# DNA vaccination against SPDV: suppression of viraemia, protection, and individual monitoring

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The Scottish Government

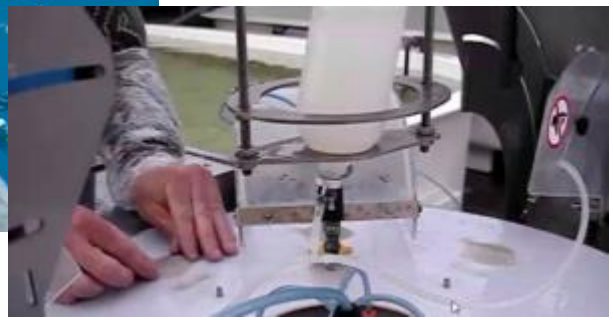
marinescotland science

PD Trination Aberdeen 2016

# Targeted disease prophylaxis in European fish farming

targetfish

http://targetfish.eu/



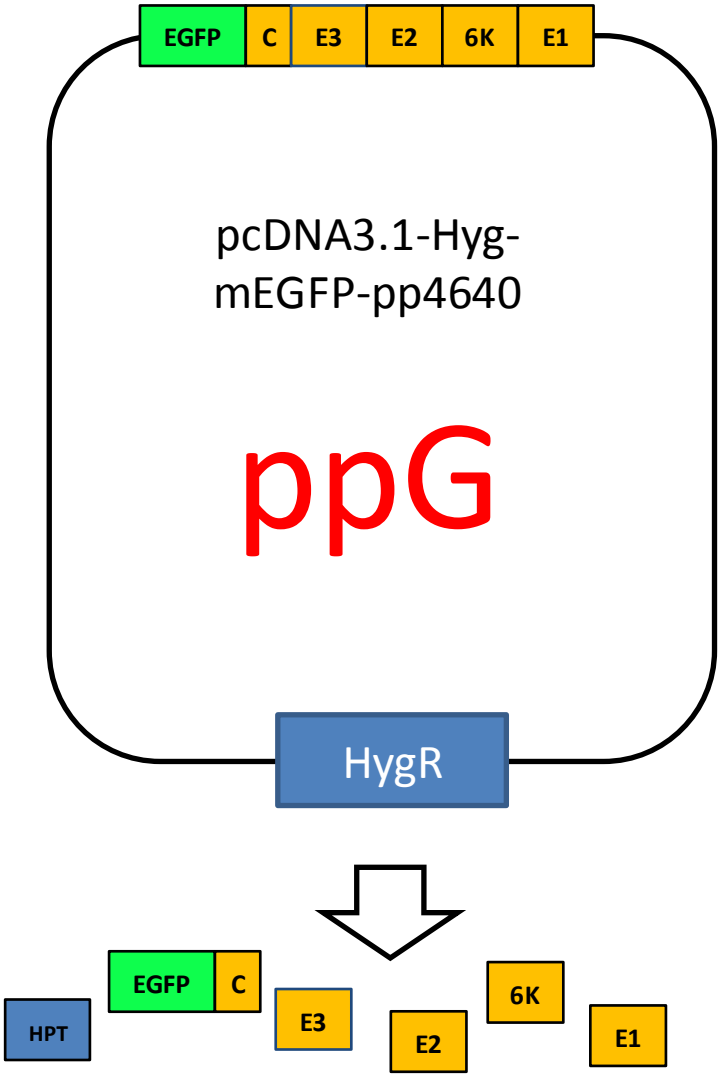
**Establish a generic knowledge-base for rational development of next generation fish vaccines and their application: efficacy, safety and delivery route.**

- develop improved vaccine for SPDV
- understand the basis for DNA vaccine protection/efficacy
- improve challenge model for SPDV
- improve sampling/monitoring methodologies

