

FISH hybridization and detection

A. Hybridization

I. Separate Denaturation

1. Incubate slide in 0.05M NaOH/2X SSC at room temp. for no more than 4 min.
(be careful not to over-denature--shorter times may be better)

2. Incubate in ethanol series:

70% ethanol	5 minutes	room temp
Absolute ethanol	5 minutes	room temp
Air dry slide		

3. Denature probe:

20 λ biotin labeled probe
80 λ Hybridization Buffer
5 λ sheared salmon sperm DNA

incubate for 10 min @ 70-75°C

4. Add 100 λ probe mix to slide
gently place coverslip on top
seal coverslip with rubber cement

5. Incubate overnight at 37°C in a sealed humidified chamber.

II. Co-Denaturation

1. Add probe (resuspended in hybridization buffer) to slide
gently place coverslip on top

2. Place sealed slide onto 80°C heat block for 30 sec to 5 min

3. Seal coverslip with rubber cement.

4. Incubate overnight at 37°C in a sealed humidified chamber.

B. Washing

1. Carefully remove rubber cement and coverslip
2. Wash 2X 5 minutes at 45°C in 2XSSC (pH7)/50% formamide
3. Wash 2X 5 minutes at 45°C in 2XSSC (pH7)
4. tap dry (do not let slide completely dry)

C. Detection-Biotin

FITC

1. Add 200 λ FITC-Avidin DCS (Vector) diluted 1:250 in TNFM
2. Float coverslip and incubate 1 hour at room temp in darkened, sealed humid chamber
3. Wash 3X 10 minutes at room temp in 2XSSC/0.05% Tween 20
4. Add 200 λ fluorescent anti-Avidin (Vector) diluted 1:125 in TNFM
5. Float coverslip and incubate 1-2 hours at room temp in darkened sealed humid chamber
6. Wash 3X 10 minutes at room temp in 2XSSC/0.05% Tween 20

Texas Red

1. Add 200 λ TR-Avidin (Vector) diluted 1:200 in TNFM
2. Float coverslip and incubate 1 hour at room temp in darkened, sealed humid chamber
3. Wash 3X 10 minutes at room temp in 2XSSC/0.05% Tween 20
4. Pre-mix Goat α -Avidin 1:125(unconjugated; Vector) and TR- α Goat 1:125 in TNFM. Add 200 λ premix to slide
5. Float coverslip and incubate 1-2 hours at room temp in darkened sealed humid chamber
6. Wash 3X 10 minutes at room temp in 2XSSC/0.05% Tween 20

D. Detection-Digoxigenin

1. Add 200 λ Fluorescein conjugated anti-Dig(Roche) diluted 1:250 in TNFM
2. Float coverslip and incubate 1 hour at room temp in darkened, sealed humid chamber
3. Wash 3X 10 minutes at room temp in 2XSSC/0.05% Tween 20

4. Add 200 μ l Fluorescein conjugated anti-sheep(Vector) diluted 1:125 in TNFM
5. Float coverslip and incubate 1-2 hours at room temp in darkened sealed humid chamber
6. Wash 3X 10 minutes at room temp in 2XSSC/0.05% Tween 20

E. Mounting and storage

7. Add Vectashield with DAPI and seal coverslip with nail polish.
8. Store at -20°C.

SOLUTIONS

Denaturation solution

0.05M NaOH
2X SSC

Hybridization Buffer

2X SSC
50% formamide
10% Dextran sulphate
0.15% SDS

TNFM

4X SSC
0.05% Tween 20
5% powdered milk