FISH on *C.elegans* gonads

Solutions:

20xSSC – (NaCl 175.3g, NaCitrate 88.2g, bring up to 1L and autoclave)
100% formamide
Hybridisation mix – (4xSSC, 20% dextransulfate)

Sample preparation:

Prepare the sample as for the immunofluorescence. After washing 3x5min with 1xPBST, add 50µl of 1M NaSCN, cover with the plastic cover-slip, and incubate for 10min at 78°C in the hybridisation oven.

Transfere slides to 1xPBST and wash 3x5min ant room temp.

Dehydrate in the series of alcohol dilutions: 30%, 50%, 70%, 96%, 96% - each 5min at RT and air dry.

Such prepared sample is ready for FISH

Probe labelling:

Nick translation:

Cosmid DNA (very clean) 2µg
2mM dNTPs(GCA) 5µl
dUTP (dig, Cy3, FITC, …) 1-1,5µl
NT buffer - 5µl - (10x NT buffer: 0,5M TRIS pH=8, 50mM $MgCl_2$, 0,5mg/ml BSA)
B-mercaptoethanol (0.28M) 1.5µl
DNAse I (1mg/ml) diluted 1:500 – 2µl
Polymerase I (Biolabs) 1µl
ddH_2O to 50µl

Incubate for 2h at 16°C. Check 5µl on the gel for the fragments size (you should get a smear between 150bp-500bp). If the size is out of this range add 2µl of DNAse and incubate another 2h at 16°C. Check again on the gel.
Stop reaction by adding 0,5M EDTA – 1µl and incubate at 65°C for 10min.

PCR labelling of 5SrDNA:

10x PCR buffer – 5µl
2mM dNTPs (w/o dTTP) – 5µl
2mM dTTP – 3,8µl
Dig-dUTP – 3,8µl
$MgCl_2$ – 2,5 µl
Prim. 5SrDNA-F (0,1µg/µl) -1µl (stocks at 1µg/µl are in the Boehringer box with AB)
Prim. 5SrDNA-R (0,1µg/µl) -1µl
gDNA – 1µl
polym 1µl
H_2O to 50µl

PCR programm:

94°C -2 min
30x:
94°C – 30sec
55°C – 30sec
72°C – 50sec

72°C – 5min
4°C – forever

Run 5µl on the gel. You should get a band of about 300bp

Hybridisation mix with probe and hybridisation:

Take approximately 100-200ng of the labelled probe per sample. Dry it in the speed-vac.
Add 15µl of formamide – shake for 30min at room temp.
Add 15µl of hybridisation mix – shake for 30mni at room temp.

Denature the probe for 5min at 95°C or in boiling water, and put on ice for 5min.
Spin down and apply onto the slide

Cover with the cover-slip (smallest possible) and seal generously with fixogum.

Denature the specimen at 80°C for 10min and let hybridise over night at 37°C in the humid chamber.

Stringent washing:

Prepare 3 copplin jars with 1x, 0,2x, 0,1x SSC and worm them up at 42°C in the water-bath.

Peal-off the fixo-gum and was the coverslips off in the 2xSSC at room temp. Transfer slides into pre-warmed sequence of SSC dilutions at 42°C.

Wash the slides in 1xPBST for 5min at room temp.

If you work with directly labelled probes add DAPI in vectashield, cover slip and check for the result at the microscope.

Detection:

Block the slides like for the immunostaining.
Put the anti-dig-FITC or Cy3 for digoxigenin labelled probes. Use avidine-FITC or Cy3 for biotine labelled probes. Cover with the plastic cover slip.
Incubate for 1h at room temp. in the humid chamber.

Wash 3x5min with 1xPBST at room temp.
Put DAPI in vectashield, cover with the glass cover-slip and seal with the fixogum.

Pawel's protocol:

Cut worms in 5ul 1XPBS
Add 5ul of 7.4% formaldehyde
Cover slip
Liquid nitrogen (freeze and crack)
5 min in methanol (-20C)
5 min in methanol:acetone 1:1 (-20C)
5 min in acetone (-20C)
3 times 1X PBS-T washes (5 min each @ Rm temp)
in humid chamber --
block with 3% BSA (regular blocking buffer) 15min at least (no more than 30min)
primary AB (10ul of diluted AB – for Y95B8A.11's peptide antibody try 1:50 and 1:100) on with plastic cover slip (4C overnight)
3 times 1X PBS-T washes (5 min each @ Rm temp)
apply 10ul secondary AB cover with plastic cover slip (Anti Rat sth from Jantsch Lab)
(Rm temp for 3 hrs)
3 times 1X PBS-T washes (5 min each @ Rm temp)
apply DAPI vector shield
coverslip on
seal with nail polish

Josef's suggestions:

Cut worms in 5ul 1XPBS
Cover slip
Liquid nitrogen (freeze and crack)
5 min in methanol:acetic acid (3:1) (-20C) methanol & acetic acid both @ Klein Lab
3 times 1X PBS-T washes (5 min each @ Rm temp)
in humid chamber --
block with 3% BSA (regular blocking buffer) 15min at least (no more than 30min)
primary AB (10ul of diluted AB – for Y95B8A.11's peptide antibody try 1:50 and 1:100) on with plastic cover slip (4C overnight)
3 times 1X PBS-T washes (5 min each @ Rm temp)
apply 10ul secondary AB cover with plastic cover slip (Anti Rat sth from Jantsch Lab)
(Rm temp for 3 hrs)
3 times 1X PBS-T washes (5 min each @ Rm temp)
apply DAPI vector shield
coverslip on
seal with nail polish