ROSLIN

Genome editing in aquaculture species

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THE UNIVERSITY of EDINBURGH

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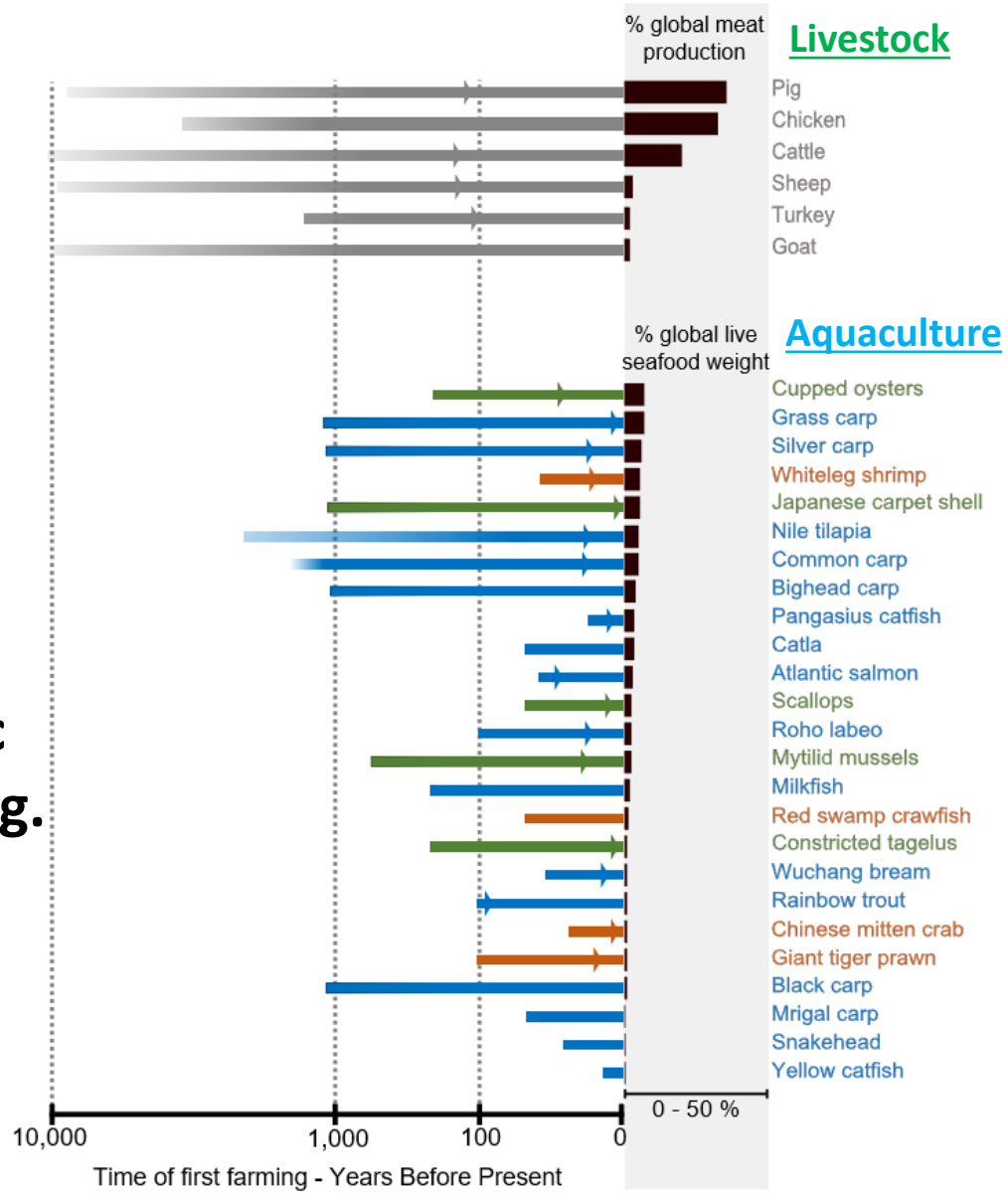
Selective breeding in aquaculture

- Domestication of aquaculture species is recent and ongoing
- Production is based on numerous and diverse species
- Family-based breeding programmes with genomic selection in few species, e.g.
  - Salmon and trout
  - Nile tilapia
  - Shrimp spp



