# Gene editing by CRISPR/Cas9: A game changer for aquaculture?

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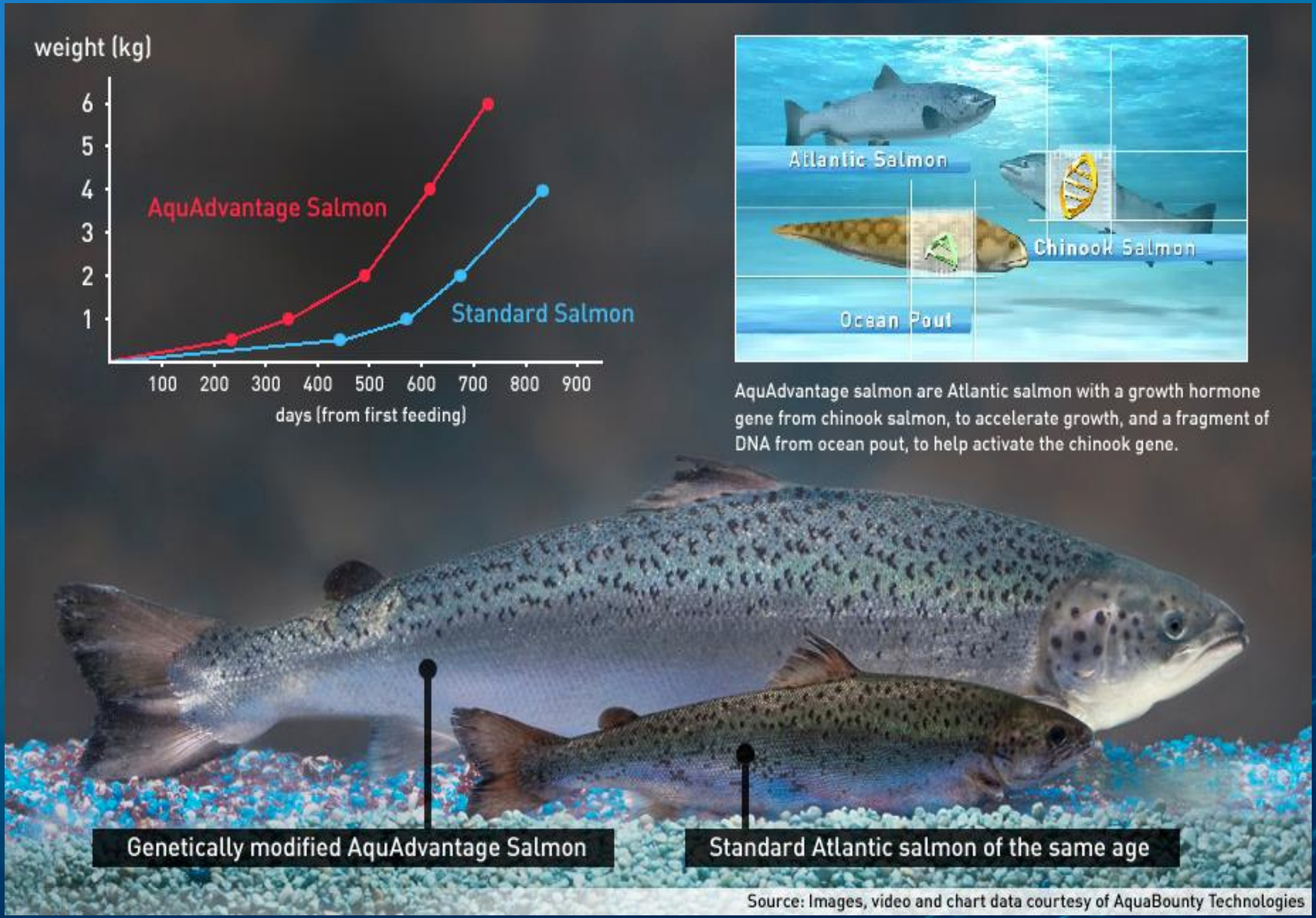
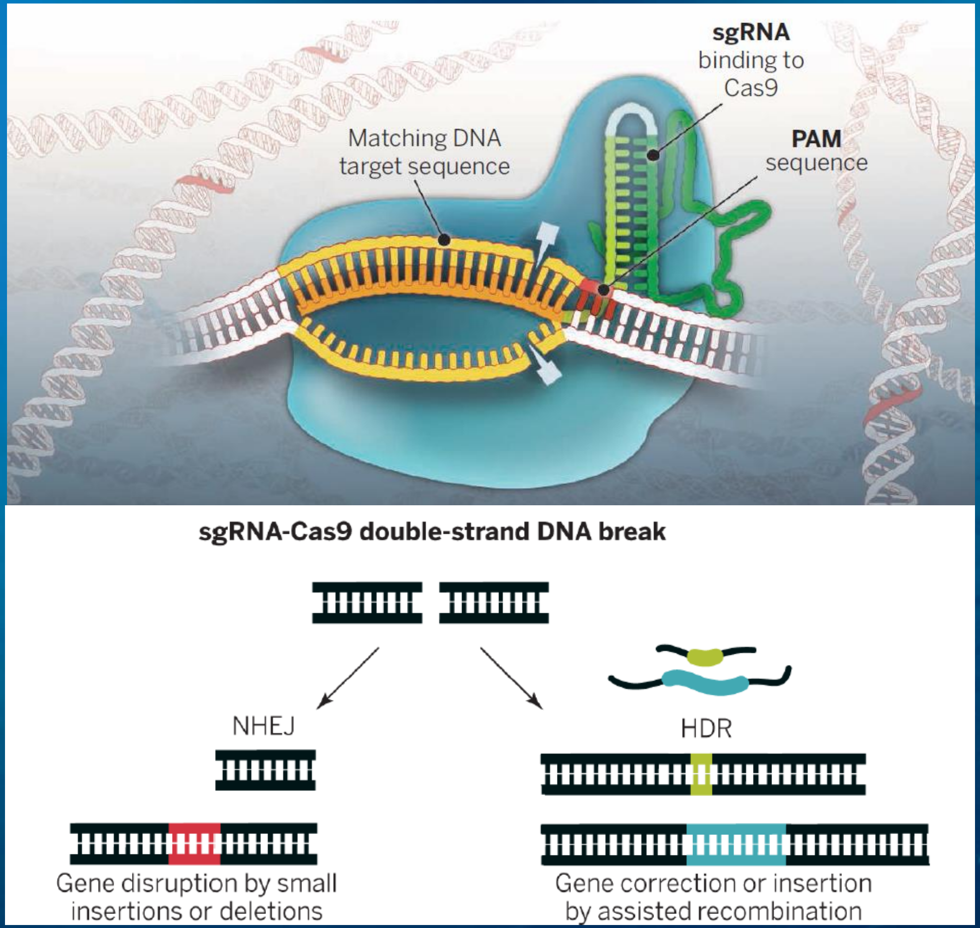
# Transgenesis



