

## Yeast Chromatin Spread

### **Growth and Spheroplasting:**

1. Grow 10 ml of yeast cells to log phase
2. Harvest and wash once with spheroplasting solution (1.2 M sorbitol, 0.1 M KPO<sub>4</sub>)
3. Resuspend cells in 1 ml of spheroplasting solution
4. add 5 microliter of  $\beta$ -Mercaptoethanol and 10  $\mu$ l of fresh zymolase (5 mg/ml of 100T zymolase in 1.0 M sorbitol)
5. Incubate at 30<sup>0</sup>C for 60 min.
6. Check the spheroplasting under phase contrast microscope.
7. Once 80-90% of the cells are spheroplasted, stop spheroplasting by adding ice cold stop solution (0.1M MES, pH 6.4, 1mM EDTA, 0.5 mM MgCl<sub>2</sub>, 1 M sorbitol).
8. Harvest the spheroplasts at 4<sup>0</sup>C (2000 rpm for 3 min)
9. Gently resuspend the spheroplasts in 1 ml of ice cold stop solution.

### **Making spread on the Slides:**

1. Put 20  $\mu$ l of spheroplasts on a clean slide
2. Add 40  $\mu$ l of paraformaldehyde solution (4% solution with 3.4% sucrose)
3. Mix gently by tilting the slide
4. Add 80  $\mu$ l of 1% Lipsol
5. Mix gently by tilting the slide
6. Add 80  $\mu$ l of paraformaldehyde solution again
7. Mix again
8. Use a tooth pick or a pipet tip to gently mix and spread the contents around the slide.
9. Let slides dry overnight at RT.

### **Immunofluorescence:**

1. Wash slides with 2 ml of 0.4% photoflow-200 (from Kodak)
2. Submerge slides in 1 X PBS for 10 min.
3. Remove excess liquid and immediately add 100  $\mu$ l of blocking solution (10 mg / ml BSA in 1XPBS)
4. Incubate for 15 min at RT
5. Shake of blocking solution and add 50  $\mu$ l of primary antibody. Just drop a coverslip on, and incubate in a humid chamber for 60 min at RT.
6. Rinse of primary antibody by submerging the slide in 1XPBS for 5 min.
7. Add 50  $\mu$ l of secondary antibody and incubate in a humid chamber for 60 min as in step 5.
8. Rinse of secondary antibody by submerging the slide in 1XPBS for 5 min.
9. Repeat step 8 once.
10. Submerge slides in DAPI (1  $\mu$ g/ml in 1XPBS) for 5 min.
11. Rinse DAPI off by rinsing slides in PBS
12. Mount slides with mounting solution.