PROTEIN/FACS ANALYSIS OF DRUG TREATED CELLS

1. Grow the desired cell line to confluency in a 10 cm dish. Trypsinize and resuspend the cells in 5.5 ml media.

2. Split the cells into 7 6 cm dishes – 3 x 0.5 ml cells, 5 x 1 ml cells.

3. Add the desired drug 12, 24 and 36 hours before harvesting. Use the 1 ml cells plates for 24 and 36 hour drug treatment. Use the 0.5 ml cell plates for untreated and 12 hr drug treatment points. Add the 36 hr drugs at approximately 9 pm on day 1, 24 hr at 9 am on day 2 and 12 hr at 9 pm on day 2. Harvesting will be at 9 am on day 3.

4. For taxol, use 10 nM final concentration. For nicodazole, use 60 ng/ml final concentration.

5. For harvesting, remove and save media. Rinse with HDF wash (save) and trypsinize. Rinse the dish with 1 ml HDF after trypsinizing. Collect all media, washes and cells together.

6. Centrifuge at 1000 rpm for 5 minutes (cold). Remove supernatant and resuspend in 2 ml PBS. Remove 0.7 ml for FACS analysis. Spin down remaining cells and remove supernatant. Store at -20°C for protein analysis. Proteins that may be probed for include BubR1, Bub1, Bub3, cdc20, MAD1, MAD2 and tubulin.

FACS

1. Spin down cells (0.7 ml). Remove most of supernatant. Resuspend cells in small remaining amount of PBS (use Yen(!) technique of dragging over rack). Add 2 ml of cold 70% ethanol slowly to tube while vortexing. Store samples at least overnight at -20°C.

2. Spin down cells. Remove ethanol and resuspend cells in 1 ml cold PBS. Transfer to a 5 ml tube (Falcon #2058). Respin and remove most of PBS. Resuspend cells in small remaining amount (~100 ul). Add 0.5 ml of FACS analysis solution. Incubate in the dark at 37°C for 30 minutes.

3. Propidium iodide FACS staining solution (per sample)
   190 ul 100mM sodium citrate pH 2.5
   9.2 ul 2.5 mg/ml propidium iodide
   1 ul 10 mg/ml RNaseA
   300 ul dH_2O.

COLLECTING DATA ON THE FACS MACHINE

CELL CYCLE DATA:

Tool bar on bottom right of screen – open **DNA ACQ.** This will launch Cell Quest Pro and open the appropriate plots

Under **Acquire**, choose **connect to cytometer**

A window open that will allow you to save your data into a folder. Do the following:

disk → users → shared → ~~facscan~~ data folder

Change directory → desktop → FACScan ~~data~~ → month → lab → your folder

Name your folder and select **Choose**

(NOTE: reset counter to 1 otherwise your first file will be #5; also delete or rename prefixes)

Under **Acquire**, choose **Counters**

Under **Acquire**, choose **Acquisition and storage** – make sure the machine is collecting 10,000 cells

Under **Cytometer**, choose **Detectors and Amps**

Under **Cytometer**, choose **Instrument Settings** **Open**

BD Mac fucc.edu — Broccoli

FACScan disk → BD Applications → CellQuestPro → Settings/Template → ~~Natasha~~ → → (Instrument) Settings → Cell Cycle → Open

Yen/Jim E8O4 copy — MPM2PI (folder) → MPM2PI

Choose **Set** and then **Done**

Change SSC to LOG mode

Acquire data by choosing **Acquire** in the acquisition control window. NOTE: Setup should be selected to allow you to adjust settings. When settings are appropriate (i.e., G1 peak on FL2-A x-axis is 200)….

Choose, **Pause**, **Abort**, deselect **setup**

Acquire for real

CD19/BRDU DATA: (TWO COLOR FAÇS)

Tool bar on bottom right of screen – open **CellQuest Pro.**

Under **File** choose **Open Document** and then do the following:

FACSScan disk → BD apps → CellQuestPro → Settings/Template → Ye CD 19

Under **Cytometer**, choose **Detectors and Amps**

Under **Cytometer**, choose **Instrument Settings**

FACScan disk → BD app → CellQuestPro → Settings/Template → BroccoliCD19

Under **Acquire**, choose **Acquisition and storage** – make sure the machine is collecting 50,000 cells

To save your file:

Acquisition/ Analysis → FACScan data → month → lab → your folder

To set parameters for collecting data, begin with a negative sample.

FL2A – set G1 at 200 then use gain to set FL2H to 200