Protocol for mouse Germline stem (GS) cells

Medium:

Culture medium for the GS cells is StemPro-34 SFM (Invitrogen#10639) supplemented with StemPro Supplement (Invitrogen#10639), 25 ug/ml insulin(nakalaitesque#19251-24), 100 ug/ml transferrin(SIGMA#T1147), 60 uM putrescine(SIGMA#P7505), 30 nM sodium selenite(SIGMA#S1382), 6 mg/ml D-(+)-glucose(SIGMA#G7021), 30 mg/ml pyruvic acid(SIGMA#P2256), 1 ul/ml DL-Lactic Acid (Sigma#L4263)), 5 mg/ml Bovine Albumin (ICN Biomedicals, #810661), 2 mM L-Glutamine(SIGMA), 5X10-5 M 2-mercaptoethanol(SIGMA), MEM Vitamin Solution (Invitrogen#11120-052), MEM Non-Essential Amino Acids Solution (Invitrogen#11140-050), 10-4 M Ascorbic Acid(SIGMA#A4544), 10 ug/ml d-Biotin(SIGMA#B4501), 30 ng/ml b-Estradiol(SIGMA#E2758), 60 ng/ml Progesterone (Sigma#P8783), 20 ng/ml mouse epidermal growth factor (Becton Dickinson#40010), 10 ng/ml human basic fibroblast growth factor (Becton Dickinson#13256-029), 103 units/ml ESGRO (murine leukemia inhibitory factor; CHEMICON#ESG1107), 10 ng/ml Recombinant rat GDNF (R&D Systems, #512-GF) and 1% fetal calf serum (JRH Biosciences, JRH#12003-78P).

TFN,insulin, progesterone, estradiol, selenite, and putrecine are prepared as aliquots and stored at –20C.
EGF,bFGF,LIF,GDNF,Stempro supplement are also prepared as aliquots (store at –20C) and should be added just before use.
Medium without EGF,bFGF,LIF,GDNF,Stempro supplement can be used for at least 3 wks.

Culture of GS cell.

It is recommended to maintain GS cells on mitomycin-C treated mouse embryonic fibroblasts (MEF).
MEF feeder should be prepared according to the conventional ES cell culture method.

Wash twice with PBS, and add 0.25% trypsin , and incubate at 37C for 4 minutes.

Add Iscove's+BSA 5mg/ml to stop the reaction.

GS cells should be plated at the density of 3x105 cells/well in 6-well culture plate. Medium should be changed every 3 days(half medium change). Culture should be passaged at the frequency of every 4-6 days dependent on the proliferation. Established GS cells can continue to proliferate >2year without losing stem cell activity.

It is important to spread GS cells uniformly on MEF feeder. Otherwise, the cells will aggregate and differentiate. The cells that are starting to differentiate will detach from the feeder layer. In this situation, remove the medium and replace with fresh medium. In most cases, differentiating cells are removed and the stem cells revive with this treatment.