Liver Perfusion and Hepatocyte Isolation

Prepare Solutions:
1. Liver Digest Media (Gibco) – 20 ml aliquot
   - keep cold until ready to use then warm to 37°C.
2. Hepatocyte Wash Medium (Gibco), 4°C
3. Liver Perfusion Medium (Gibco) – 20 ml aliquot
4. Transport Medium: Hepatozyme (Gibco) + 1.0% BSA
   - prepare 50 ml conical + 0.5g BSA
   - distribute so that 2 conicals each contain 25 ml
5. Hepatozyme + 10% FCS

Liver Perfusion:
- oxygenate and prewarme Liver Perfusion Medium
- canulate portal vein and make incision in inferior vena cava
- perfuse for 10 min at 0.7 ml/min (20% pump setting) with Liver Perfusion Medium
- Oxygenate and prewarme Liver Digest Medium
- remove liver and place in 25 ml of ice cold Transport Medium in sterile 50 ml conical

Hepatocyte Isolation:
- in TC hood place liver into sterile 10 cm dish with 10 ml Hepatocyte Wash Medium
- using sterile forceps and scissors cut open the liver and gently scrape off the cells
- dissociate cell clumps by gentle pipetting the solution up and down using a 25ml pipette
- filter the solution through 100 µM nylon caps atop 50 ml conicals
- rinse filter twice with 10 ml Wash Medium each time
- centrifuge at 50g (~1000 rpm) for 3 min.
- gently resuspend pellet in 10 ml Wash Medium using 25 ml pipette and repeat centrifugation
- resuspend cells in Hepatozyme + 10% FCS for plating or assay buffer for immediate use
- determine cell concentration/viability with trypan blue exclusion assay
- for plating place cells in tissue culture treated plates (collagen coated) and replace medium with Hepatozyme + 1% Pen/Strep/Glut after 2h