DIY Spin Column Protocol

Description

A spin-column DNA extraction method using homemade buffers and silica spin columns/plates from Epoch in the USA, costs around 20cents AUD per sample.

What you need

Spin columns 

or 96 well silica plates 

available from Epoch

Ethanol
Tris
EDTA
SDS
Ammonium Acetate
Proteinase K
RNAse
Clean water
96 well square deep plate (2ml) to collect waste Example
96 well round deep plate (1ml) to elute DNA into for storage Example

Protocol

It's easiest to do 200 individuals at a time in two 96 well plates. If you do this they stay balanced and you don't need to discard the flow through after each step.

96 well version

1. Homogenise tissue in the beadmill.
2. Mix 40ml of Lysis buffer and 400µl of Proteinase K (10mg/ml).
3. Add 200µl of Lysis buffer/ProK mix to each sample.
4. Incubate at 50^{o}C for 2hrs/overnight
5. Add 2µl RNAse (10mg/ml) to each sample
6. Incubate at 37^{o}C for 30mins
7. Add 200µl Binding Buffer and 200µl Ethanol to each sample
8. Tranfer all 600µl of Lysate Binding buffer and Ethanol to the 96 well silica plate (sitting on top of a 2ml deepwell plate)
9. Spin on high speed for 4 minutes
10. Dispose of flow-through and add 500µl of wash buffer
11. Spin on high speed for 2 mins
12. Discard flow through and add another 500µl of wash buffer
13. Spin on high speed for 15 mins to make sure membrane is dry
14. Platce the silica plate on the 1ml deep well plate for elution

15. Add 100µl of elution buffer to each well and incubate @ room temperature for 2 mins
16. Spin on high speed for 1 min

Individual columns

1. Homogenise tissue in the beadmill.
2. Add 200µl of Lysis buffer and 2µl ProK
3. Incubate at 50^{o}C for 2hrs/overnight
4. Add 2µl RNAse (10mg/ml) to each sample
5. Incubate at 37^{o}C for 30mins
6. Add 200µl Binding Buffer and 200µl Ethanol to each sample
7. Tranfer all 600µl of Lysate Binding buffer and Ethanol to the column/collection tube
8. Spin on high speed for 1 minutes
9. Dispose of flow-through (tip out of collection tube) and add 500µl of wash buffer
10. Spin on high speed for 1 mins
11. Discard flow through and add another 500µl of wash buffer
12. Spin on high speed for 1 min
13. Place the spin column on a new collection tube / eppie
14. Spin on high for 2 mins
15. Place the spin column on a clean eppie
16. Add 100µl of elution buffer to each column and incubate @ room temperature for 2 mins
17. Spin on high speed for 1 min

Recipes

Lysis Buffer (1 litre)

10mM Tris 2mM EDTA 1% SDS

1M Tris-HCl pH 7.5	10ml
0.5M EDTA	4ml
SDS (sodium dodecyl sulphate)	100ml of 10%
make up to 1L with clean H_2O	

Binding buffer (1 litre)

3M GuHCl 3.75M NH_4Ac pH 6

Guanidine Hydrochloride	573.18g
Clean H_2O	500ml
7.5M Ammonium Acetate	500ml
Adjust pH to 6 using glacial Acetic acid	

See HERE for notes on different binding buffer options.

Wash Buffer (1 litres)

10 mM Tris-HCl pH 7.5, 80% ethanol

1M Tris-HCl pH 7.5	10ml
96% ethanol	800ml
Clean H_2O	190ml

Elution Buffer (500ml)

10mM Tris-Cl, pH 8.5

1M Tris-HCl pH 8.5	5ml
Ultrapure DNAse and RNAse free H_2O	495ml

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