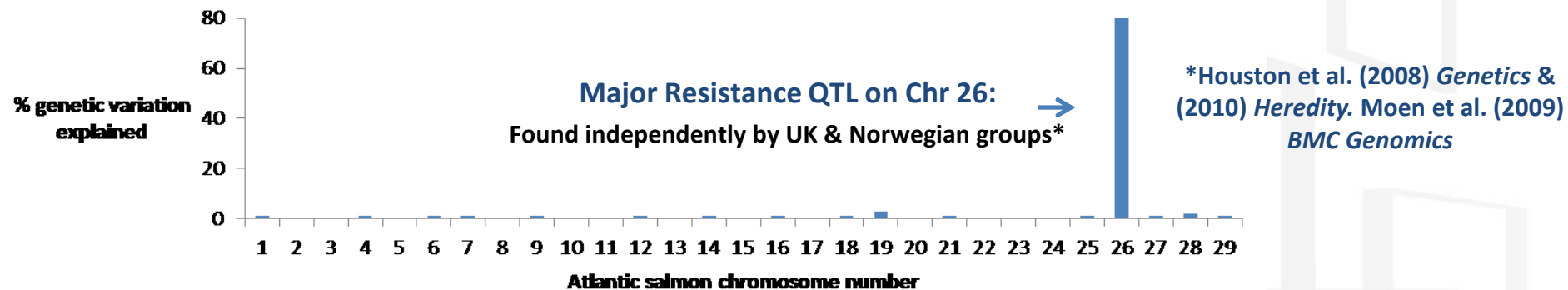
- Infectious diseases = serious problem for salmon farming
- Host resistance is universally heritable



**Selective breeding can be applied to improve disease resistance**

- Aquaculture species potentially useful models to understand genetic basis of host resistance to pathogens

- IPN – endemic birnavirus causes mortality at two windows
- Stage 1: QTL Mapping Resistance Loci - Single locus explains most genetic variation in resistance in fresh & sea water



- Marked contrast in mortality level between RR and SS homozygotes

		Sire haplotype	
Dam haplotype		R	S
R		0%	2%
S		1%	63%

- **Stage 2: Goals:**
  - SNP markers to accurately predict QTL genotype @ population level
  - Understanding of underlying biological mechanisms
- **Advances in genomic tools and technology:**
  - Reference genome sequences for A. salmon and rainbow trout
  - Microarrays and RNA Sequencing
  - Tools for high density SNP genotypes
    - i. RAD genotyping by sequencing & SNP array
    - ii. Whole genome sequencing

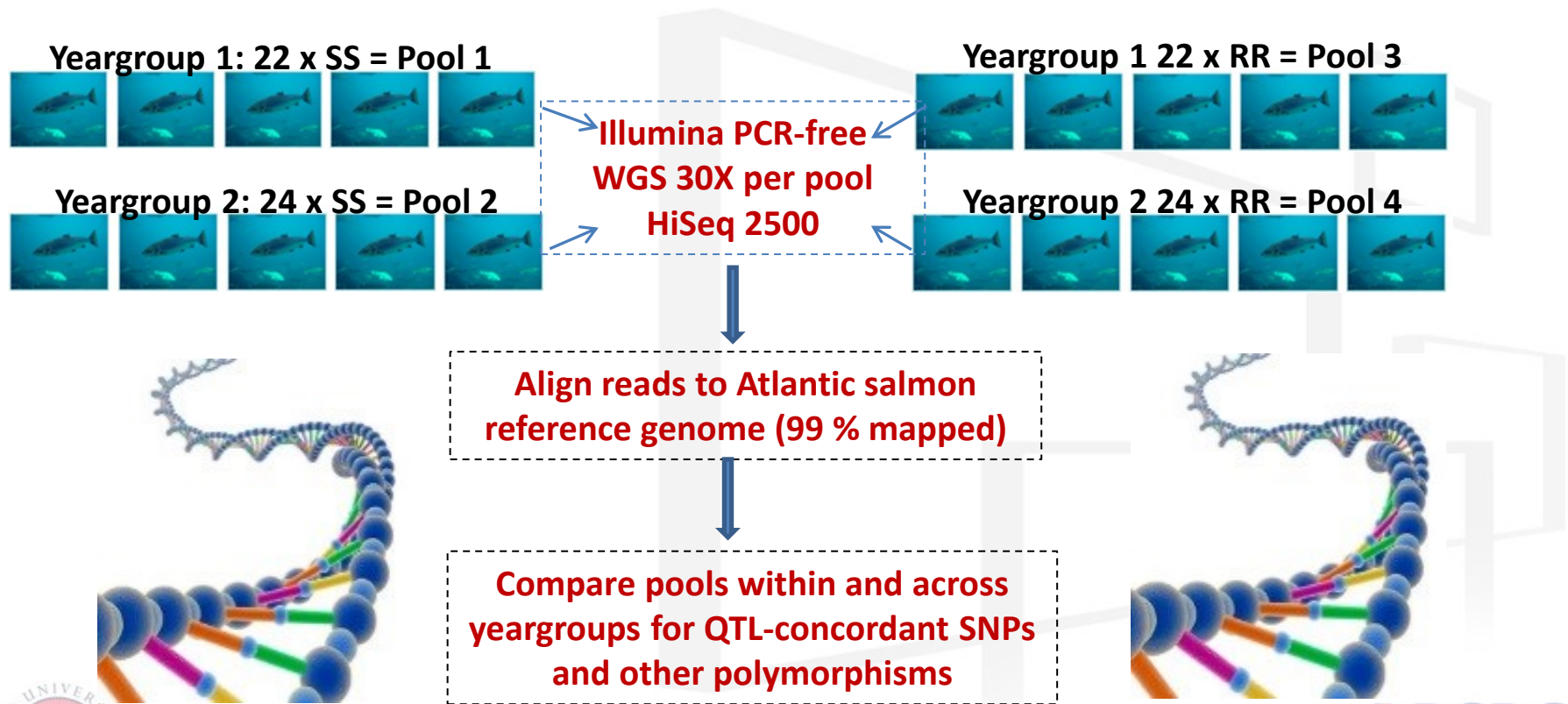
Population	Mortality Rate (SE)		
	RAD01 SNP Genotype		
	RR	RS	SS
TEST	0.10 (0.03)	0.17 (0.01)	0.60 (0.01)
VALIDATION	0.11 (0.01)	0.25 (0.01)	0.63 (0.01)

