MS-5 preparation for neural induction

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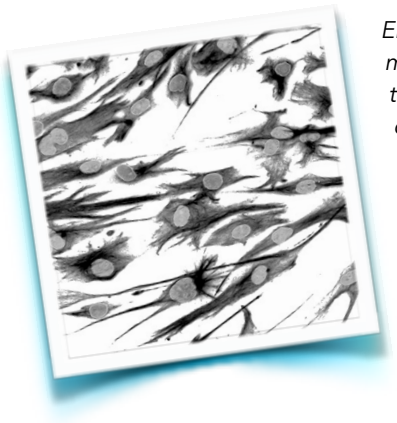

Embryonic stem cells can be directed to neural cell fates by co-culture with MS5 bone marrow stromal cells (Barberi et al. 2003). MS5s (and the similar PA-6 line) are not transformed although they have been in culture since the 80's.As such, individual cultures change over time. One should always keep good notes about the lineage of MS5s, since occasional passages (sublines) will stop working well. MS5s should not grow to confluence: this will reduce their neuralizing capacity. Passage 1:6-1:10 when the cells have reached 60-70% confluence. I prefer to grow large lots of MS5 before irradiation, counting and freezing.

[MS5 expansion]

1. Expand MS5s in 225 flasks (no gelatin needed for expansion). Cells are typically split every ~3 days.
2. Rinse cells with PBS, and trypsinize.Verify that most of the cells have detached on the microscope. Spin down with twice the volume of alpha-MEM (200 xg for 5 minutes).
3. Resuspend and count. Expose cells to 60 Gy (gamma or X-ray) irradiation to mitotically inactivate the cells. Alternatively, you can use mitomycin C if you do not have access to an irradiator.
4. Freeze down cells in 10% DMSO in alpha-MEM. I prefer to freeze down 3.4 million cells per vial, enough to plate 2 x 6 cm dishes.

[Plating irradiated MS5 before differentiation]

1. Gelatinize (0.1% in PBS) for 15 minutes.
2. Thaw cells quickly in a 37˚C water bath.
3. Spin down in alpha-MEM to remove DMSO. Resuspend pellet in alpha-MEM. For example, I add 6 ml alpha-MEM to each vial (3.4 million cells), and plate 3 ml per 6 cm dish (1.7 million total per 6 cm dish).
4. Aspirate gelatin and add cells directly to dish. Leave at least 24 h, but not more than 3 days, before co-culture with ES cells.

[Plating ESCs to begin neural induction]

1. Aspirate alpha-MEM and wash once with SRM to remove residual serum. Serum inhibits neural induction.
2. Plate 5000 mouse ESCs resuspended in SRM per 6 cm dish. For human ESCs, split 1:20 - 1:30 onto MS5s in SRM. Noggin can also be added to hESC cultures to enhance neural induction (250-500 ng/ml, R&D Systems). Make sure ES cells (particularly the mouse) are not resuspended in serum containing media at any time.

SR (Serum Replacement) Medium for 1 L:

820 ml Knockout DMEM (Invitrogen-Gibco, 10829-018)
150 ml Knockout Serum Replacement (Invitrogen-Gibco, 10828-028)
10 ml L-glutamine (Invitrogen-Gibco, 25030-081*)

**thaw once to make 10 ml aliquots, then refreeze. Do not reuse after second thaw*

10 ml MEM Non-essential amino acid solution (Invitrogen-Gibco, 12383-014)
1 ml 2-Mercaptoethanol (1,000x) liquid (Invitrogen-Gibco, 21985-023)

alpha-MEM (for MS-5 culture) for 1 L:

900 ml alpha-MEM (Invitrogen-Gibco, 12571-063)
100 ml FBS (Invitrogen-Gibco, 16140-071)

**do not be tempted to try Glutamax containing media. I've had some sublines (but not all) of MS-5 that died after a few days in Glutamax but not Glutamine.According to the company, not all cell types express the enzyme necessary to break down the dipeptide.*

Barberi T, Klivenyi P, Calingasan NY, Lee H, Kawamata H, Loonam K, Perrier AL, Bruses J, Rubio ME,Topf N, Tabar V, Harrison NL, Beal MF, Moore MA, Studer L. 2003. Neural subtype specification of fertilization and nuclear transfer embryonic stem cells. Nat Biotechnol. 21:1200-1207.

Perrier AL,Tabar V, Barberi T, Rubio ME, Bruses J,Topf N, Harrison NL, Studer L. 2004. Derivation of midbrain dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci USA 101:12543-12548.