Culturing McManus Lab Immortalized Dicer Floxed MEFs

June 1, 2010

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Description of Cells/Genotype:
The McManus Lab has prepared an SV40-immortalized mouse embryonic fibroblasts (MEFs) line containing a floxed allele for Dicer (loxP sites flanking the downstream RNAse III domain of Dicer). Several cell lines have been made, some which have wild-type, heterozygous, or homozygous Dicer configurations[1], and some lines also contain a ROSA26-CreERT[2] allele and/or a ROSA26-lox-stop-lox YFP allele[3]*. In the presence of Cre recombinase, the RNAse III domain of Dicer is removed, and the YFP allele is activated because of the removal of the early stop codon.

Growth Medium:
Dulbecco's Modified Eagle Medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS), 1% L-Glutamine, and 1% Pen-Strep.

Culture Conditions:
Split every three days to a fraction of 1/3 to 1/5. These cells grow best when grown at high-confluency (80-150%), and their growth rate may be compromised at low confluency (less than 5-10%).

Flow Cytometry:
These cells are very sticky, so take care if using flow cytometry to analyze YFP expression (trypsinize them well and filter immediately before flow analysis). Note that over-grown cells are more difficult to trypsinize completely.

Ablation of Dicer by Cre:
Ablation of Dicer can be achieved using Cre-containing adenovirus (or by the addition of OHT when using the CreERT-containing lines). Cre-adenovirus can be obtained from several academic and commercial sources; we usually use an MOI of 0.3, but recommend that you test a range of titers in your own hands. The cells will not be happy after the ablation of Dicer. Two to four days after the introduction of Cre, cells will slough off the bottom of the plate at an increased rate. After 1-2 weeks, surviving cells are mainly derived from the outgrowth of wild-type cells that escaped infected with the Adeno Cre virus.

**We recommend that you make sure to conduct the appropriate PCR of the potential alleles to ensure your correct genotype, as many different lines have been passed around from hand-to-hand in the scientific community.*

References:

1. *The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb.* Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ. Proc Natl Acad Sci U S A. 2005 Aug 2;102(31):10898-903. Epub 2005 Jul 22.

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3. *Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus.* Shankar Srinivas, Tomoko Watanabe, Chyuan-Sheng Lin, Chris M William, Yasuto Tanabe, Thomas M Jessell, Frank Constantini, BMC Dev. Bio. March 27, 2001