Houston et al. 2012, BMC Genomics

**→ Commercial application:  
SNP panel used as genetic test to  
select resistant broodstock**

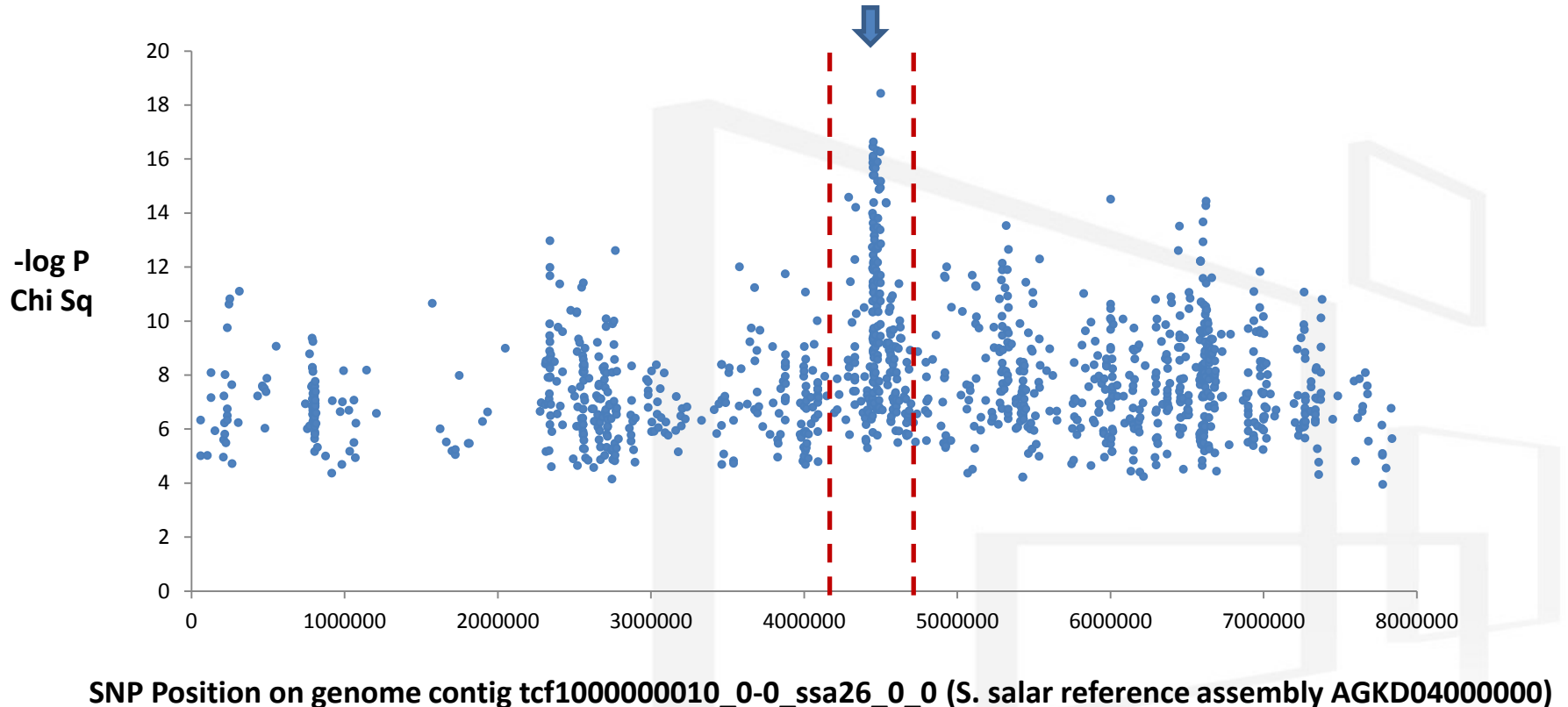
# IPN Fine Mapping

## (iii) Whole genome sequencing pools of RR and SS fish



# IPN Fine Mapping

- (i) Linkage mapping QTL Peak
