DNA Isolation from ES Cells

From 96-well plate

1) Grow ES cells in a 96-well plate to be over-confluent. You don't need LIF in your media.
2) Quickly invert the plate over to dump media.
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 overnight at 60 ^{o}C.
7) Allow the plate to cool to RT.
8) Add 100 uL ice-cold Ethanol/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 on paper towels.
11) Add 150uL cold 70% EtOH and spin 10 minutes at 3000 rpm to rinse the pellet. Decant supernatant.
12) Repeat washing step (#11) and air-dry DNA pellet until there is no detectable Ethanol 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 ^{o}C ~10 mins

Bradley Lysis Buffer

Stock Solutions	Final Conc	for 50mL
1M Tris-HCl (pH 7.5)	10 mM	500 uL
0.5M EDTA	10 mM	1 mL
10% SDS	0.5 %	2.5 mL
5M NaCl	10 mM	100 uL
H2O		45.9 mL

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

EtOH/NaCl Mix

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