

Transgenic Core Facility
Institute of Molecular Biology, Academia Sinica
2789-9312, 2652-1438

TCF PROTOCOL

ES Cell Genomic DNA Handling Tips

1. The genomic DNA (gDNA) of ES cells is extracted and precipitated by experienced staffs in TCF.
2. The gDNA pellets of ES cells are suspended in 70% ethanol at -20°C, and are transferred to user right away. (The gDNA can be stored at this condition for days.)
3. Washing the gDNA pellet once with 70% ethanol at RT by centrifugation at 13,000xg for 5 minutes. (The 70% ethanol should be prepared by high quality ethanol and sterilize ultra-pure water. Never use ethanol solutions lower than 70% to wash gDNA.)
4. Discarding the ethanol solution and drying the gDNA pellets at RT for minutes.
(Vacuum drying is strictly prohibited.)
5. Dissolving the gDNA in 10 mM Tris-HCl, pH7.6, containing 0.1mM EDTA at 55°C overnight with occasionally agitation. (The Tris buffer solution should be sterilized by autoclave before used.)
6. Running standard agarose DNA gels to confirm the quality and quantity of gDNA.
(Random selecting a few samples for analysis.)
7. The gDNA samples are now ready for screening by PCR and/or Southern blot analyses.
(The gDNA could be stored at 4°C for days. For long term storage, the gDNA should be kept in a non-defrost refrigerator below -20°C.)
8. Owing to the limitation of storage space, the period of genotyping should NOT be longer than 2 months.