

Making male meiotic spreads

Adapted from Peters lab protocol (Peters et al Chromosome Research 1997)

<u>Hypo buffer:</u>	<u>for 10 mL:</u>
Tris-HCl pH 8.2, 30 mM	500 ul [600 mM stock]
Sucrose pH 8.2, 50 mM	5 ml [100 mM stock]
Sodium citrate, 17 mM	1.7 ml [100 mM stock]
ddH ₂ O to bring up volume	2.8 ml

100 mM sucrose pH 8.2:
3.42 g sucrose (MW 342) in 97 ml ddH₂O
3 ml 600 mM Tris-HCl at pH 8.2
can store aliquots at -20C

1% PFA with 0.15% Triton (PFA-T):
Open fresh vial of PFA.
For 100 ml:
6.25 ml 16% PFA
10 ml 50 mM borate pH 9.2
80 ml ddH₂O
→ pH to 9.2
bring up to 100 ml with ddH₂O

1. Dissect 1 testis and remove tunica in DMEM. Tease apart tubules and tear apart to release cells.
2. Transfer to 15-ml Falcon tube in 7 ml total. Let big chunks settle to bottom of tube and transfer cell suspension (sup) to fresh tube.
3. Spin in TC centrifuge 8 min, 1000 rpm. Remove sup.
4. Add 1 ml hypo buffer and mix carefully. Incubate 7 min, RT.
5. Transfer to ~5 Eppendorfs (200 ul each) and spin 8 min, 1000 rpm, RT. Remove sup.
6. Resuspend in 160 ul of 100 mM sucrose. Wait exactly 2 min.
While waiting, dip 4 pre-cleaned slides outlined in wax pen in 1% PFA + 0.15% Triton. Lay slides down in humid chamber.
7. After 2 min, drop 2 drops of 20 ul (for adult testis) onto each slide. Repeat for each Eppendorf (total of 20 slides).
8. Dry in humid chamber ~2h.
If necessary, remove lid and air dry another 1-2 h until dry.
9. Dilute Photo-Flo 1:250 in PBS. Put slides in 24-slide rack, dip in Photo-Flo ~1 min, then freeze at -80C (no need to dry after Photo-Flo).

References

Peters AH, Plug AW, van Vugt MJ, de Boer P. A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Res.* 1997;5(1):66-68.

Alpatov R, Lesch BJ, Nakamoto-Kinoshita M, et al. A chromatin-dependent role of the fragile X mental retardation protein FMRP in the DNA damage response. *Cell.* 2014;157(4):869-881.