S. pombe in situs: indirect immunofluorescence

Growth and fixation of cells:

1. Grow cells into log phase in YES (10 mls). Fix by adding an equal volume of 8% PFA in 250 mM Hepes buffer pH 7.4. Fix for **15-90** minutes at rt (For preparation of PFA, see below).

2. Wash cells x3 with PBS.

3. Resuspend in 1 ml PBSSorb containing 0.5 mg/ml zymolyase and 0.1 mg/ml Novozym 254. Incubate at 37°C until the cells become somewhat phase dark. (takes 15-90 minutes). It is probably not a good idea to overdo the digestion.

4. Spin down and resuspend in 1 ml PBSSorb + 1% Triton X-100 for 1-10 minutes. Wash x3 in PBS.

5. Resuspend in about 200 ul PBS. Apply 5 ul cells to polylysine coated cover slips, and spread carefully with a tip. Drain off excess cells with a kimwipe, and allow to dry.

6. Extract with methanol, 6 min, -20°C
acetone, 30 secs, -20°C

(Cells can be stored at this stage at –20°C). This step may not be necessary for detection of some antigens (eg microtubules may be detectable without Me/Ac step)

7. Transfer coverslips to a petri dish, wash in PBS for 5 mins.

8. Replace PBS with PBSBAL; incubate for 30 mins at rt (can rock gently).

6. Drain off PBSBAL, and add 5 ul primary antibody (diluted in PBSBAL) to coverslip. Incubate for 1 hour at rt.

7. Wash 3 x 5 mins in PBSBAL. Apply secondary anitbody (5 ul) and incubate for 1 hour. It's a good idea to spin the secondary antibody to get rid of any fluorescent precipitate before use.

8. Wash 2 x 5 mins in PBS. Drain off PBS and mount in DAPI mountant (in antifade if preferred).

9. Optional step: Seal coverslip with nail varnish and allow to dry completely before examining.

Tip: The intensity of fluorescence is supposed to be higher if the stages after the addition of the secondary antibody are carried out in the dark

Storage: Coverslips (dry) can be stored at -20°C (after step 6).

Solutions needed:

8% PFA

Add 16 g paraformaldehyde to 100 ml water. Heat to 60°C and stir to dissolve. (Vapour is toxic! Use sealed tube or do in hood).
To get all the pfa to dissolve it will be necessary to add a few drops 1 M NaOH, but I have been assured that it is important to keep the NaOH to an absolute minimum. When dissolved add 100 ml 0.5 M HEPES buffer, pH 7.4, filter (0.2 u) and store in 10 ml aliquots at -20°C.

PBS

PBSSorb= PBS + 1.2 M sorbitol (S. pombe) + 1.0 M sorbitol (S. cerevisiae)

PBSBAL = PBS + lysine + azide + BSA:

100 mM lysine hydrochloride, pH 6.9
0.01% NaN_3
1% essentially fatty acid free BSA (sigma)

made up in PBS

Cover slips:

Soak in 0.1 % poly L lysine (5-15 mins). Drain, dry, rinse in H_2O, dry.