Isolation of bone marrow

(contributed by Chris Jackson (chris.jackson@bris.ac.uk))

A protocol I have used to isolate rat bone marrow is:

1. Kill the rat and dissect out the femurs, cutting the bones close to the chondyles. Keep the femurs on ice.
2. Load a 5ml syringe with ice-cold PBS.
3. Fit a blunted 21 gauge needle (nip the bevel off with pliers).
4. Squirt about 3ml of the ice-cold PBS down the shaft of each excised femur.
5. Catch the tissue fragments in a hemispherical 80-mesh stainless steel sieve perched on top of a plastic tube.
6. Rub the tissue fragments through the sieve using the bottom of another plastic tube, and rinse through with the remaining ice-cold PBS.
7. Centrifuge the sample.

Isolation of bone marrow (contributed by David Mullin)

Purification of genomic DNA from bone marrow. For each animal examined, the head of a single femur was cut off and marrow was removed by aspirating dounce buffer (14 mM sodium phosphate pH 8.0, 137 mM NaCl, 3 mM KCl, and 10 mM EDTA) through a puncture in the other end of the bone using a 27 gauge needle and syringe. High molecular weight genomic DNA was purified from marrow using a previously described method (Kohler et al. 1991). No douncing is needed. The marrow recovered from one femur was suspended in 3 ml of dounce buffer and an equal volume of lysis solution (2 mg/ml proteinase K, 2 % (w/v) SDS and 0.1 M EDTA pH 7.5) was added. After a 1 hr incubation at 50oC the nucleic acids were extracted twice with phenol-chloroform and once with chloroform. Genomic DNA was precipitated with ethanol, combined with 500 ml of TE buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA), and incubated at room temperature in the dark for at least one week to facilitate solubilization.