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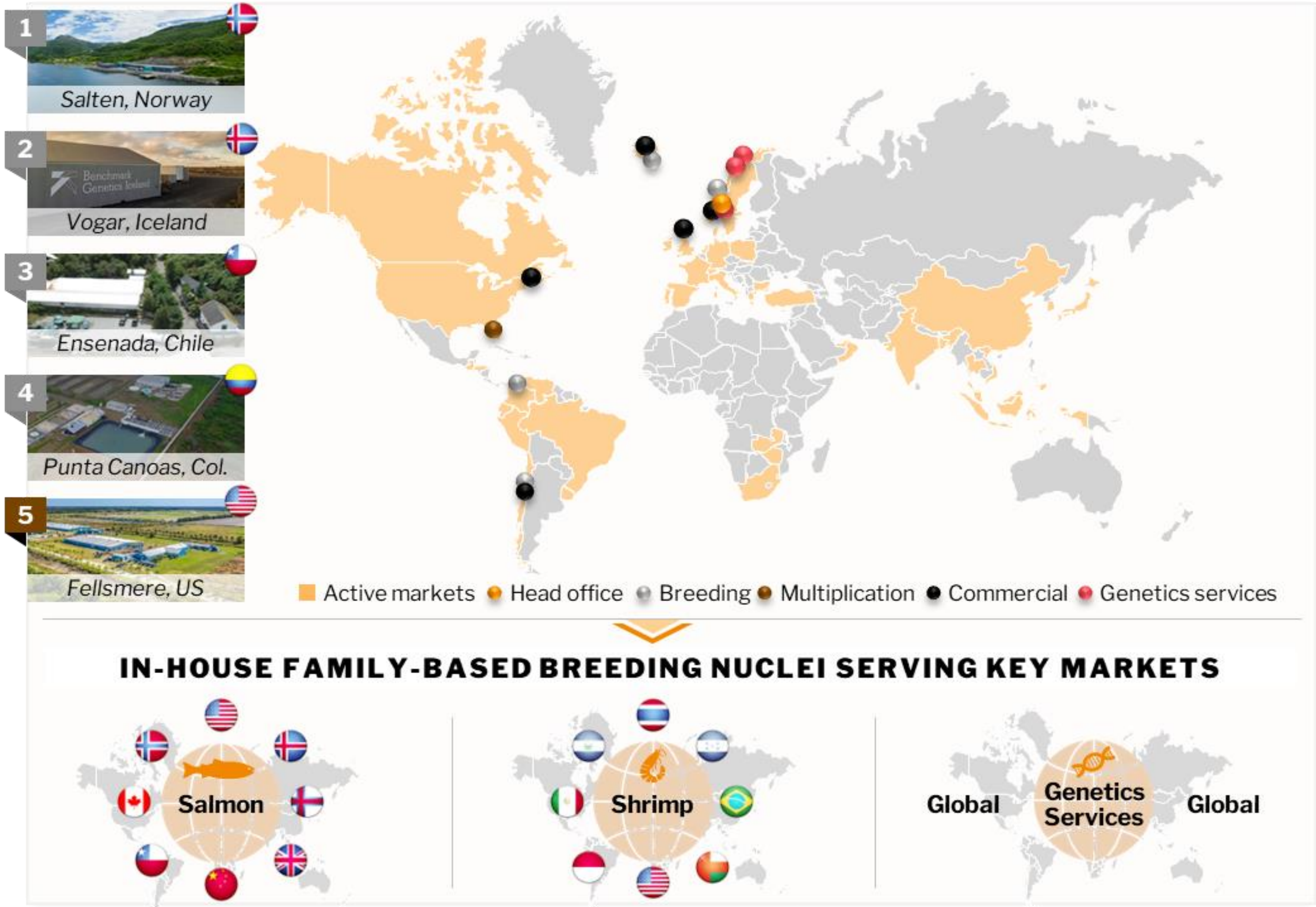
Commercial Scale Gene Editing in Aquaculture: Opportunities and Challenges

Ross Houston, Director of Genetics & Innovation

GENETICS | ADVANCED NUTRITION | HEALTH

Benchmark Genetics

- **Benchmark Genetics provides genetically-improved strains of salmon and marine shrimp for global markets, and external genetic & genomic services for several other species**



