BISHOP LAB
LIPSOL CHROMOSOME SPREADS AND IMMUNOSTAINING PROTOCOL
Updated July 2006. See helpful hints on page 3 for further explanation of protocol.

SPHEROPLASTING

1. Harvest 5mL cells from yeast culture in 15mL conical tube
2. Spin for 3 minutes at 5000rpm
3. Resuspend pellet in 1mL ZK buffer
4. Add 40μL 1M DTT
5. Incubate for 2 minutes at room temperature with gentle mixing
6. Spin and process as in steps 2 and 3
7. Add 5μL zymolyase 100T solution
8. Incubate for 20-30 minutes in 30ºC incubator on rocking platform or rotator
9. Spin as in step 2
10. Pour off supernatant and wash pellet with 2.5mL cold MES
11. Gently resuspend pellet in 300-400μL cold MES

SPREADING CHROMOSOMES

1. Spot 20μL cell suspension on a clean slide
2. Add 40μL PFA solution, swirl
3. Add 80μL 1% Lipsol, swirl
4. Incubate from 30 seconds up to 2 minutes, monitoring lysis using a light microscope
5. Once 80-90% of spheroplasts are lysed, immediately add another 80μL PFA solution
6. Remove slide from microscope stage
   Note: Be careful! There is a lot of liquid on the slide.
7. Pass a clean glass Pasteur pipet lengthwise across the entire slide to spread the DNA
   Note: There should be liquid covering the entire slide after spreading
8. Let slides dry in hood for at least 4 hours to overnight
9. Store slides in slide box at -20ºC

IMMUNOSTAINING

1. Dip slides in 0.2% photoflo (Kodak) for 30 seconds to remove the sucrose coating
2. Transfer slides to TBS for 5 minutes
3. Drain liquid from slide and wipe off back of slide with Kimwipe (Do not let the slide dry out!)
4. Add 350μL TBS+BSA to each slide and incubate in a wet chamber for 15 minutes
5. Drain as above and add 80μL primary antibody in TBS+BSA (at appropriate dilution)
6. Put on coverslip
7. Incubate 4 hours to overnight at 4ºC in wet chamber
8. Dip slides in TBS to remove coverslips
9. Wash slides 2x10 minutes in TBS

All Further Steps: Keep slides in the dark!

10. Drain slides as in step 3, add 80μL secondary antibody in TBS+BSA (at appropriate dilution)
11. Put on coverslip
12. Incubate slides for 2 hours at 4ºC
13. Remove coverslips and wash as above in step 9
14. Air-dry slides completely (3-4 hours in the hood)
15. Add 30-40μL Vectashield + DAPI (1.5μg/mL), put on coverslip, and seal shut with nailpolish
16. Slides can be stored in the dark at 4ºC for several days

1M DTT
This can be made ahead of time and stored in 1mL aliquots at –20ºC

Zymolyase Solution
20mg/mL Zymolyase 100T
2% Glucose
50mM Tris, pH 7.5

Make fresh on the same day as making spreads (can be stored at 4ºC until ready to use)

Paraformaldehyde (PFA) Solution
3% Paraformaldehyde
3.4% Sucrose

Make fresh on the same day as making spreads
Wearing gloves, put 1.5g paraformaldehyde into 125mL flask
Add 45mL dH_20 preheated to 60ºC
Put in clean stir bar and begin mixing (no heat)
Add 150μL 1N NaOH
Stir until clear (if not clear in 15 minutes, add another 50μL NaOH)
Adjust pH to 7.0 with HCl
Filter in 0.2μM filter unit, put in clean flask
Add 1.7g Sucrose and mix until dissolved
Cover and store on ice, discard any leftovers after spread production

MES Wash Solution
1M Sorbitol
0.1M MES, pH 6.5
1mM EDTA
0.5mM $MgCl_2$

Mix, store at 4ºC

ZK Buffer
25mM Tris, pH 7.5
0.8M KCl

Mix, filter sterilize, and store at room temperature

1% Lipsol
Make dilution from 100% stock, store at 4ºC – this stuff is hard to come by, so don't waste any!

Helpful Hints for Protocol

Spheroplasting

- During zymolyase incubation, cells can be checked for spheroplasts on the phase contrast or light microscope after about 20 minutes. Two volumes of water should lyse most cells.
- Be gentle with spheroplasted cells–do not vortex and resuspend gently using a cut-off pipet tip.
- When working with the pellet of spheroplasts, plastic Pasteur pipets are useful for removing all excess liquid from the pellet before resuspending it for spread production.

Spreads

- Wash glass slides in water and soak in ethanol before making spreads. The day of spread production, dry slides in a slide rack in the hood, and polish with lens paper before using.
- Make a few extra spreads per sample in case slides look bad or break.
- PFA takes a while to make, so take this into consideration if timing is critical. Remember to start heating water to 60ºC before setting up to make the PFA solution.
- Adjusting the pH of PFA can be tricky because it is extremely sensitive to ion concentration. To start, use one or two drops of 1M HCl and continue adjusting pH hereafter by adding drops of increasingly diluted HCl.
- Zymolyase will not completely go into solution, so be sure to pipet up and down or vortex the zymo solution when using it.
- If you are doing a timecourse, it may be easier to keep cells from early timepoints on ice until all of the samples are harvested. Bottom line: once you have started the protocol and made spheroplasts, you should make the spreads.
- The dissection scope (with needle apparatus removed) is excellent for monitoring lysis.
- Dedicate a pipetman adjusted to the proper volume for each reagent for the spreading process so you don't have to change volumes on pipets while making spreads.
- It is difficult to make spreads with gloves and there will be a lot of liquid on the slides, so be careful not to get paraformaldehyde on your fingers!
- When making the actual spread, pass the glass pipet from one end of the slide to the other without pressing on the glass of the slide. The liquid will spread underneath the pipet.
- To prevent overspreading of the DNA, pass the glass pipet over the slide only one time. This will be easier to do if you keep all of the liquid toward one end of the slide while monitoring lysis. Then when ready, you can pass the pipet one time from the side that has the liquid to the side that does not have liquid in order to cover the entire slide.
- Be careful when transferring slides to or from the hood – they easily slide off of plates.

Immunostaining

- In all steps involving coverslips, try to prevent any air bubbles from forming under the surface of the slip. A good method is to lay one end of the slip down and allow the liquid to "wick" underneath it. Then slowly lower the other end of the slip onto the slide, allowing the liquid to follow the slide, which pushes air bubbles out. This can also be done with forceps, which allows more leverage of the coverslip.
- Preventing photobleaching is critical after addition of the secondary antibody to the slides. Incubation and washes should be done under a box or in chambers covered with foil, etc.
- Vectashield is an excellent antifade reagent and samples with Vectashield will be good for several days if stored in the dark at 4ºC.