ImmunoFISH

Reagents required

20 X SSC		TNT buffer	TNB buffer:
175.3 g	NaCl	0.1 M TrisHCl (pH7.5)	0.1 M TrisHCl (pH7.5)
88.2 g	sodium citrate	0.15 M NaCl	0.15 M NaCl
adjust pH with HCL		0.05% Tween 20	0.05% Blocking Reagent
1 L	H2O		

50% Formamide in 2 X SSC		70% Formamide in 2 X SSC	55% Formamide in 2 X SSC
Formamide	50 ml	70 ml	55ml
H2O	40 ml	20 ml	35 ml
20 X SSC	10 ml	10 ml	10 ml

10% Dextron sulfate

Probe Synthesis with Renaissance Random Primer Biotin Labeling Kit

To generate biotin-N6-dATP labeled DNA probe from random hexamers primer;
Provide net synthesis of labeled DNA;
Probe is typically 300~600 bases;
Signal can be amplified up to 1000 fold;
Can be stored in -20 C for reuse. *** denature before use.

1. Thaw all labeling components except for the Klenow Fragmemnt and place on ice. Slightly spin down.
2. Dilute1 ug of template DNA with water to a final volume of 19 ul. Denature DNA at 95 C for 10 min and quickly chill the DNA on ice for 5 min.

3. Mix 19 ul of denatured DNA with:

Random primers and reaction buffer mix	5 ul
Biotin nucleotide mix	5 ul
Klenow Fragment	1 ul
Total	**30 ul**

4. Mix well and incubate overnight at room temperature/37 C 1 hr.
5. Terminate the reaction by adding 5 ul 0.1 M EDTA (pH 8.0). If the probe is not used immediately it can be stored at -20 C. The probe can be stable for at least 1 year at -20 C. Or precipitate the probe in 30ul TE.

Template DNA	25 ng	100 ng	250 ng	500ng	1 ug
Synthesis DNA	27 ng	70 ng	270 ng	408 ng	710 ng

Making denatured probe

1. Take 9 ul of probe from stock and add 21 ul of hybrid mix solution.

Formamide 50%	10.5 ul
2 X SSC	3.0 ul
10 X Dextron sulfate	7.0 ul

2. Denature the probe at 70 C for 8 min.
3. Incubate the probe at 37 C for 90 min.

Fluorescence in situ hybridization

1. Chromosome spread was aged for 3 days at RT.
2. Apply 200 ul of RNase (100ng/ul in 2 X SCC) to each slide and cover with coverslip. Place in a moisture chamber for 10 min at RT.
3. Rinse the slides with 4 changes of 2 X SCC (pH 7.0) at RT.
4. Dehydrate the slides in ethanol series (70, 85 and 100%) for 2.0 min each at RT.
5. Denature the target DNA by immersing the slides in denaturing solution. Heat to 70 C for exactly 20 min.
   denaturing solution: 70% Formamide in 2 X SCC (pH 7.0)

6. Dehydrate the slide once again in chilled ethanol series.
7. Air dries the slide.
8. Warm the slide on 37 C hot plate.
9. Add 15 ul of denatured probe onto each slide and cover with glass slab.
10. Seal the slide and coverslip with hail polish.
11. Incubate the slides at 37 C for overnight.

Post hybridization Wash

1. Remove the coverslip gently.
2. Wash the slides in 55% Formamide in 2 X SCC for 5 min X 3 at 42 C.
3. Wash the slides in 0.1 X SCC three times for 5 min each at 60 C.
4. Wash the slides in 4 X SCC for 5 min at RT.
5.

Blocking and Conjugate Incubation

*** Never left the slide dry after this step

1. Prepare blocking solution in 4 X SSC/ 3%BSA.
   50 ul 4 X SCC, 150 ul 20% BSA
2. Add 200 ul to each slide and incubate at 37 C for 30 min.
3. Wash the slides once in TNB buffer.
4. Prepare streptavidian-HRP conjugate in TNB (dilute).
5. Add 200 ul to each slide and cover the coverslip. Incubate at 37 C for 45 min.
6. Wash the slides 3 times in TNB buffer for 5 min each with agitation.
7. Pipette 100 ul of fluorescence Tyramide Amplification onto each slide and incubator at RT for 8 min.
8. Wash the slides 3 times in TNT buffer for 5 min each with agitation.
9. Wash the slides with PBS for 30 min.
10. Add serum against LANA (human serum)/NuMA (1:1000, goat anti-LANA) and proceed for immunofluorescence.