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Analyzing basic motility of your engineered Dicty:

You want to analyze basic motility of all strains that have an HO number. Let's take (at least) two movies on two different days.

Prepare one 12 well plate and one 24 well plate (you can share with others) - each of your strains sitting in one well - for your movies.

Besides the strains you are actually interested in you will need:

To make sure you can detect fluorescence:
You will use the 24 well plate for scanning through your strains to check for fluorescence.
You want a negative control (HO46 if that is your parent strain; NB: use a different negative control if your parent strain is different, e.g. PTEN- (HO12) or PI3K1/2- (HO2)) and a positive control (HO179 gives nice RFP signal at the plasma membrane; you can use the same positive control for different parent strains).

For taking movies:
In your 12 well plate you want to have a 'wildtype' control strains: Either HO46, HO2 (PI3K1/2-) or HO12 (PTEN-), depending on which strain you put your construct into.

Prepare cells:

- Start with your strain in a tissue culture dish below 80% confluency (little to no floating cells)
- Resuspend cells in your tissue culture dish
- Take 1ml out and count the cells
- Take 1x10^5 cells out of that 1ml and add them to 1ml of media in a new tube
- Immediately put that mix into a well of a 12 well plate (do the same thing for a 24 well plate at the same time)*(=10^5 cells/ 3.8cm2)(8well chamber Lab-Tek II:0.7cm2)*
- Let your cells sit down for >30 minutes (you can leave them for the whole day in that state)

You can set cells up in the evening the day before you want to image them. In that case I suggest using 3.5x10^4 cells/ well (they will divide over night).

Before taking a movie:

- Replace media with 2ml KK2 and wait 30 minutes before starting movies

(during iGEM09 the cells have been sitting in buffer for 0.5-3 hours, each strain for a different amount of time on different days; also during iGEM09 the thermostat in the microscopy room showed 63-65F=17-18degC, kids say that was a constant)

At the microscope:

Compare fluorescence of your cells in the 24 well plate to your negative and positive control When scanning through the plate, note down which strains are clearly RFP positive (remember: your constructs are fused to RFP so that we can detect their expression), which ones you are unsure about and which ones you think are RFP negative.

Taking movies:

Switch to the 12 well plate: take brightfield movies (move the 'wheel of fortune' all the way to the left, set focal plane such that cells appear uniformly dark – slightly out of focus) for 10 minutes - one frame every 15 seconds. The area in the middle of the well will give best image quality.
Use camera settings with 2ms exposure time (20x, 2x2 binning = ca.500X500px; we measured pixelsize ; at these settings data out of the analysis are px/15 sec: multiply by 3.21 for um/min (px to um conversion factor = 0.8025; per 15sec to per min = 4).

Next take one picture of the same field of view using fluorescent RFP settings. This will allow us to identify the interesting cells should we need to
Use camera settings with 500 ms exposure time.

Save movies in your folder clearly labeled so that you will be able to find them again.

→ ***export them as .tif files and proceed to Matlab analysis.***