Megakaryocyte Culture from Mouse Fetal Livers

The protocol for culturing mouse megakaryocytes is essentially as described in the following publication:
Lecine et al., Characterization of the transcription factor NF-E2 .....
J. Biol. Chem. 273:7572-7578 (Issue of March 27, 1998)

Briefly, whole livers are recovered aseptically from mouse fetuses between embryonic days 13 and 15. We've typically had best results with E13.5 cultures, which yield a purer population of MKs; although the overall cell yield is higher with E14.5 cultures, the total number of recoverable MKs is not much higher. Fetal liver is a significantly better source than adult bone marrow or spleen. Typically, outbred strains of mice produce larger litters with larger livers than do inbred mouse strains; for most purposes, either source is OK.

Single-cell suspensions are prepared by successive passage of the whole liver through 18-, 20-, and 22- or 23-gauge needles.

Fetal liver cells are then cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin (we no longer add DMEM non-essential amino-acids), and 1% tissue culture supernatant from a murine c-Mpl ligand producer cell line. The latter is now our preferred source of thrombopoietin, but almost any alternate source is probably OK at a concentration between 0.02 and 0.1 microgram/mL.

By the third day, large polyploid megakaryocytes will begin to dominate the culture, and by the fifth day over 50% of the cell mass will be mature megakaryocytes, many of which will be producing proplatelets, as described in the following paper:
Lecine et al., Mice lacking transcription factor NF-E2 .... Blood 1998, 92:1609-1616 (September 15, 1998 issue)

We no longer separate MKs immunologically as described in the above JBC paper; rather, we use a one-step albumin gradient, exactly as described in Drachman et al., Blood 89: 483-492 (1997). We use 1.5% albumin over 3% and sediment at 1Xg for 35-40 minutes at room temp.

Both the megakaryocyte and non-MK fractions thus obtained are useful for further culture or for isolation of total or nuclear protein and total or poly(A)+ RNA.