FISH for FACS applications, using Quantigene probes (BFF)

This is a draft space to keep some notes. Don't try this at home, without consulting Darach (you will probably have some better ideas on how to do stuff and I don't want to miss out on helpful suggestions).

Growth & Sampling

Grow your cells however you like. To collect samples, quickly filter them down onto the same nylon filters we use for RNA sampling. Immediately flash-freeze into liquid nitrogen.

You probably want to collect > 100e6 cells per sample.

Fixation & Permeabilization

Yeast cells need to get into a state where they can survive all the hybridization steps but also be permeable enough to hybridize to probes. This differs for different states of physiology (especially during shifts!), which means different states of balanced or unbalanced growth respond differently to these manipulations.

Do the Filter & PFA fix, lyticase digest, etOH permabilization. As described in that protocol, the PFA fixation time depends on the growth condition. Do a series of fixes with polyA positive control probes to determine where's overfixing and where's underfixing. In my hands, if they survive exhaustive digestion for polyA (with 1 hour zymolase, as in protocol), then they can probably survive Quantigene just fine.

Hybridization

I've adapted the directions from the Quantigene kit, below.

Temperature control is critical. It must be at 40C, not 41C, not 39C. I would error lower than higher because you can correct an erroneous earlier hybridization with correct temperatures later (probably). Use the special thermometer for this.

- Cells should be in 70% etOH, 4C. Aliquot out sample to new eppendorfs, the ones that come with the kit. (appropriate cell number not tested, <100e6 works) Spin RT 1200g 3min.
- Aspirate all and R/S in Quantigene kit solution D, 1ml. Centrifuge at 1200g 3min RT.
- Prepare a 1/20 dilution of the Target Probe in the Target Probe Dilutent.
- Aspirate all and R/S in 25ul of Solution D. Add 25ul of the diluted Target Probe (or just Dilutent for a negative) from above, do not put on ice. Vortex briefly to mix.
- Put in 40C incubator for 2 hours. Interrupt at around 1 hour mark to vortex mix.
- At 2 hours, wash the cells as in the washing step:
  - (The washing step:
    - Add 500ul of Quantigene Wash Buffer to the hybridizations, invert to mix. Spin samples 800g 3min RT.
    - Aspirate all to waste, R/S with 500ul Wash Buffer. Spin 800g 3min RT.)
- Aspirate all to waste. R/S in 25ul Wash Buffer. 
- Add 25ul of pre-warmed Quanitgene Pre-Amp mix (green lid). Incubate at 40C for 1.5 hours. Do the washing step. 
- Add 25ul of pre-warmed Quanitgene Amp mix. Incubate at 40C for 1.5 hours. Do the washing step.
  - Prepare a 1 in 100 dilution of the Label Probe in the Label Probe Dilutent, keep under foil and prewarm. 
- Add 25ul of pre-warmed Quanitgene diluted Label Probe mix. Incubate at 40C for 1 hours.
  - Prepare (per sample) 500ul of Wash Buffer with 5ng/ml DAPI. 
- Add 500ul of Quanitgene Wash Buffer to the hybridizations, invert to mix. Spin samples 800g 3min RT. 
- Aspirate all to waste, R/S with 500ul Wash Buffer with the 5ng/ml DAPI, let sit at RT under foil for 5min. 
- Spin 800g 3min RT. 
- Aspirate all to waste. R/S in 500ul of Storage buffer. Spin cells 3min 800g RT. 
- Aspirate all, R/S with 100ul Storage Buffer.
  - Keep cool from now on out. Store at 4C, keep on ice at bench.

Just because there's a pellet doesn't mean cells are intact! Just because they're no pellet doesn't mean there's no cells (just not very many)! That being said, pellets are really great for morale.

**Microscopy**

Put ~5ul of PBS-suspended cells onto a (optionally poly-L coated) coverslip. Let settle onto coverslip ~20min, try putting on a chemwipe in a petri dish in a box to protect against dust and light. Flip onto coverslip, seal with nail polish. Image on Hochwagen microscope.

**FACS**

Store at 4C. Just before measuring on FACS, dilute to >200ul in flow-cytometry grade (filtered) 1xPBS and sonicate with standard settings. Keep on ice! Keep on ice! 

Bring your samples and controls on ice and extra PBS to dilute. Go FACS with Pui-Leng. You'll need 0.25ml eppendorf tubes for sorting.