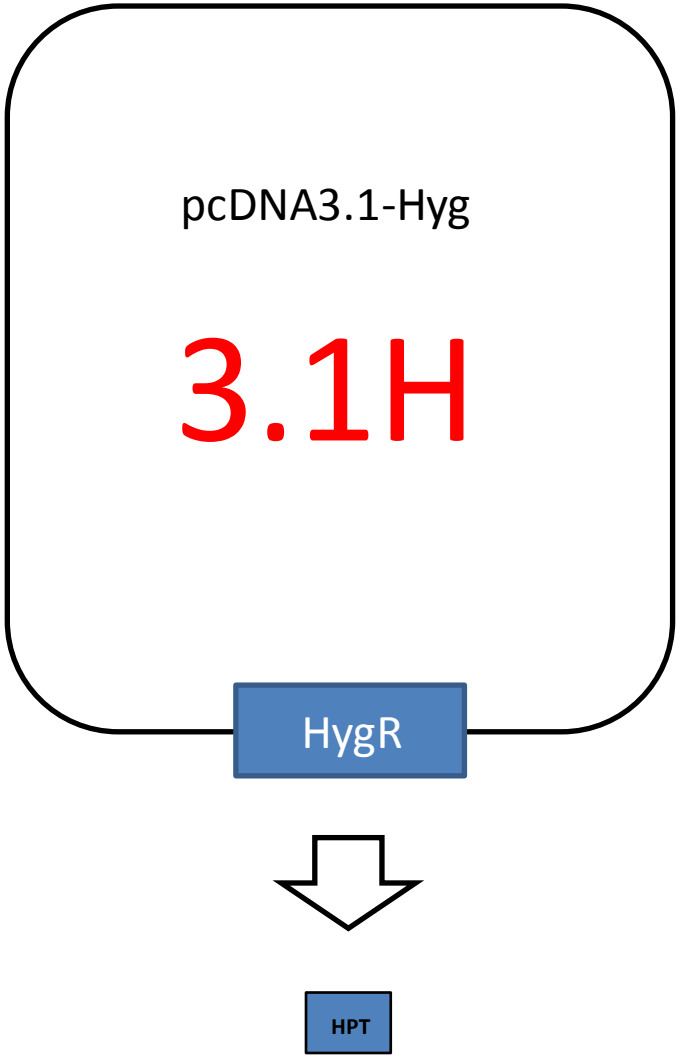
# Generation of DNA Vaccine

# SPDV DNA vaccine

**Subtype 1**
**Structural proteins (all)**



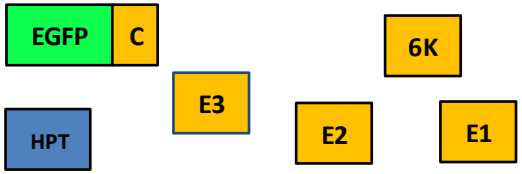

# Placebo

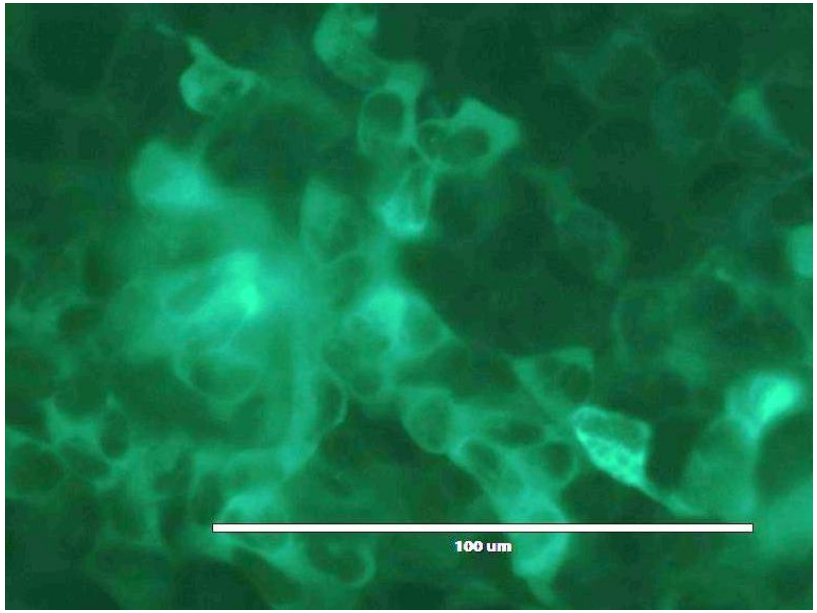


# *In vitro* verification of DNA vaccine expression

**Genetically engineered stable CHSE cell line (CHSE-ppG).**

- RNA transcription of the SPDV DNA vaccine construct confirmed
- Protein expression of SPDV polyprotein confirmed (EGFP as marker)
- Cleavage of SPDV polyprotein indicated (EGFP-capsid protein in cytoplasm)