Selective breeding in aquaculture

- **High fecundity and external fertilisation**

Nile Tilapia
(*Oreochromis niloticus*)

Atlantic salmon
(*Salmo salar*)

Gilthead sea bream
(*Sparus aurata*)

Pacific oyster
(*Crassostrea gigas*)

Approx number of offspring per mating

10^3 10^4 10^6 10^8

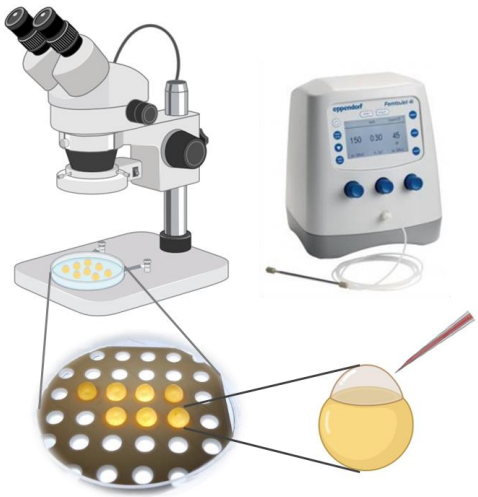
- **Advantageous for genetics and genome editing research and application**
  - **Easy access to large numbers of embryos for editing**
  - **Large-scale experimental disease challenges feasible**
  - **Rapid dissemination of improved / edited germplasm**

Target traits for improvement

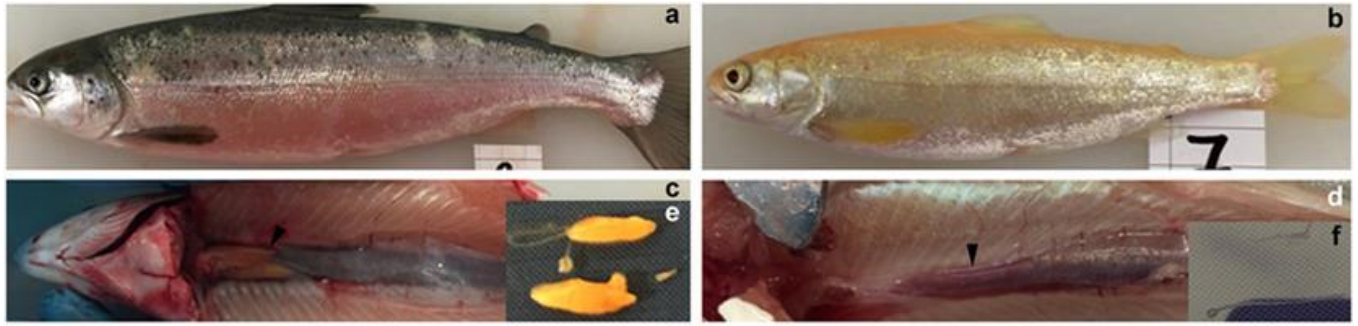
- **Infectious disease presents a major threat to aquaculture**
  - Major economic, animal welfare, and environmental concern
  - Vaccination, biosecurity or other control measures often infeasible
- ➢ **Breeding and genome editing technologies have high potential to improve disease resistance in aquaculture**
- **Other target traits**
  - Production efficiency; growth, FCR
  - Adaptation to vegetarian diets
  - Sterility and monosex populations

Development of *in vivo* editing

- **Methods of *in vivo* CRISPR editing in aquaculture species**
  - **Species-dependent, but generally embryo microinjection**
  - **First examples in Atlantic salmon focussed on sterility traits**



Large fish embryos for hand-held microinjection



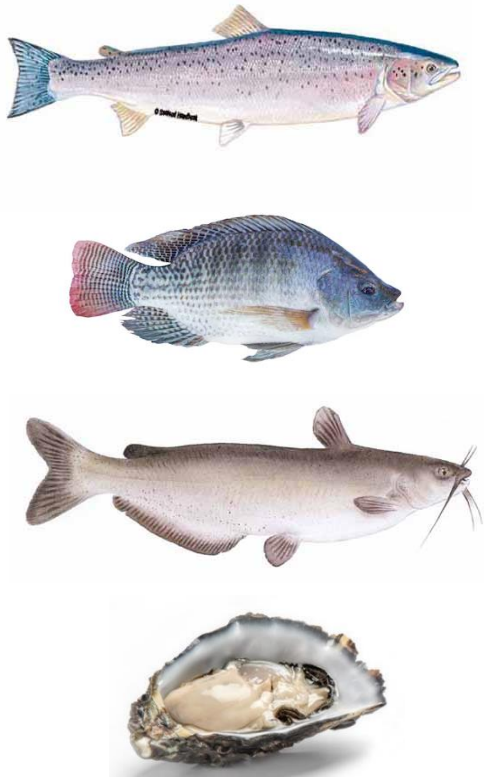
Knockout of *dnd* gene to induce germ cell ablation and sterility in salmon. Concurrent knockout of *slc45a2,* albinos as a 'tracer' (*Wargelius et al. 2016*)