# Gene editing by CRISPR/Cas9

## Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9




**NHEJ**=
Non-Homologous End Joining

**HDR**=
Homology Directed Recombination

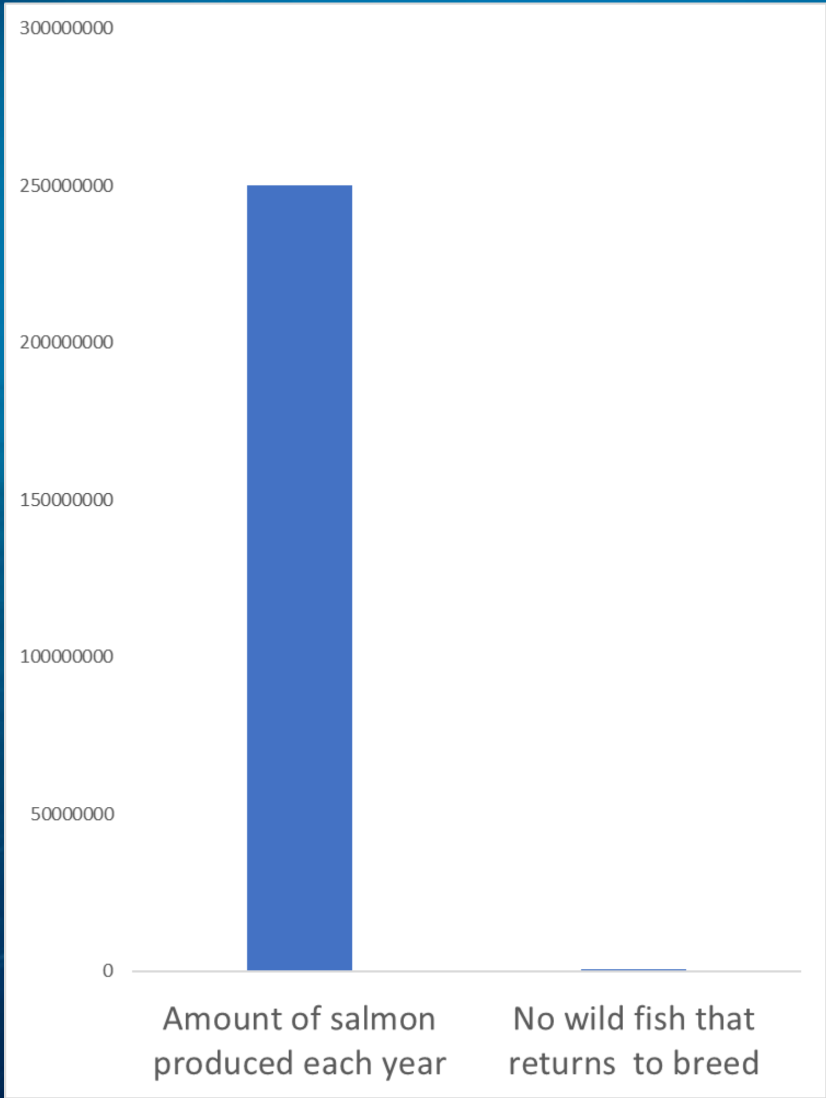

**The new frontier of genome engineering with CRISPR-Cas9**
Jennifer A. Doudna and Emmanuelle Charpentier
*Science* **346**, (2014);
DOI: 10.1126/science.1258096

# CRISPR/Cas9
# Research tool and possible industrial applications

- Genetic containment of wild populations (salmon)
- Filet quality (salmon)
- Disease resistance (pig)
- Welfare (cow)
- Move traits between strains (salmon)

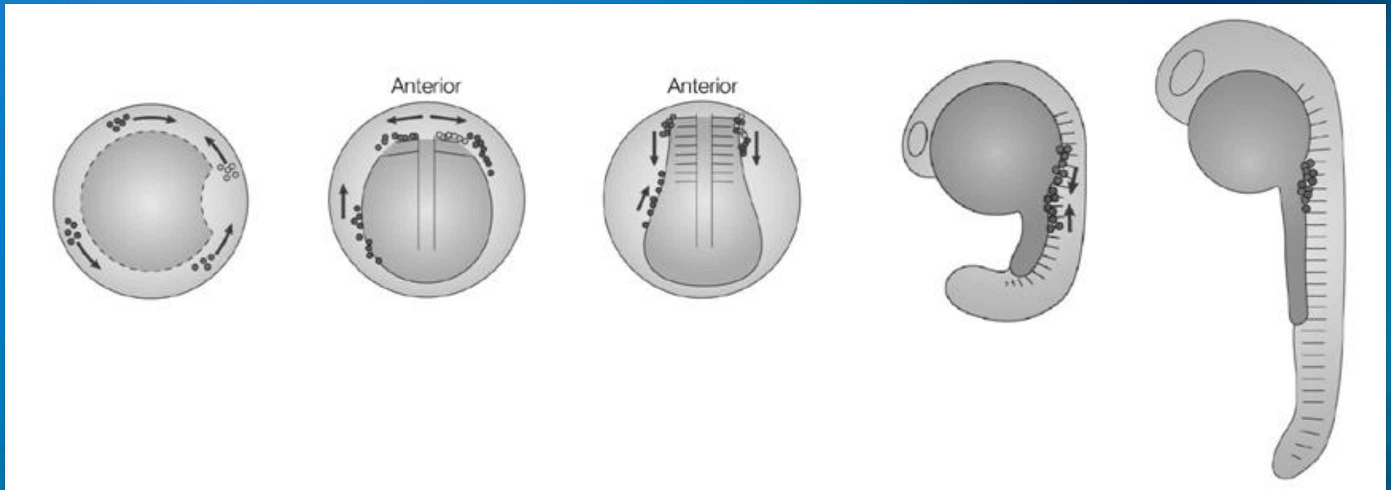
# Environmental challenges – escaped salmon

