Meiotic Spread

(Spreads adapted from Luis's 'Modesti/Giroux', immunostaining from Koshland)

Solutions

KS buffer (make fresh each time, only because it's easy and will be clean)

- 1.2 M sorbitol (from 2% filtered stock)
- 2% KAc (from 20% filtered stock)

Spheroblasting buffer (make fresh)

- KS buffer + 1/100th volume 1M DTT + 1/100th volume zymolyase (from 10mg/ml 100T stock)

MS buffer (make fresh each time, only because it's easy and will be clean)

- 1.2 M sorbitol
- 0.1 M MOPS (from 0.5 M filtered stock)
- 1 mM EDTA (from 500 mM filtered stock)
- 0.5 mM $MgCl_2$ (from 1 M filtered stock)

Add 1 mM PMSF just before use (from 250X stock).

Fixative (make in fume hood)

1. Put 2 g paraformaldehyde in 50 ml dH_2O and 200 ul 1 M KOH.
2. Shake in 50 ml Falcon ~1 hr at 30° C until dissolved.
3. Add 1 ml 0.5 M MOPS.

Detergent

- 1% Kodak Photoflo (Hard to get — original protocol used 1% NP-40, which gives similar results.)

Filter sterilise and keep. Vary concentration to increase spreading. (You have to increase it considerably, 5-10%, to increase spreading so try to improve the physical spreading etc. instead.)

Blocking buffer (make fresh each time)

- 0.1 g dried milk powder
- 0.25 g BSA
- 5 ml PBS

Procedure

Preparing the cells

1. Take 1.5-3 ml sporulating culture and keep on ice.*
2. Spin down in small centrifuge 1 min at 5000 rpm.
3. Resuspend in 1 ml (ice-cold) KS buffer.**
4. Spin down 2 min at 6000 rpm.
5. Resuspend in 300 ul spheroblasting buffer.
6. 20 min at 37° C.
7. Check cell wall digestion.***
8. Spin down spheroblasts 3 min at 4000 rpm.
9. Gently resuspend in (ice-cold) MS buffer.
10. Spin down 3 min at 4000 rpm.
11. Gently resuspend in 60-100 ul MS buffer.****

Preparing the slides

1. Boil ~5 cm water in a large glass beaker in microwave.
2. Add 1/100th volume of 1M HCl (for 0.01M).
3. Set up over Bunsen burner flame and stand slides up inside beaker (matt end up).

4. Bring to the boil (may have to cover top).
5. Boil for ~10 min.
6. Use forceps to take out slides and rinse them in 100% ethanol.
7. Lay out to dry — keep dust free!

Spreading (done in fume hood)

1. Pipette 20 ul gently resuspended spheroblasts onto centre of slide.
2. Prepare 3 full pipettes to carry out steps 3-5 as quickly as possible.
3. Add 80 ul fixative.
4. Add 40 ul detergent in a swirling motion — can try waiting 1 min here too.
5. Add 80 ul fixative is a swirling motion.
6. Use final pipette on its side to spread central area of slide without touching slide.
7. Remove bubbles.
8. Dry flat in fume hood.*****

Immunostaining

1. Wash 10 min in PBS.
2. Drain slide (just 2 s), add 200 ul blocking buffer to central area of slide.
3. Incubate 10 min at room temperature in humidity chamber.
4. Drain slide, add 200 ul 1° antibody in blocking buffer.
5. Add plastic square, incubate 1 h in humidity chamber.
6. Remove plastic square, wash twice ~10 min in PBS.
7. Repeat steps 4-6 for 2° antibody (incubate and wash in dark!).
8. Drain slide, add ~2 ul DAPI/antifade to centre of slide.
9. Add coverslip, cover slide with a tissue and press gently to spread DAPI and dry.
10. Paint edges of coverslip with nail varnish.

N.B. If you are not immunostaining, it is that you wash spread 30 min in PBS before proceeding to DAPI step. Also, the original protocol said to rinse dried spreads in 0.2% Photoflo. This is not necessary, but it may be if you are not immunostaining.

* You can get away with less but aim for 2 ml as you do seem to lose cells and it's easier at the last stage if there are more cells — wait at this stage until the end of the time course.

** The original protocol has 3 washes in 1ml KS buffer but this is unnecessary and loses more cells — sometimes wash in less volume if cells are particularly sticky and don't spin down well (seems to be more of a problem at 5-7 h).

*** Mix a 2 ul sample (from the bottom of the Eppendorf) with 2 ul 2% SDS on a slide, add coverslip and check >95% cells are lysed (usually just see a whole lot of cell debris swirling around — make sure you mix properly or it's difficult to tell). Usually 20 min is enough for me but do it for longer if necessary. The only times the cells were not well digested was when MS buffer was used instead of KS, in which case, had to start again!

**** You can leave it at this stage overnight on ice if you want!

***** Original protocol says 2 h but always seems to need longer — do at least overnight, often over the weekend! Don't worry if they do not look dry after overnight drying as they have a 'wet-look'! Also, sometimes they look crystalline, which may seem worrisome, but there is not a noticable difference at the microscope stage. You can store spreads long term no problem in slide boxes at −80° C.