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Version 1

Non-invasive Detection Method for *Bonamia ostreae* in *Ostrea edulis* using the Franklin qPCR machine V.1

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We use this protocol and it's working

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Abstract

This is a comprehensive protocol for using the Franklin qPCR (Biomeme Inc.) machine to detect the presence of *Bonamia ostreae* parasite in European flat oyster (*Ostrea edulis*). Previous methods relied on sacrificially dissecting animals to perform histology. While sensitive, these methods were destructive and untested animals carrying *Bonamia ostreae* are not detected. Here, we present a non-invasive alternative method to detect *Bonamia ostreae* from *Ostrea edulis* pseudofaeces/faeces using a portable qPCR machine.

Materials

1. Container to hold oysters
2. Aeration tubing, airstones, and pumps
3. Pasteur pipette
4. 1.5 ml Eppendorf tube for sediment
5. Lysing matrix A bead-beating tube
6. qPCR tubes
7. Biomeme M1 Sample Prep Cartridge Kit
8. Franklin qPCR machine
9. 1uM pre-filter
10. qPCR primers (*B.ostreae*, *O.edulis*)
11. Taqman qPCR probe (*B.ostreae*, *O.edulis*)
12. 1uM Barb column filter
13. 1ml syringe
14. Bleach



Sample Collection

18h

- 1 Collect oysters and wash to remove any excess mud/sediment on their shells.
- 2 Separate collected animals and cover with seawater (approx.  1 L for every  200 g of live animal).

Note

- 1) The volume of seawater used is a ballpark figure, which should allow for quite a bit of variation.
- 2) Ensure that animals from separate testing sites are held separately, with strict bio-security protocols in place to prevent cross contamination.
- 3) Ensure animals are aerated and kept in a place with minimal disturbance to ensure they filter and produce faeces.

- 3 Aerate buckets overnight for  16:00:00 at ambient temperature on sampling location (see Figure 1).

16h



Figure 1: Set up for incubation process

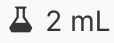
- 4 Collect the sediment remaining in the bottom of each bucket (faeces and pseudofaeces) using a Pasteur pipette into a  1.5 mL Eppendorf tube.

Note

- 1) Allow the sediment to settle for  00:05:00 minutes before removing the excess supernatant.
- 2) Leave approximately  1 mL ml of sediment with some water to aid in the transferring process when using a Pasteur pipette.

- 5 OPTIONAL: Store samples collected at  -20 °C until further use.
- 6 Disinfect all equipment with working strength bleach according to biosecurity SOPs.

DNA Extraction

- 7 Extract DNA from the sediment samples by physical beating in  **2 mL** **Lysing matrix** **A** tubes and filter through a 1 μ M barb column filter.
- 8 Use a syringe with a 1 μ M pre-filter to remove the supernatant without debris.
- 9 Make two holes in the first M1 chamber and transfer sample into the Biomeme M1 cartridge.
- 10 Process filtered sediment through the Biomeme M1 extraction cartridge (see Figure 2), **according to the manufacturer's protocol (refer to link below)**.

[M1 Sample Prep Cartridge Techniques on Vimeo](#)



Figure 2: Biomeme M1 extraction cartridge

On-site Franklin qPCR assay

1m 20s

- 11 Store DNA at  **-4 °C** until further use.
- 12 Transfer  **20 μ L** of extracted DNA to Biomeme Go-Strip assay tube (see Figure 3).



Figure 3: Transferring extracted DNA into Biomeme Go-strips assay tube

Note

The qPCR mastermix consists of: $\text{20 } \mu\text{L}$ sample, $\text{1.8 } \mu\text{L}$ primers, $\text{0.5 } \mu\text{L}$ *B.ostreae* probe, $\text{0.45 } \mu\text{L}$ *O.edulis* probe, and $\text{1.85 } \mu\text{L}$ nuclease free water.

Note

The three assays consist of *B. ostreae* (Marty et al., 2006) to assess for presence of the pathogen, *Ostrea edulis* (Sanchez et al., 2014) to check the quality of the DNA extraction, and a technical internal positive control (IPC) assay (provided by Biomeme Inc) to ensure the reaction has worked.

- 13 Follow the instructions on connecting the qPCR machine to the phone using the Biomeme app. Choose the LyoDNA test (Figure 4) and follow the on screen instructions.

