

Isolation of DNA from ES cells in 6-well plate

Reagents

confluent 6 well plates, or larger dishes (adjust volumes)

PBS

proteinase K (10 mg/ml in water, Merck)

lysisbuffer ("tail buffer": 50 mM Tris/Cl pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS)

saturated NaCl

isopropanol

70 % (v/v) ethanol

TE

Procedure

- aspirate medium, wash once with PBS
- add 0.8 ml lysisbuffer, return to incubator for 1 hour
- transfer lysed cells with cut off blue pipett tip to labelled 1.5 ml Eppendorf tube. Freeze lysates or proceed.
- Add 20 ml proteinase K solution. Mix well and incubate at 56°C overnight. Freeze lysates or proceed.
- Add 0.28 ml saturated NaCl. Place on mixer for 5 min.
- Centrifuge for 10 min at room temperature.
- Transfer 0.75 to 0.85 ml supernate to fresh Eppendorf tube.
- add 0.5 to 0.6 ml isopropanol. Place on mixer for 3 min.
- Centrifuge 3 min at room temperature.
- Aspirate supernate. Wash the pellet once with 1 ml 70% ethanol.
- Air dry the pellets. Resuspend in 0.08 to 0.12 ml TE, depending on the size of the DNA pellet. For Southern blot analysis, use 20 to 40 ml per digest.