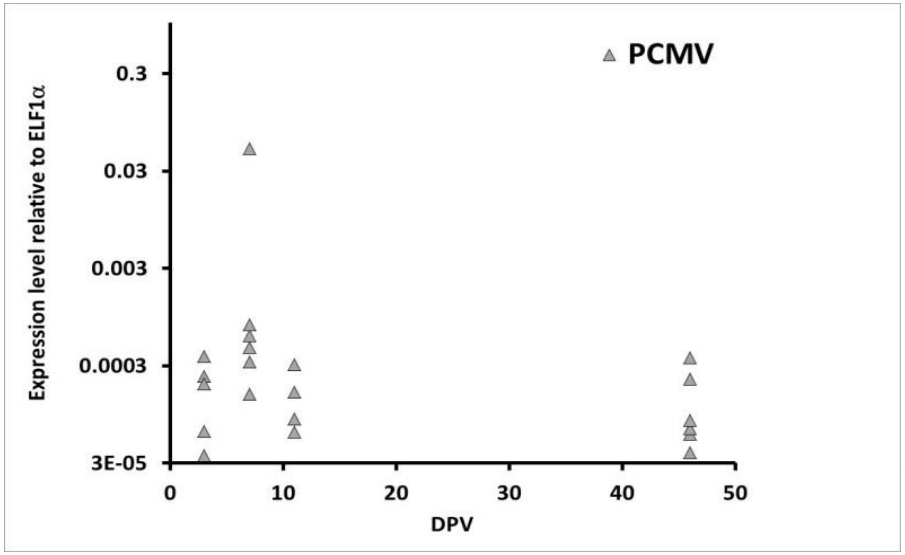

Visualisation of cytoplasmic mEGFP-capsid fusion protein in CHSE-ppG

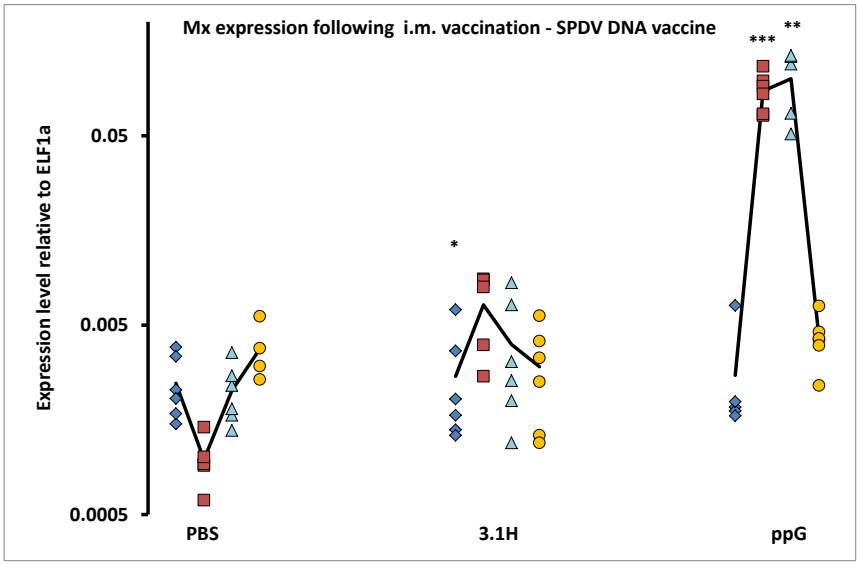
# *In vivo* verification of DNA vaccine expression

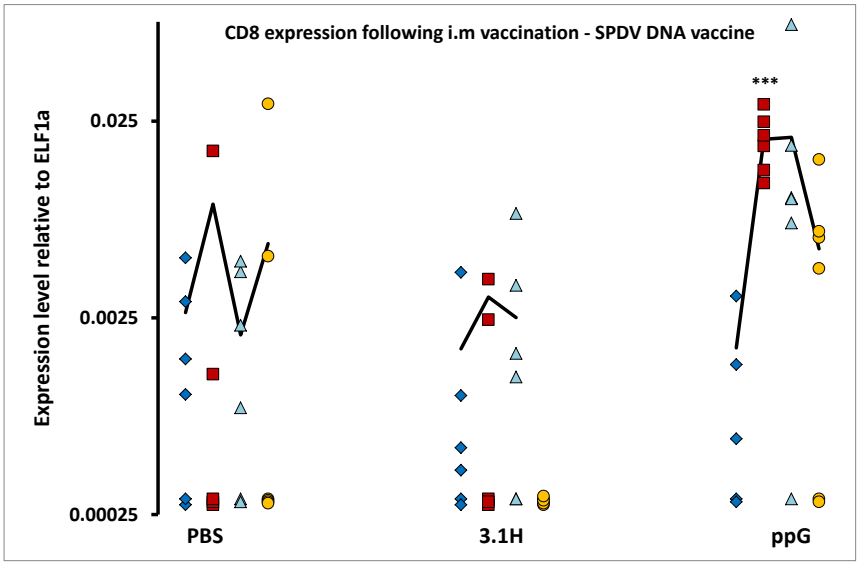
**Vaccination – (no challenge)**
50g parr salmon; i.m. injection; samples (n=6) at 3, 7, 11, (smoltification) 46 dpv.

