Trialling the use of a portable Real-Time PCR machine for onsite rapid detection of pathogens in Atlantic salmon aquaculture.

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DRIVERS:

- In 2019, looked into the possibility of doing rapid in house PCR testing for pathogens.
- at the time it could take up anything up to 8 days to get results and post wasn't always reliable.
- Speed up the turn around time on sampling to results
- Get an instant picture/indication of health status on site before potential handling & husbandry procedure.

- COVID-shortage of machines so it was put on the backburner.

- Re-visted in 2022 and, with the assistance of BIM, purchased a Genesiq q16 Real-Time PCR Instrument to investigate the suitability and practicalities of implementing it’s use in salmon aquaculture.

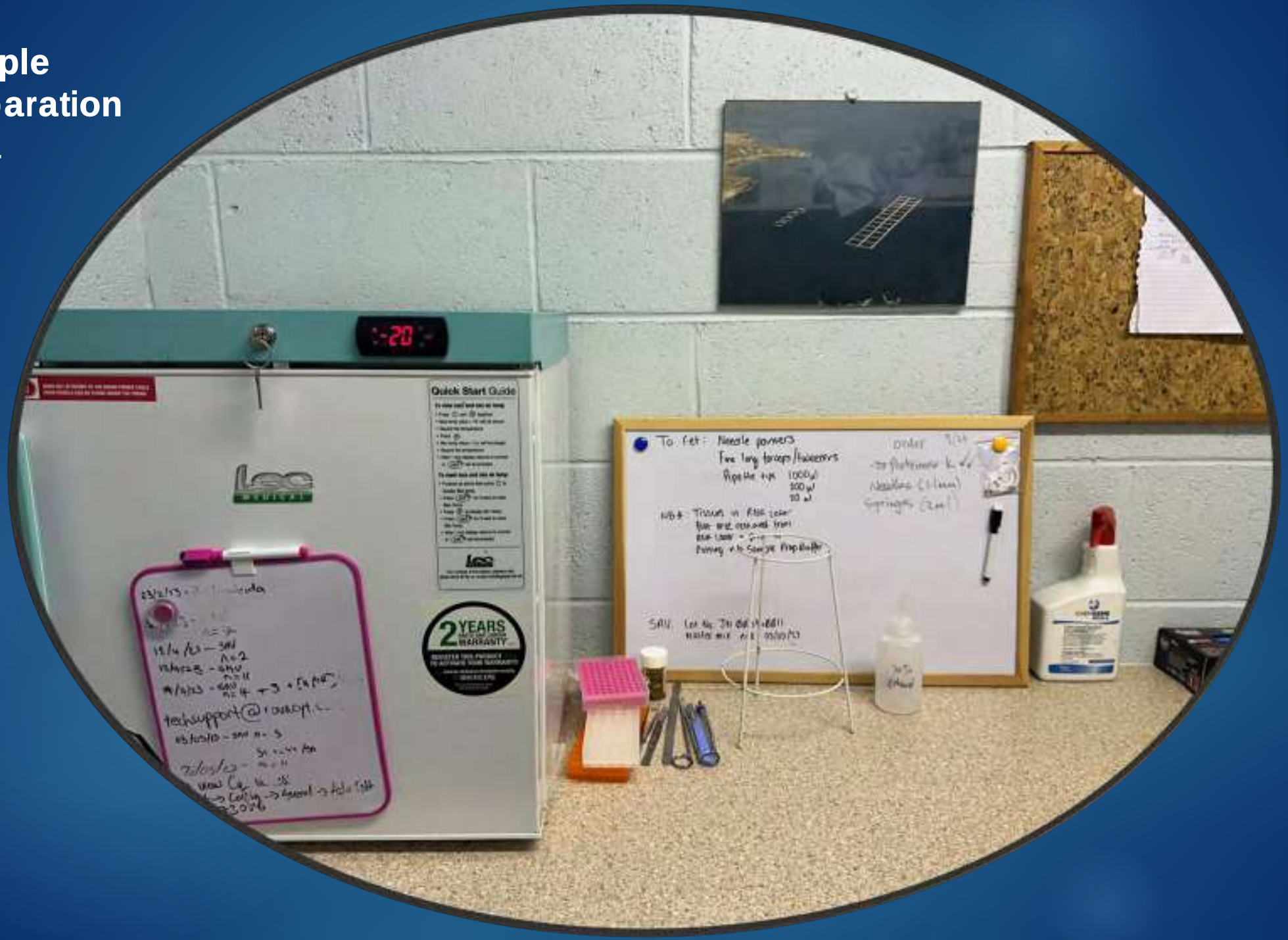
genesig® q16

- Making real-time PCR **accessible** to everyone, everywhere
- Small, **portable**, silent
- genesig **EASY** kits are optimised for q16 – with universal cycling conditions
- **Fast** – Template to result in c100min
- **Automatic** data analysis and **interpretation**
- **Technical training and support**

SET UP LAB WITH DEDICATED AREAS FOR:

