Cell Cycle Analysis

1. Test principle

Yeast cells are fixed in ethanol. A washing step is followed by the incubation with RNAse A and the staining with propidium iodide (PI). Afterwards the cells are analysed by flow cytometry.
Susan Forsburg's method for *Schizosaccharomyces pombe*

2. Specimen

Yeast cells in suspension

3. Materials and reagents

- 5ml Falcon polypropylene Round-Bottom test tubes (12x75 mm)
- cooling centrifuge
- sterile-filtered Phosphate-buffered saline (PBS)
- 70% ethanol, ice-cold
- 0.5 M Na citrate stock (filtered), 50mM diluted stock.
- 10 mg/ml RNase A
  Dissolve 50 mg RNAse A (Sigma, R-4875) in 5 ml of PBS and adjust to 0.1% Tween-20.
  Place solution into a 95°C waterbath for 30 min.
  Allow the solution to cool on ice for 1 hr. Remove precipitate by filtering through a 0.2 μm filter.
  Solution can be stored for up to 6 months at 4°C or in aliquots be frozen at -20°C.
- 4 mg/ml Propidium iodide (PI) solution (filter and store in dark at -20°C).
  Dissolve PI (Sigma, P 4170) in dH_2O at 4 mg/ml.
  Store aliquots at -20°C for up to 2 years. Aliquots that are frequently used can be stored at 4°C for up to 2 months. Discard solutions of PI that have been exposed to room temperature for more than 48 hr, or if they appear dark red.

4. Controls

- Untreated cells stained with PI

5. Procedure

1. Spin down 10^7 cells from an exponentially growing culture - 2000 rpm for 5 mins. Pour off supernatant.

2. Vortex tube while adding(**dropwise**) 1.0 ml **ice-cold** 70% EtOH. Store at 4 °C (cells keep indefinitely).

3. When you want to process the cells, take 0.3 ml (this will be 2-3 x 10^6 cells, assuming a little loss in the washing) and add to 3 ml 50 mM Na citrate in a 5ml Falcon tube. Mix and spin 2000 rpm for 5 mins.

4. Discard supernatant and resuspend pellet in 0.5 ml 50mM Na citrate containing 0.1 mg/ml RNase A. Leave in 5 ml Falcon tube and put in 37 °C room for 2 h.

5. For staining:
   Add 0.5 ml 50 mM Na citrate containing 8 µg/ml PI, so that final concentration in the sample is 4 µg/ml. There can be non-specific staining of yeast ends at higher concentrations if cells are starved, or spores. Cells can be processed immediately or conveniently stored overnight at 4°C in the dark before processing the next day. If necessary, cells can be stored at this stage for a maximum of a week (4°C in the dark). Check them under the fluoresence microscope (red channel) to verify staining.

6. (Optional) Just before processing the cells, sonicate for 45 s again leaving cells in the 5 ml Falcon tubes. Sonication prevents doublets of cells which give spurious peaks and is particularly useful if your cells have varying DNA contents and will clean up spores or wee mutants.

7. Analyze samples by flow cytometry.

Note:

- PI is a nucleic acid-specific, red-fluorescent dye. It is also a suspected carcinogen, so employ appropriate safety precautions when working with this dye. This dye will bind to both DNA and RNA, however, treatment of the ethanol-fixed cells with RNAse A, as described above, will digest all of the RNA so that the PI will fluorescently label the DNA, specifically.
- You can fix more than 10^7 cells, but don't process many more than $5x10^6$ fixed cells. Using too many cells can lead to incomplete staining and artifacts.
- You can make controls representing 1, 2 and 4C DNA contents. Use nitrogen starved haploid cells, exponentially growing haploids and exponentially growing diploid cells respectively. You can fix large numbers of cells and use them over many months. It's helpful to include a control sample in each series of samples that you process.
- If you are dealing with particularly fragile cells (e.g. very elongated cells) there may be a problem with lysis when cells are washed in water before fixation. This can be avoided by washing with 1M sorbitol. You can even fix cells in 70% ethanol, 30% 1 M Sorbitol. If you have problems with lysis even in the culture medium, then 1.2 M sorbitol can be included here as well. Wash out the sorbitol before flow cytometric analysis because it destabilizes the sample stream resulting in high CVs.
- Learn how to use the 'Live Gate' option. This allows you to reduce the background in your samples (which may be caused by anything from particles of medium to bacteria or other contaminants) and will improve your data. It also gives you the option of focusing on a particular subpopulation that you may be interested in.

General reference: Sazer and Sherwood (1990) J. Cell Sci **97**:509-516