Development of *in vivo* editing

Gratacap et al. (2019),
Trends in Genetics, 35:672-84

- **CRISPR editing successfully applied in diverse aqua species**

Species	Target gene[a]	Trait of interest
Atlantic salmon, *Salmo salar*	*tyr/slc45a2*	Pigmentation
	dnd	Sterility
	elov-2	Omega-3 metabolism
Tilapia, *Oreochromis niloticus*	*dmrt1/nanaos2-3/foxl2*	Reproduction
	gsdf	Reproduction
	aldh1a2/cyp26a1	Reproduction
	sf-1	Reproduction
	dmrt6	Reproduction
	amhy	Reproduction
	wt1a/wt1b	Reproduction
Sea bream, *Sparus aurata*	*mstn*	Growth
Channel catfish, *Ictalarus punctatus*	*mstn*	Growth
	ticam1/rbl	Immunity
	LH	Sterility
Southern catfish, *Silurus meridionalis*	*cyp26a1*	Germ cell development
Common carp, *Cyprinus carpio*	*sp7a/sp7b/mstn(ba)*	Muscle development
Rohu carp, *Labeo rohita*	*TLR22*	Immunity
Grass carp, *Ctenopharyngodon idella*	*gcjam-a*	Disease resistance
Northern Chinese lamprey, *Lethenteron morii*	*slc24a5/kctd10/wee1/soxe2/wnt7b*	Pigmentation/development
Rainbow trout, *Oncorhynchus mykiss*	*igfbp-2b1/2b2*	Growth
Pacific oyster, *Crassostrea gigas*	*mstn*	Growth



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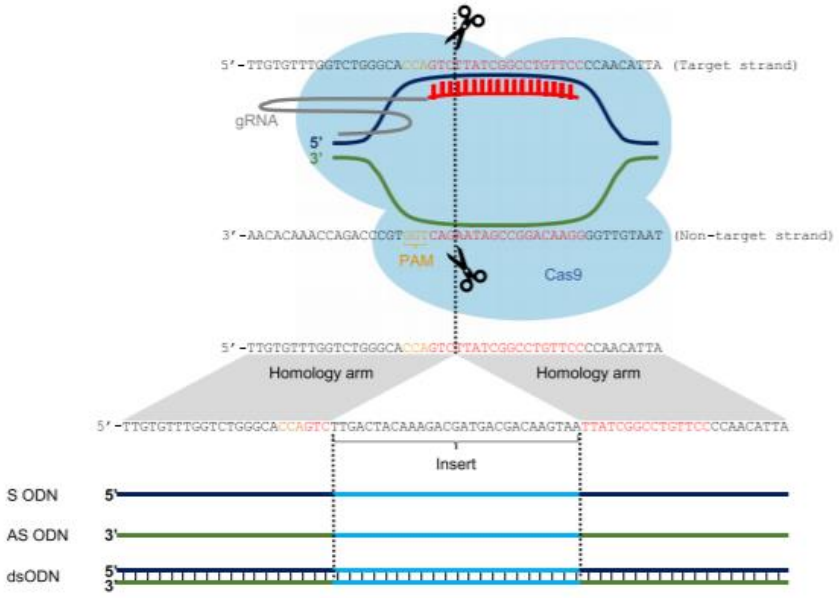
Development of *in vivo* editing

- **Methods of *in vivo* CRISPR editing in aquaculture species**
  - **Most studies have focused on gene knockout using NHEJ repair**
  - **Homology-directed repair has been successfully trialled**