Salmon
- Norway strain
- Iceland strain
- Chile strain

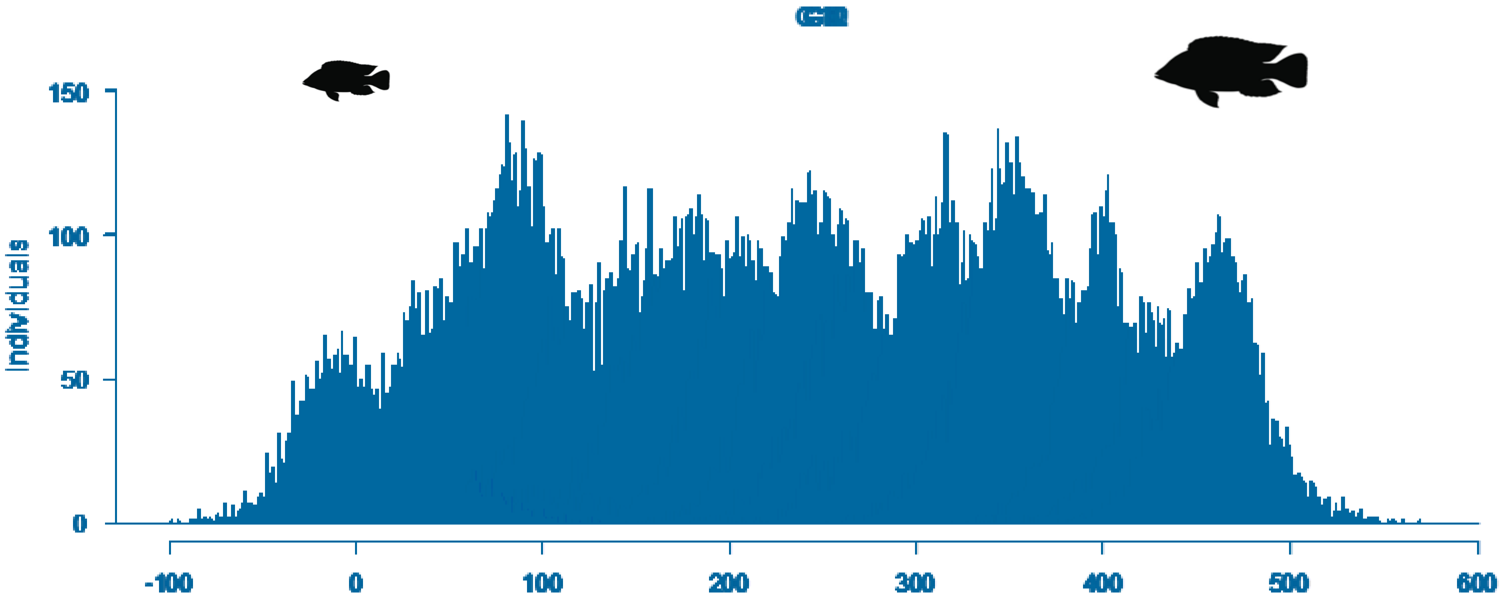
Shrimp

~30% global salmon egg supply

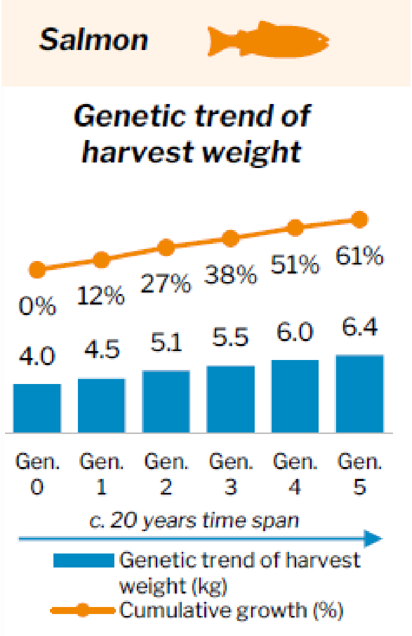
The Joys of Aquaculture Breeding

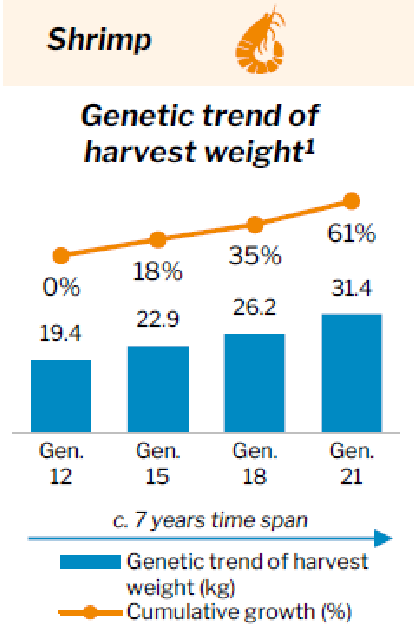
- Aquaculture species have many favourable characteristics for genetic improvement
- External fertilization, flexible mating designs
- High fecundity
- Early in domestication = large amount of genetic variation

- Rapid, cumulative, sustainable genetic gains achieved with well-managed selective breeding programs
- Benefits of selective breeding observed throughout the production and supply chain



Growth EBVs, Nile tilapia population





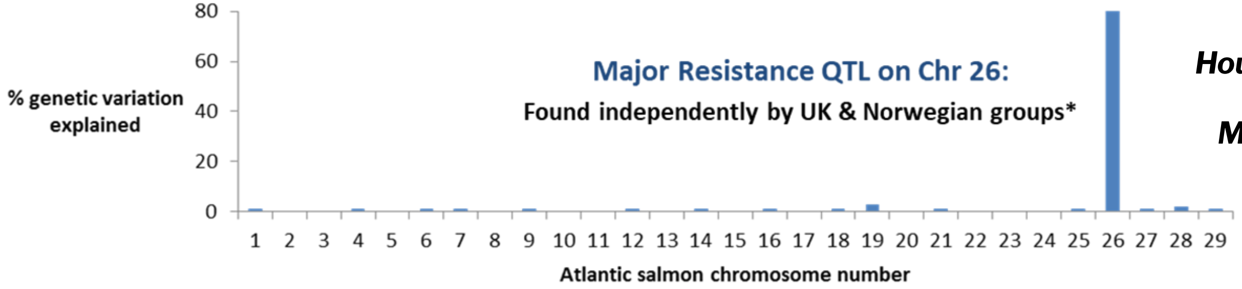
Genetics as the ultimate disease prevention tool

Infectious disease presents a major threat to all aquaculture systems

- Vaccination, biosecurity, treatment measures not feasible in many cases
- <1% fish vaccinated globally, generally not possible in invertebrates
- A key difference to terrestrial livestock production

➢ <u>Genetic innovations have particularly high potential to tackle disease in aquaculture</u>

