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% dextran sulfate)

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.

Transfer 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 MgCl2, 0.5mg/ml BSA)
B-mercaptoethanol (0.28M) 1.5μl
DNase I (1mg/ml) diluted 1:500 – 2μl
Polymerase I (Biolabs) 1μl
ddH2O 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
MgCl2 – 2.5 μl
Prim. 5SrDNA-F (0.5μg/μl) –1μl (stocks at 1μg/μl are in the Boehringer box with AB)
Prim. 5SrDNA-R (0.1μg/μl) –1μl
gDNA – 1μl
polym 1μl
H2O to 50μl
PCR programme:

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 30min 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 5'/each time

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 coplin jars with 1x, 0.2x, 0.1x SSC and warm them up at 42°C in the water-bath.
Peal-off the fixo-gum and was the coverstips 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)
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