Care and Feeding of JSL1 cells

To thaw cells:

1. Thaw bullet quickly (in hand or water bath) and wipe down outside with Ethanol.
2. Add contents of bullet to 10 mls media+10% FCS (see below) and spin 5 min at 1 K.
3. Aspirate off supernatant and resuspend cell pellet in 5-10 mls media+10% FCS.
4. Transfer to a well of a 6 well plate or a T25 flask and let recover for several days in incubator

To maintain/grow cells:

Once growing well (should approximately double every 24 hrs.), split every 2-3 days and keep at density of 0.2-1.5 million cells/ml. Cells should be maintained in media +5% serum. If cells get above a density of 1.5 million cells/ml split back to approx. 0.5 million/ml and let recover for a day before doing any transfection or analysis.

To Freeze cells:

1. Grow approximately 50-100 mls cells in media with 5-10% FCS to a density of ~1 million/ml.
2. Spin down cells in sterile conical tubes. 5 min at 1 K. Aspirate supernatant.
3. Resuspend cells in 1 ml Freezing media (see below) for every 10-20 mls of original culture.
4. Aliquot 1.5-2 mls in cryovials. Put in –20 degrees for 1-2 hours then move to –80 freezer.

Media

RPMI 1640 w/ glutamine (we get our from Fisher/Mediatech cat# MT 10-040-CV) supplement with Penicillin, Streptomycin (100 U each final) and Glutamine (2 mM final) and desired concentration of FCS (fetal calf serum)

Freezing Media

50% “media” (see above)

40% FCS (regardless of how much serum is in your “media”)

10% DMSO