

Isolation of genomic DNA from ES Cells

1. Store pellet at -80°C
2. Resuspend cells in storage liquid once cells are thawed.
3. Add $600\mu\text{l}$ Cell Lysis Solution and pipet up and down a few times. Solution should be very viscous. Incubate at 37°C for 20 minutes to solubilize particulate matter if necessary but this is not usually necessary.
4. Add $1.20\mu\text{l}$ RNase A (10mg/ml) and mix the sample by inverting the tube 25 times. Incubate at 37°C for 30 minutes.
5. Add $200\mu\text{l}$ Protein Precip. Soln. (5M NH_4OAc) to the cell lysate.
6. Vortex vigorously at high speed for 30 seconds.
7. Centrifuge at $\sim 11,000$ rpm for 10 min to pellet proteins.
8. Pipet off supernatant and put into a new eppendorf tube.
9. Add $600\mu\text{l}$ IsoOH and invert 25 - 40 times to precipitate genomic DNA.
10. Centrifuge at 2,000rpm for 5 minutes to pellet the DNA. Sometimes it sticks to the side of the tube and doesn't pellet.
11. Discard the supernatant and add $600\mu\text{l}$ 70% ETOH. Invert the tube until the pellet is no longer stuck to the tube.
12. Centrifuge at 2000rpm for 5 minutes to pellet DNA. Discard supernatant and allow to air dry 10 minutes. A chemwipe which has been rolled into a needle shape may also be used to dry area surrounding pellet.
13. Add $60\mu\text{l}$ TE and rock overnight to resuspend. If you're in a hurry, carefully loosen the pellet with a pipetman and incubate at 65°C for 1 hour.
14. Store samples at 4°C .