TRANSFECTION

DNA

1. Digest DNA to be used for transfection. Digest 100 ug of each DNA type with 100-150u Eco R1 (or other enzyme to release vector) for 1 to 1.5 hours in volume of 500 ul. Check on gel to ensure comparable amounts of DNA are present.

2. Extract with equal volume phenol/chloroform (500 ul) and then extract again with equal volume (500 ul) chloroform alone. After each step pull off bottom layer with "long tip" micropipette.

2a. Use 10 ug EcoA, 25ug SvNaeZ, 50 ug Sal EC (42 kb released) for precipitation (see #3).

2b. Remove pipetteman ejectors and clean pipetteman with 70% ethanol.

3. Add appropriate amounts of DNA to a sterile eppendorf and precipitate with 0.1 volume 3M NaAcetate and 2-3 volume 100% ethanol. (Can use room temp. ethanol, let sit for about 10 min. and then spin for about 10-15 min.)

3a. Use sterile technique.

4. Wash with 70% ethanol- spin 10-15 min. and use long tip pipette to draw off ethanol (do in hood for sterility). (No need to dessicate.)

5. Resuspend DNA in RPMI (or sterile water) in 400 ul volume. Let sit for ~1hr or pipette to dissolve.

P3HR1 CELLS

1. Use cells in log phase growth (800,000-1million cells/cc)

2. Aliquot 10 million cells separately into 15cc tubes.

3. Spin 1400 rpm x 10min (room temp.)

4. Pull off supernatant with pasteur pipette.

5. Wash with 5 cc sterile PBS or media and spin 1400 rpm x 10min. (room temp)

6. Draw off all supernatant using pasteur pipette with micropipette tip on the end. Remove all liquid from sides of test tube.

7. Add DNA (400 ul volume) to cells and pipette up and down.

ELECTROPORATION

1. Put cuvettes in rack.

2. Add the 400 ul aliquots of cells/DNA to cuvettes and let sit for 10 min.

3. While waiting, put 10-20cc RPMI into T25 flasks (want cells to be ~500,000cells-1 million cells/cc).

4. Tap bottom of cuvettes to stir cells and then place in holder and slide into electroporator.

5. Turn electroporator on (button in back). Set to 200volts (0.20).

6. Push 2 buttons simultaneously and stop at the beep. (Flashes chg) Write down the time constant (shown at end- should be ~30 for 400ul). Continue with next sample (no re-set needed).

7. Use pasteur (or 1cc) pipette to remove cells/DNA and place into T25 flasks (see #3). Rinse cuvettes with about .75cc of the media in T25 flasks to ensure all cells are removed and added to the T25.

8. Incubate for 3-4 days with T25 flasks standing up.