FISH protocol

Put slides from freezer[1] in 37°C cupboard for 30 min to warm up and avoid moisture built up on slides.

Prepare humid chamber[2] with paper towel and sterile water.

Once dry, place slides on glass with dark background and number sequentially (2 slides per probe).

Prepare probe:

Solution	slide #, slide #
Formamide (1/2)[3]	30
Dextran Sulphate (1/5)[4]	12
20X SSC (1/10)	6
Salmon sperm[5]	2
H_2O	to 60 µl total
Cy3 (red) probes	up to 200 ng = 9µl (Invitrogen NT kit)
SG (green) probes	up to 200 ng = 10µl (VysisInc kit)

Denature at 75°C for 10 min and immediately put on ice. Mix and spin. Keep in fridge until use.

Prepare RNase solution 1ml 2X SSC + 1 µl 100ng/µl Qiagen RNase[6].

Breathe on slides to visualise the spread material, immediately apply 40 µl RNase solution and cover with plastic coverslip. Incubate in humid chamber at 37°C for 1 hr.

Prepare 3 coplin jars with 2X SSC.

Turn on waterbath to 70°C.

[1] Third freezer from left.

[2] Top shelf above FISH sink.

[3] SigmaUltra cat # F5786, on fridge door.

[4] Sigma solution in H_2O. In -20°C freezer box.

[5] -20°C freezer box.

[6] Qiagen # 1006671, stock 100 mg/ml. Final 100 µg/ml In fridge, in box, inside a small plastic bag.

Remove coverslip with forceps before putting in coplin jar and wash slides 3 x 4 min in 2X SSC on shaking incubator.

Prepare pepsin solution: Add 1 ml 10mN HCl in 5 µl pre-aliquoted pepsin[7].

Place last wash coplin jar on paper towel, place slides vertically on paper supported by coplin jar to dry. Wipe bottom of slides with paper and place on dark background.

Separate plastic coverslips so they are ready to use.

Prepare 3 coplin jars with 2X SSC.

Add 45 µl pepsin solution at 10 sec intervals to the slides, and immediately add a coverslip. Treat for 2 min.

Wash 3 x 4 min in 2X SSC in shaking incubator.

Prepare slide denaturation solution: 24.5 ml formamide[8] + 10.5 2X SSC (7:3 ratio) directly in whilte coplin jar. Mix with thermometer, close lid and place in waterbath at 70°C. Turn on fume hood.

Prepare formaldehyde solution (FAD): 10 ml 37% formaldehyde + 90 ml 1X PBS[9]

Incubate slides in FAD solution for 12 min.

Wash 3x 5 min in 2X SSC.

Put slides through ethanol series (50%, 70%, 100%)[10], 2 min each. Dip slide in and out to ensure all water is initially mixed from slide. Dry in black rack on fan.

[7] Sigma, stock 10 mg/ml. 1:200 dilution. In -20°C freezer.

[8] SigmaUltra # F5786. In gel room toxic cupboard.

[9] FAD in gel room toxic cupboard, Merck formaldehyde for microscopy # 1.03990.1000, free from acid, 37%. 1:10 dilution to 3.7%. Use fume hood.

[10] On shelf above FISH bench. Reuse the ethanol.

Check temperature in white coplin jar to be 68°C, denature slides for 1.5 min.

Put slides through -20°C ethanol series for 2 min each. Dry with fan on black rack.

Apply 30 µl probe per slide, cover with glass coverslip, seal with rubber cement, put in humid chamber at 37°C overnight.

DAY 2

Turn on waterbath at 42°C. Prepare 4 coplin jars with 2X SSC and 2 with 0.1X SSC (stringent wash - 1 ml 20X SSC in 200 ml H_2O prepared in flask).

Wash 2x 5 min (sharp) 2X SSC, 2x 0.1X SSC, 2x 2X SSC in 42°C waterbath with shaking and the lids on the coplin jars. Remove last jar from waterbath during last wash to room temperature incubator, with shaking.

Wash one more time at room temperature with 2X SSC, 5 min with shaking.

Wash slides in 4X SSC 0.1% tween[11] for 7 min, without shaking.

For µsats, replace all of above with: 2x 5 min 2X SSC, 2x 5 min 1X SSC at room temperature without shaking.

[11] Use magnetic stirrer for 30 min to prepare.

Briefly wash in 1X PBS and drain. Clean back side of slide with tissue paper, add 25-30 µl Vectashield with DAPI[12] on each, and use forceps to slowly lower a glass coverslip on the slide.

Press on the flat surface of the dissecitng microscope with tissue paper to remove excess mounting solution.

Nick translation

Need 1 µg of DNA per reaction, typically start with 100 ng labelled DNA per slide.

Cy3 - Red

Invitrogen Nick Translation system # 18160-010.

dNTP no dTTP mix	5
DNA	x
H_2O	38-x
Cy3 dTTP	0.6
PolI/DNaseI mix	5

SG - green

Vysis Inc kit # 32-801300

DNA	x
H_2O	17.5-x
0.2mM SG	2.5
0.1mM dTTP	5
0.1mM dNTP	10
Buffer	5
Enzyme	10

In both cases mix and spin and incubate overnight at 15°C on PCR machine. Stop the reaction the next morning with 5µl 0.5mM EDTA (Ambion)[13].

[12] Ready made solution in special freezer compartment. To make dilute 1 µl 500mg/ml DAPI stock from freezer box into 500 ml VectaShield from fridge door.

[13] Storage room in box that fits 8 such bottles.