The case of Infectious Pancreatic Necrosis (IPN) in salmon



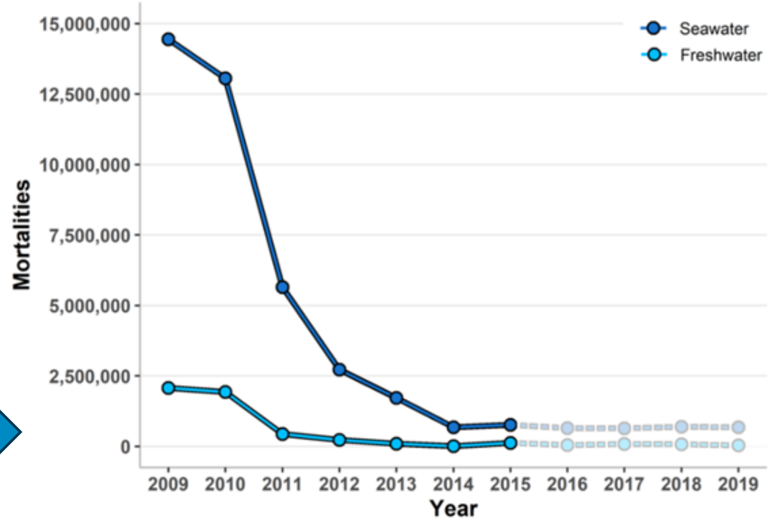
Houston et al. 2008

Moen et al. 2009

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

Marked contrast in mortality levels between QTL genotypes

Global application of MAS

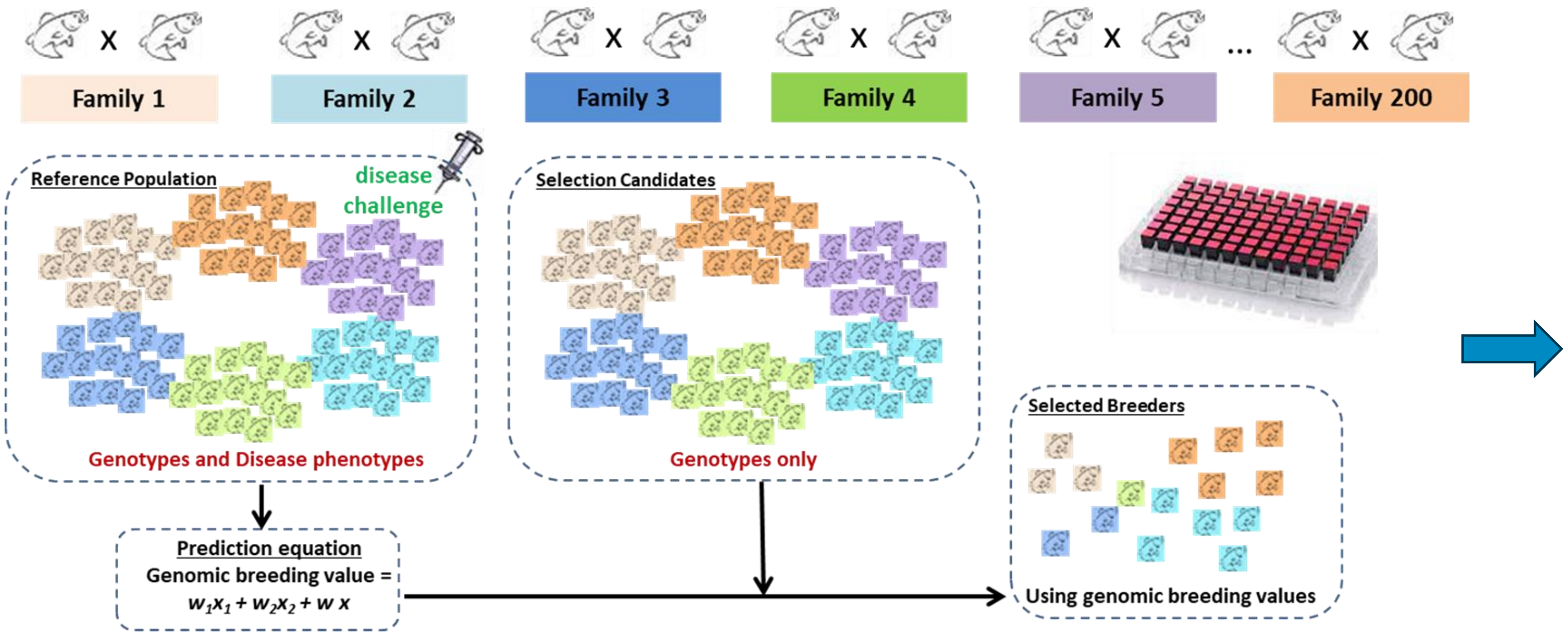


Reduction in mortality due to IPN on Mowi farm sites in Norway

Genomic selection is a key technology for improving disease resistance

Genomic selection for resistance to Cardiomyopathy Syndrome (CMS) in salmon

- **Annual disease challenge testing to measure resistance on siblings of selection candidates**
- **Validation of genetic selection using field testing in presence of disease pressures**

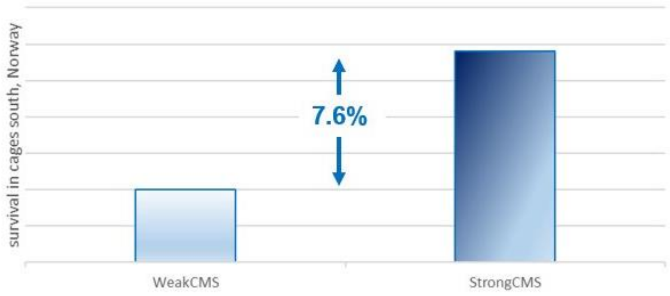


Adapted from Hayes and Goddard, 2009

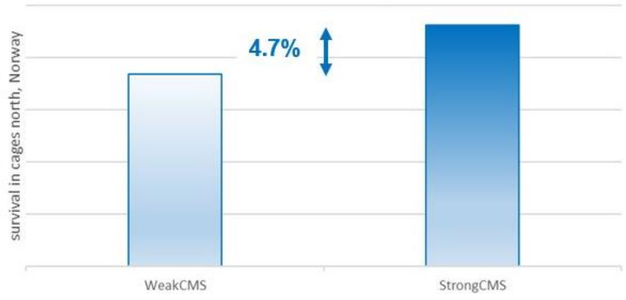
- **Genetic and genomic innovations continuously delivering improvements in production, health and welfare to the salmon industry – so what are the next frontiers?**

Documentation field test/survival:

Higher survival rate south Norway



North Norway

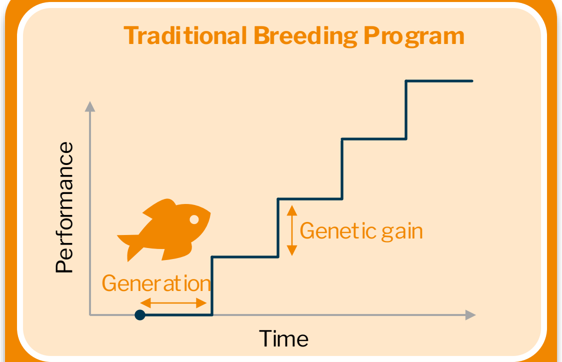


The next innovation frontiers in aquaculture breeding

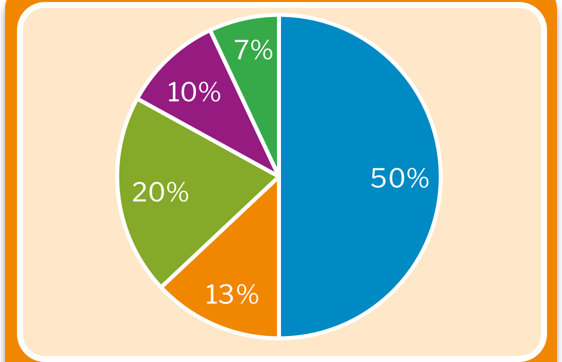
Achieving sterility in production fish has several direct and indirect benefits

Prevents interbreeding with wild fish in escapes (also important for future gene editing applications)

Prevents maturation of production fish



Allows faster generation interval by not having to select for late maturation

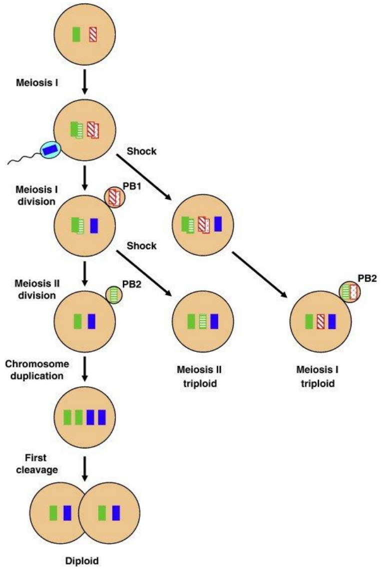


Removes a trait from the breeding goal, allowing more improvement for other key traits