- (ii) Most significant SNPs from RAD-Seq and SNP chip
- (iii) SNPs in *cdh-1-1* (Moen et al 2015, Genetics)

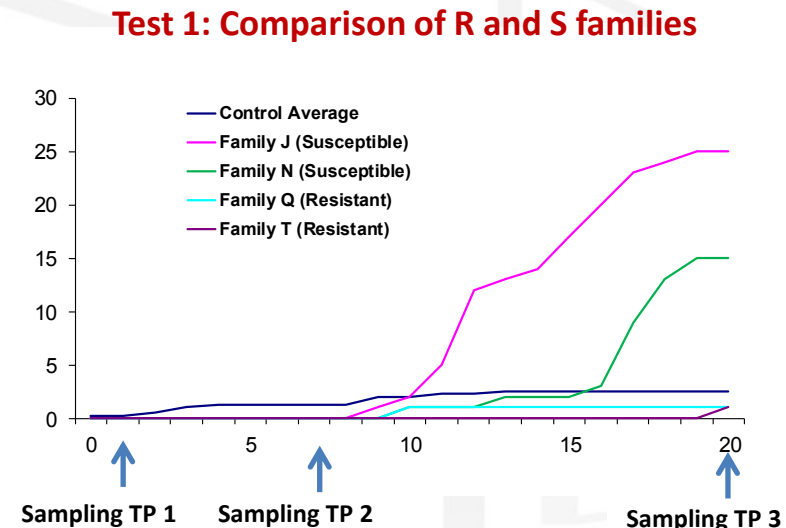
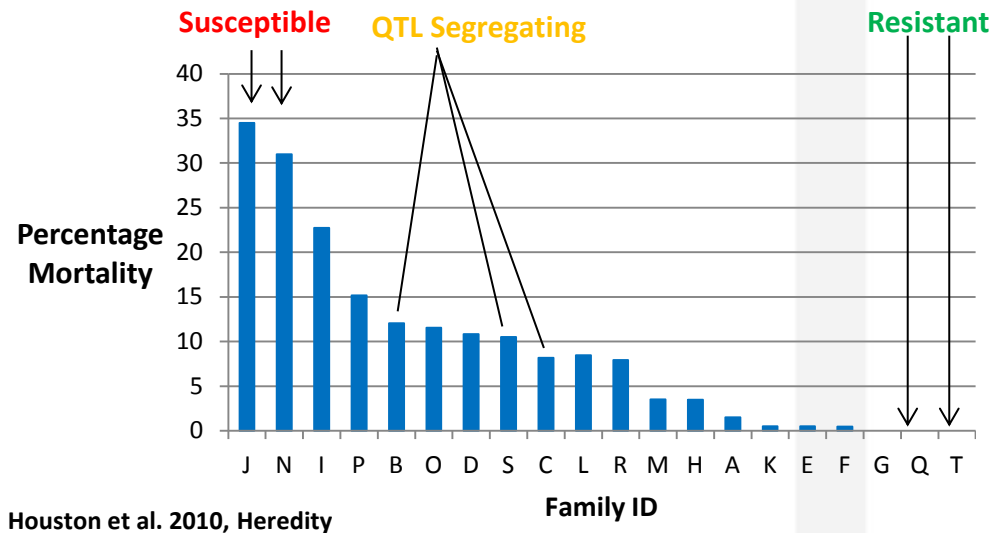


## Markers (nearly) concordant with QTL type;

- QTL SS fixed for susceptibility SNP allele
- QTL RR almost fixed for resistance SNP allele

