Fluorescence in situ hybridization to detect DNA sequences

FISH can also be used to detect specific DNA sequences or gene loci (4, 15). To make DNA accessible to the probe, the cellular DNA must be denaturated by incubation in denaturation buffer.

Equipment and reagents

- Glass coverslips, 22x22mm
- 6-well plates
- 4% freshly made paraformaldehyde (Electron Microscopy Sciences) in PBS, pH 7.4
- Triton X-100 (Sigma)
- Parafilm
- Forceps, Dumont, GG (Electron Microscopy Sciences)
- Avidin-DCS-Texas Red or –fluorescein (Vector)
- Filter paper
- Mounting medium (Molecular Probes)
- Microscopy coverslide
- Deionized formamide (Ambion)

Method

1. Fix sub-confluent cells grown on glass coverslips in 3.7% paraformaldehyde/5% acetic acid in PBS for 15 min at room temperature[a]
2. Wash the cells three times with PBS for 5 min each at room temperature
3. Wash the cells twice with 2x SSC for 5 min each at room temperature
4. Denature coverslips in 70% formamide/2x SSC for 7 min at 85ºC[b]
5. Prepare the probe: Denature 2 μl nick translated probe in 10 μl deionized formamide for 8 min at 95ºC
6. Place immediately on ice
7. Add hybridization buffer to give 50% formamide, 2x SSC, 10% dextran sulfate,1 mg/ml tRNA
8. Place hybridization mixture onto each coverslip and seal with rubber cement

9. Put the slide into a chamber moistened with 2x SSC and incubate for 12-16 h at 37ºC
10. After hybridization wash four times in 2x SSC for 20 min each at room temperature
11. Wash twice in 4x SSC for 10 min each at room temperature
12. Incubate with avidin-conjugated with fluorochrome (2 μg/ml) in 4x SSC for 1 h at room temperature
13. Wash four times in 4x SSC for 15 min each at room temperature[c]
14. Mount the coverslip in mounting medium

[a]Acetic acid permeabilizes membranes; therefore, no detergent permeabilization is required

[b]Make sure the solution is at 85ºC when you add it to the coverslips. The most convenient way of doing this is to use a small water bath and to float the coverslips in dishes.

[c]If background is a problem add 0.1% Triton X-100

- FISH is compatible with detection of proteins by indirect immunofluorescence. Perform the IF first and then proceed to the FISH.