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Phenol-chloroform DNA purification

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1 Works for me dx.doi.org/10.17504/protocols.io.re6d3he

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ABSTRACT

Because of the influence of salts on enzymatic reactions, Qiagen extractions can be purified before making RAD tags. First, it is necessary to perform a chloropane extraction (phenol / chloroform 50/50) by adding SDS to the aqueous phase (final conc. 0.1%), this eliminates contaminants introduced by the matrix of the column. Then, perform an ethanol precipitation followed by a wash with 70% ethanol. This is to eliminate the salt that was used to bind the nucleic acids to the column and also to remove EDTA (inhibitor!) which is 0.5 mM in the buffer AE. Eluting with water is nonsense as the pH is not optimal and thus yield is poor and the DNA is not buffered and there is always too much salt.

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MATERIALS

NAME	CATALOG #	VENDOR
Phenol-chloroform-isoamyl alcohol 25:24:1 (PCI)	15593049	Invitrogen - Thermo Fisher
EtOH 100% at -20ºC		
EtOH 70% at 4ºC		
20% Sodium dodecyl sulfate (SDS)		
Na acetate 3M pH 4.8 or 5.2		

Phenol-chloroform extraction

1 To 100ul eluted DNA, add 0.5 ul of 20% SDS (*this may not be necessary as there are no cells) and 100 ul of phenol-chloroform.

2 Vortex well.

3 Centrifuge at room temperature for 5 min, at full speed (14,000 rpm).

4 Pipette the aqueous phase (upper phase, aprox 80 ul, it is better to leave some DNA than to pipette phenol) to a new labeled tube.

5 Discard original tube.

The tube contains phenol-chloroform.

6 Proceed to ethanol precipitation.

Ethanol precipitation

7 Add 1/10 volume Na acetate 3M pH 4.8 or 5.2 (ie 8ul for 80 ul DNA solution)
add 2 volumes ETOH 100% (storage -20°C) = 176 ul

Total volume: 264ul, possible with 264 ng of starting DNA - otherwise, you must add a carrier: tRNA, glycogen or linear acrylamide.

8 Vortex.

9 Put on dry ice for 30 min. or overnight at -20°C

10 Centrifuge at 4°C for 30 min at 14,000 rpm.

11 Discard the supernatant.

12 Wash with 500ul EtOH 70% (storage 4°C)

13 Centrifuge at 4°C for 5 min at 14,000 rpm.

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14 Discard the supernatant

15 Quick spin

16 Pipette out the last drop of EtOH

17 Speed Vac for 3' or 5-7 min at room temperature.

18 Resuspend in 20ul of water ot Tris 10 mM pH 7.5 or 8.0

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