

Hepatocyte ploidy  
FACS analysis/sorting

**Hoechst loading and FACS**

1. Isolate primary or cultured cells. This protocol works well for the following cell types (and most likely other cell types): primary mouse hepatocytes, cultured mouse hepatocytes, primary human hepatocytes and mouse kidney cells.
2. Adjust the concentration to 2 million total cells/mL with loading medium. Lower cell densities are OK.
3. Add Hoechst (final concentration = 15 ug/mL) and Reserpine (final concentration = 5  $\mu$ M). Hoechst is actively pumped out of cells by the ABC transporter, ABCG2. Reserpine selectively inhibits ABCG2, thereby preventing the cells from pumping Hoechst out.
4. Incubate cells in the dark for 30 min at 37°. This step “loads” Hoechst into the cells.
5. Optional antibody stain.
  - Spin down cells (800 rpm x 3 min x 4°). Depending on the number of cells you have, the volume at 2e6/mL may be too large
  - Add antibodies and incubate on ice.
  - Wash and spin down cells (800 rpm x 3 min x 4°).
  - Add secondary antibody (if needed), incubate, wash and spin down.
  - Resuspend at concentration 2e6 total cells/mL.
6. Add PI (final concentration = 5 ng/mL).
7. Keep cells on ice and in the dark until sorting/analysis.
8. For sorting on the Influx, use the 150  $\mu$ m nozzle. Sort into FACS collection medium.
  - Hoechst is excited with the UV laser.
  - You can identify cell populations based on the number of nuclei/cell. Sort putative populations onto a slide and count nuclei. 2n cells are all mono-nucleated. 4n and 8n cells are typically 10-50% mono-nucleated.
  - Check the purity of your sort by analyzing a small portion of the sorted cells (remember to add PI for the purity check). You do not need to reload with Hoechst or reserpine.
  - Expect approximately 25-50% of the cells to be dead.

**Reagents***Loading medium*

DMEM, high glucose  
10% FBS  
HEPES buffer, 10 mM

*FACS collection medium*

DMEM, high glucose  
50% FBS  
HEPES buffer, 10 mM

*Reserpine*

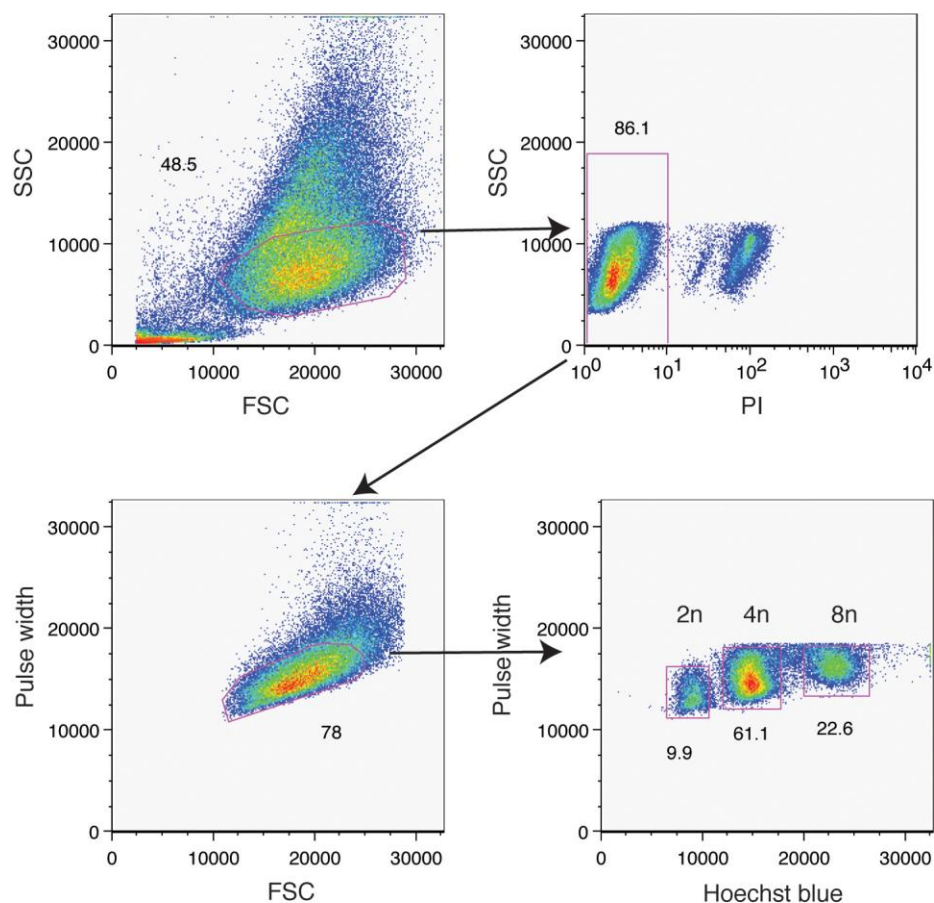
Sigma, cat# R0875-1G  
FW = 608.69  
Stock concentration = 5 mM = 1000X  
Working concentration = 5  $\mu$ M  
Preparation of 5 mM Reserpine: dissolve  
30 mg in 10 mL DMSO  $\rightarrow$  filter sterilize  
 $\rightarrow$  aliquot  $\rightarrow$  store in freezer.

*Hoechst 33342*

Invitrogen/Molecular Probes, Cat # H3570  
Stock conc = 10 mg/mL in water = 667X  
Working conc = 15  $\mu$ g/mL

*Propidium Iodide (PI)*

Stock = 1 mg/mL = 200X  
Working conc = 5  $\mu$ g/mL

**Ploidy gating strategy**

Hepatocytes are from 8 mo Male 129.