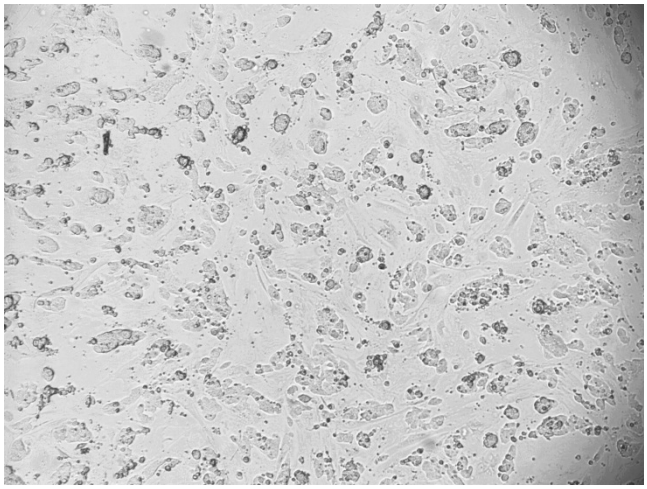
1. **What reagents should I have on hand to successfully culture mES cells?**

   Refer to the Murine Embryonic Stem Cell Culturing Protocol for detailed information. The table below provides a list of reagents necessary for culturing mES cells. There are no preferred vendors. Reagents can be obtained from a variety of vendors; the list below is given for convenience only.

Reagent	Potential Sources
6-well Plates	Nunc, BD biosciences, Corning
0.1% Gelatin	Millipore, Stem Cell Technologies
DR4 Mouse Embryonic Fibroblasts (MEFs)	Thermo Fisher, Applied Stemcell, Open Biosystems
DMEM (High Glucose)	Thermo Fisher Scientific , Sigma, Hyclone
ES Cell Fetal Bovine Serum	Applied Stem Cell, ATCC, Millipore, Invitrogen
Glutamax	Thermo Fisher Scientific
B-mercaptoethanol	Thermo Fisher Scientific , Sigma
Non-essential Amino Acids	Thermo Fisher Scientific , Mediatech
Sodium Pyruvate	Thermo Fisher Scientific , Mediatech
Leukemia Inhibitory Factor	Millipore, Stem Cell Technologies, Stemgent
Doxycycline	Clontech
Puromycin	Thermo Fisher Scientific

2. **I have thawed my mES cells according to the Murine Embryonic Stem Cell Protocol. What should my cells look like 24 hours after plating?**

   The picture below is of a cell line 24 hours after thaw. Note the round colonies and even distribution of the cells throughout the dish.



3. **How do I know when it is time to passage my mES cells?**

   Typically, mES cells passage every 2-3 days and plate at a density between 1.0 X 10^6 and 1.8 x 10^6 cells per well of a 6-well plate. The cells should be passaged when they are between 70-80% confluence using split ratios ranging from 1:4 to 1:10. Refer to the specific Certificate of Analysis for the cell line you have ordered for more details.

4. **Why do you recommend that I cryopreserve a small number of vials of stem cells for my laboratory?**

   Establishing a master stock is critical so that a vial can be recovered and expanded if a problem arises with the growing stem cells such as: contamination, spontaneous differentiation and/or changes in chromosomal integrity. If any of these occur, you can recover a vial of your master stock and expand it to make a working stock for the laboratory.

5. **Initially my cells were growing and passaging as expected. Now my cells are not growing and seem to be lifting off the plate. What is happening?**

   The sudden change in growth kinetics of a cell line can be indicative of problems with media components. Specifically, puromycin concentration as well as FBS and MEF quality can impact the growth of your cells. Testing new lots of reagents prior to using them in growth medium for your cells can decrease the probability of observing negative effects on the growth of your cells. For information, we recommend testing new lots of MEFs to determine the optimal plating density, see the protocol "Determining Optimal Plating Density for Mouse Embryonic Fibroblasts (MEFs)". We further suggest performing a puromycin kill curve as the concentration can vary slightly with each lot of antibiotic. More trouble shooting tips can be found in Table 2 of the Murine Embryonic Stem Cell Culturing Protocol.