- Sample preparation
- DNA/RNA Extraction
- Sample Analysis
- -20 deg.C. Freezer
- Doesn't have to be a big space, as long as each area is kept separate with it's own equipment.
- NO CROSS CONTAMINATION BETWEEN EACH AREA AND EQUIPMENT

Sample preparation area



Extraction area

Analysis Area

TRAINING

- Technical support team came over for 2 days training at end of Feb.2023 and the follow up technical support has been excellent.
- Extraction and sample kits are very simple to use:
- Bottles are colour coded and there is an easily followed step by step procedure

1.4 Quick guide – DNA/RNA extraction

See main body of handbook for detailed instructions of use, tips and troubleshooting

genesig Easy DNA/RNA extraction			
Step		Lab-in-a-box pipette	
1	Combine: • 200µl sample • 20µl Tube 1 • 5µl Tube 8* • 200µl Tube 2 • 10µl Internal extraction control DNA/RNA†		Shake Wait 15 minutes
2	Add • 500µl Tube 3		Shake Wait 5 minutes Magnetise! Remove all liquid
3	Add • 500µl Tube 4		Shake Wait 30 seconds Magnetise! Remove all liquid
4	Add • 500µl Tube 5		Shake Wait 30 seconds Magnetise! Remove all liquid
5	Add • 500µl Tube 6		Shake Wait 30 seconds Magnetise! Remove all liquid Air dry for 10 minutes with the lid open
6	Add • 200µl‡ Tube 7		Shake Wait 30 seconds Magnetise!
DNA/RNA is in the liquid!			

* Addition of Carrier RNA is only required if extracting RNA from your sample.
† If using a standard or advanced genesig kit instead of EASY genesig kit, use 4µl of the internal extraction control template.
‡ Between 50µl and 200µl of elution buffer can be used, see full protocol for details.

TRAINING

- Spent a day with Sam and Steph in the Marine Institute Fish Health Unit, involving sample preparation, extraction, PCR reactions- Although methods were different, they advised on BEST PRACTICE for all processes

- Protocols needed to be adapted for sample types, preservation etc.

- Worked with Primer Design and MI to develop procedures and protocols specific to our needs

TESTING:

- Once satisfied with methods, commenced testing
- Initially tested for: SAV, *Aeromonas salmocida*, *Piscirickettsia salmonis*
- Took duplicate samples and results were cross referenced and validated by Pharmaq Analytiq and FHU.
- All 16 test runs were successfully validated with exception of one run for Rickettsisa in Sept.2023
- FHU got very low positives and Genesig q16 results were negative.

BACK TO THE DRAWING BOARD:

- Initial extraction kit/method may not be sufficient to break down the bacterial cell walls

- On discussion with the technical support group and FHU, a new extraction method was recommended.
- This involved using a thermomixer to provide thermal digestion, breaking down cell walls and releasing DNA/RNA.
- Again, worked with Sam and Steph to develop a new method for more sensitive detection.
- Started using this new method 12 months ago.

STEPS:

1.SAMPLE PRESERVATION

- Sample Type (heart, blood, kidney)
- RNAlater
- -20deg.C Freezer

2. SAMPLE PREPARATION

- Homogenise tissue
- Buffers
- Thermal digestion in the thermomixer for 3 hours

3. SAMPLE EXTRACTION

- Magnetic beads/buffer to trap DNA/RNA
- Multiple washing to remove contaminants
- Elution buffer to elute highly pure DNA/RNA
- Can be analysed straight away or frozen for later analysis

STEPS (cont):

4. PCR ANALYSIS

- Add in master mixes/primer/probe mixes
- Can run 14 samples plus positive and negative controls
- Put in machine
- Set up the programme on the computer
- Wait for 100 minutes

5. RESULTS

- Quantitative
- Alerts for failed samples

genesig q16 PCR software - 2.10

Open Experiments: Rickettsia analysis 2024-09-04 16.38.ppf (Rickettsia analysis 2024-09-04 16:38) | New | Open | Save | Save As... | Close | Configuration

GENESIG

Stages: Setup | Results

Notes | Details

Name and Details

Rickettsia analysis 2024-09-04 16:38

Kit type: genesig® Easy Target Detection kit
Instrument Id.: NPW-FC9-P6P
Run Completion Time:

Notes

R10 Moribund fish hanging around the edge
Sampled 03/10/2024
Kidneys and lesions analysed for Rickettsia.
Mag DNA extraction-2hr digestions
Samples from same fish: 6950 & 6949
6948 & 8961
8960 & 8959
6942 & 6943
6941 & 6940
8953 & 8952

Lot No. JN00383-0011 exp. 1/11/25

Samples

Color	Name	Note
	PCR 6950 lesion	
	6949 kidney	
	6948 lesion	
	8961 kidney	
	8960 lesion	
	8959 kidney	
	6942 lesion	
	6943 kidney	
	6941 lesion	
	6940 kidney	
	8953 lesion	
	8952 kidney	
	8951 kidney	
	control	

Tests

Color	Name	Note
	R10 moribund	

Well Contents

Pos.	Test	Sample
1	R10 moribund	Negative Control
2	R10 moribund	Positive Control
3	R10 moribund	PCR 6950 lesion
4	R10 moribund	6949 kidney
5	R10 moribund	6948 lesion
6	R10 moribund	8961 kidney
7	R10 moribund	8960 lesion
8	R10 moribund	8959 kidney
9	R10 moribund	6942 lesion
10	R10 moribund	6943 kidney
11	R10 moribund	6941 lesion
12	R10 moribund	6940 kidney
13	R10 moribund	8953 lesion
14	R10 moribund	8952 kidney
15	R10 moribund	8951 kidney
16	R10 moribund	control

Run

Run Status

End of experiment.

