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TaqMan qPCR INRAE eWHALE

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for the TaqMan assay used by INRAE team in eWHALE ring test study

Materials

- Pipettes : monochannel p20, p100, p200, p1000, multichannel p20 and corresponding filter tips
- 1.5 mL microcentrifuge tubes

Before start

Lab work:

Put all equipment (micropipettes, disposable tips, microtubes, molecular grade water, qPCR plate) in UV cabinet and

light UV for 20 mins.

Thaw, vortex and spin samples.

Thaw and vortex Master Mix environnemental TaqMan™ 2.0.



Master mix preparation

- 1 Determine the number of reactions, including negative controls and NTCs. Add 2-3 reactions to have margin for pipetting error.

Prepare the Master mix according following this volume for 1 reaction :

4 μL of Molecular Grade Water

10 μL Master Mix environnemental TaqMan™ 2.0

1 μL Probe at [M] 0.13 micromolar (μM)

1 μL Forward primer at [M] 0.5 micromolar (μM)

1 μL Reverse primer at [M] 0.5 micromolar (μM)

Vortex and spin.

Plate preparation

2m

- 2 Add 17 μL of Master mix.

2m

Add 3 μL of Sample .

Seal the plate with qPCR plate seal and centrifuge briefly (00:02:00 at 2000g).

qPCR

11m 20s

- 3 Put the plate in the thermal cycler. qPCR is performed with an initial denaturation at 95 °C for 00:10:00 followed by 49 cycles of 00:00:30 at 95 °C and 00:00:50 at 60 °C .

11m 20s