How can mass-scale sterility be achieved in Atlantic salmon

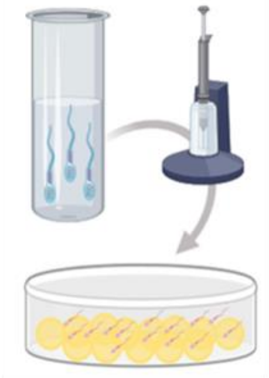
There are three main categories of methods for achieving sterile production fish

Triploidy

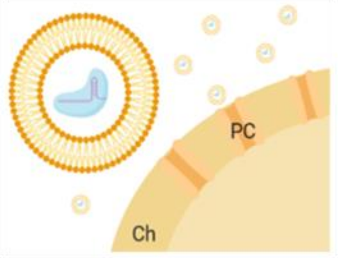


- Available commercially now
- Standard methods for application & validation
- Applied directly to production eggs
- Triploids have specific production requirements
- Less robustness to some environments

Germ-cell inhibition (non-GM)



Sperm-mediated transfer by incubation/electroporation



Egg-mediated transfer by lipofection

- Specific inhibition of germ cell development leading to sterility using antisense molecules
- Applied directly to production eggs
- Normal development & production conditions
- Methods in development
- Regulatory environment uncertain

Germ-cell inhibition (gene editing)

- Specific inhibition of germ cell development leading to sterility using CRISPR knockout
- Normal development & production conditions
- Methods in development
- Major regulatory barriers
- Heritable changes to germline

Sterility is a challenging trait for aquaculture breeding programs

Our progress in genetic selection depends on the breeder's equation

(Selection intesity) (Accuracy) (Genetic variation)

$$\Delta G = \frac{i \times r_{TI} \times \sigma_A}{L}$$

(Genetic gain) (Generation interval)

Reduced generation interval has major potential to improve gain in breeding nuclei for species with long generation interval (e.g.) salmon

Age at maturation – Conflicting targets!

<u>Sterility achieved through molecular methods</u>

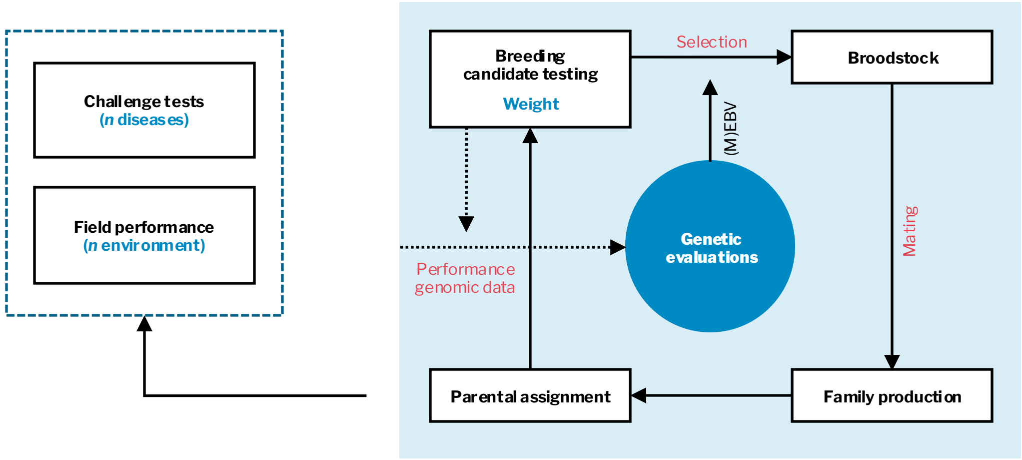
Breeding Nucleus

Rapid/ early maturation to potentially reduce generation interval

Commercial Production

Delayed / late maturation to avoid precocious maturation in production, and prevent risk of interbreeding with wild populations

Freedom to focus on *<u>reducing generation interval</u>*, with major impact on genetic gain in nucleus

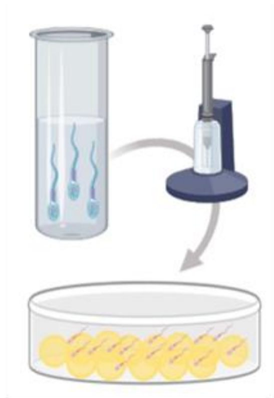


Gene editing in aquaculture species

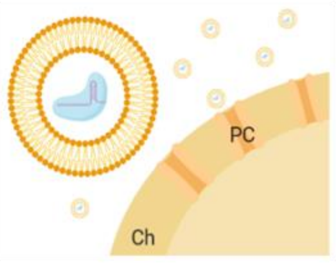
Gene editing allows targeted and precise changes to the germline

- Key difference to ‘traditional’ GMO is it does not involved transgenesis
- Targeted changes typically could have occurred naturally, or have occurred naturally

Microinjection by handheld injection system



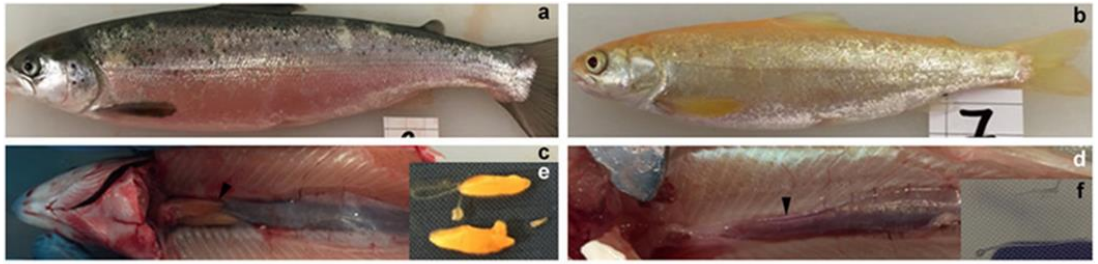
Sperm-mediated transfer by incubation/electroporation