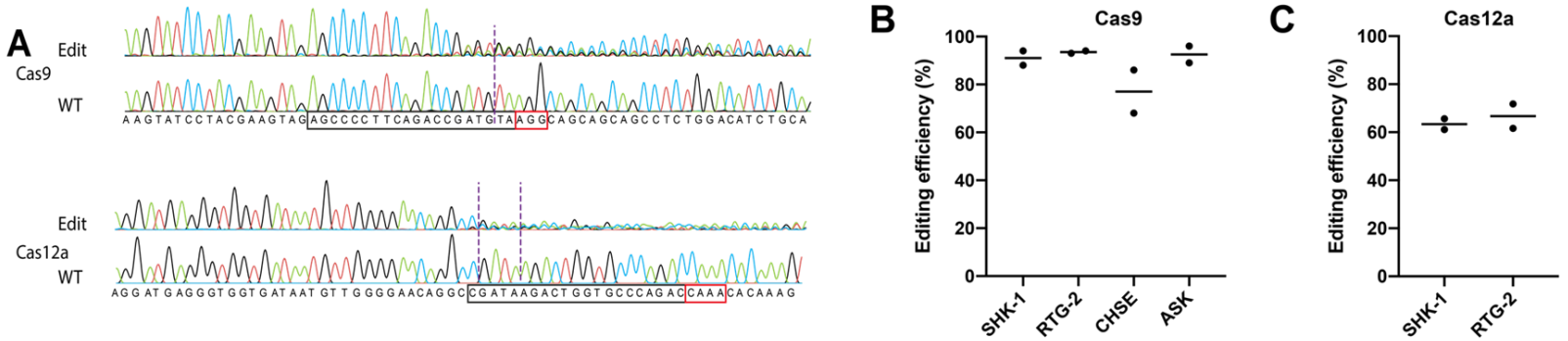
27% Knock-in efficiency in Atlantic salmon F0 embryos (Struame et al. 2020, Sci Rep, 10:409)

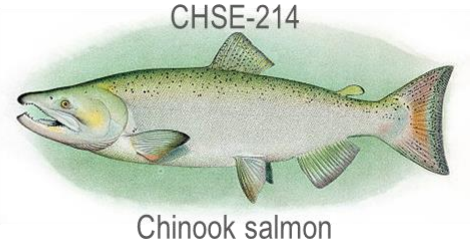
- **Knockout with NHEJ and knock-in with HDR achieved in salmon**
- **Efficiency, survival, mosaicism ongoing challenges to be tackled**

Development of *in vitro* editing

Gratacap et al. (2020),
Mar Biotechnol 22, 717–724

- **Cell line editing needs development in aquaculture species**
  - **Paucity of cell lines and lack of methods for cell line editing**
  - **Progress recently, esp RNP CRISPR approaches in salmonid cell lines**





- **Platforms to test edits before *in vivo* applications**
- **Suitability for pathogen challenges and CRISPR screens**

Applications of genome editing

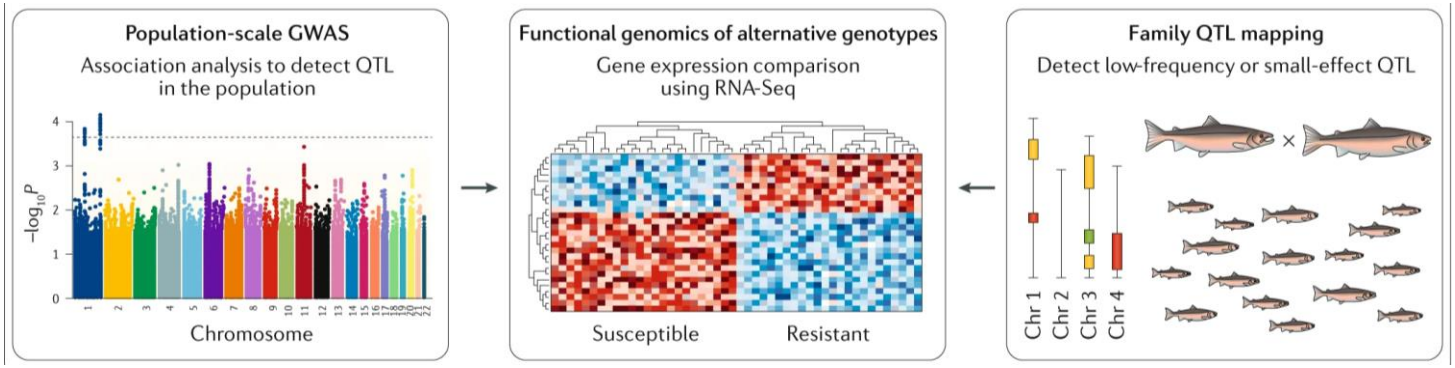
- **Genome editing opens up possibilities to expedite genetic improvement in aquaculture species:**

