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

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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

Keywords: Bonamia ostrea, Ostrea edulis, qPCR, Real-Time PCR, Environmental DNA, in-situ detection

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

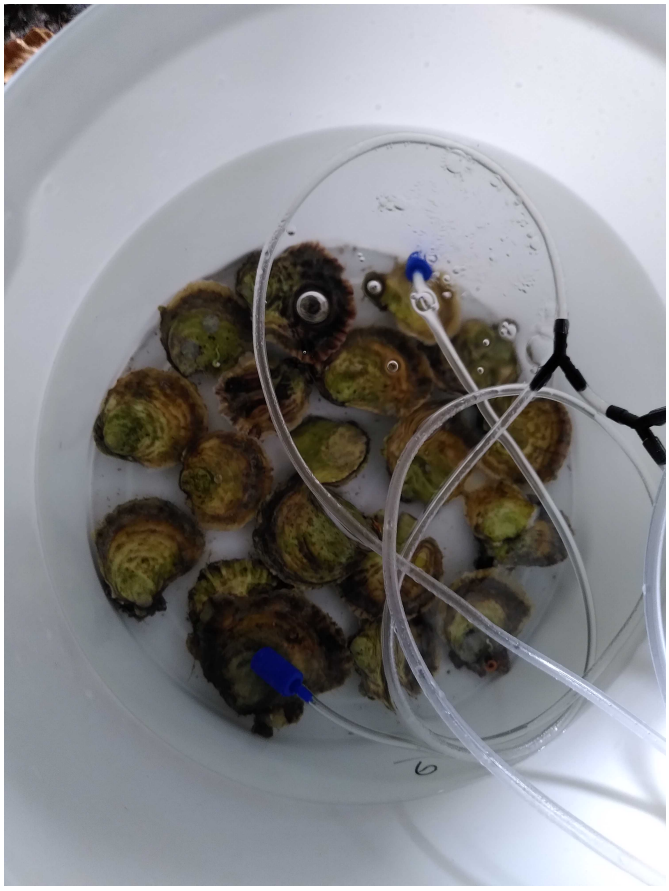

Figure 1: Set up for incubation process

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 uM barb column filter.

8 Use a syringe with a 1 uM 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 µL of extracted DNA to Biomeme Go-Strip assay tube (see Figure 3).