Egg-mediated transfer by lipofection

Mass-scale editing in development, but mosaicism remains a key issue

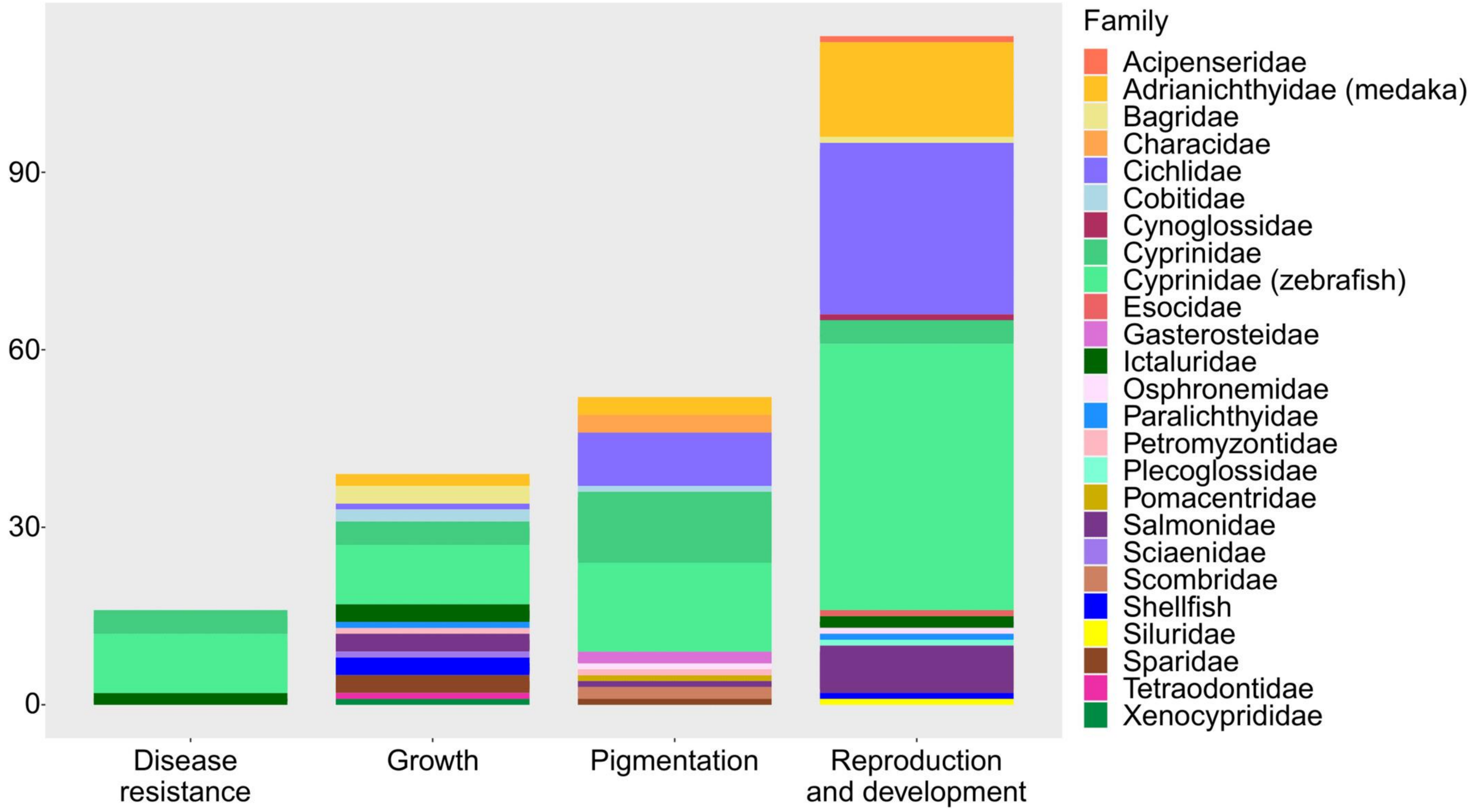
➢ **Both knockout through non-homologous end joining (NHEJ) and precise replacement through homology-directed repair (HDR) have been performed successfully in Atlantic salmon**



Knockout of *dnd* gene to induce germ cell ablation and sterility in salmon. Concurrent knockout of *slc45a2*, albinos as a tracer (*Wargelius et al. 2016*)

Gene editing across diverse aquaculture species

- **Gene editing has been successfully performed for many species and traits in R&D projects**

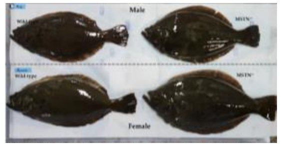


Moran *et al.* (2024)

But few commercially-available gene edited aquaculture lines

Tiger pufferfish (*Takifugu rubripes*). Leptin Receptor KO for higher growth. Japan

Red seabream (*Pagrus Major*). Myostatin KO for higher yield. Japan.



Olive flounder (*Paralichthys olivaceus*). Leptin Receptor KO for higher growth. Japan.

Nile tilapia (*Oreochromis niloticus*). Myostatin KO for higher yield. Argentina and Brazil

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Gene editing is a game-changer for future performance

Genetic and breeding technologies form a key part of disease prevention

- Genetic selection provides solution for certain diseases already (IPN, CMS)

Gene editing has transformative potential to deliver complete disease resistance

- Gene editing involves specific targeted changes to the germline, which could have occurred naturally
- Early successes (e.g. PRRSV in pigs) give prescedent, and salmon equivalents will come soon
- Disease resistance brings concurrent animal welfare, environmental, and economic benefits

Benchmark Genetics' strategy focuses on 3 parallel pillars to achieve future gene edited products in aquaculture

Gene editing targets

Benchmark focus on gene editing targets for resistance to Infectious Salmon Anaemia Virus, Pancreas Disease, and Sea Lice from ongoing R&D programs

Scalable delivery methods

Our Reproductive Technology team focus on tools and methods to develop accurately edited commercial product lines within a commercial breeding program

Regulatory Approval

Benchmark actively engage in dialogue with stakeholders to promote appropriate regulatory change in key markets

Germ cell and surrogate broodstock technologies

Dr Diego Crespo, Benchmark Genetics

- Cells4Traits project: isolation, culture, editing, and transplantation of salmon germ cells

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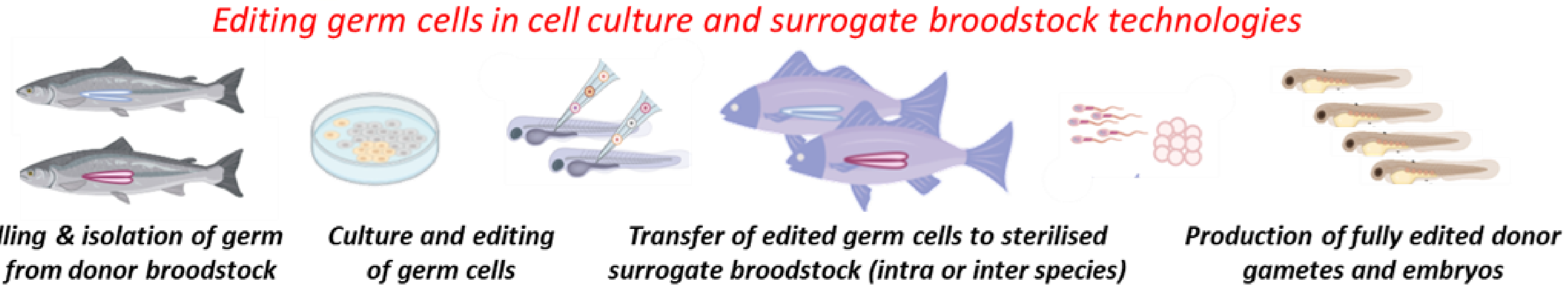
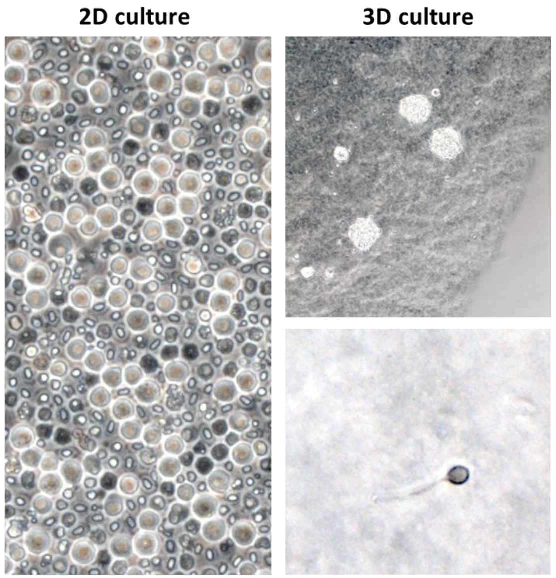


