polyA staining using singly-labeled FISH

(Darach: if you’re starting to use this for a project, why don’t you check if you can do the hyb faster, a la TurboFISH from the Raj lab, as described in timeseries in www.cell.com/biophysj/fulltext/S0006-3495(17)30162-5 )

This protocol is designed to stain yeast cells with a poly-dT probe to quantify mRNA abundance in single cells using flow cytometry.

This protocol is based on the screen from Amberg, Goldstein, and Cole 1992

Prior to staining perform a Filter & PFA fix, lyticase digest, etOH permabilization on cells. Following that procedure, cells should be stored in 70% ethanol at -20C.

Time: This protocol requires one overnight incubation and can be completed in two days.

(note: the protocol seems to be fairly robust to cell number, i.e. ~1/100th the cells seems to result in similar staining. As long as you have a pellet, you should be ok. However, the absence of a visible pellet does not typically result in robust results.)

Procedure

A. Sample collection and fixation

Collect and Fix cells using the Filter & PFA fix, lyticase digest, etOH permabilization protocol. Following that procedure, cells should be stored in 70% ethanol at -20C.

B. Staining

1. Take aliquots of ~5e6 cells per sample into eppendorfs and place on ice.

2. Spin down cells and remove ethanol. Resuspend in 2xSSC.

3. Spin 800g for 5min. Aspirate all liquid away from the cells. Resuspend cells in 100ul of hybridization buffer with 1ul 10uM probe (DGO1453). Vortex briefly, then place on 37C roller drum O/N in dark (16+ hours).

4. Add 1ml 2x SSC (room temperature) to each sample, invert, spin 10 minutes RT at 800g.

5. Aspirate buffer. Resuspend in 2xSSC. Put back on the 37C roller drum for 15min.

6. Spin 5min at 800g. Aspirate buffer. R/S in 2xSSC.

7. Spin 5min at 800g. Aspirate buffer. R/S in filtered 1x PBS, > 200ul.

8. Sonicate samples (note: this step is essential for efficient flow cytometry analysis).

   (note: Dilute with 1x PBS to a reasonable concentration if necessary (ie just barely noticeable turbidity), this works for our machine but you will need to familiarize yourself with acceptable density for your cytometer.)

9. Analyze on microscope or flow cytometer.

Possible controls:

1. No stain containing all reagents except poly-dT probe (this should be included for each strain or condition tested)

2. RNase treated cells
   a. Spin cells at 800g for 5min.
   b. Resuspend in 1x PBS + 200ug/ml RNaseA
   c. Incubate at 42C for 30min.
   d. Spin 800g for 5 minutes and aspirate
   e. Wash with 2xSSC
2. Spin 800g for 5 minutes and aspirate
3. rpb1-1 allele, shifted to the restrictive temperature. Consistent with other results in the literature, inhibition of PolII doesn’t eliminate all mRNA, but will diminish their abundance.

Reagents

Probes

(dT)_{80}+ V 5’-labeled with Alexa 488 (DGO1453), ordered from IDT. Stock concentration of 100uM. Working solution of 10uM. Protect from light!

Hybridization Buffer

To make: add dextran sulfate to ~ 4.5mL of all other reagents, use vigorous vortexing and a pipette to break up dextran if necessary, then bring it to 5mL total volume. Make a batch and freeze aliquots at -20C.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>dextran sulfate</td>
<td>500mg</td>
</tr>
<tr>
<td>20x SSC</td>
<td>1mL</td>
</tr>
<tr>
<td>ecoli tRNA (50mg/ml)</td>
<td>10uL</td>
</tr>
<tr>
<td>salmon sperm ssDNA (2mg/ml)</td>
<td>625uL</td>
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<tr>
<td>nuclease-free H_{2}O</td>
<td>up to 5mL</td>
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20x SSC

1. In ~400ml of MilliQ H_{2}O, dissolve 87.65g NaCl and 44.10g Na Citrate.
2. pH it to 7.0 using 1M HCl, shouldn’t take too much.
3. Bring to 500ml volume with MilliQ, checking pH.
4. Aliquot into small bottles and sterilize via autoclaving.