HotSHOT DNA extraction

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Abstract

Rapid, cheap, and effective DNA extraction for PCR. Easily scaled up to 96-well plates. This originates with Truett et al (2000) PMID:10907076

This protocol is very well tested by many labs for subsequent PCR amplification. For PCR it does not matter that DNA also has protein, is in low concentration, and is fragmented. This is a very robust DNA preparation method.

It is not difficult to do 960 DNA extractions per day (10 plates), the major determinant is tissue handling.

Alkaline Lysis Solution (pH 12)	For 100 ml add
25 mM NaOH	25 ml of 100 mM NaOH
0.2 mM Na2EDTA	0.4 ml of 50 mM Na2EDTA (pH 8)
	74.6 ml of ddH2O

Neutralizing Solution (pH 5)	For 100 ml add:
40 mM Tris-HCl	630 mg of Tris-HCl
	100 ml of ddH2O

Notes:

- Prepare alkaline lysis solution fresh each day
- Neutralizing solution is stable at room temperature for long periods (months-years)
- Tris-HCL is NOT Tris-base
- The main cause of failure is too much tissue for the volume of alkaline lysis solution

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Protocol

Place tissue in alkaline lysis solution

Step 1.

Add 50ul of alkaline lysis solution into 0.2ml PCR tubes, or 96 well PCR plate, and add tissue.

Solutions are detailed in the Description section

The volumes of solution and tissue are the major determinant of how well your DNA extraction will work. Too much tissue is the most common cause of PCR failure following HotSHOT. Sub-visible amounts up to tissue the size of a full stop . try 25ul. Tissue the size of a 10pt Arial font o might need 75ul. Begin by trying a few tissue to solution ratios until you are familiar. Too much tissue is the most common cause of failure for HotSHOT beginners.

SAFETY INFORMATION

It is important to wear eye protection when dealing with extremely alkaline solutions

Heat tissue 95°C for 30 mins

Step 2.

Incubate at 95°C for 30 minutes (in a PCR machine).

DURATION

00:30:00

Neutralize

Step 3.

Add 50ul (an equal volume) of neutralizing solution

Store or use

Step 4.

Store at 4°C or -20°C for long periods. Use 1-5ul in a PCR reaction

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