

Isolation of erythroblasts and megakaryocytes from mouse fetal livers.

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Fetal livers are extracted from embryonic day E14.5 mouse embryos and mechanically dissociated. Progenitor cells are purified using a cKit-positive magnetic bead selection (StemCell EasySep Kit #18757). The progenitor cells are expanded in IMDM, 10% FBS in the presence of mSCF and TPO for 7 days. Normally, up to 20-fold expansion is achieved. On day 7 the cells are spun down and washed 4 times to remove mSCF and cultured in IMDM, 10% FBS in the presence of TPO for 5 more days to allow for terminal megakaryocyte differentiation. After a total 12-13 days of culture, the mature megakaryocytes are purified using an anti-CD41 antibody coupled to magnetic beads (StemCell EasySep Kit #18554). The purified CD41-positive megakaryocytes are fixed according to Hardison lab CHIPseq protocol.

Erythroid cells are obtained by enriching fresh E14.5 fetal liver preps for Ter119-positive cells using an anti-Ter119 antibody coupled to magnetic beads (StemCell EasySep Kit #18554). The purified Ter119-positive megakaryocytes are fixed according to Hardison lab CHIPseq protocol.