Fluorescence in situ hybridization

Equipment and reagents

- Glass coverslips, 22x22mm
- 6-well plates
- Paraformaldehyde 4% (Electron Microscopy Sciences) in PBS, pH 7.4
- Parafilm
- Triton X-100 (Sigma)
- Forceps, Dumont, GG (Electron Microscopy Sciences)
- Avidin-DCS-Texas Red or –fluorescein (Vector Laboratories)
- Filter paper
- Mounting medium (Molecular Probes)
- Microscopy coverslide
- Deionized formamide (Ambion)
- Yeast tRNA (10 mg/ml) (Sigma)
- Rubber Cement

Method

1. Cells grown on a glass coverslips are fixed in 4% paraformaldehyde in PBS for 20 min at room temperature[a]
2. Wash the cells with PBS three times for 5 min each at room temperature
3. Permeabilize the cells with 0.2 % Triton X-100 in PBS on ice for 5 min
4. Wash the cells three times with PBS for 5 min each at room temperature
5. Wash the cell with 2x SSC for 5 min at room temperature
6. Add the following to a 1.5 ml microcentrifuge tube at room temperature 100 ng biotinylated nick-translation probe or anti-sense riboprobe, 20 μg yeast tRNA[b,c,d]
7. Dry it under vacuum
8. Resuspend the pellet in 10 μl deionized formamide. Incubate the tube for 10 min at 75ºC. Put in water-ice slurry immediately for 5 min

9. Add at room temperature 2 μl 20 x SSC, 2 μl water, 2 μl 50% dextran sulfate, 2 μl 10 mM EDTA (pH 8.0), 2 μl 0.1 M Tris-HCl (pH 7.2). Mix the components and centrifuge briefly
10. Place 20 μl hybridization mixture onto each coverslip and seal with rubber cement
11. Put the slide into a chamber moistened with 2x SSC and incubate for 12-16 h at 42ºC
12. After hybridization, remove coverslips and wash three times in 2x SSC for 15 min each at 37ºC
13. Wash one times in 1x SSC for 15 min each at room temperature
14. Incubate with avidin-conjugated to a fluorochrome (2 μg/ml) in dilution solution (4x SSC/0.25% bovine serum albumin) for 1 h at room temperature
15. Wash three times in 4x SSC for 10 min each at room temperature
16. Wash three times in PBS for 10 min each at room temperature[e]
17. Mount coverslips in mounting medium[f,g]

[a]Pre-extraction before fixation in CSK buffer with 0.5 % Triton X-100 for 3 min on ice before fixation in paraformaldehyde (18) can increase the detection of target sequence

[b]The following recipe is for a volume 20 μl, which is sufficient for one hybridization reaction covering an area of 22 x 22 mm

[c]If no signal is detected, the probe concentration can be increased up to 400 ng/20 μl.

[d]Hybridization without labeled probe should be performed as a control with each experiment

[e]Optional. Wash briefly in water. Wash briefly in absolute ethanol and air dry

[f]FISH is compatible with detection of proteins by immunofluorescence It is recommended that, after rinsing of cells in PBS, incubate the cells with primary antibody and then rinse in PBS and incubate with appropriate secondary antibody (17).

[g]If background is a problem add 0.1% Triton X-100 to all wash solutions.

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