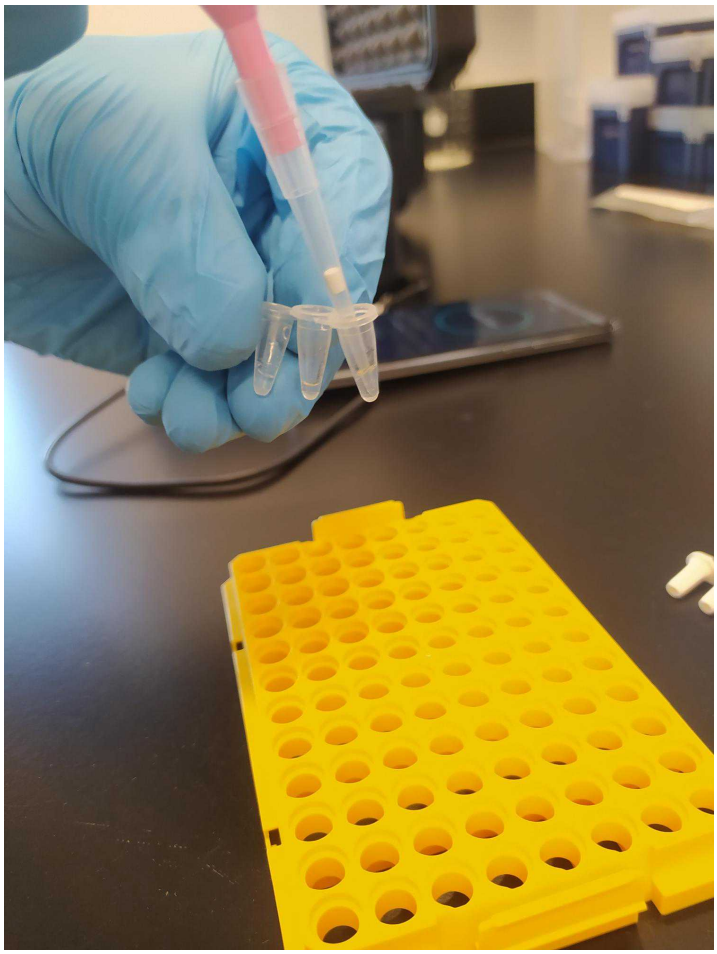

Figure 3: Transferring extracted DNA into Biomeme Go-strips assay tube

Note

The qPCR mastermix consists of: 20 µL sample, 1.8 µL primers, 0.5 µL *B.ostreae* probe, 0.45 µL *O.edulis* probe, and 1.85 µL 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.

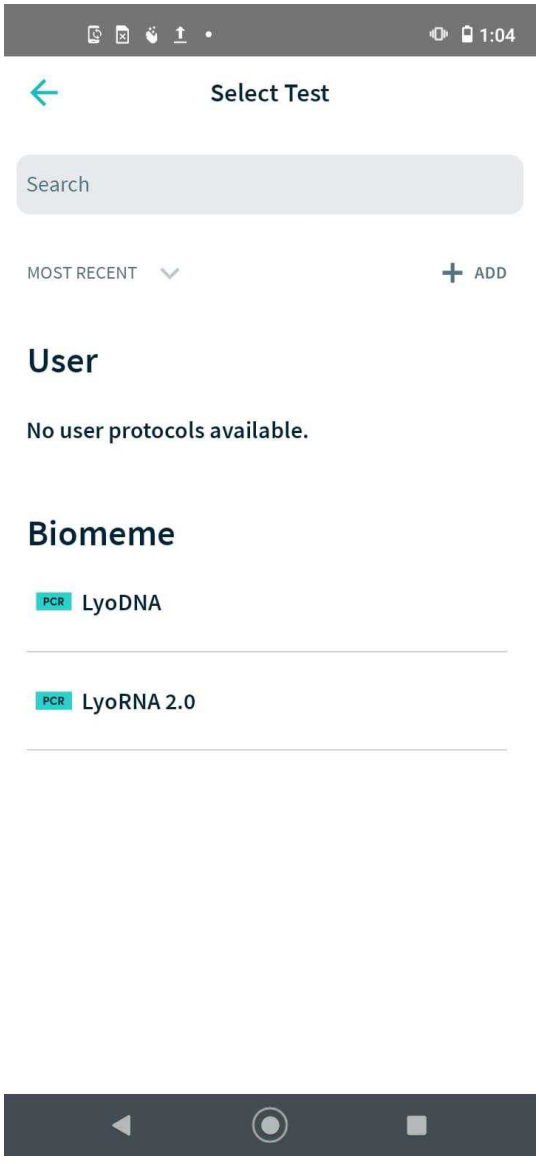

Figure 4: Test Selection on the Biomeme App

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	CCCGGCTTCTTAGAGGGACTA	Marty et al (2006)
	Bon18S_F_Marty	Reverse Primer	ACCTGTTATTGCCCCAATCTTC	
	Bon18SFAM_Marty	Probe	FAMCTGTGTCTCCAGCAGAT-BHQ1	
	OEDU16S_F	Forward Primer	GGCGCCCCACCTAAAAAT	Sánchez et al (2004)
	OEDU16S_R	Reverse Primer	AGACCCCGTGCAACTTTTAAAG	
	OEDU16S_P	Probe	[TxRd]TGAAACTCCTAAACAAGTTG[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 1m 20s 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.

