9. Protocol for Preparation of ES Cells for Blastocyst Injection

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Injection Media Recipe: DMEM with hepes and 5% fetal bovine serum

-Invetrogen (Gibco) DMEM high glucose & 25 mM HEPES, 500ml (smallest size) Cat #12430-054

-1 ml frozen aliquots of (inactivated) ES compatible FBS

Pipet 19 mls injection DMEM to sterile conical and add 1 ml FBS mix. Syringe filter through .2um filter (discard first ml or so). Expel remaing to new tube, cap and set on ice.

Tuesday

1.) Thaw and plate MEF's to a gelatinized 6 well plate

2.) Thaw and plate one vial of ES cells onto 1 or 2 wells of the above 6 well plate.

Wednesday Feed those cells.

Thursday

At 9 am (about 1.5 hours before you need the cells for injection), prep them:

1. Aspirate the medium.
2. Rinse three times with 2 ml PBS (without $CaCl_2$ and $MgCl_2$).
3. Add 5 drops of 0.05% trypsin-EDTA with a 5 ml pipet.
4. Incubate 5-7 minutes at 37 ^{0}C in incubator.
5. Neutralize with 1 ml **ES media**, pipette the cells 30 times with a P1000 at 500ul to get single cell suspension.
6. Pre-plate suspension in a fresh 6 well plate for 20-25 minutes in incubator (no gelatin or MEF's).
7. After 20-25 minutes, carefully remove the supernatant and aliquot into a labeled eppy (Supe) on ice. The MEF cells re-adhere to the plate while ES cells will lightly associate. Gently wash plates with 1 ml of **injection media** to rinse off remaining ES cells. Put in labeled eppy (Rinse) in ice ** use these to inject. Removal of the MEF cells is the most important step of the preparation: MEF cells are large and sticky and make for a very difficult injection day.

NOTES:

Please clearly label your eppies with your name, your PI and the name of your construct/clone # and "rinsed" or "supe" before submitting to the core for injection.

Cells must be received by 11 a.m. the Thursday of injection or you will lose your reserved time slot: NO EXCEPTIONS!

To ensure your cells are in the best condition for injection:

1. Cells should be in log phase 40-60% confluence on day of injection.
2. Never centrifuge them after trypsinization.
3. Resuspend them sufficiently but gently.