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 3hr 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	0.25ml	0.5% Igepal
Tween-20	0.25ml	0.5%Tween-20
20mg/ml RNase A	1.0ml	400µg/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	**Final concentration**
20mg/ml Proteinase K (>20U/mg)	1.2mg/ml
made up in 10mM Tris pH8.0, 40% glycerol – store at -20°C - Invitrogen)	

Protocol

1. Add 100µl GenLysis buffer to each well of the plated PBS washed ES cells and then add 3µl 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 58°C from 3 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 RNAseA (20mg/ml), shake for 30sec using a plate shaker and incubate for 30min at 37°C.
7. Add 5µl 5M NaCl.
8. Shake for 30sec using a plate shaker.
9. Transfer 70µl to a 96 well round bottom polypropylene plate
10. Add 80µl 100% Isopropanol.
11. Centrifuge for 1hr at 2,800xg and 4°C and then tip off isopropanol
12. Add 150µl 70% ethanol.
13. Centrifuge 5min at 2,800xg and 4°C
14. Tip off ethanol.
15. Repeat steps 15 -17
16. With plate still inverted, place on a Whatman blotting paper and centrifuge up to 30xg (~15sec) and stop.
17. Add 50µl 0.1xTE and seal.
18. Shake for 30sec, using a plate shaker.
19. Incubate for 30 min at 58°C.

20. Shake for 30sec, using a plate shaker.
21. Leave overnight on the bench
22. For QC use 4µl on a 1% agarose gel.
23. Seal with a foil seal and store at -20°C.