- Successful production of donor-derived gametes from surrogate broodstock, including (i) gene edited intra-species transfer, and (ii) inter-species transfer of donor germ cells
- Focus on optimizing germ cell culture methods and conditions
  - ➢ 2D cell cultures suited for germ cell propagation
  - ➢ 3D cell cultures suited for germ cell differentiation

➢ Method for generating gene-edited gametes *en masse* (including sterility)

➢ Amplification and dissemination of elite germplasm

➢ Alternative method for monosex production without hormones



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Gene Editing Targets: the Infectious Salmon Anaemia Virus example

- **Targets for gene editing have been developed through extensive collaborative R&D programs**
  - **Genome-wide CRISPR screens and comparative biology to identify host cell targets:**

BBSRC bioscience for the future

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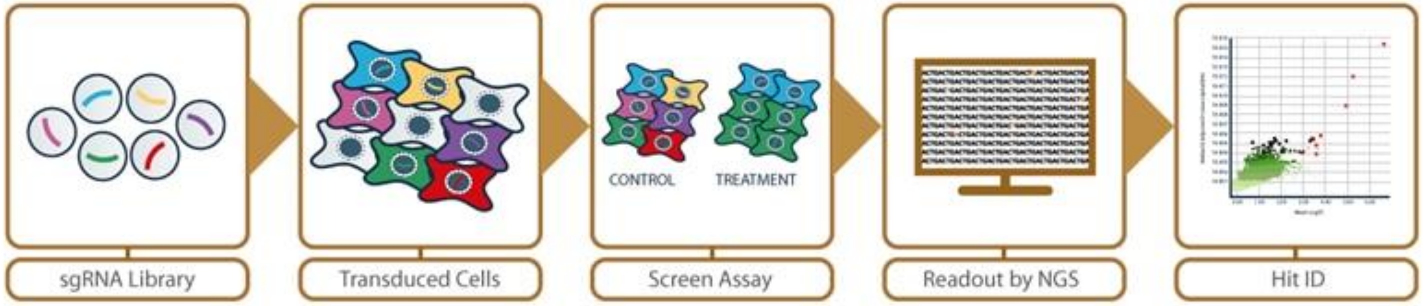
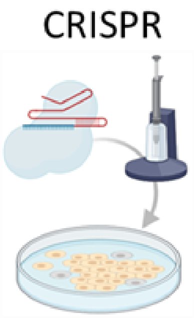
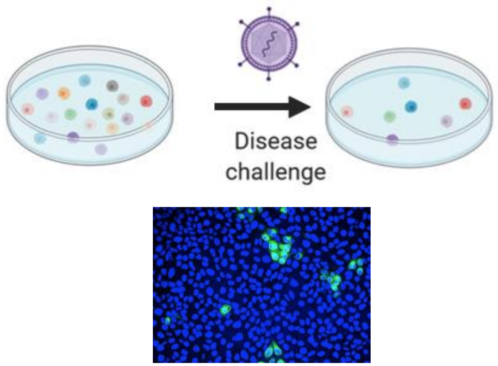
Benchmark® Genetics



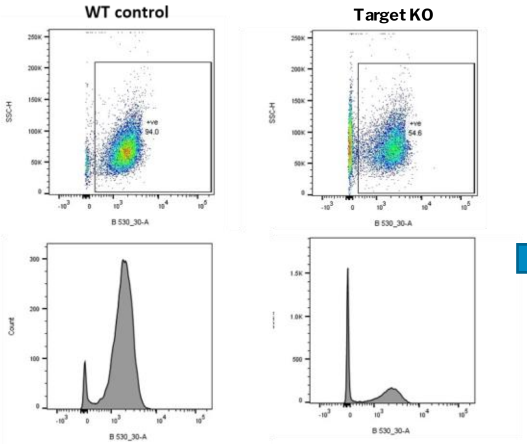
Essential host genes for human influenza replication identified using genome-wide CRISPR screen for evaluation for ISAV resistance



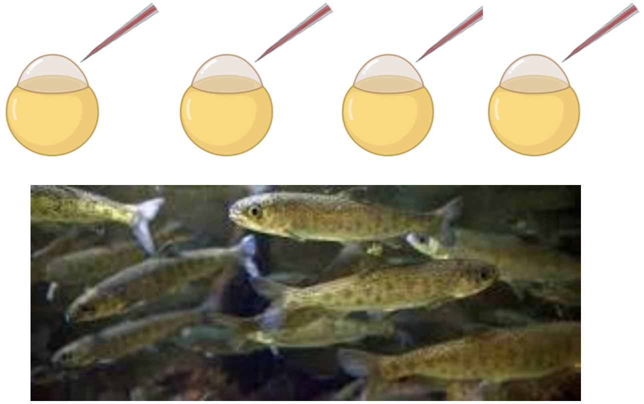
CRISPR knockout of host target genes in salmon cell culture



Assessing impact on viral infection using immunofluorescence



Knockout of candidate gene causes major reduction in ISAV infection



Gene edited Benchmark salmon for evaluation of ISAV resistance and other traits

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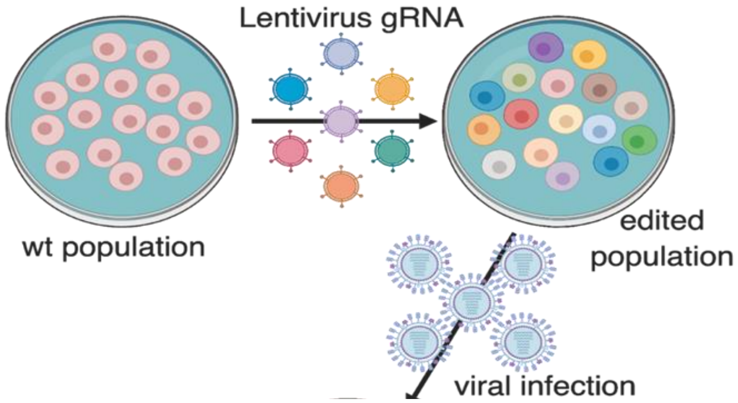
Gene Editing Targets: CRISPR screen in an aquaculture cell line

- Pooled CRISPR screens in cell culture offer a promising avenue to identify disease resistance targets
  - Proof of principle applied to Infectious Pancreatic Necrosis (IPNV) in Atlantic salmon



› Genomics. 2021 Nov;113(6):3842-3850. doi: 10.1016/j.ygeno.2021.09.012. Epub 2021 Sep 20.

