

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

TCF PROTOCOL

DNA Quality and Quantity Required for TCF Pronuclei Microinjection Service

1. The quantity of DNA required for pronuclei microinjection service is at least 5 µg dissolved in less than 100µl. For transgene size less than 3 kb or more than 10 kb, DNA quantity should be increased to 10µg dissolved in less than 100 µl.
2. Circular DNA should be reasonably fresh. (no more than two months)
3. The quality of the DNA preparation should be Qiagen grade or better. (Don't use the Mini preparation kit to extract DNA)
4. DNA preparation needs to be free of contaminant. (CsCl₂ banding, commercial Midi and Maxi preparation kits are preferred)
5. DNA digestion should be fresh and complete. (no more than 1 week)
6. The digested DNA should be kept at -20°C and referred to TCF right away. (Enzyme inactivation and DNA purification are not required)
7. The isolated DNA fragment for pronuclei microinjection should be free of vector sequence. The size of the vector band(s) should be at least 1kb differs from the transgene.
8. TCF perform DNA elution/extraction according TCF standard procedure to maintain consistency
9. TCF will test DNA quality and quantity before scheduled microinjection. If the submitted DNA does not fit the requirement of TCF's specification, TCF will not process the scheduled service.