**Site of ppG intramuscular injection**

- transcription of ppG observed
- induction of IFN type 1 in ppG group
- increase in CD8 marker in ppG group



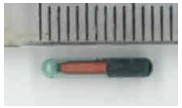

DPV 3 DPV 7 DPV 11 DPV 46

# Experimental Trials & Sampling

# Non lethal, same animal, sampling

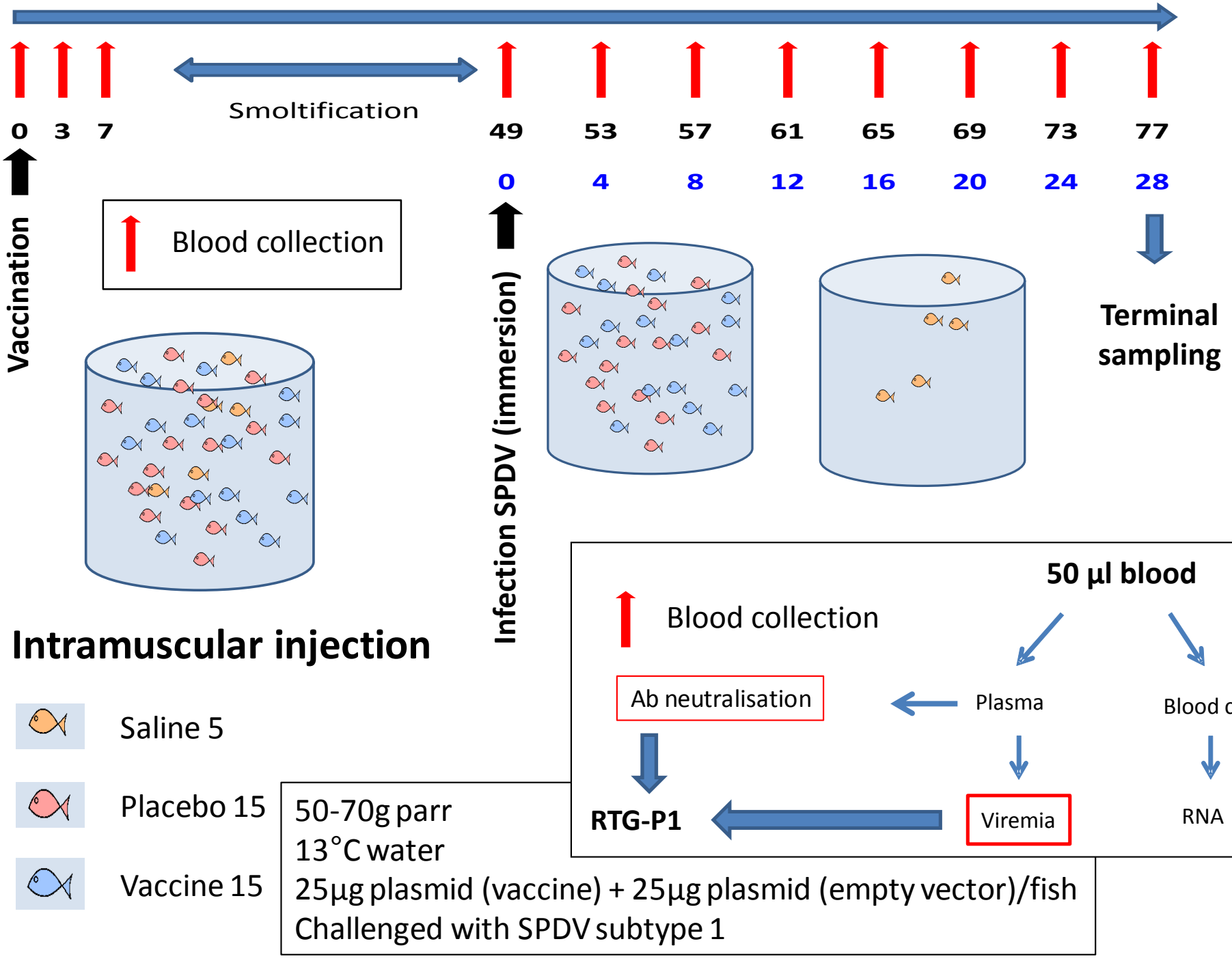
Individual tagging (PIT)