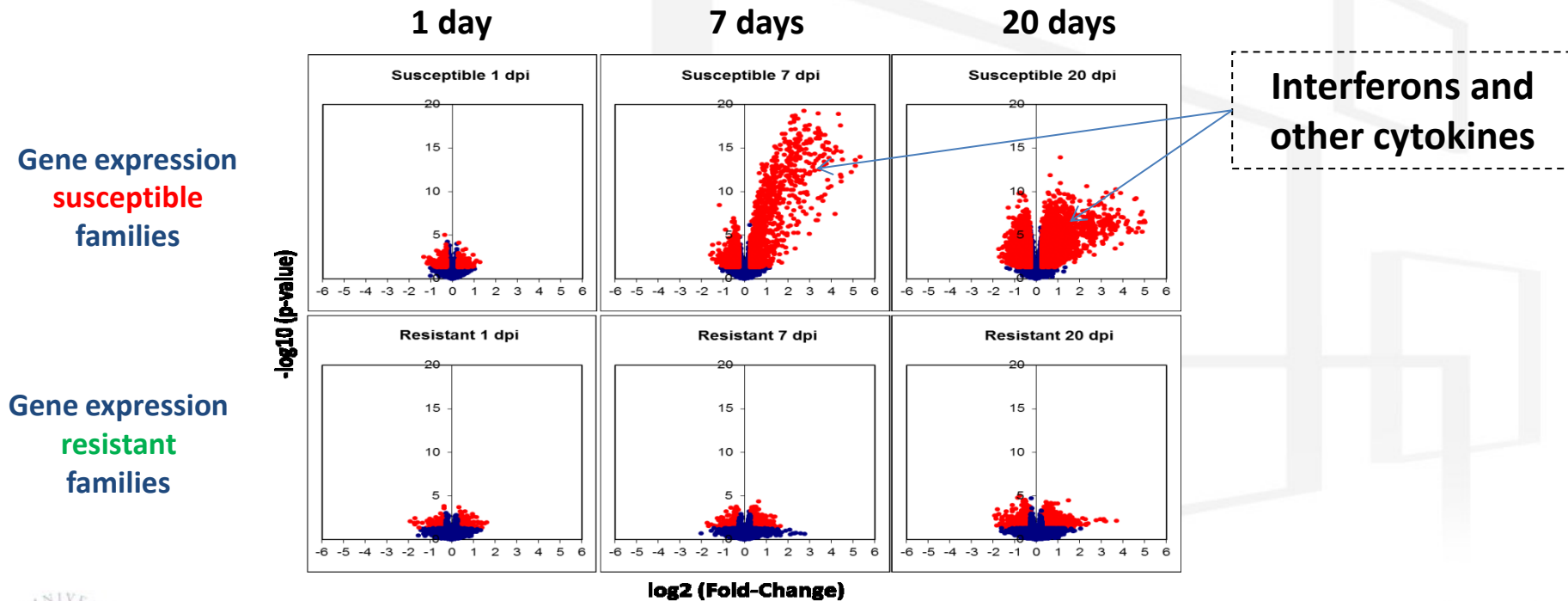


- Gene expression contrast between resistant and susceptible fry
  - Families of fry given bath challenge of IPNV



- Replicated challenge & control tanks for each TP
- Whole fry homogenate (pools of 6 biological replicates per condition); RNA extracted and hybridised to Agilent 44K array

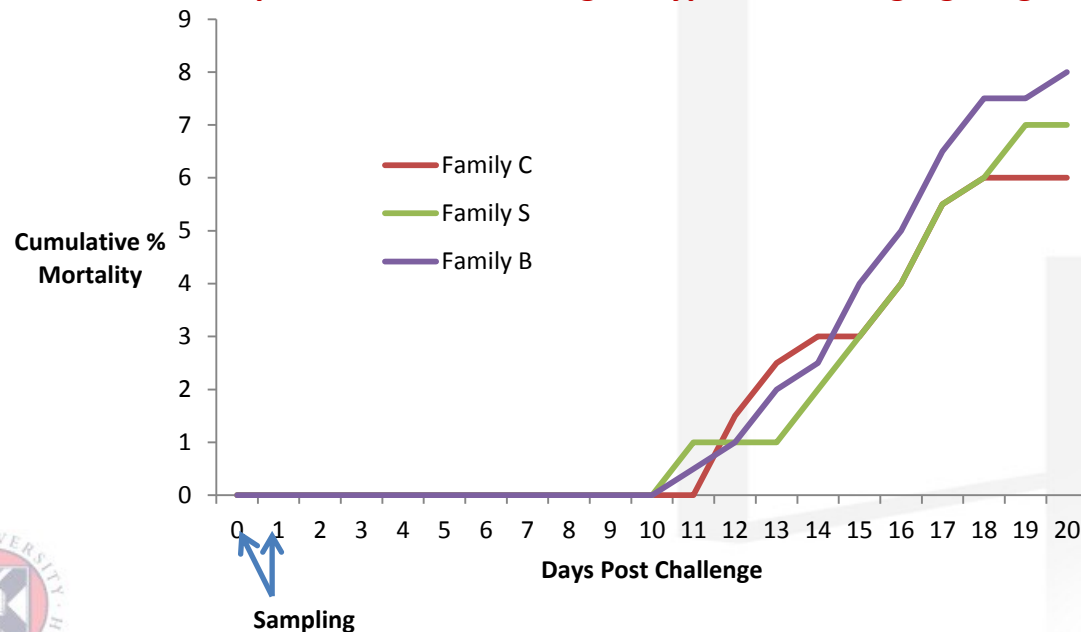
- Microarray comparison of host response to infection in resistant and susceptible families





