DNA Isolation for Generation of Transgenic Mice

Important: Plan to use very careful ("RNA") techniques in manipulations, and use tips and tubes from new or seal boxes. This method uses QIAEX II Kit.

1. Digest 10 µg plasmid DNA in 100 µl 1X restriction digestion buffer with 3 µl of appropriate enzymes at an appropriate temperature for at least 2 hrs. Mix the sample occasionally during the incubation.

2. Run 1 µl on an analytical agarose gel to check the digestion while continuing the digestion. If necessary, add 2 µl enzyme(s) and digest for another hour and repeat the analytical agarose gel. Confirm the digestion is complete.

3. Run the sample on a 0.8% agarose gel (ultra pure agarose WITHOUT Ethidium Bromide) in 1X TAE buffer made by using the isolation comb (or you fuse the teeth with tape to make a fused well so that you can load 100 µl of sample).

4. Stain the gel in 0.001% crystal violet (Aldrich C0775) in ddH_2O for 3 hrs (NOT Ethidium Bromide!!!). Use visible light from a light box (turn on the regular light, not the UV light, in the UV box) to visualize the bands, which will avoid UV nicking. Sensitivity is much lower, but on a preparative gel, 10 µg plasmid DNA should be plenty.

5. Excise the DNA band of interest with a clean scalpel. The gel slice can be stored at -20°C before isolation.

6. Weigh the gel slice. Add 3 volumes of Buffer QX1 (from the QIAEX II Kit for DNA fragments 100 bp-4 kb). For example, if the gel weighs 0.5 g, add 1.5 ml of Buffer QX1. If necessary, cut the gel slice into two equal pieces and proceed with the isolation in two tubes.

7. Resuspend QIAEX II beads by vortexing for 30 sec and add 30 µl QIAEX II mixture (of beads) to the sample in Buffer QX1 (if you divide the gel slice into two tube, add 15 µl to each tube).

8. Incubate at 50°C for 10 min to solublize the gel and let the DNA to bind. Mix by vortexing every 2 min.

9. Spin the sample for 30 sec in a microfuge and remove the supernatant with a pipette.

10. Wash the pellet with ~500 µl of Buffer QX1 (If the isolation is started in two tubes, combine them at this point).

11. Wash the pellet twice with 500 µl of Buffer PE.

12. Air-dry the pellet till it becomes white. It takes about 30 min in room temperature.

13. Add 20 µl of pre-warmed (65°C) sterile TE (10 mM Tris/1 mM EDTA, pH 7.4, provided by transgenic core facility) and vortex to elute the DNA.

14. Spin for 30 sec and carefully pipette the supernatant into a clean tube (KEEP the supernatant which contains the DNA!). Please try to avoid the beads being transferred.

15. Repeat steps 13 and 14 and combine the supernatants in a clean tube.

16. Spin the supernatant in the clean tube for 30 sec and transfer the liquid to another clean tube again to remove all the insoluble particles. You can afford to lose some DNA, but try not to get the beads or other particles in the final DNA solution, which will clot the microinjections needles.

17. Measure OD260 by using Nanodrop.

18. Check the recovery and concentration of the isolated DNA on an analytical gel by taking 2 µl of the isolated DNA in a serial dilution of 1:5, 1:10, 1:20, and 1:40.

19. Run an analytical agarose gel by loading 5 µl of 10 kb marker (the concentration of each of the bands would be 50 ng) and 4 µl of each of your diluted samples mixed with DNA dye.