HotSHOT genomic DNA extraction

This protocol is published without a DOI.

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COMMENTS 0

DEC 10, 2022

WORKS FOR ME 1

ABSTRACT

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

PROTOCOL CITATION

FishFloorUCL 2022. HotSHOT genomic DNA extraction. **protocols.io** https://protocols.io/view/hotshot-genomic-dna-extraction-ckbhusj6

CREATED

Dec 09, 2022

LAST MODIFIED

Dec 10, 2022

PROTOCOL INTEGER ID

73801

Materials

1 Prepare the BASE 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

2 Prepare the NEUTRALISATION solution (50X)

- 63.04 g Tris-HCL (2M final concentration); also called Trizma HCl
- ddH2O to 200 mL total volume

30m

Procedure

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

4 If larvae: add larvae one per well with Pasteur pipette. Take up all the liquid using a P200 pipette.
If finclip: add finclip directly to well (this can be done while finclipping).

5 Add 50 µL of 1x BASE Solution into each well of the plate.

30m

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

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 sealed plate
or
-20 °C for long-term storage. This will also prevent the samples from evaporating.