- **RNA-Seq focus on early timepoints (prior to infection and 1 day post infection)**
  - Offspring genotyped to determine underlying QTL status (RR and SS)
  - RNA extracted from whole fry and sequenced on Illumina HiSeq 2000

## Test 2: Comparison of RR and SS genotypes within segregating families

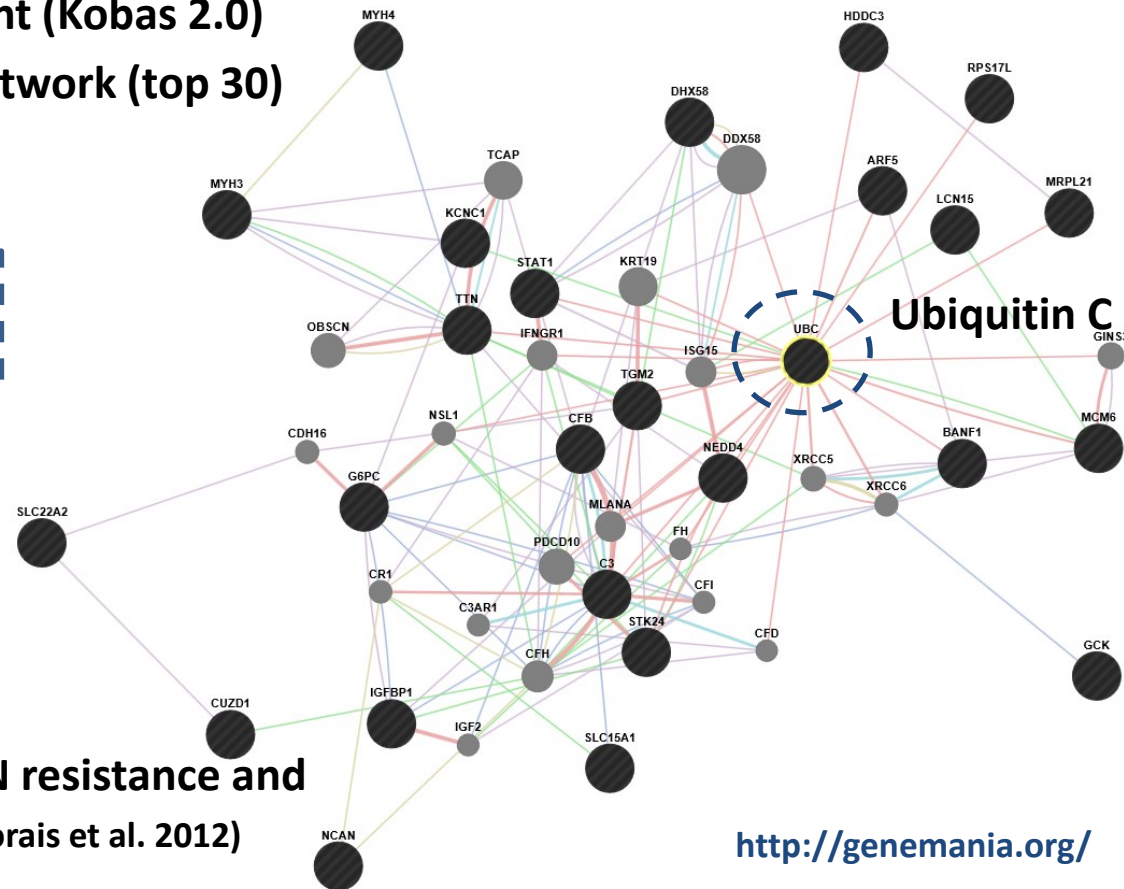


- 3 segregating families
- 2 timepoints (0 and 1 DPI)
- 2 genotypes (RR & SS)
- 6 fry per condition
- **72 fry transcriptomes**

- **Atlantic salmon fry transcriptome assembled**
  - 1.6 bn reads → aligned to ICSASG\_v1 genome assembly
  - ~113K transcripts from ~80K putative genes
  - ~63% annotated, covers 88% of publicly-available ESTs
- **Differential expression RR vs SS**
  - i. Genome-wide comparison of gene expression
  - ii. Identification of human homologues → pathway enrichment
  - iii. QTL region differential expression

- **Pre-infection (Timepoint 0) comparison of RR vs SS**
  - 628 significantly differentially expressed transcripts
  - KEGG pathway enrichment (Kobas 2.0)
  - Functional connection network (top 30)