The nedd-8 activating enzyme gene underlies genetic resistance to infectious pancreatic necrosis virus in Atlantic salmon

Jon Pavelin [1], Ye Hwa Jin [1], Remi L Gratacap [1], John B Taggart [2], Alastair Hamilton [3], David W Verner-Jeffreys [4], Richard K Paley [4], Carl-Johan Rubin [5], Stephen C Bishop [1], James E Bron [2], Diego Robledo [1], Ross D Houston [6]

Pooled CRISPR screen in salmon cells for IPNV Resistance

- 90K guide library developed for chinook salmon genome
- Transduced into CHSE214-EC: cell line with integrated Cas9
- IPN challenged versus mock-challenged control
- Survival of cells used as screening method
- Sequencing of surviving cells versus control used to assess enrichment of guides highlighting resistance-associated genes

sgrna	Gene	Control reads	Treatment reads	LFC	FDR	Impact
26388	nae1	916.21/975/887.07	1568.5/1779.6/1646.9	0.84527	4.24E-31	Resistance

- Nae1 near top of list provides two-way validation; (i) method is effective, (ii) unbiased evidence for QTL causative gene
- Multiple other genes also light up → value of approach to identify resistance candidates without any QTL variation

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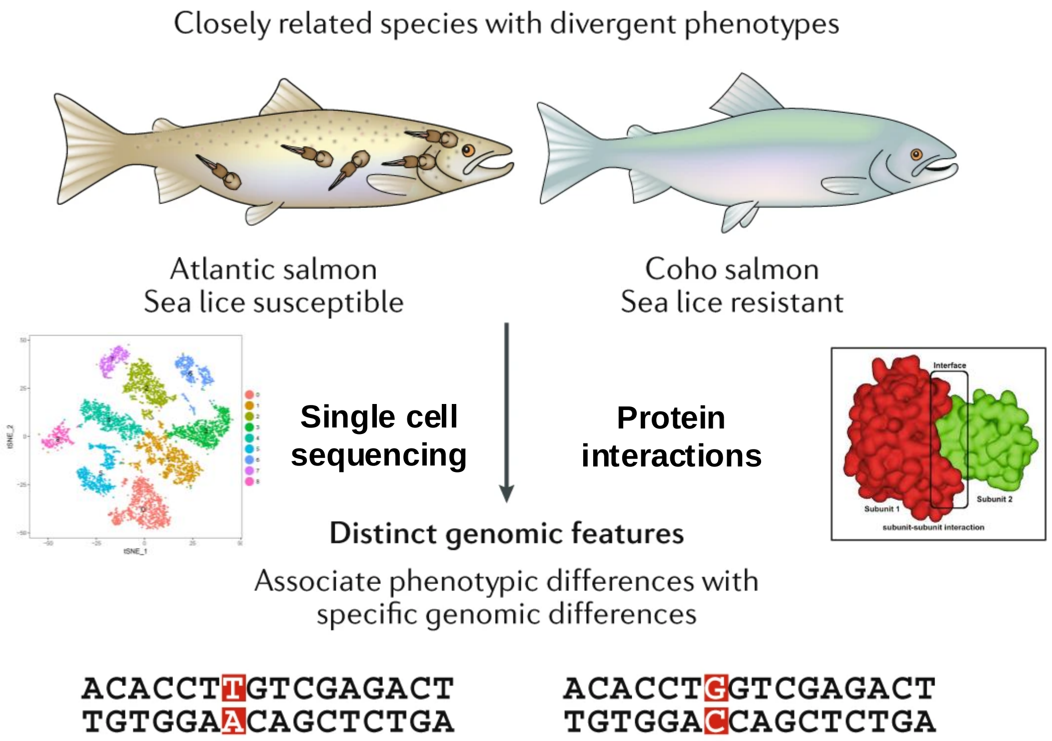
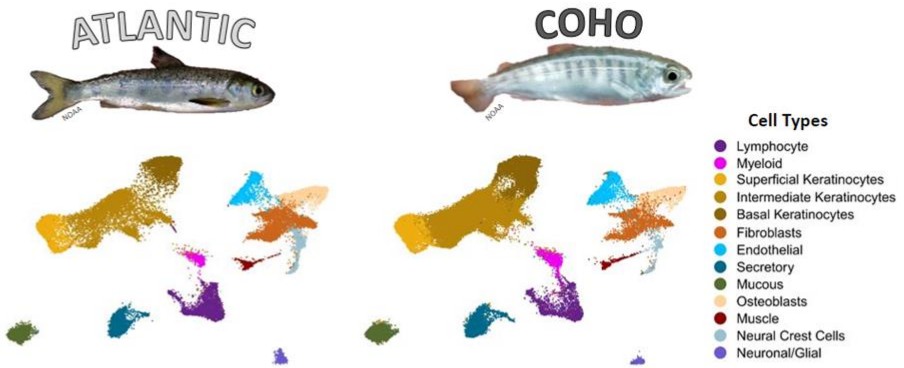
Gene Editing Targets: Resistance to sea lice

- Targets for gene editing have been developed through extensive collaborative R&D programs
  - **Detailed comparison of lice-resistant coho salmon to lice-susceptible Atlantic salmon**
  - **Host response to infestation during early days post-infestation using single cell sequencing**



CrispResist Project
Led by Nick Robinson, Nofima



Gene editing targets have been identified via detailed comparison of host response in specific cell types post-lice-attachment. Gene edited lines developed and phenotyping using lice challenge experiments underway.

