Preparation of Fetal liver cells for reconstituting chimeras

1)- Take pregnant female at day 14 or 15, (but not before or after for the hematopoiesis peaks in the liver at this time, then move to the spleen and to the bone marrow after birth).

2)- Harvest the embryos, and put them in a small petri dish containing some liver cell buffer.

20 ml RPMI	1 bottle (500ml) RPMI
2 ml FCS (final 10%)	50 ml FCS (final 10%)
400 ul antibiotics Peni-Strep	10 ml antibiotics Peni-Strep
200 ul Glutamine	5 ml Glutamine

3)- Cut the head off. Store the tail in an eppendorf tube and number it for genotyping.

4)- Dissect the liver put it in an eppendorf tube containing 1ml of RPMI Peni-Strep FCS buffer. Numbered them, as the tails, to keep track of them.

5)- Prepare a cell suspension by pipetting up and down each liver with a P1000 first and then P200.

USE FILTERED TIPS to avoid cross contamination between samples!

6)- Let the cells settle 5min on ice. Remove the supernatant and avoid taking the debris that have sedimented at the bottom of the tube. Transfer in a new eppendorf.

NOTE: If you want to freeze the fetal liver cells.
*To freeze the cells down --> Spin the cell suspension. Resuspend in **COLD** **FCS 10% DMSO**. Transfer into a cryotube and put on ice and bring to the -80°C **STRAIGHT AWAY**!*
To thaw the cells --> Transport the cryotubes containing the frozen fetal liver cells in dry ice, or in liquid nitrogen. Slightly thaw the cryotube between your hands to release a ice cube that can be pour in a 50ml falcon tube.
*Add up to 50ml **COLD** PBS drop by drop agitating the falcon tube constantly.*

*Spin the cells down to remove the DMSO. Resuspend the foetal liver cells in 50ml of PBS and leave them on the bench to release the maximum of DMSO at RT for 30min. Wash once in **COLD** PBS.*

7)- One foetal liver is used to reconstitute up to **three** lethally irradiated mice.
Check cell viability with trypan blue. Adjust volume to 200ul per mouse. FILTER and inject cells iv.

NOTE: Here is a tip to help the reconstitution.
Add 500.000 bone marrow cells from a TCRβδ-/- mouse for each reconstitution → so 1.5x 10^6 for 3 recipients. This provides the lethally irradiated recipient with a "ready" hematopoeitic system.