



ES cell genomic DNA purification in 96 well format

The protocol below is a quick purification of ES cell genomic DNA. The protocol uses a 4hr digestion of the ES cells, followed by isopropanol precipitation of the DNA.

Reagents (All reagents are from Sigma)

GenLysis Buffer

Stock concentration	volume (ml)	Final concentration
1M TrizmaHCl pH8	1ml	20mM Tris/HCL pH8
0.1M EDTA pH8	0.5ml	1mM EDTA
5M NaCl	0.05ml	5mMNacl
20% SDS	2.5ml	1.0%SDS
Igepal	0.5ml	0.1% Igepal
Tween-20	0.5ml	0.1% Tween-20
20mg/ml RNase A	1.0ml	100ng/ml RNase A
(stock made up in 1mM TrizmaHCl/15mM NaCl – store at -20°C)		
HPLC Water	43.95ml	

Store at 4°C

Proteinase K

Stock concentration	volume (ml)	Final concentration
20mg/ml Proteinase K	2ml	0.8mg/ml Proteinase K

(stock made up in TE/50% glycerol (1:1) – store at -20°C)

Add Fresh to GenLysis Buffer before addition to ES cells.

Protocol:

1. Add 50µl GenLysis buffer to each well of the plated PBS washed ES cells (if you have not added the Proteinase K to the GenLysis Buffer then add 2µl Proteinase K to each well)
2. Shake for 30sec using a plate shaker.
3. Centrifuge up to 50xg and stop
4. Seal the plate with a sticky seal and incubate at 55°C from 4 hrs to overnight in a sandwich box containing wet towels.
5. Remove the plates from the box and shake for 1min using a plate shaker.
6. Add 5µl 5M NaCl.
7. Shake for 30sec using a plate shaker.
8. Add 55µl 100% Isopropanol.
9. Shake for 30sec, using a plate shaker.
10. Transfer 130µl to a 96 well round bottom polypropylene plate.
11. Centrifuge for 10min at 2,800xg and 4°C
12. Incubate for 10min at room temperature.
13. Centrifuge for 20min at 2,800xg and 4°C, and then tip off isopropanol.
14. Add 100µl 70% ethanol.



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15. Centrifuge 5min at 2,800xg and 4°C
 16. Tip off ethanol.
 17. Repeat steps 14 -16
 18. With plate still inverted, place on a Whatman blotting paper and centrifuge upto 30xg (~15sec) and stop.
 19. Leave to dry for 10min at room temperature
 20. Add 50µl HPLC water.
 21. Shake for 30sec, using a plate shaker.
 22. Incubate for 20 min at 55°C.
 23. Shake for 30sec, using a plate shaker.
 24. For QC use 4µl on a 1% agarose gel.
 25. Seal with a foil seal and store at -20°C.