PROTEINASE K DIGESTION OF EMBRYO TISSUE

REAGENTS REQUIRED

Digestion buffer (100mM Tris-HCl, pH8.8; 5mM EDTA, pH8.0; 0.2% SDS; 200mM NaCl)

Proteinase K (20mg/ml) in 10mM Tris, pH 8.0; 20mM CaCl2; 50%(v/v) glycerol

Stored at -20°C

1. Excise tail and hind limbs of an embryo using a razor blade and place in a pre-labelled microtube. Wash razor with 95% EtOH between embryos.
2. Prepare lysis solution. Make a single mixture of 5µL proteinase K and 195µL lysis buffer per sample. Aliquot 200µL lysis solution into each microtube. Vortex briefly and spin down in microcentrifuge (30seconds).
3. Incubate samples in 55°C water bath overnight (~16hours)
4. Vortex tubes for 5 minutes at 15,000RPM to pellet undigested tissue and remove solution from the lid of the tube
5. Dilute digest 1 in 5 (ie. Add 800µL) with ddH2O
6. Vortex tubes briefly and centrifuge at 15,000RPM for 5 minutes
7. Use 1µL digest per PCR reaction