✓ **Harnessing naturally-occurring variation:**
  - Increase frequency or fix desirable alleles at existing QTL
  - 'Introgression' of alleles from different strains / species

✓ **Creating *de novo* mutations / alleles with desirable effects:**
  - Knowledge of the biology of the trait
  - Genome-wide CRISPR screen approaches

Targeting naturally-occurring variants

- **Genome editing to identify and utilise causative variants for QTL affecting production traits (e.g. disease resistance)**



Genome-wide association studies

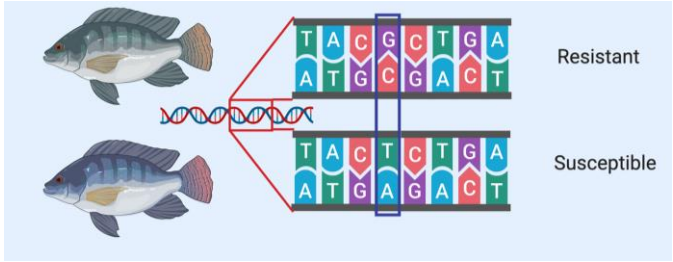
Sequencing and functional annotation

Gene expression comparison

Editing functional variants in cell culture

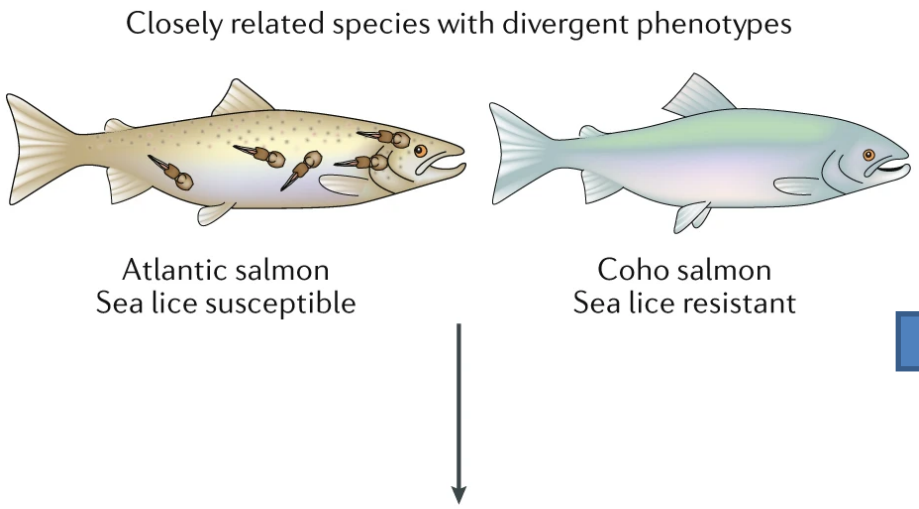
Editing of functional variants *in vivo*

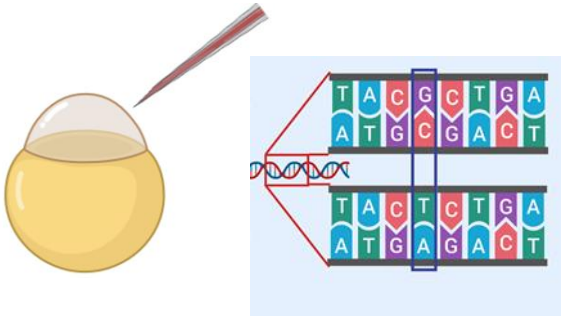
Number of candidate functional variants



Targeting naturally-occurring variants

- **Genome editing to transfer favourable alleles from different strains or species**