Genetic introgression with wild populations

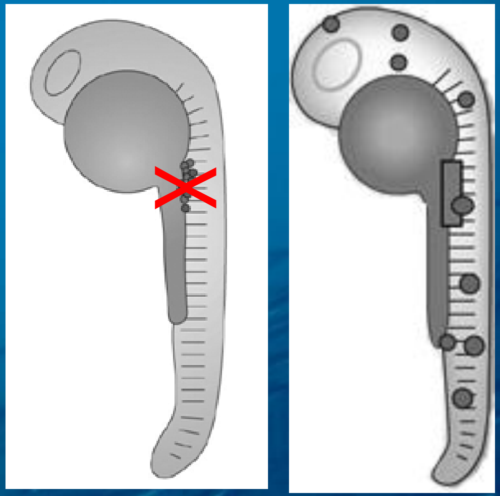




*Genetic containment of wild populations*

# Sterile salmon through germ cell ablation




*Disrupt the migration of primordial germ cells*



*No germ cells will form = sterility*

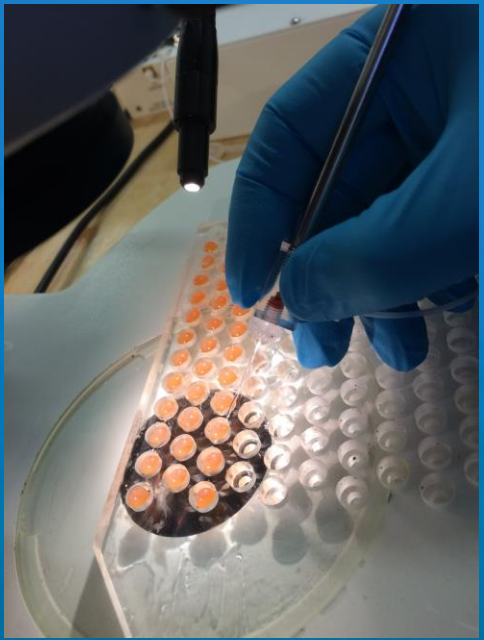
***<u>Dead end (dnd) gene:</u>***

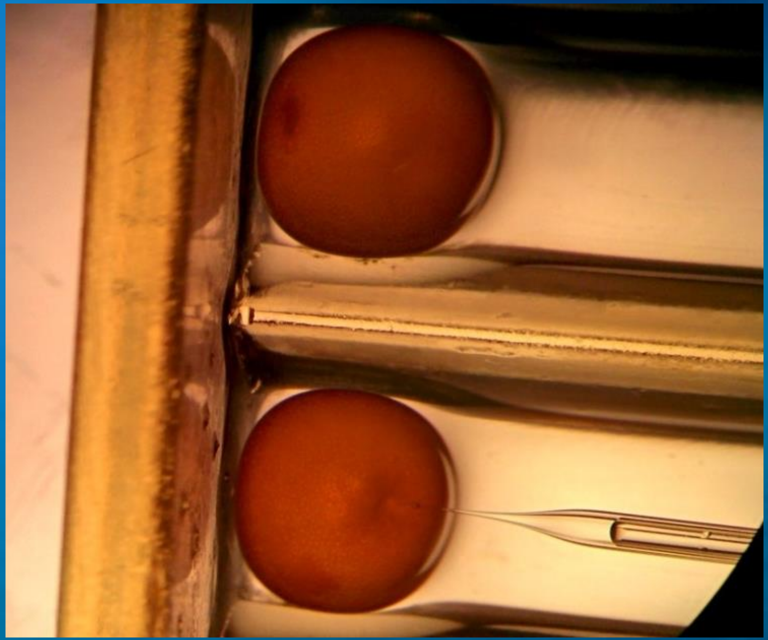
- RNA binding protein
- *dnd* mutation in mice
- *dnd* knockdown in fish
  (zebrafish, medaka, pond loach, rainbow trout, starlet)

**Lack of primordial germ cells**

***Genetic containment of wild populations***

# Exploring the CRISPR/Cas9 methodology in salmon





Injection of guide RNA + Cas9 in newly fertilized eggs
Targeting a pigmentation gene: *slc45a2* (*albino*)