- PIT tag inserted into abdominal cavity



Repeated blood samples
1 µl/g fish every 4 days

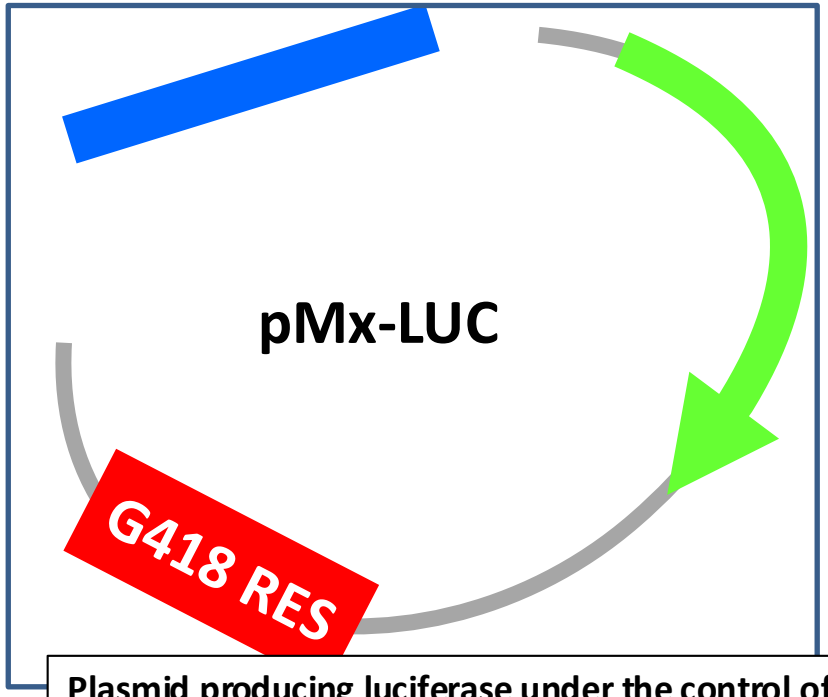
Syringe 0.3X12.7mm


Smoltification
0
3
7
49
53
57
61
65
69
73
77
0
4
8
12
16
20
24
28
Vaccination
Blood collection
Infection SPDV (immersion)
Terminal sampling
Intramuscular injection
Saline 5
Placebo 15
Vaccine 15
50-70g parr
13°C water
25µg plasmid (vaccine) + 25µg plasmid (empty vector)/fish
Challenged with SPDV subtype 1
50 µl blood
Blood collection
Ab neutralisation
Plasma
Blood cell
RTG-P1
Viremia
RNA

# RTG-P1 stable cell line: reporter cell-based assay for SPDV viraemia and Ab neutralisation test

Rainbow trout genome


Promoter region incorporated into plasmid, upstream of luciferase gene.




**Plasmid producing luciferase under the control of the rainbow trout *mx1* gene promoter**

- modified plasmid incorporated into reporter cell line
- when cell line exposed to virus, promoter is activated and in turn activates luciferase
- luciferase breaks down luciferin added to cells in assay
- break down of luciferin gives off luminescence
- luminescence indicates presence or replicating virus
- level of luminescence related to virus load

**Genetically engineered stable cell line RTG-P1 ATCC CRL-2829**

# Analysis

# Assessment of SPDV DNA vaccine efficacy

**Viraemia**
All time points

RTG-P1 cells were incubated for 14 days @ 14°C with individual plasma samples.

Luciferase activity was measured as estimation of virus levels.

**QPCR**
DPI 28/DPV 77

TaqMan quantitative PCR for nsP1 (Hodneland & Endresen 2006).
Pooled muscle and heart tissue homogenate.

**Histopathology**
DPI 28/DPV 77

| Tissue | Score | Description |
|---|---|---|
| Heart | 0 | Normal appearance |
| | 1 | Focal myocardial degeneration ± inflammation (<7 fibres affected) |
| | 2 | Focal myocardial degeneration ± inflammation (<15% of heart affected) |
| | 3 | Multifocal myocardial degeneration ± inflammation (>15 & <50% of heart affected) |
| | 4 | Severe diffuse myocardial degeneration ± inflammation (<50% of heart affected) |
| | R | Repair |
| Red & White skeletal muscle | 0 | Normal appearance |
| | 1 | Focal myocytic degeneration ± inflammation |
| | 2 | Multifocal myocytic degeneration ± inflammation |
| | 3 | Severe diffuse myocytic degeneration ± inflammation |
| | R | Repair |

