S.cerevisiae immunostaining

As written protocol assumes fluorescent detection

NOTE 1: You're going to look at these yeast under a microscope, so you need very few cells compared to what we usually do. We're talking 10mls of a low density culture max, and most steps after that are in ependorff tubes.

NOTE 2: Require polylysine coated slides, a diamond tipped pen to etch them and a DAKO-pen (to draw circles around the intended spots). **(i.e)**

NOTE 3: You will need to make nice with a microscopist. They have specialized (and expensive) equipment you need access to, and a ton more expertise than this protocol will give you (**Part A** is easy; you need the advice for **part B**).

As used in: Keogh *et al* (2006) *Nature* **439:**497.
Exponential phase cultures (10ml, $OD_{600} \approx 0.3$) were gamma-irradiated (γIR) (40Gy). This dose was chosen as it induces DSBs in 86% of cells in the population, with an average of two DSBs per cell [13]. Repair was allowed to proceed at 25°C with samples taken at the indicated time points. Samples (1ml) were formaldehyde crosslinked (5% final), washed with PBS and spheroplasted with lyticase. Cells were applied onto polylysine-coated slides and fixed by successive washes in methanol and acetone. For immunofluorescence first and second antibody layers were applied in PBS / 0.1% Triton X-100 / 2% BSA. To facilitate the visualization of nuclei, DAPI was applied at 10µg/ml in PBS.

A. Permeabilize / Fix cells:

A.1. Grow yeast cells under condition of interest. Remove aliquot to ependorff tube and add formaldehyde (HCHO) to 5%. Incubate RT°, 15mins. Collect by centrifugation (flash spin in a microfuge up to 10K) and wash twice with PBS. (**NB.** usually have a packed cell volume (pcv) < 20µl).

PBS: 10mm Sodium Phosphate pH 7.4
0.9% NaCl

A.2. Resuspend in 100µl lyticase digestion solution. Spheroblast 30°C, 30min. Add 900µl PBS, collect by centrifugation (microfuge: 2K, 5m, 4°C) and repeat PBS wash.
NOTE 4: Be careful – spheroblasts are more fragile than cell-walled yeast

A.3. Resuspend cells pellet 1/20 in PBS (10µl pcv : 200µl PBS). Spot 10µl of this onto precoated slides within the DAKO spot (see **NOTE 2**). Leave for 20 min.
NOTE 5: Adding more will NOT help – it will just make it harder to find isolated yeast for pictures.

Lyticase Digestion Solution (in PBS)
100µg/ml Lyticase (Sigma L4025; 50kU $45; 500U/mg solid)
3µg/ml PMSF
2µl/ml β-ME (13.8M stock at 4°C)

A.4. Wash slide in PBS (2 x dips, 4°C)
Fix slide in MeOH (5 min, -20°C)
Fix slide in Acetone (20 min, -20°C)

Dry slides overnight (RT°C)
Place in sealed bag to exclude moisture at store at –20oC until ready to immunostain (used within a week)

B. Immunostaining

B.1. Wash slide in PBS (2 x dips, 4°C)
Wash slide in PBS / 0.1% Triton X-100
Apply block (2% BSA, 0.1% Tween20 in PBS) directly within the DAKO spot
Block for 60min, 4°C.

B.2. Remove block and add 1st layer Ab (appropriate dilution in PBS / 2% BSA, 0.1% Tween20). Incubate 1 – 2 hrs RT° (**NB.** humidified chamber)

B.3. Remove the majority of Ab. Wash slide in PBS (10 x dips, 4°C)
Add 2nd layer Ab (appropriate dilution in PBS / 2% BSA, 0.1% Tween20). Incubate 1 – 2 hrs RT° (**NB.** humidified chamber). **If a fluorophore, incubate in dark**.
Remove the majority of Ab. Wash slide in PBS (10 x dips, 4°C)

B.4 Add DAPI (10µg/ml) to stain nuclei (binds DNA). Incubate 5 min RT°.
Wash slide in PBS (2 x dips, 4°C)
` Remove liquid. Add mounting agent (**speak to a microscopist**)
Add coverslip: glass **NOT** plastic (autofluorescent)
Seal slip in place
Store at 4°C until ready to visualize.