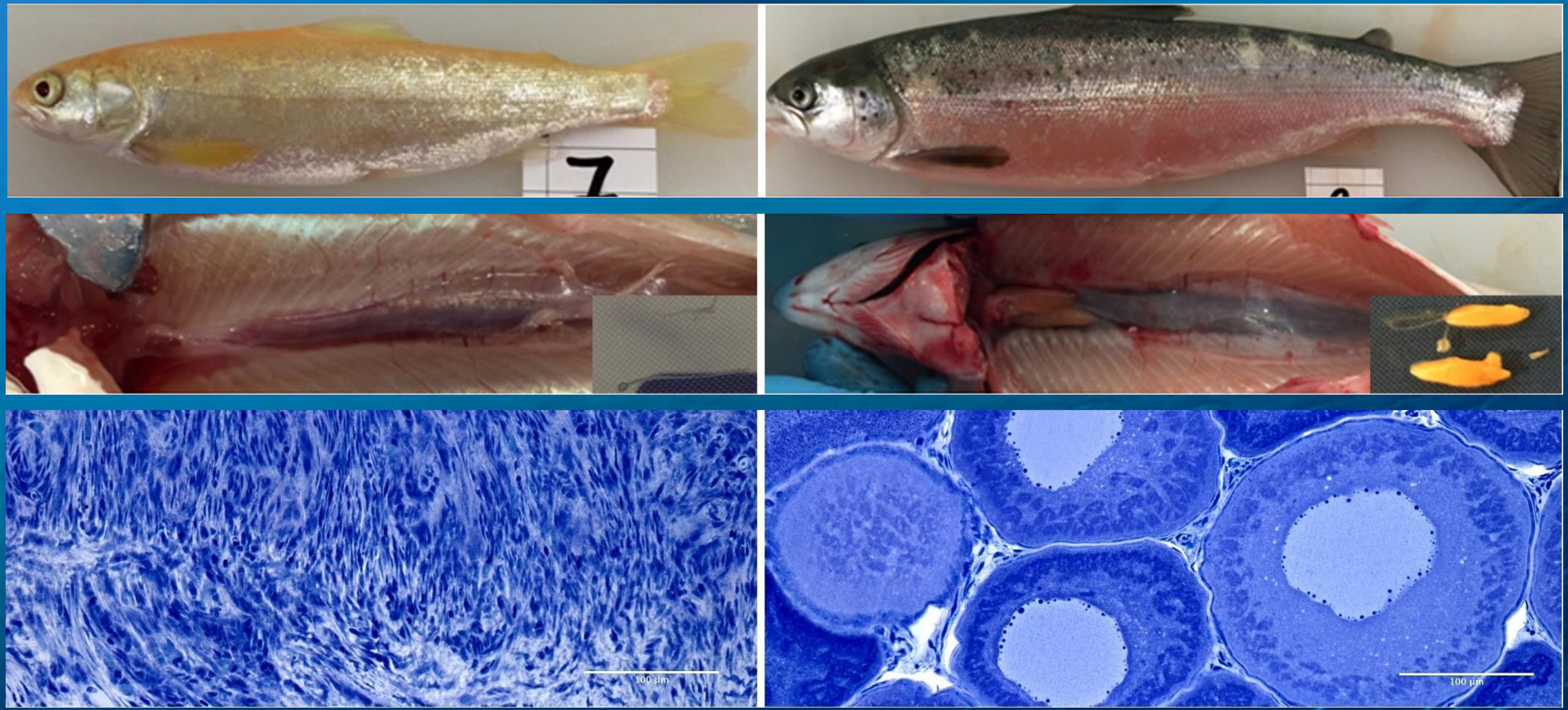
Successful editing = albinos

***Genetic containment of wild populations***

*Edvardsen et al., Plos One (2014)*

# Germ cell-free (sterile) salmon

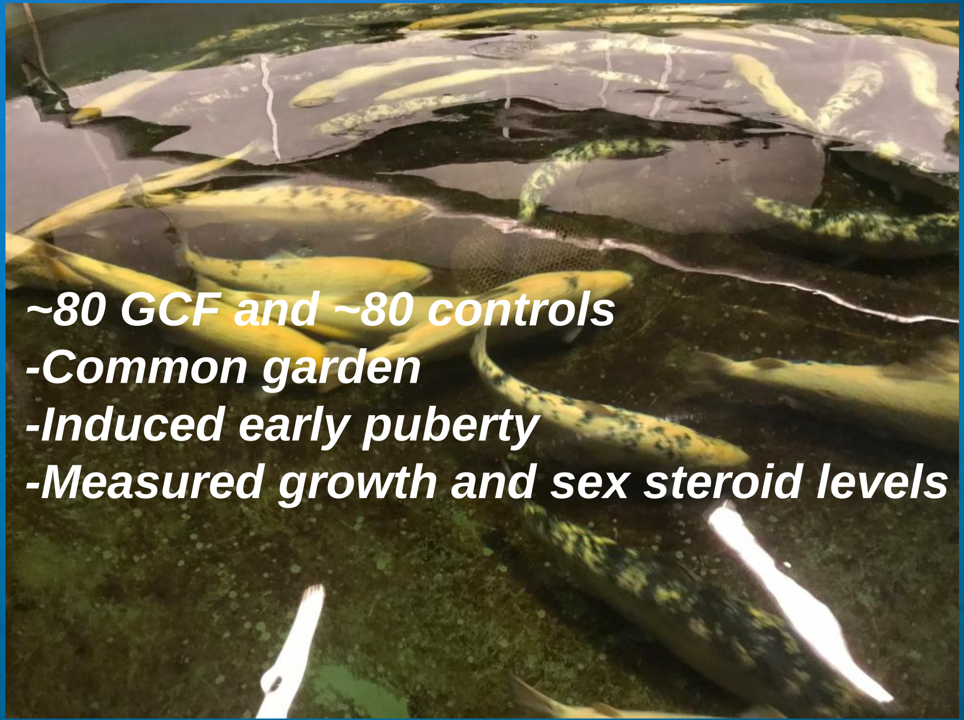
***dnd* + *albino* Knockout female** | **Control female**




