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Version 1

HotSHOT genomic DNA extraction V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

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



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

Procedure

30m

- 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.
- 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.

30m

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.