Electroporation of targeting construct

E14 ES cells are feeder-independent ES cells, derived from 129. We use 5 million cells with 5 ug of linearized KO construct and pick only 48-96 colonies.

Materials:

- Bio-rad GenePulser
- Gene Pulser Cuvette (1652088)

Methods:

1. Change media 2-4 hours prior to electroporation. Cells must be about 80% confluent and healthy.

2. Rinse the cells with 10ml of pre-warmed PBS and add 2ml of pre-warmed trypsin. Be sure to cover the entire plate by carefully swirling it.

3. Incubate in a 37C incubator for 5 minutes and check under the microscope. If you see big clumps of cells, incubate additional 2-5 minutes. Do not over-trypsinize!

4. Once majority of the cells are uniformly dispersed into small clumps or single cells, inactivate trypsin by adding 10 ml of medium. Gently pipette up and down using 10ml pipette 10-15 times without making bubbles.

5. Transfer the cells into a 50ml Falcon tube. Spin down the cells by centrifuging for 5 minutes at 1000rpm. Aspirate off supernatant. While waiting, count the cells.

6. Wash the cells once with 50 ml of PBS. Centrifuge for 5 minutes at 1000rpm and spirate off PBS.

7. Resuspend the pellet in 0.9ml of PBS. Add 5ug of DNA into cells and mix gently. Incubate at room temperature for 5 minutes.

8. Transfer the mixture to the electroporation cuvette (Bio-Rad, light blue cap). Try to avoid touching the metal plates. Prepare another cuvette as a test cuvette with 0.9ml of PBS.

9. Set the Bio-rad GenePulser at 250V, 500uF (requires the capacitance extender). Place

the test cuvette and press the two red buttons to electroporate. The machine will flash and will beep when electroporation is complete, at which time you should release the buttons. The time constant should be between 5.6-8.0 ms.

10. If Bio-rad GenePulser is working correctly, you should see small bubbles along the metal plate. If you see the bubbles, then place your actual sample cuvette and perform electroporation. If you do not see any bubbles, check the machine before electroporating your cuvette.

11. Leave the cuvette at room temperature for 5 minutes and then remove the cells from the cuvette to a 50ml falcon tube containing pre-warmed fresh media. Wash out the cuvette once or twice with media to get all the cells. Plate the cells onto five 10cm plates.

12. Place the plates in a 37C incubator. Change media next day.

13. Start drug selection (G418, 250ug/ml), 36-48 hours after electroporation and change media everyday. Continue drug selection until colony picking.