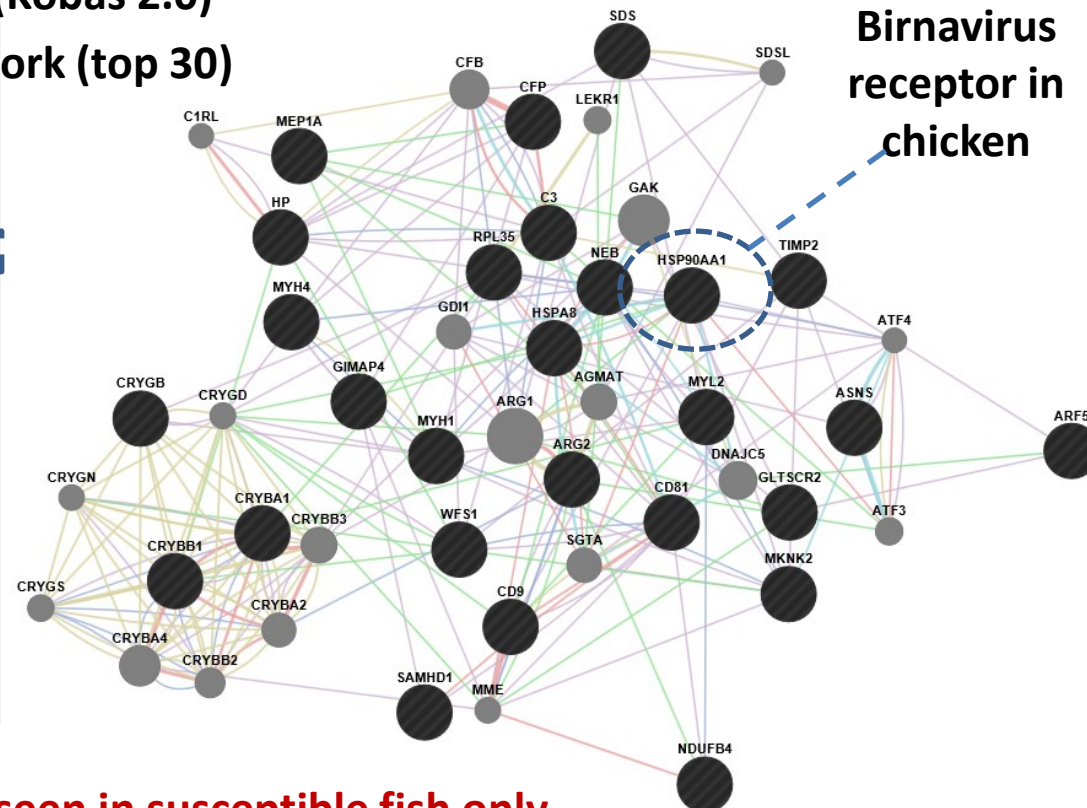
KEGG Pathway	P-Value
Protein processing in endoplasmic reticulum	0.001254
Fatty acid metabolism	0.001257
Influenza A	0.003696
Fatty acid biosynthesis	0.004733
PPAR signaling pathway	0.007884
Fructose and mannose metabolism	0.010228
Hepatitis B	0.011012
AMPK signaling pathway	0.011752
Biosynthesis of unsaturated fatty acids	0.017246



- **Possible link between IPN resistance and fatty acid metabolism (Morais et al. 2012)**

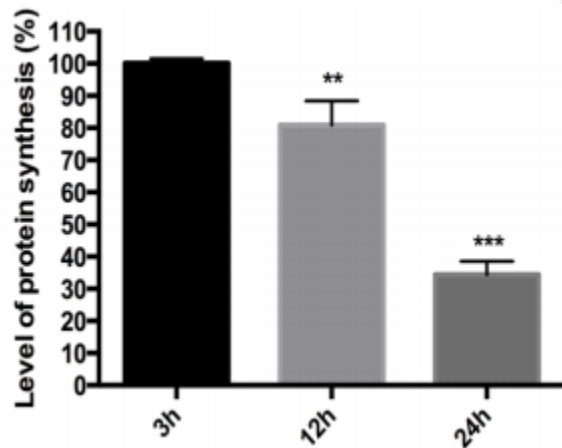
- One day post-infection comparison of RR vs SS
  - 316 significantly differentially expressed
  - KEGG pathway enrichment (Kobas 2.0)
  - Functional connection network (top 30)

KEGG Pathway	P-Value
Tight junction	1.51E-05
Ribosome	3.45E-05
Protein digestion and absorption	0.000350864
Pancreatic secretion	0.003120276
Alanine, aspartate and glutamate metabolism	0.006104826
Biosynthesis of amino acids	0.007011666
Mineral absorption	0.013506223
Complement and coagulation cascades	0.031222676
Hypertrophic cardiomyopathy (HCM)	0.048987447

