Heather P. Harding Revised 11/5/03

Immortalization of MEF with SV40 T antigen

Reagents:

Medium:

- DMEM
- 10% FCS
- 1X Pen/strep
- 1X Glutamine
- 1X Non-essential amino acids
- 55uM beta-mercaptoethanol

Tag expression vector
Fugene
Serum free dMEM

Method:

•Split primary MEF at pass 2-4 into 6 well dishes.
•Make 2 dilutions ~1/4 and 1/6 for each genotype.
•Grow overnight in 37C incubator
•Transfect ~25% confluent well with 2ug Tag expression vector using Fugene:
    •Change medium 1 hr before transfection. Use 2ml for each well.
    •Mix 2ug Tag expression vector with 20ul serum free dMEM in 1.5 ml tube.
        (Make a mix if you will be transfecting cells from several mice.)
    •In a separate 1.5ml tube, place 200ul serum free dMEM and then add 6ul
        Fugene (prewarmed to room temperature for 15 min).
    •Add DNA to Fugene mix and let stand for 15min at room temp.
    •Add mix dropwise to cells.
•Incubate cells 14-16hrs (overnight) in incubator.
•Change medium.
•When cells are just confluent (~2 days after transfection) split them into a 10cm dish=P1.

Additional splitting: The goal is to get cells that have been split 1/10 at least 5 times (i.e. a 1/100,000 fold splitting of the original cells) but if a low number of cells were transfected in the initial experiment then there may be few surviving cells from the early 1/10 splits. Because of this it is best to make a low density and high density plate at each pass. If it is clear that the low density (1/10) pass is growing, then the high density (1/4) plate is frozen as a backup or discarded. Often this 1/4 plate must be split before you can tell if the 1/10 plate is going to grow. (The P2-P3 low density plates can take 3-10 days to grow.)
****Note that the 1/10 split is a strong negative selection against non-transformed cells whereas non-transformed cells can survive many 1/4 splits so that even if you keep the

high density plates as a back up, you will only get rid of the non-transformed cells by multiple rounds of 1/10 splitting. About 85% of the time I can use straight 1/10 splitting, but a few times I have used the 1/4 plate from pass 2 or 3 followed by 4-5 more passes of 1/10. By P6-100,000 (see below) the cells should grow to confluence in ~3-4 days after a 1/10 split.