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HotSHOT genomic DNA extraction V.2

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FishFloorUCL[1]

[1]University College London

FishFloorUCL

Francois Kroll
Sorbonne Université

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Abstract

How to extract genomic DNA from larvae or finclips using the HotSHOT method.

Materials

1 Prepare the BASE stock solution (50X):
- 14.03 g KOH crystals (1.25M final concentration)
- 4 mL of 0.5M EDTA (10 mM final concentration)
- ddH2O to 200 mL total volume

Can be stored at room temperature for up to four years

2 Prepare the NEUTRALISATION stock solution (50X)
- 63.04 g Tris-HCL (2M final concentration), also called Trizma HCl
- ddH2O to 200 mL total volume

Can be stored at room temperature for up to four years

Procedure

30m

3 Prepare fresh 1X BASE and 1X NEUTRALISATION solution in nuclease-free H2O.

4 Transfer finclips or culled larvae to individual wells of a 96-well plate.

Note

(Finclips) Transfer directly to a 96-well plate during fin-clipping
(Larvae) After culling:
- Pipette technique: Use a pipette set to 5µL to transfer larvae with as little fish water as possible
- Forceps technique: Transfer individual larvae using blunt round-ended forceps. This technique is only possible if you have already loaded the plate with 50µL 1X BASE solution (step 5)

5 Add 50 µL of 1X BASE solution into each well of the plate

6 Seal the plate and place on PCR block 95 °C 00:30:00

30m

7 Cool at Room temperature

8 Add 50 µL of 1x NEUTRALISATION solution into each well of the plate.

9 Note, the extracted DNA concentration is often *too high* for downstream applications like PCR or KASP.

Storage

10 Store at 4 °C if you will use the DNA in the next few weeks. Beware, the samples will slowly evaporate from a sealed plate. Alternatively, -20 °C for long-term storage. This will also prevent the samples from evaporating.