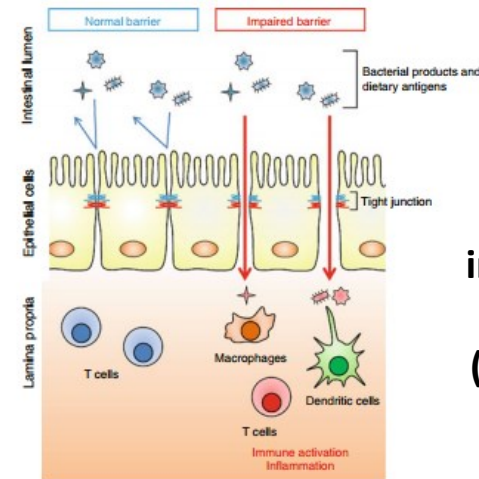


**May reflect responses to virus seen in susceptible fish only**

- **Resistant and susceptible genotypes differ in their gene expression in pathways related to:**
  - Cellular protein turnover
  - Tight junction regulation



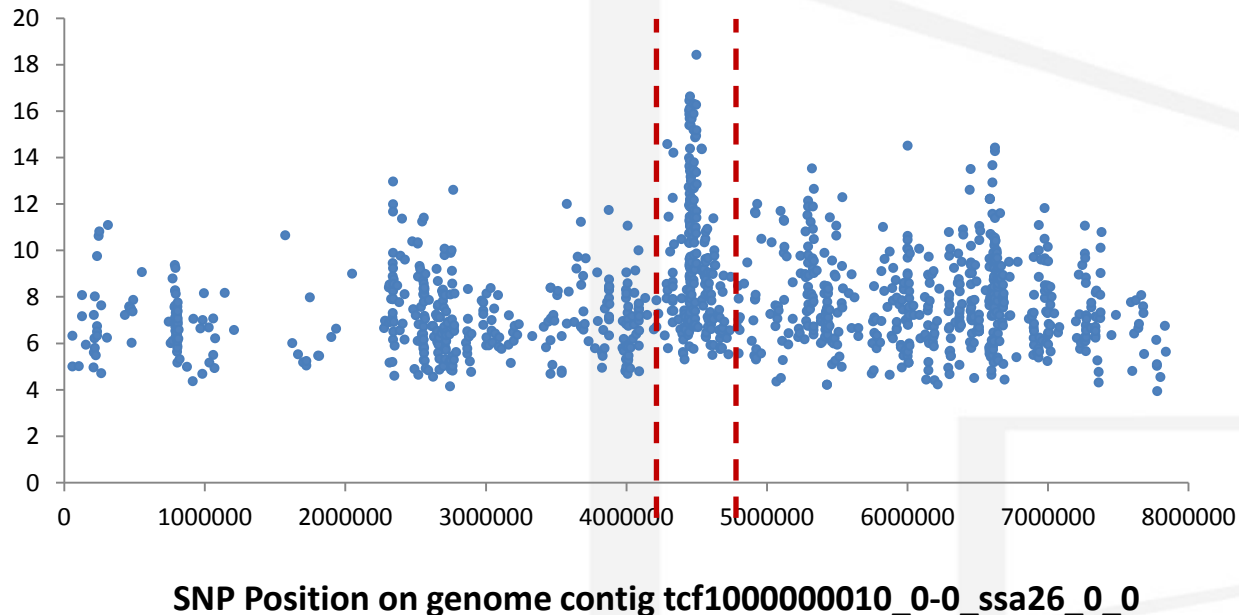
Gamil et al. 2015, Viruses



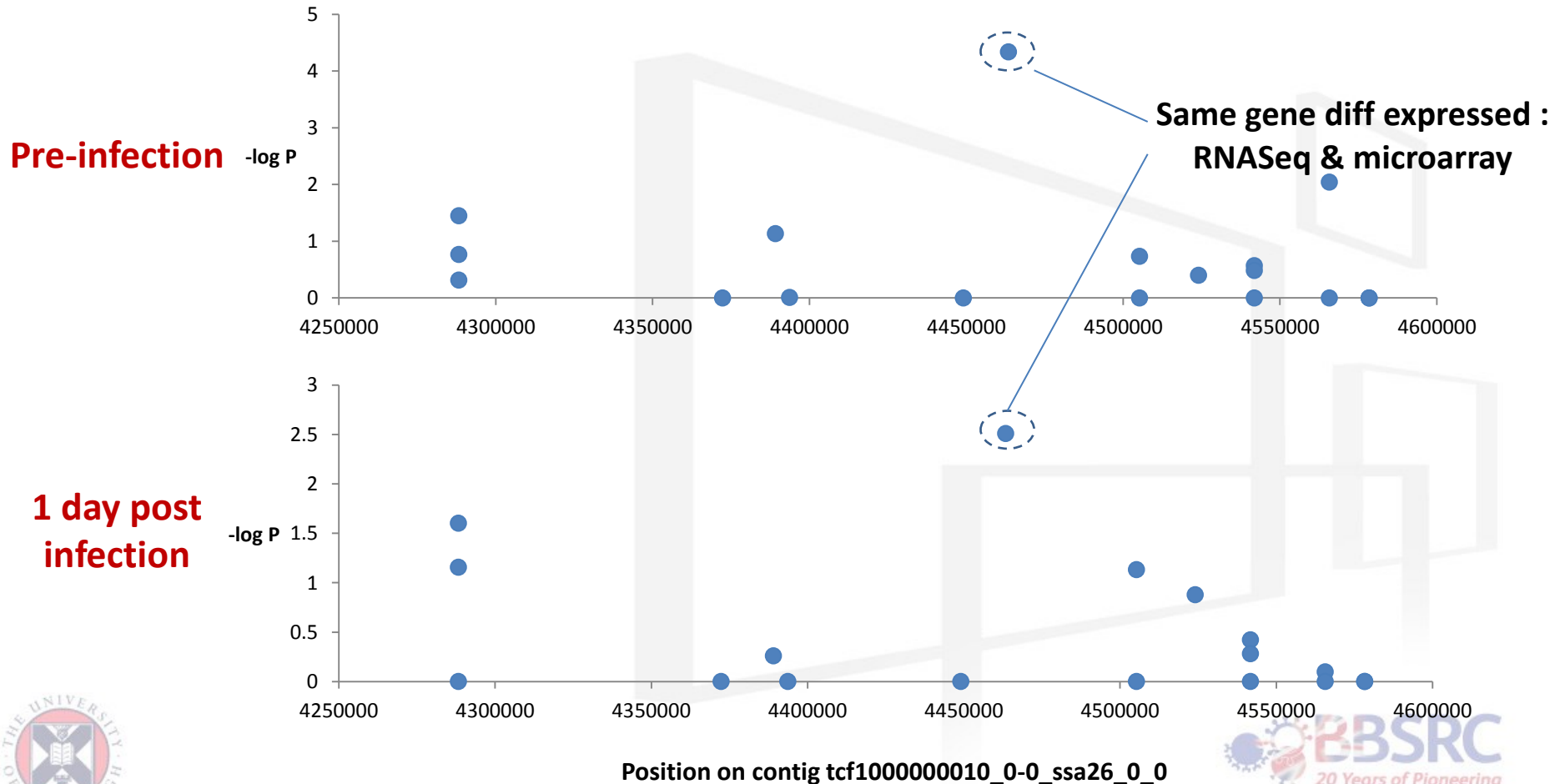
IPNV modulates intestinal epithelial barrier for entry (Sundh et al. 2011)

Suzuki 2013, Cell Mol Life Science

- **Differential expression of genes in QTL region (RR vs SS)**
  - **Positional and functional candidates**



- Differential expression of genes in QTL region (RR vs SS)





# Conclusions



- i. **30 X WGS of homozygous resistant and susceptible pools  
→ detailed polymorphism map of narrow QTL region**



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- ii. **Microarray analysis of 1,7&20 DPI suggests resistance mechanisms early in viral infection process**



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- iii. **RNASeq assembled transcriptome with 80 K transcripts → comprehensive coverage of existing EST database**



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- ii. Microarray analysis of 1,7&20 DPI suggests resistance mechanisms early in viral infection process
