Supplies:
**Matrigel (stock at -20)**
Matrigel™ Basement Membrane Matrix (BD 354234)  -20  10ml
Thaw on ice and aliquot to single use sizes in eppendorf tubes store at -20

**HBSS** no Ca2+, no Mg2+, no phenol red (Life Technologies 14175-079)  +4  1L

**TrypLE™ Express** (Life Technologies 12605-010)  RT  100ml

**EDTA** (SIGMA E7889-100ml)  RT  100ml

**DMSO** Hybri-Max (SIGMA D2650-100ml)  RT  100ml

**L-cell Medium (kept at +4C)**
DMEM high glucose (SIGMA D6429-500ml)  +4C  500ml
100x Penicillin/Streptomycin (SIGMA P4333-100ml)  -20C  5ml
FBS (Heat inactivated)  -20C  50ml

**Primary Culture Medium (kept at +4C, make fresh weekly)**
Advanced DEM/F12 (Invitrogen 12634-010)  +4C  500ml
100x L-glutamine (SIGMA G7513-100ml)  -20C  6.25ml
100x Penicillin/Streptomycin (SIGMA P4333-100ml)  -20C  6.25ml
FBS (Heat inactivated)  -20C  125ml

**Washing Medium (kept at +4C, keep up to 1 month)**
DMEM/F12 with HEPES (SIGMA D6421-500ml)  +4C  500ml
100x L-glutamine (SIGMA G7513-100ml)  -20C  5ml
100x Penicillin/Streptomycin (SIGMA P4333-100ml)  -20C  5ml
FBS (Heat inactivated)  -20C  50ml

**Freezing Medium**
Washing Medium  +4C  8ml
FBS (Heat inactivated)  -20C  1ml
DMSO (SIGMA D2650-100ml)  RT  1ml
OR use:
Primary Culture Medium  +4C  9ml
DMSO (SIGMA D2650-100ml)  RT  1ml

**Supplements**
The CM contains unknown factors that can antagonize a factor of interest therefore, for short-term experiments (1-2 days) spheres can be formed by adding Wnt-3a (100ng/ml) and R-spondin 1 (500ng/ml) in primary cell culture medium.

**R-spondin 1** (Stock Solution: 250ng/ul)
Dissolve 50 ug R-spondin 1 (R&D 3474-RS-050) in 200ul PBS (+0.1%BSA)
Make aliquots (20-40ul) Keep at -80°C
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Wnt-3a (Stock solution: 100ng/ul)
Dissolve 10 ug human Wnt-3a (R&D 5036-WN-010) in 100ul PBS (+0.1%BSA)
Make aliquots (10-20ul) Keep at -80C

Chemicals
G418 (SIGMA G8168-10ml) 50mg/ml
Hygrogold/Hygromycin (InvivoGen ant-hg-1) 100mg/ml

ROCK Inhibitor
Addition of 10 uM Y-27632 improves cell viability by inhibiting anoikis.
Y-27632 (SIGMA Y0503-1mg) Stock 10mM in water
Dissolve 1mg in 312ul water to make 10mM stock of rock inhibitor, keep at -20C
Use at 1:1000 in 50% CM

TGFbetaIR Kinase inhibitor
Addition of 10 uM SB-431542 improves sphere formation by neutralizing TGFb from Matrigel
SB-431542 (EMD Millipore 616461-5mg) Stock 10mM in DMSO
Dissolve 5mg in 1.189ml DMSO to make 10mM stock of TGFb inhibitor, keep at -20C
Use at 1:1000 in 50% CM

(We don’t currently use) Collagenase solution
Washing Medium +4C 10ml
Collagenase type I (Invitrogen 17100-017) +4C 20mg
Gentamicin (Invitrogen 15750060) RT 10uL: final 50ug/ml