TRANSDUCTION EFFICIENCY: GFP FLOW CYTOMETRY

Calculating the percent efficiency of Spinoculation of Viral β-constructs into MEF's

Reagents: 1x Trypsin, 1x PBS, GFP cells (2wells), control cells (1well), 5ml pipette and pipette aid and (3) Falcon 12x75 mm (#35-2058) plastic tubes.

Cell Culture

1. Aspirate off Media and wash monolayer x 1 with 3 ml PBS, aspirate off PBS
2. Add 1 ml of 1x trypsin to the cells and let sit for 2-3 min.
3. Aspirate off trypsin and let monolayer sit for another 2-3 min.
4. Blast off cells in 3ml of 1xPBS and transfer to Falcon 12x75mm (#35-2058) tube
   - 3ml/well
5. Repeat for other wells to be collected (typically 2 GFP wells and 1 control well)

Cytometer- 4th floor flow cytometer

- Click on McIntosh HD on Desk top
- Open Pekala Lab folder → GP2-293 GFP Template
  - Click on Acquire on menu→select Connect to cytometer (this will bring up the acquisition control box, make sure the setup box is checked)
  - Click on Cytometer from menu → on keyboard hold down the apple key + press 1,2,3 (this will bring up Detectors & amps, Threshold boxes, drag these out of the way)
  - Click on Cytometer on menu→ select Instrument Settings →open →select McIntosh HD from drop down box → select Pekala lab folder…select the most recent data file→click open, click set and done
  - Click Acquire on menu→ select acquisition/storage→ under collection criteria make sure parameters are as follows: 10000 counts and G1-R1. storage gate "all" → Click OK
  - Click Acquire→select Parameter Description (this will open parameter Description box) select Folder→ select Pekala folder from drop down menu → click New→ create a new name for file (ex. MEF 6.26.06) Click Create, then click the selected data at bottom of box, under the parameter description box →click file → under Data collect, File count change to start at 001
  - On the Cytometer Turn machine to RUN
  - Place control Sample under wand
  - In the Acquisition control box click Acquire (this will start collecting data for adjustments, when you have enough cells to form a peak press Pause…put R1 box around the mass of cells and adjust FSC FL1 in the Detectors & Amps box the peak should be centered under the M1 gate → Click restart to collect more data points to visualize changes. Repeat if necessary until peak is where you want it
    - Click Pause→ click abort, and uncheck setup box
  - Click Acquire on the acquisition control box(this will start collecting control data into Data File 001) Wait for the beep, that denotes the cytometer has collected 10,000 cells
  - Switch tubes and acquire data for the two GFP tubes
  - Close all windows and don't save changes
  - Switch machine back to Standby
  - Take sample out and replace it with the Isoton II solution

Analysis

- Click on Macintosh HD
- Select Pekala's file → select GFP analysis template
- Click apple + 1,2 (this will open Detectors and Amps and Threshold boxes set all thresholds to 0 and leave FSC-H selected and set FL2 and FL3 Detectors & Amps to 150volts.)
- Click apple + A (this selects all data plots)
- Click on Plots from menu → select Change Data File→ Select Mac HD from drop down box→ select Pekala Lab folder in box→ find and select today's folder (want ever you named it) → select Data File 001 → click open
- Adjust parameters of first plot so that R1 encompasses the most cells in the middle portion
- Adjust Second Plot (histogram) Move the vertical gate of M1 towards the peak so that it is selecting 1% total cells (M1 %total = 1)
- Adjust the third plot (scatter) Move the horizontal gate so that the UR quadrant in %total = 1
- Once all parameters for the control are set...click Batch under menu→ select Run (this will print all data in the selected folder according to the parameters you just set)
- Close out all windows without saving
- Login Acquisition time in Excel program
    - Fill in Month, Day, PI name, Your initials, Time in, Time out, time spent on machine
    - Close window and click save changes

Calculations

- Find the %Gated data in histogram (Marker M1) and scatter (Quad UR) in both GFP Data folders (002 & 003). Subtract 1 from each number; take a mean of the 4 values.
- This mean is the % efficiency of the transduction