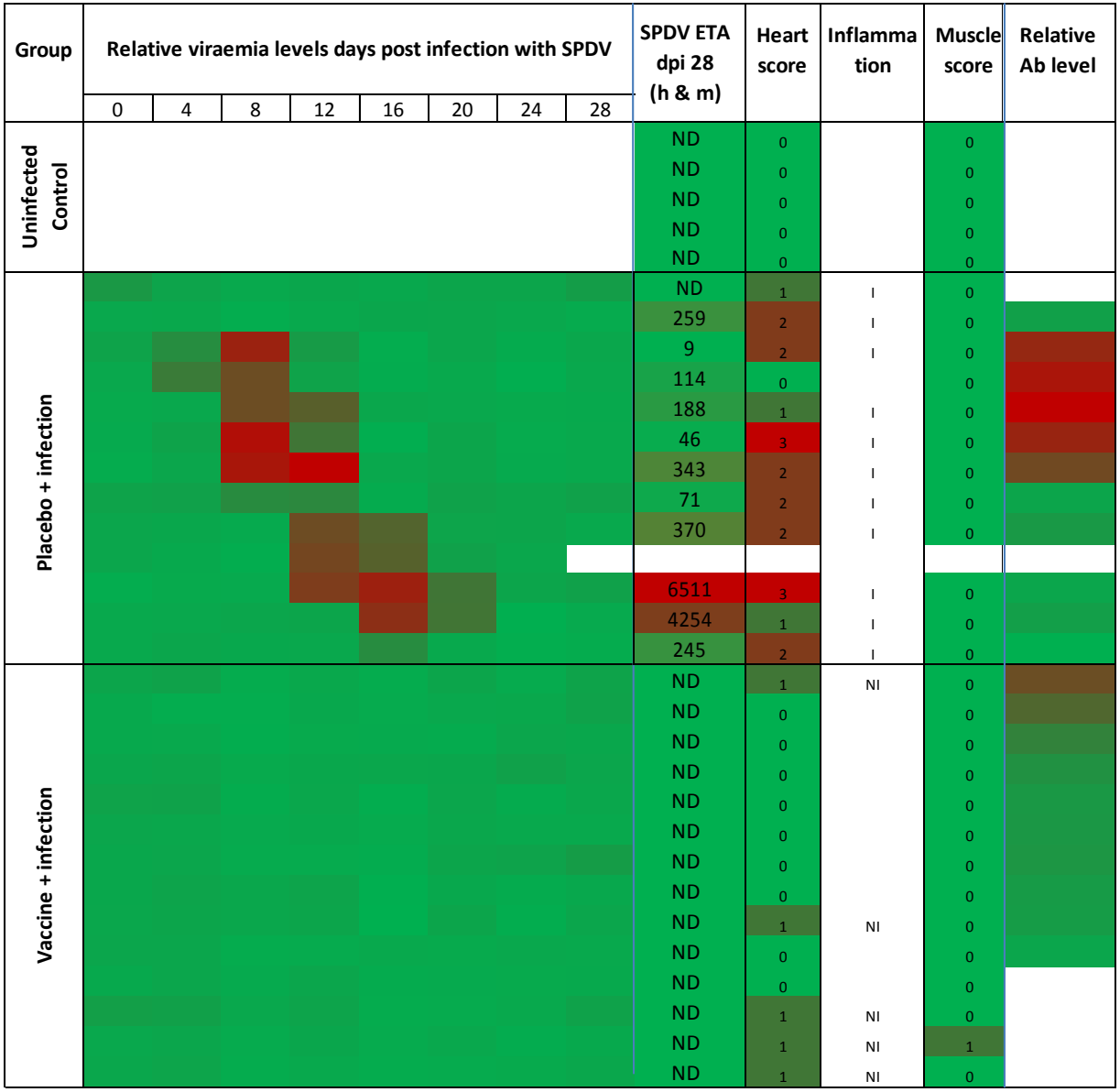
Graham et al., J. Fish Diseases 2011, 34, 273-286

**Ab neutralisation**
DPI 28/DPV 77

RTG-P1 cells were incubated for 7 days @ 14°C with SPDV in the presence or absence of individual plasma samples.

Luciferase activity was measured and the neutralisation levels were estimated.

