Splitting MEF's:

Thomson Lab, 2004

1. Remove MEF medium*.
2. Wash cells with 5 ml of PBS w/o CaMg (to get rid of trypsin inhibitors).
3. Add 1.5 ml Trypsin/EDTA (0.05% Trypsin) to each flask and allow to sit for about 5 minutes.
4. To loosen cells, either tap flask against the heel of your hand or pipet the cells off.
5. For every 1 ml of Trypsin/EDTA added, add at least 1 ml of MEF medium to neutralize the trypsin reaction.
6. Add the cell suspension to a 15 ml conical tube and pipet several times to individualize the cells.
7. Add 10 ml MEF medium to new T75 flasks.
8. Divide the cell suspension appropriately amongst the new T75 flasks and place at 37∞C**.

Notes:

**MEF medium is:*

89% DMEM (Gibco #12100-046)
10% FBS (Gibco #16000-044)
1% NEAA (Gibco #11140-050)

***MEFs will grow slower with each passage. It is possible that you may begin by splitting the first passage 1:5, but end up splitting the last usable passages (p4 and p5) only 1:2.*