ESC Passage (on feeder layer of mouse embryonic fibroblasts)

Preparation:
At least two hours before passing ES or iPS cells:
- Pre-coat wells for plating with 0.1% gelatin in H2O; sit 5 mins RT
- Plate appropriate number of feeder cells/flask or well (see mef plating protocol)

When ready to pass cells:
-warm PBS, 0.05% Trypsin/EDTA, and 15% FBS/IMDM

Protocol

1. Aspirate old media
2. Gently wash with pre-warmed PBS: 5cc x 1; remove PBS
3. Trypsinize: 1mL 0.05% Trypsin/EDTA x 2 min at 37 degrees C
   Gently tap plate and see if cells starting to lift.
4. Pipette up and down with P1000 pipette (or 5cc pipette)
   Quickly check cells under microscope:
5. Stop trypsin by adding 1mL 15% FBS/IMDM to well; mix by swirling plate
   Place cells in appropriately-sized conical
   Spin cells at 300g, 4C, 5 min
   Aspirate supernatant, leaving pellet behind
6. Resuspend cell pellet in 1mL of working complete ES media (+LIF) and pipette up and down for
   single-cell suspension
9. Add appropriate volume of working media to mef-coated well
   Add desired volume of cell suspension dropwise to well; swirl plate to thoroughly disperse cells
   evenly throughout plate (especially if using P-35)