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

TCF PROTOCOL

DNA Extraction From Tail Sample for PCR or Southern Blot

- 1. Tail samples will be collected by TCF and each sample will be put in a 1.5ml boil-proof microtube
- 2. Add 500µl of NTES solution to each tube
- 3. Incubate at 55°C with gentle shacking overnight or for more than 6 hrs
- 4. Spin down for seconds.
- 5. Add 120µl 5M KOAc (Potassium acetate) to each tube, mix gently by rotating the tube
- 6. Incubate at 4 $^{\circ}$ C for 1 hour
- 7. Centrifuge 13,500g at 4 $^\circ\!\mathrm{C}$ for 30 min
- 8. Remove the supernatant to another tube and discard the debris
- 9. Add 2.5 volumes ice cold 100% EtOH to the supernatant, mix gently by rotating the tube
- 10. Centrifuge 13,500g at 4 $^\circ\!\mathrm{C}$ for 5 min
- 11. Wash the DNA pellet with 1ml 70% EtOH
- 12. Centrifuge 13,500g at 4 $^\circ\!\mathrm{C}$ for 2 min
- 13. Air dry the pellet
- 14. Dissolve DNA with 50µl TE buffer or water
- > <u>NTES solution</u>: (DNase, RNase and other contamination should be avoided)
 - o 100 mM NaCl
 - o 50mM Tris-HCl
 - o 50mM EDTA
 - o 1% SDS
 - o 200µg/ml proteinase K