# Results

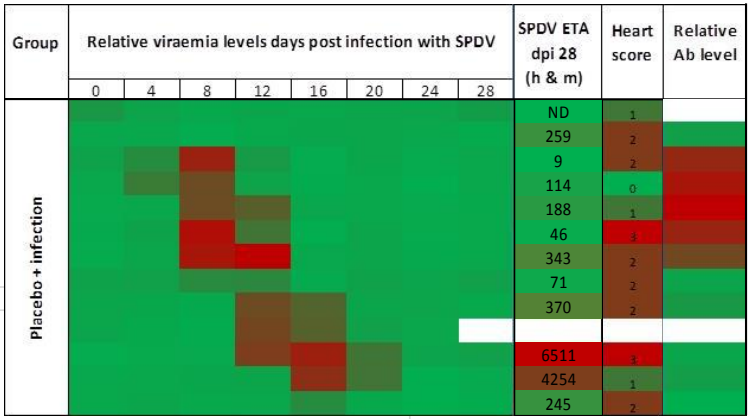


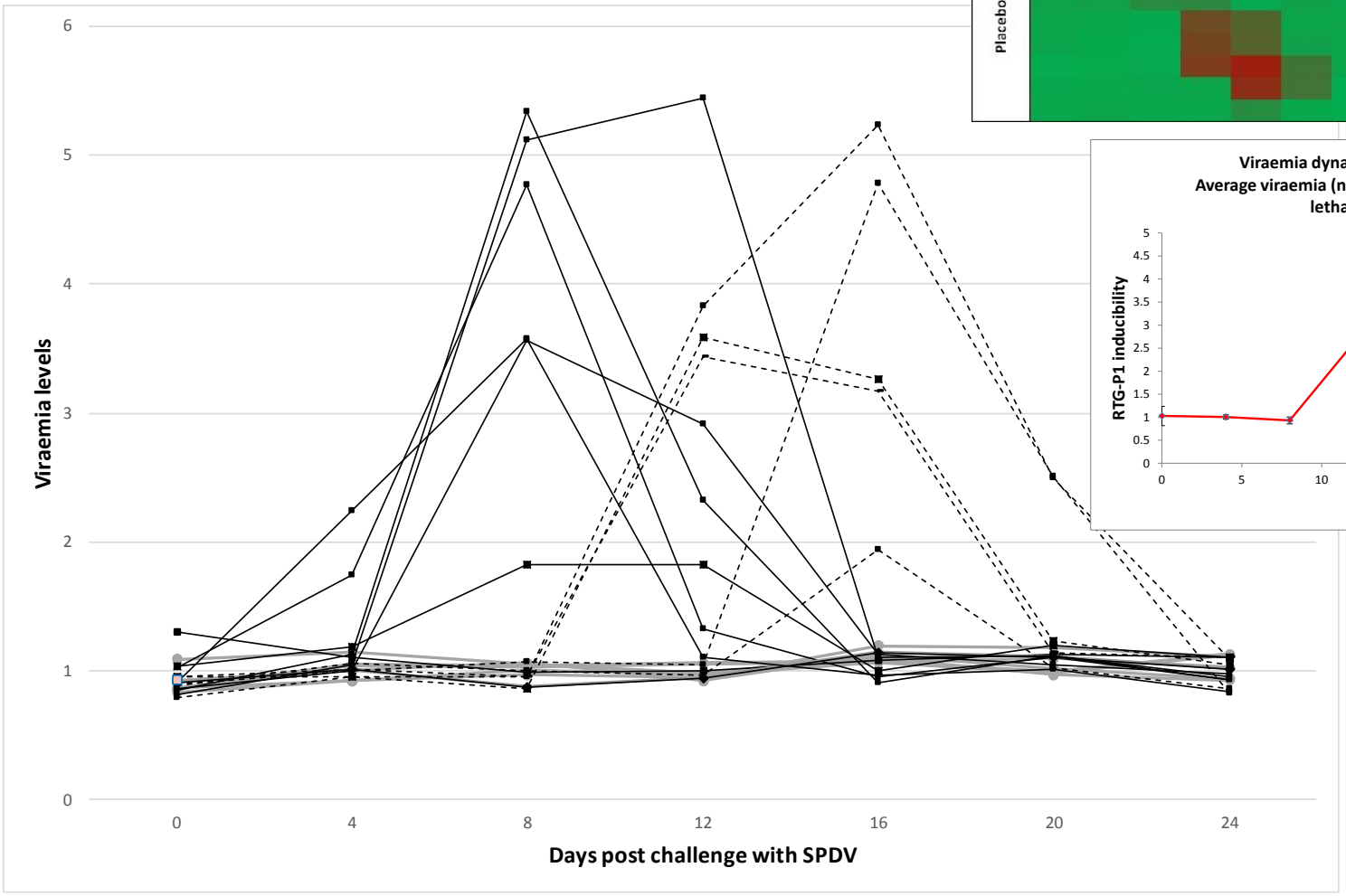

**I**: inflammatory; **NI**: non-inflammatory; **ND**: not detected

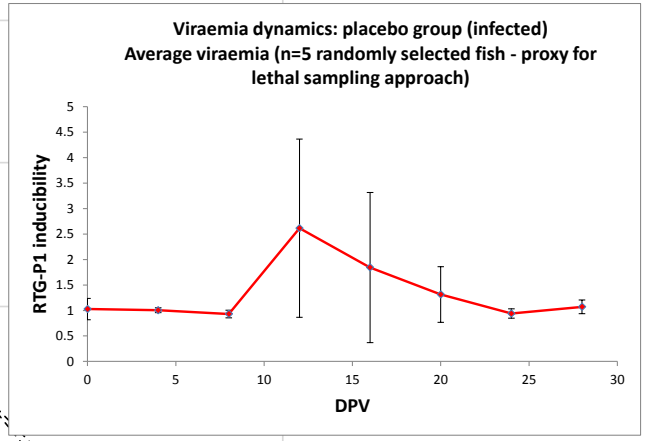
# Differential Infection Dynamics & Response in Challenged Fish

# Viraemia: inter-individual fish variation

- early viraemia
- late viraemia
- no observed viraemia



# DNA Vaccine Safety

# DNA vaccine safety

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

**2016**

recommends marketing authorisation for CLYNAV: Salmon pancreas disease vaccine (recombinant DNA plasmid)

Report sent

efsa
European Food Safety Authority

mandate to assess new data. Dec 2016 expected response

Discussion with EFSA in 2016

- very little data on which to consider safety
- main concern is integration of full plasmid or plasmid fragments into fish genome
- previous data did not sufficiently address plasmid fragments

**- all additional data welcomed to support decisions**

# Analysis of Integration Events

## Elimination of free plasmid

Electrophoresis

↓

Long/circular PCR to detect remaining intact free plasmid

## Targeted enrichment of integrated plasmid

DNA : site of DNA vaccination

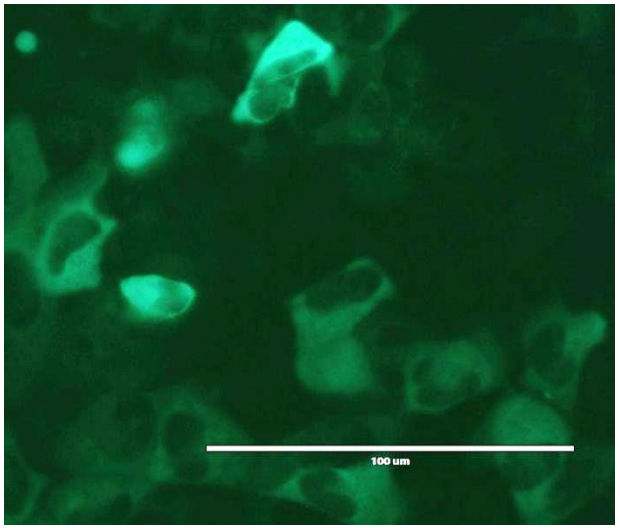
↓

Probe hybridisation Bead capture, Post capture amplification

↓

Sequencing illumina/PACBIO

## Limit of detection of method




### Stable cell lines: CHSE-ppG

- 100% ppG plasmid integration
- cell DNA sheared to generate small fragments – plasmid fragments
- used to spike host DNA – low-high

# Summary

**DNA Vaccine**
(within the conditions tested)

- Complete suppression of viraemia due to DNA vaccine
- No propagation of virus detected in target organs
- No myocardial degeneration nor inflammation in heart tissue

**Challenge**

- Sea water bath immersion challenge (more natural/controlled)

**Sampling**

- Non-lethal sampling highligths differences in infection dynamics/response between individual fish
  - better interpretation of findings/predicted outcome
  - selection of fish for response type

**Basis for Protection**

- Evidence for IFN type 1 and CD8 response to vaccine
  - further gene analysis ongoing
- Ab involvement in protection uncertain
  - analyse earlier plasma samples from ppG and placebo gps

# Acknowledgements

**Aquarium staff**
Mark Paterson

**Statistical advice**
Malcolm Hall

**Assistance with sampling and sample processing**
Louise Feehan
Mark Fordyce
Carina Duarte

**Assistance with sample analysis**
Mercedes Reguera

**Thank you for listening**



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