**Genetic containment of wild populations**

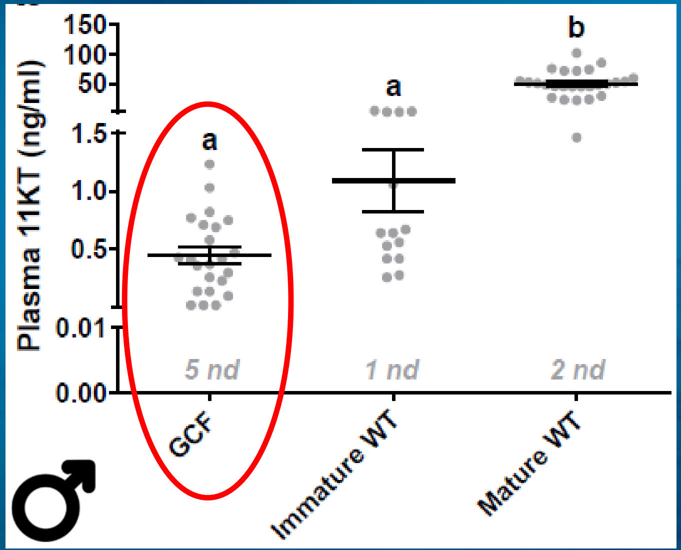
*Wargelius et al., Sci. Rep. (2016)*

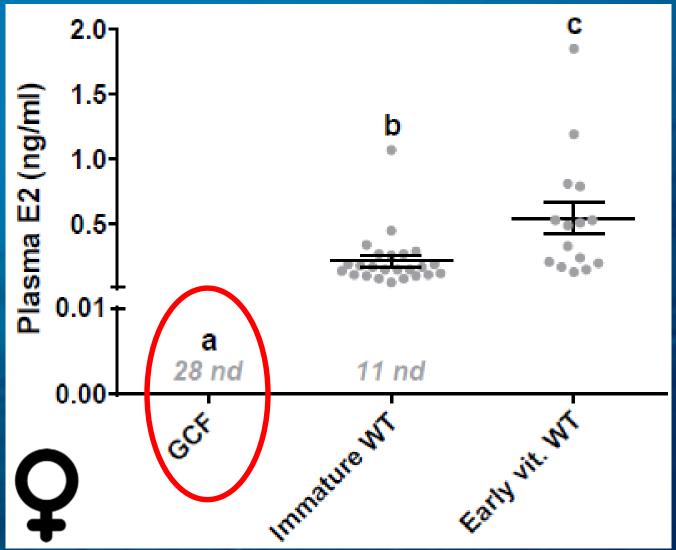
# Germ cell-free (GCF) salmon



*~80 GCF and ~80 controls*
*-Common garden*
*-Induced early puberty*
*-Measured growth and sex steroid levels*

## Growth and sexual maturation




Very low or no production of sex steroids

## Ongoing: New, long-term growth experiment



*Kleppe et al., Sci. Rep. (2017)*

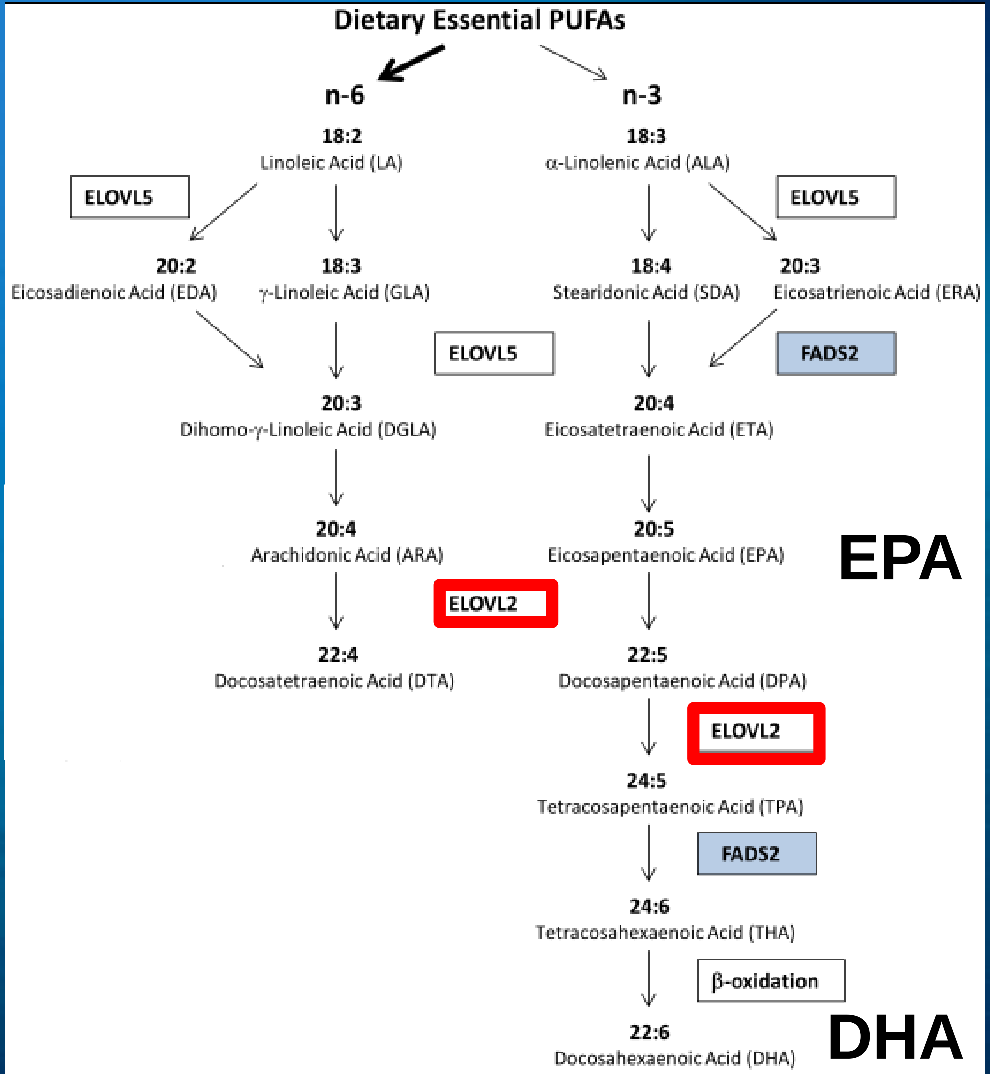
# Environmental challenges – feed resources

Marine resources are still needed to produce salmon feed

Increasing proportions of plant based ingredients
→ Less omega-3 fatty acids both in feed and filet

