Transfection of DT40

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1) Linearize about 25-50 microgram (about 50 microliter) of plasmid DNA outside the gene of interest by digesting with an appropriate restriction enzyme. Digest in 500 microliter total volume. Check the digestion by analyzing an aliquot of 5 microliter on an agarose gel.

2) Precipitate the DNA by adding 1 ml of 100% Ethanol. Spin down for 10 min at 4 C. Decant the supernatant, add 1 ml of 70% Ethanol and spin down for 5 min. Decant the supernatant and dry the pellet in the speed vac.

3) Resuspend the pellet in 500 microliter PBS. Vortex briefly to loosen the DNA pellet from the wall of the tube, and shake the tube in an eppendorf shaker to dissolve the DNA.

4) Determine the density of live cells in the culture that you want to use for transfection. Add a volume containing about 10 million cells to a 50 ml tube and spin down for 10 min at 1500 rpm, 4 C.

5) In the meantime prepare a 50 ml tube containing 20 ml of chicken medium, label two 96 well flat-bottom microtiter plates, and place an electroporation cuvette on ice.

6) Once the centrifuge has stopped, take the tube, remove the supernatant and resuspend the cells in 5 ml of PBS.

7) Spin down for 5 min at 1500 rpm, 4 C.

8) Remove the supernatant and resuspend the cell pellet in 300 microliter of PBS. Add the 300 microliter of the cell suspension and the 500 microliter of the DNA solution into the electroporation cuvette.

9) Keep the cuvette on ice for 10 min. Electroporate the cuvette using the BioRad apparatus using 25 mF and 550 volts. Keep on ice for another 10 min.

10) Add the cell/DNA solution to the tube containing 20 ml of chicken medium and distribute into the wells of the microtiter plates adding 100 microliter into each well.

11) The following day add 100 microliter of selective medium (containing twice the final concentration of the drug) to each well.

12) Leave the plates for about ten days in the incubator. Drug resistant colonies should be visible by then.