

#### IV. Preparation of ES clones for blastocyst injection

1. Once ES clones with appropriate recombined alleles have been identified, and verified by DNA analysis of clones thawed from master plates, the cells can be prepared for injection into blastocysts. A T25 flask is coated with gelatin, and feeder fibroblasts are added to this flask. This can be done on the Monday of the week of injection.
2. 2-3 days before injection, one frozen vial of amplified ES cells is thawed and plated into the T25 flask. The media is ES media (with G418). Change the media every day.
3. On the day of injection, the media is changed to ES media, without G418 and LIF, 1-2 h before the cells are needed. The cells are then trypsinized, pelleted, and resuspended in 10 ml of DMEM supplemented with 20 mM HEPES, pH 7.3, and 10% FCS. The cells are stored on ice while injections are performed.