Gene Editing: Implementation

- **Gene editing has transformative potential to tackle major production, health and welfare, and environmental challenges in salmon farming**
- **But what risks need to be considered?**

Risks of the technology itself	Risks of unwanted effects of edit
Health and welfare of animals undergoing editing process and their offspring – already well tested	Impact of target edit on other traits – testing prior to implementation, e.g. →
Off-target effects due to unwanted editing at other genomic locations – testable and quite simple to exclude	Escapees interbreeding with wild salmon – gene editing or non-GE methods can achieve sterility on mass scale

ELSEVIER
Aquaculture
Volume 560, 15 November 2022, 738456

Full production cycle performance of gene-edited, sterile Atlantic salmon - growth, smoltification, welfare indicators and fillet composition

L. Kleppe [a], P.G. Fjelldal [b], E. Andersson [a], T. Hansen [b], M. Sanden [a], A. Bruvik [a], K.O. Skaftnesmo [a], T. Furmanek [a], E. Kjærner-Semb [a], D. Crespo [a], S. Flavell [b], A.Ø. Pedersen [b], P. Vogelsang [a], A. Torsvik [a], K.A. Kvestad [b], S. Olausson [c], B. Norberg [c], R.W. Schulz [a, d] … A. Wargelius [a]

"In conclusion, germ cell free [gene edited] salmon performed to a large extent similarly to their WT counterparts but had the clear advantage of never maturing."

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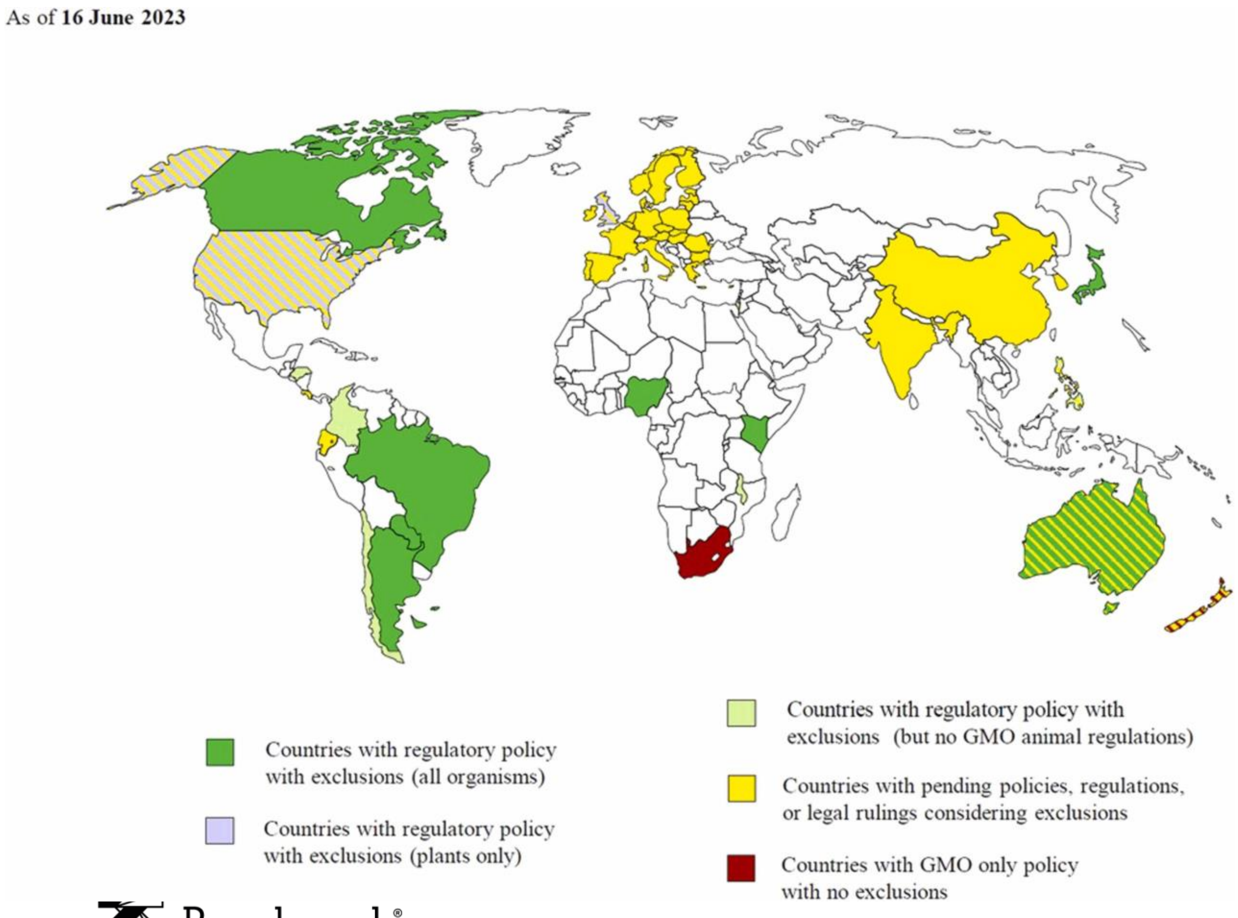
Gene Editing: Implementation

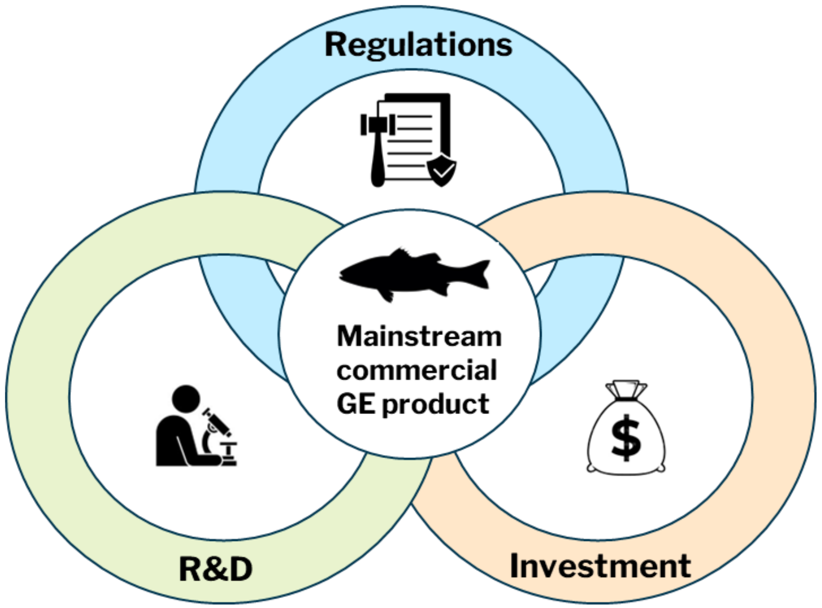
- Gene editing has transformative potential to tackle major production, health and welfare, and environmental challenges in salmon farming
- Uncertainty of regulatory environment is a major barrier to investment, innovation, and application



Hallerman *et al.* 2024

- Current GMO legislation is largely prohibitive to commercial application
- Gene Editing has prompted many countries to review regulatory paradigms
- Some consensus around light-touch regulation for 'Cisgenic' gene edited changes – which could have occurred by natural mutation

Scientific progress, investment, and progressive regulatory change are all closely interrelated



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Benchmark Genetics' extensive R&D programmes focus on applying the latest technologies towards improved fish health, welfare, and performance for customers