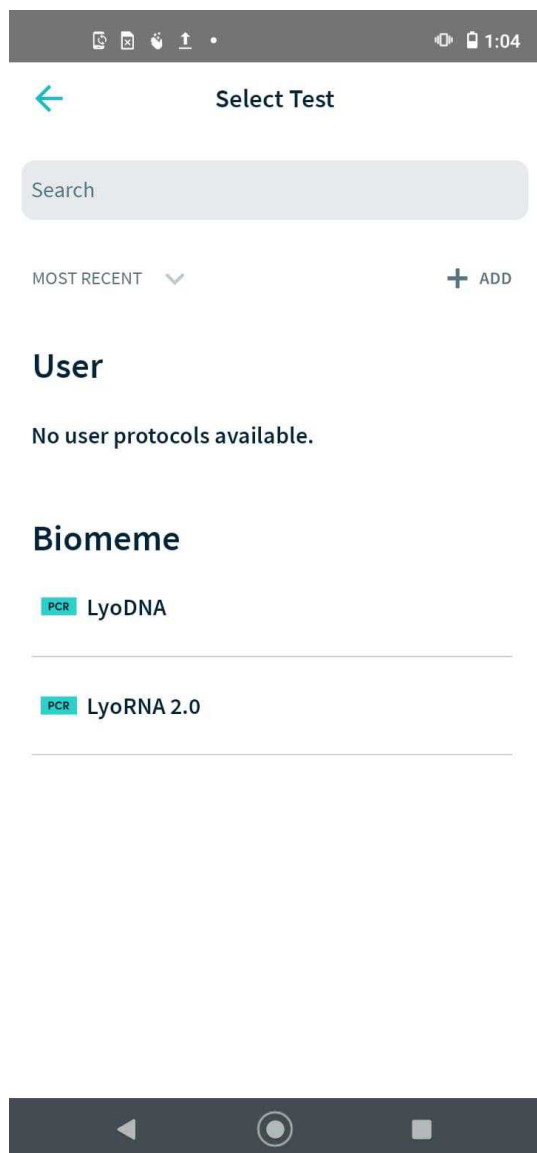


Figure 4: Test Selection on the Biomeme App

- 14 Place the Biomeme Go-Strip assay tubes into the Franklin device in the correct orientation (see Figure 5).



Figure 5: Biomeme Go-Strip assay tubes placed in the correct orientation according to instructions

- 15 Run a qPCR assay using the Franklin qPCR machine against the three probe-based assays. Primers and probes used in this assay is given below (Table 1).



Note

	A	B	C	D
	Name	Designation	Primer Sequence 5'-3'	Reference
	Bon18S_F_Marty	Forward Primer	CCCGGCTTCTTAGAGG GACTA	Marty et al (2006)
	Bon18S_F_Marty	Reverse Primer	ACCTGTTATTGCCCCA ATCTTC	
	Bon18SFAM_Marty	Probe	FAMCTGTGTCTCCAGC AGAT-BHQ1	
	OEDU16S_F	Forward Primer	GGCGCCCCACCTAAAA AT	Sánchez et al (2004)
	OEDU16S_R	Reverse Primer	AGACCCCGTGCAACTT TTAAAG	
	OEDU16S_P	Probe	[TxRd]TGAAACTCCTAA ACAAGTTG[BHQ2]	

Table 1: Real-time qPCR assays used

- 16 The Franklin qPCR protocol consisted of  00:01:00 minute heat activation and 45 cycles of  95 °C for 1 second and  60 °C for  00:00:20 .
- 17 Use the Biomeme app on the phone to analyse the qPCR results to determine the presence of *Bonamia ostreae*. Refer to Figure 6 for an example of positive *Bonamia ostreae* presence.

1m 20s

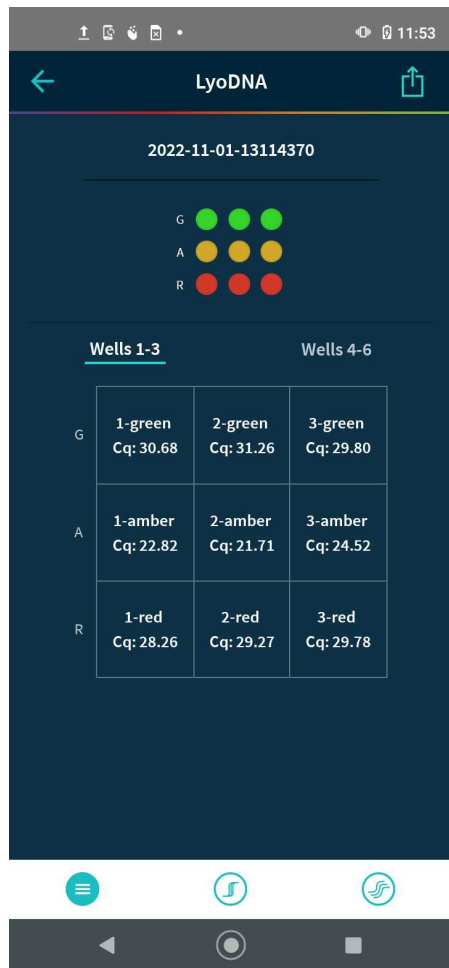


Figure 6: The green dots and the Cq values in the first row indicate positive *Bonamia ostreae* detection (based on a Cq value cut-off point of 35)

18

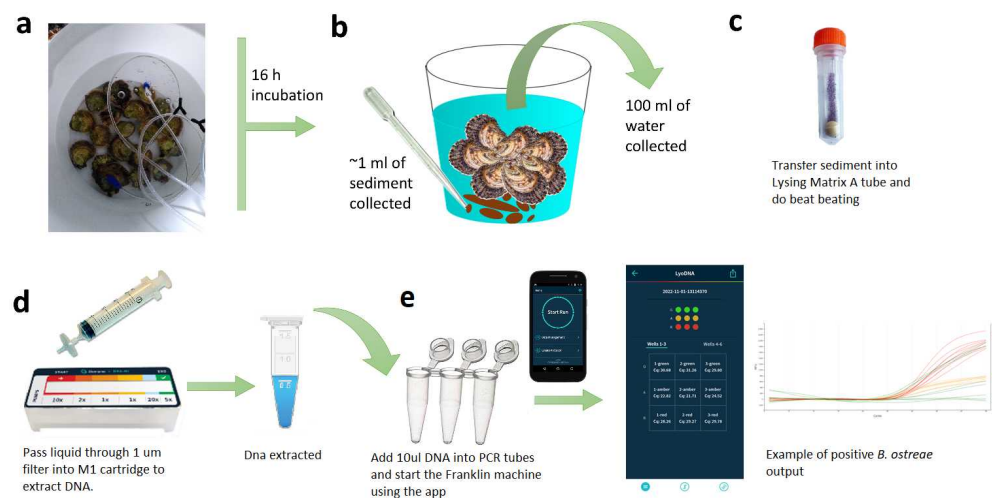


Figure 7: Schematic of *Bonamia ostreae* detection process