Show full log...

Run Control

Abort Run | Start Run

genesig q16 PCR software - 2.10

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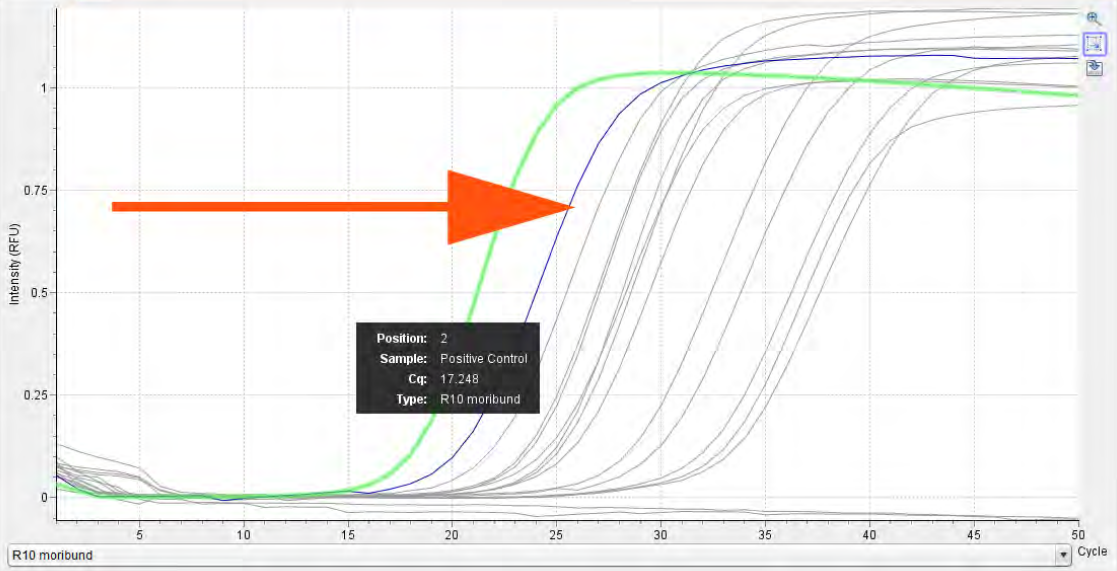
GENESIG

Stages: Setup | Results

Summary | Details



Notes

Sample Results | Details

#	Test	Sample	Status	Copy Number	Cq (Test)	Cq (I.C.)
1	R10 moribund	Negative Control	PASS	n/a	n/a	n/a
2	R10 moribund	Positive Control	PASS	n/a	17.25	17.26
3	R10 moribund	PCR 6950 lesion	POSITIVE	190,600	19.64	25.72
4	R10 moribund	6949 kidney	POSITIVE	10,293	23.85	17.78
5	R10 moribund	6948 lesion	POSITIVE	4,532	25.03	19.48
6	R10 moribund	8961 kidney	POSITIVE	249	29.22	21.44
7	R10 moribund	8960 lesion	POSITIVE	19,552	22.92	20.10
8	R10 moribund	8959 kidney	POSITIVE	7,242	24.36	22.26
9	R10 moribund	6942 lesion	POSITIVE	8,959	24.05	22.50
10	R10 moribund	6943 kidney	POSITIVE	29	32.33	22.99
11	R10 moribund	6941 lesion	POSITIVE	65,709	21.18	22.64
12	R10 moribund	6940 kidney	POSITIVE	19	32.91	23.49
13	R10 moribund	8953 lesion	POSITIVE	22,505	22.72	22.56
14	R10 moribund	8952 kidney	POSITIVE	48	31.58	24.07
15	R10 moribund	8951 kidney	POSITIVE	620	27.90	24.38
16	R10 moribund	control	NEGATIVE	n/a	n/a	22.85

Export...

OUTCOMES-

- Turn around time-from sampling to results = 12 hours
- Allows for independent screening of pathogens
- Instant results, particularly during periods of high demand on external laboratories
- Provides instant information directly to site managers who can then make decisions on fish husbandry practices, whilst prioritising fish health and welfare.
- Eg. If PD is suspected, can test for SAV – plan fish handling accordingly (although it can only test for the virus; not antibodies); still need to send away serology samples.

NOT A DIAGNOSIS!!!!

- disclaimer from suppliers that “this product is developed, designed and sold for research purposes only”
- Rickettsia need a diagnosis from a vet, however, this gives a heads-up and a head start on the lengthy time it takes to secure therapeutic feed mixes.
- A new rapid extraction method is now available, which may reduce turnaround time by several hours. Plan to trial summer 2026.

Thanks to
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