**Can we make the salmon produce more of its own omega-3 fatty acids?**

# Synthesis of omega-3



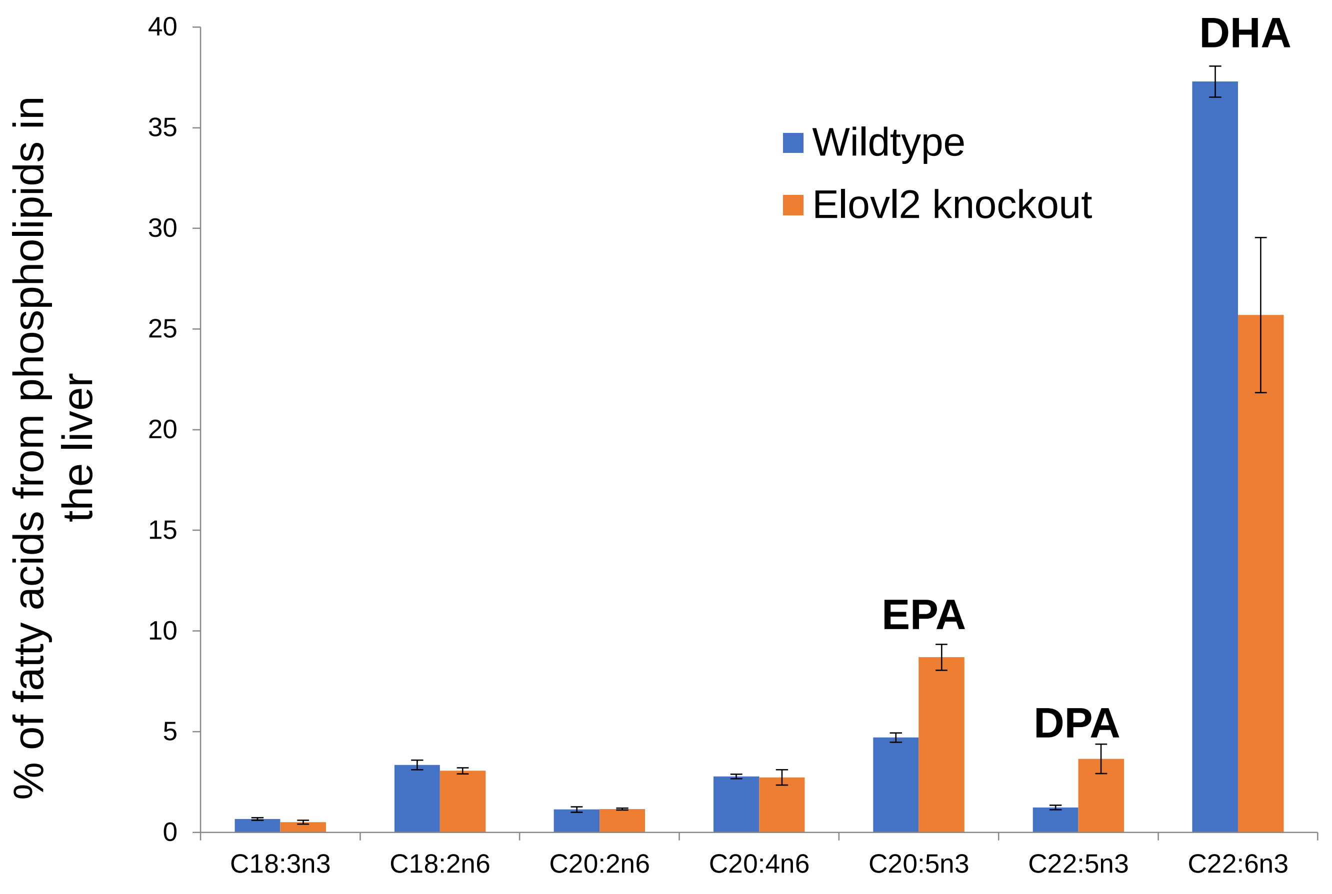

**Control**

***elovl2* + *albino* knockout**

***Filet quality***

*Datsmoor A, Winge P in prep.*

# Synthesis of omega-3




| | C20:5n3 | C22:5n3 | C22:6n3 |
|---|---|---|---|
| p-values | 0,0043 | 0,027 | 0,031 |

*elovl2* knockout inhibits elongation of EPA and DPA

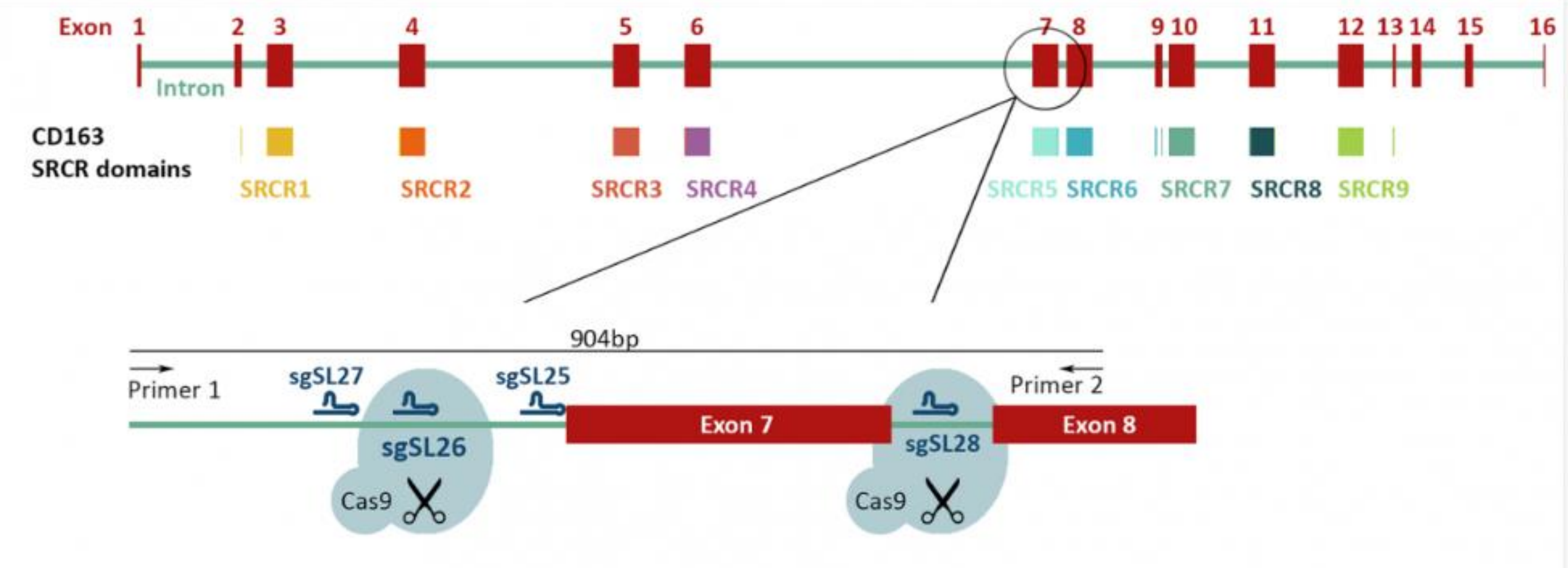
*Filet quality*

*Datsmoor A, Winge P in prep.*

# Prrsv resistant pig

## Porcine reproductive and respiratory syndrome virus (PRRSV)

costs the United States swine industry around $644 million annually, and in Europe about 1.5b€ every year.




