DNA Isolation from ES Cells -96 well plate

Bradley Lysis Buffer without Proteinase K

Stock Solutions	Final Conc	for 500mL
1M Tris-HCl (pH 7.5)	10 mM	5ml
0.5M EDTA	10 mM	10 mL
10% SDS	0.5 %	25 mL
5M NaCl	10 mM	1ml
H2O		bring up to 500ml

ProteinaseK (20mg/mL)
:add just before use as 1mg/ml final concentration

EtOH/NaCl Mix

100% EtOH	394 mL
5M NaCl	6 ml
Total volume	400mL

Methods:
1. Grow ES cells in a 96-well plate to be over-confluent.
2. Quickly invert the plate over to dump media and remove excess liquid by blotting on paper towels.
3. Wash cells 1x with 150 uL PBS, then dump PBS.
4. Add 50 ul of Bradley Lysis Buffer containing proteinase K.
5. Replace lid and seal the plate with parafilm. Put the plate into a humidified chamber
6. Incubate in the humidified chamber O/N @ 60 degree.
7. Allow the plate to cool to RT

8. Add 100 uL ice-cold EtOH/NaCl mix to precipitate DNA and mix well. Then incubate the plate about 30 minutes at RT
9. Spin in 96-well plate holder centrifuge, 3000rpm 20 minutes
10. Invert the plate to decant liquid. Blot the plate on paper towels to remove access
liquid.
11. Add 150uL cold 70% EtOH and spin 10minutes at 3000 rpm to rinse the pellet. Decant supernatant onto paper towels.
12. Repeat washing step (#11) and air-dry DNA pellet until there is no detectable EtOH smell (approx 10-15 minutes)
13. Add 30 uL of warm TE pH 8.0
14. Cover Plate with wax cover and incubate at 56 degree ~10 mins

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