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Culture and Fixation of Cells

Culture (sterile conditions, in hood)

1) Use sterile coverslips (autoclaved) in your well/plate.
2) Plate appropriate concentration of cells to well/plate with coverslips.
3) Let your cells stick down and treat them the same way you would a normal culture (e.g. incubation & feeding). You don't want to grow them too long on the slips before fixation, because you'll get colony like piles of cells which are hard to focus on under the microscope, and they tend to slough off when too dense…its always best to have as much of a monolayer as possible (we usually plate at densities that we can fix the next day).

Fixation (non-sterile on bench top)
(For **nuclear** hybridizations)

1) On ice: 4 small coplin jars. Put two jars on ice and two on counter top at room temp.
2) In 1st jar on ice, put 10 ml of CSK Buffer

CSK Stock:

50ml	0.1M Pipes pH 7.8
50ml	1M NaCl
1.5ml	1M MgCl2
51.35g	Sucrose

Bring up to 500 ml with H2O - filter sterilize and store at 4C (just to keep things from growing in it)

3) In 2nd jar on ice put 9 ml CSK buffer and 0.5ml of a 10% triton solution (Roche), and 0.5ml of VRC (vanadyl ribonucleoside complex -NEB - RNAase inhibitor - 200mM stock). (you can substitute RNAsin instead of VRC for some picky antibodies).
4) In 3rd jar (at room temp) put in 10 ml of 4% Paraformaldehyde in 1XPBS.
5) In 4th jar (at room temp) put in ~10 ml of 70% ETOH

Procedure: We grow cells on 22x22 coverslips, so the coplin jars are small and hold about 10 mls. If your cells are on slides increase the volume proportionally.

6) Hanks Rinse: while they are still in the petri dish, rinse coverslips (cells) 2X with pre-warmed Hanks (or PBS).
7) CSK Rinse: While they are in the last rinse, remove the slips from the dish with forceps, and place in 1st coplin jar just long enough to rinse (remember to keep track of which side has the cells on it).
8) Extraction: Transfer the slips to the 2nd coplin jar for 3-5 min. (many cultured cells need 3-5 min, but its always better to use the shortest time in triton)
9) Fixation: Transfer the slips to the last jar for 8-10 min.
10) ETOH Rinse: Dip slips in the ETOH jar to rinse off paraformaldehyde
11) Storage: Transfer the slips into a plastic petri dish with 70% ETOH (keep the cell side of the slips facing up) Parafilm the sides of the petri dish, and store at 4C until used. (You can also store in PBS but you don't tend to get as good a penetration with the probe, the cells tend to come off the slides more, and "things" grow in it eventually)