Schematic of genome editing in pigs for PRRSV resistance

**The virus infects macrophages**

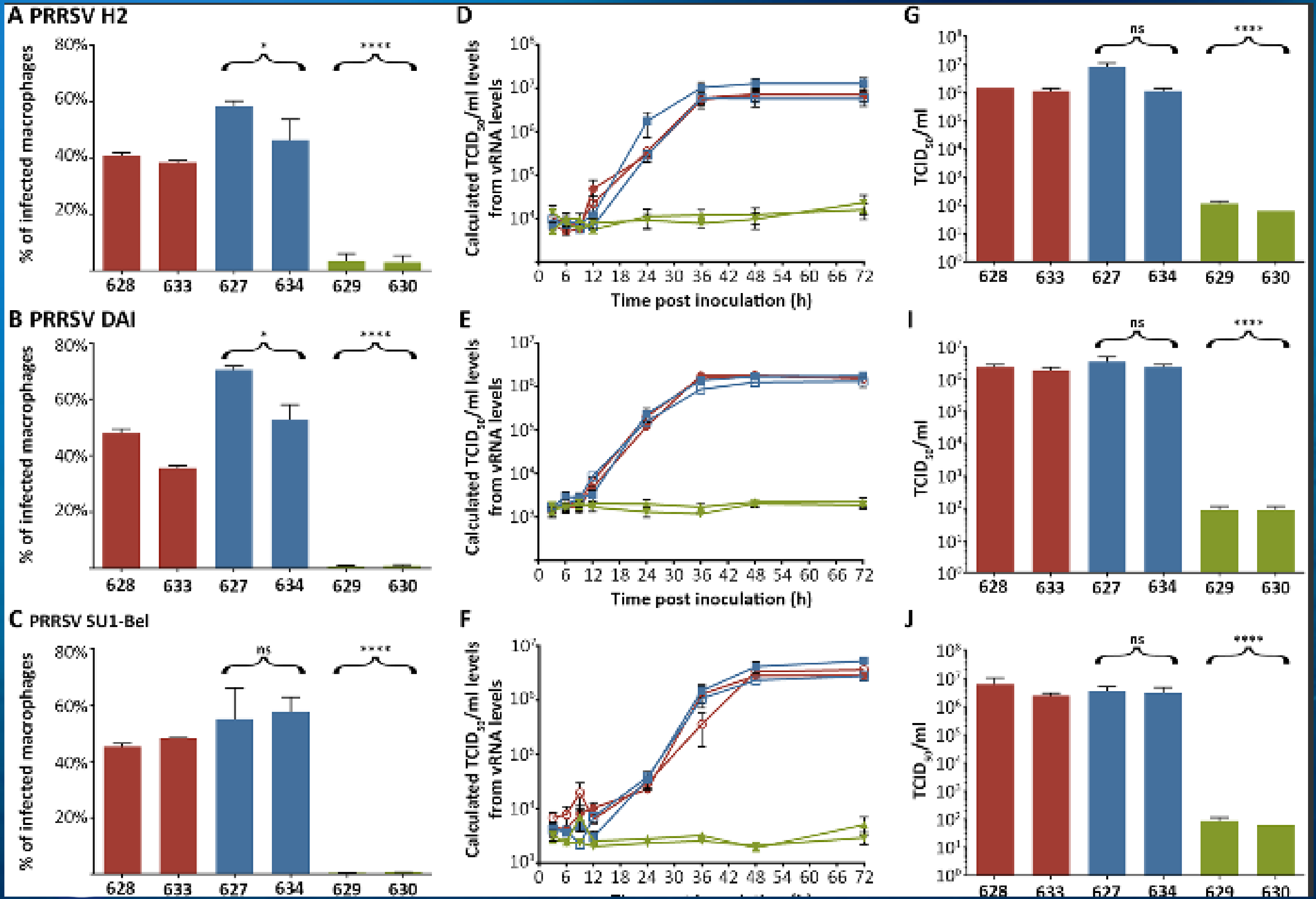
**SRCR5 domain: Interaction site for the virus**