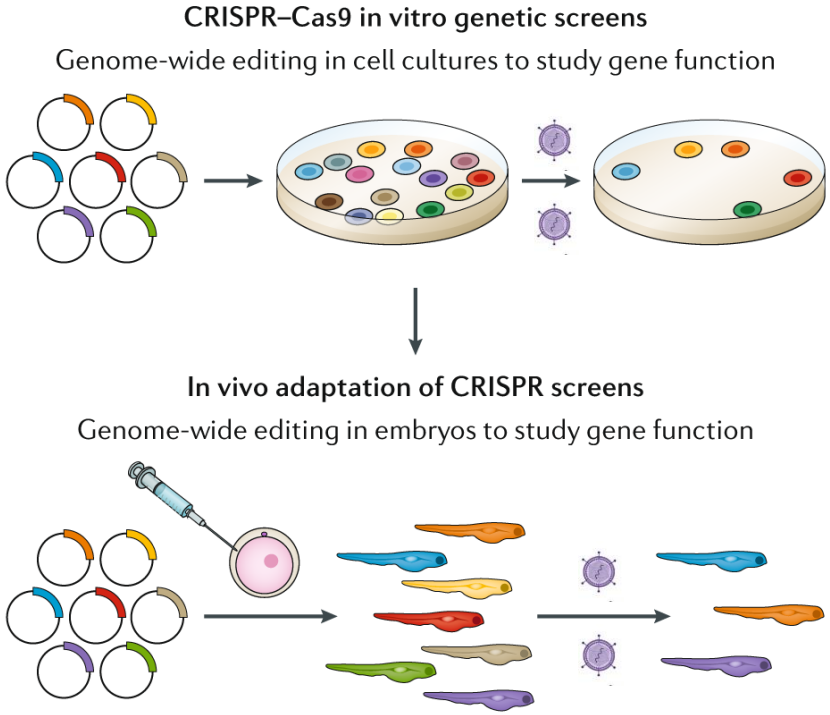


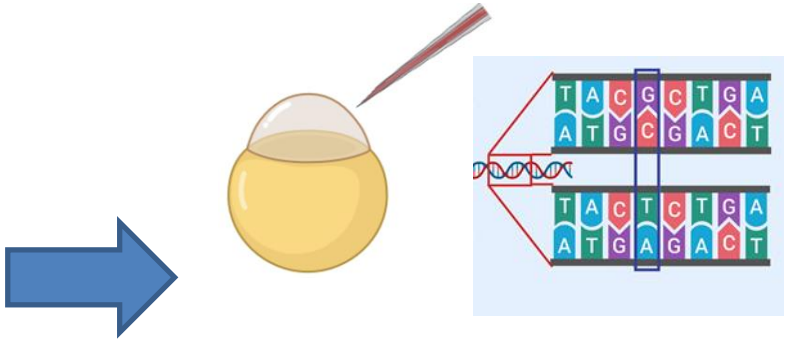
Targeted 'introgression-by-editing' of favourable functional alleles into target species

Creating *de novo* variants

Houston et al. (2020) *Nat Rev Genet* 21:389–409

- **Creation and evaluation of *de novo* alleles with favourable effects on production traits**



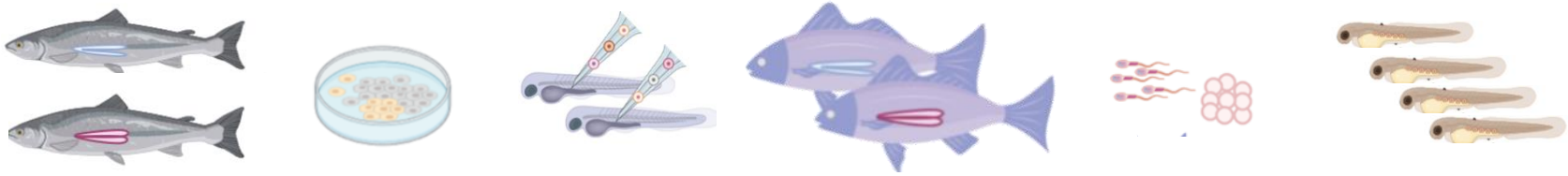


Generate *de novo* variation to perturb target disease resistance gene

Research and translation priorities

- **Improved methods of predicting functional variants is key**
  - **High quality reference genomes with functional annotation need development in most aquaculture species**
- **Improving techniques for reliable and efficient editing**
  - **Improved cell line models for *in vitro* targeted and genome-wide screens**
  - **Reducing F0 mosaicism for *in vivo* editing, alternative delivery methods**

