

# Transgene Preparation

v.3

09.13.05

NDJ

1. Verify transgene sequence by sequencing entire transgene.
2. Set-up large digestion of transgene

DNA	20ug
Enz buffer	20
Restriction Enzyme	5ul (10u/ul)
H2O	to 200ul
37°C	3 hours
Spike w/ Restriction Enzyme	4ul
37°C	ON (overnight)

3. Run ON on crystal violet gel
  - a. Pour 300ml 2ug/ml crystal violet gel (30ul (20mg/ml stock) in 300ml)
  - b. Use large comb to accommodate 200ul digest
  - c. Run ON at 20V
4. Cut out band and pour another gel around it. Run for one to two hours.
5. Extract from gel—see Gel Extraction Protocol
6. Phenol Chloroform extraction – See Phenol Chloroform Protocol
7. EtoH precipitation
8. Resuspend in injection buffer
  - i. T<sub>5</sub>E<sub>0.1</sub> (Tris 5mm 0.1mm EDTA)
  - ii. Filtered in 0.45 or 0.2uM
9. Dialyze using Slide-A-Lyzer (Pierce #66380 10,000MWCO 0.5-3mL) in filtered T<sub>5</sub>E<sub>0.1</sub> according to company protocol.
10. Estimate concentration with spectrophotometer (260 nm UV).
11. Make a 2-4ng/ul (volume 500ul) dilution of part of sample.
12. Run transgene through filter (to remove particulate that may obstruct injection needle; Millipore 0.45uM PVDF filter #SLHVRO4NL).
13. Make serial dilutions of estimated 1, 2, 5, 10ng to run on ethidium bromide gel. This extra step will verify size and concentration 10ng should be barely visible.
14. Bring 500ul (2-4ng/ul) of transgene to Mouse Genetics Laboratory for pro-nuclear injection with appropriate IRB paperwork.