**CD163 has several functions**

*Disease resistance*

*Burkard et al., Plos Pathogens (2017)*

# Prrsv resistant pig




Control
Heterozygous
Biallelic/homozygous

**H2, DAI and SU1-Bel = subtypes of the virus**

**Pulmonary alveolar macrophages were collected from lungs, and infected with PRRSV.**

**-% infected macrophages (A-C)**
**-Virus replication growth (D-F)**
**-Conc. of infectious viral particles (G-J)**

*Disease resistance*

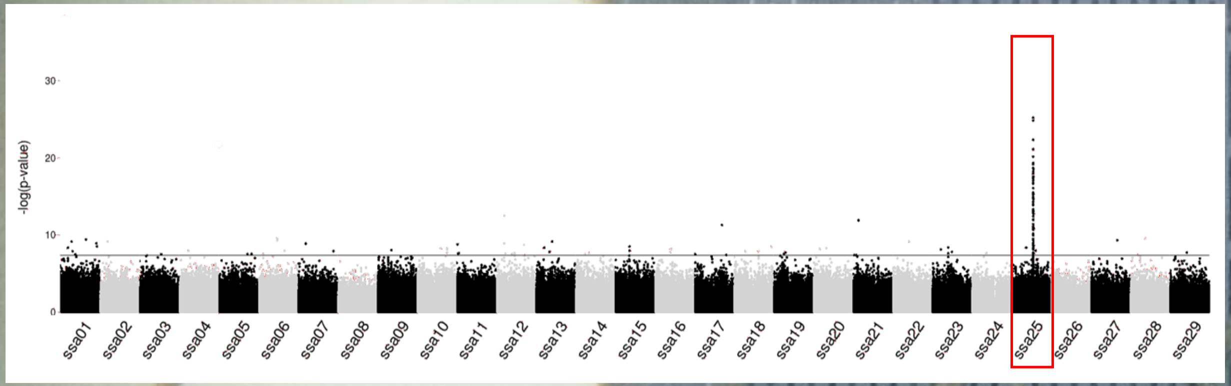
*Burkard et al., Plos Pathogens (2017)*

# <u>Knock in</u> of wild traits beneficial for salmon aquaculture

- Disease resistance
- Sea water adaptation
- Nutritional content of filet
- Time of maturity

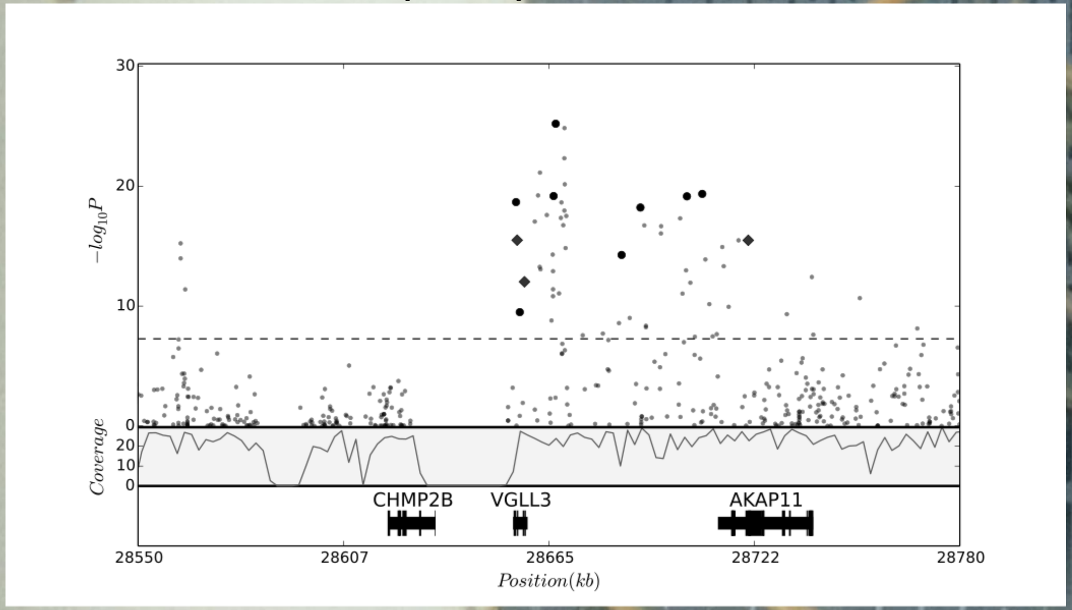
*Move traits between salmon strains*

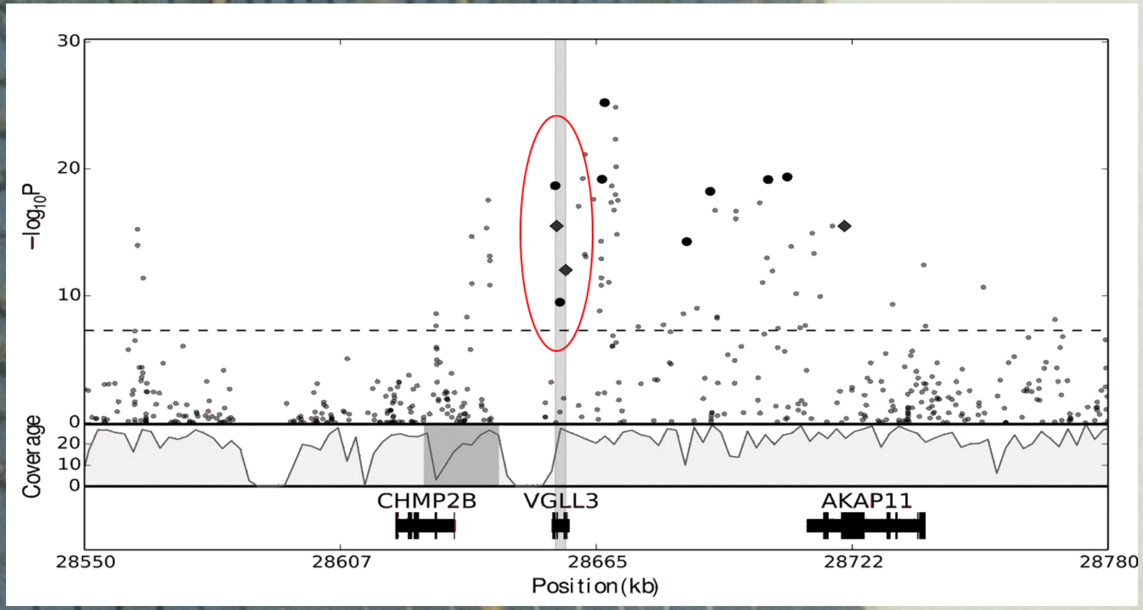
# Knock in of wild traits beneficial for salmon aquaculture




- Disease resistance
- Sea water adaptation
- Nutritional content of filet
- <u>Time of maturity</u>

*Ayllon et al., Plos Genetics (2015)*
*Barson et al., Nature (2015)*




***Move traits between salmon strains***

# Potential use of CRISPR in aquaculture

## Benefits

- Presise and fast targeting of genes
- Can knock out, correct or add DNA
- Less risk/ethical consideration than classical transgenic methods
  (do not need to mix DNA from different species)

## Risks

- Off-target activity (solved by sequencing)
- Genetic introgression of farmed fish into wild populations
  (solved by genetic containment)
- Trust

# This work was carried out by:

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