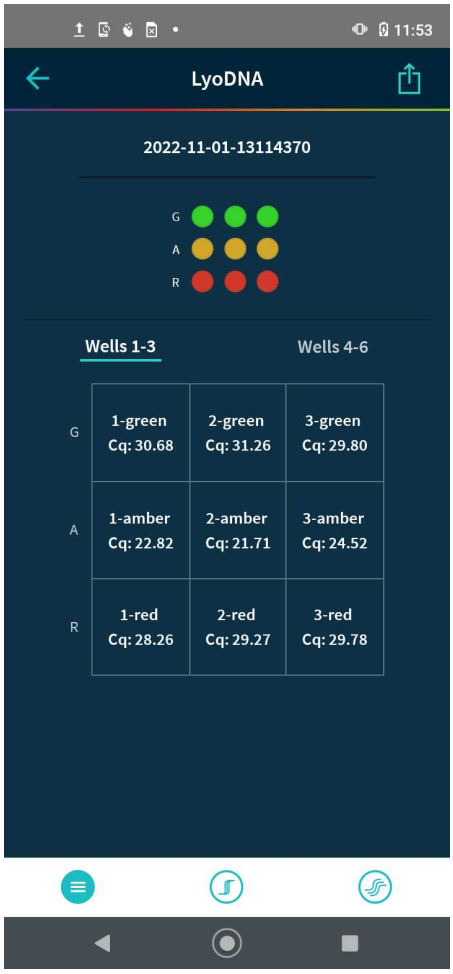

Figure 6: The green dots and the Cq values in the first row indicate positive *Bonamia ostreae detection (*based on a Cq value cut-off point of 35)

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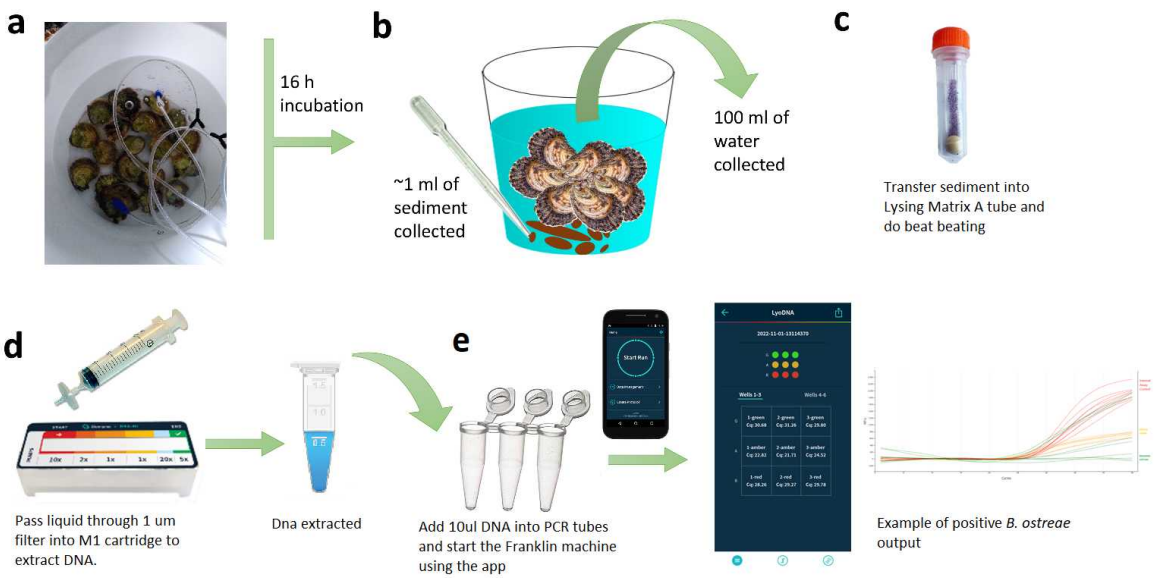

Figure 7: Schematic of *Bonamia ostreae* detection process