Editing germ cells in cell culture and surrogate broodstock technologies



Labelling & isolation of germ cells from donor broodstock

Culture and editing of germ cells

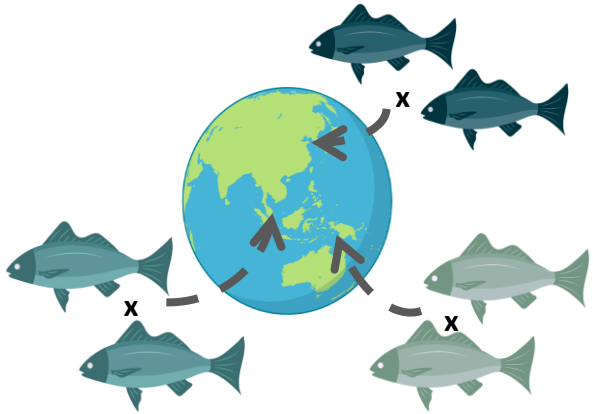
Transfer of edited germ cells to sterilised surrogate broodstock (intra or inter species)

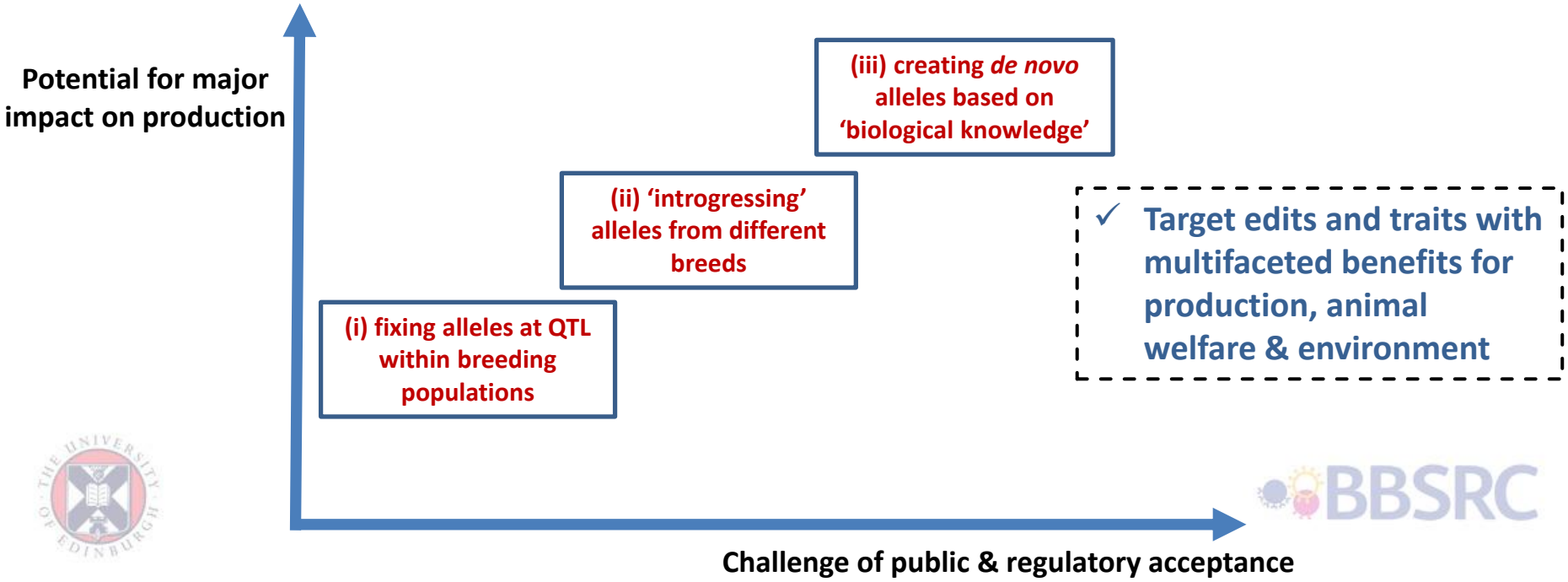
Production of fully edited donor gametes and embryos

- **Integration of editing technologies with breeding programmes**
  - **Need for sterility of production stocks carrying edits**

Future directions to application

- **Huge potential to harness high fecundity for widespread delivery of edited strains to tackle production barriers**
- ➢ **Applications dependent on public and regulatory landscape**





✓ **Target edits and traits with multifaceted benefits for production, animal welfare & environment**

Acknowledgements

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