- iii. RNASeq assembled transcriptome with 80 K transcripts → comprehensive coverage of existing EST database
- iv. **One transcript with constitutive differential expression in QTL region → potential cis-acting effect**



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- i. 30 X WGS of homozygous resistant and susceptible pools → detailed polymorphism map of narrow QTL region
- ii. Microarray analysis of 1,7&20 DPI suggests resistance mechanisms early in viral infection process
- iii. RNASeq assembled transcriptome with 80 K transcripts → comprehensive coverage of existing EST database
- iv. One transcript with constitutive differential expression in QTL region → potential cis-acting effect
- v. **Cell protein turnover & regulation of tight junction have roles in host resistance to IPNV infection**



# Acknowledgements



**Natalie Lowe**  
**Serap Gonen**



**UNIVERSITY OF  
STIRLING**

**John Taggart**  
**Michaël Bekaert**  
**James Bron**



**Derrick Guy**  
**Alastair Hamilton**  
**Alan Tinch**  
**Jose Mota-Velasco**



**David Verner-Jeffreys**  
**Richard Paley**



**UPPSALA  
UNIVERSITET**

**Carl Rubin**  
**Leif Andersson**



**edinburgh  
genomics**

**Karim Gharbi**  
**Richard Talbot**



**Professor Steve Bishop**

