In Situ Hybridization

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Abstract

Modified lightly from

Determination of gene expression patterns using high-throughput RNA in situ hybridization to whole-mount Drosophila embryos

Richard Weiszmann, Ann S Hammonds & Susan E Celniker

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Protocol

Day 1: Prehybridization

Step 1.

Take 20-40 ul of embryos into each tube

Step 2.

Re-hydrate the embryos in 3:1 methanol:formaldehyde (2.5% (vol/vol)) in 1 PBS for 2 min.

Step 3.

Re-hydrate in 1:3 methanol:formaldehyde (2.5% (vol/vol)) in 1 PBS for 5 min.

Step 4.

Post-fix in formaldehyde (2.5% (vol/vol)) in 1x PBS for 10 min.

Step 5.
Rinse 6 in PBT.

Step 6.

Add the hybridization buffer without dextran sulfate into each tube

Step 7.

Incubate by shaking at 125 r.p.m. on the Gyrotory shaker for at least 1 h at room temperature to pre-hybridize the embryos.

Prepare probes

Step 8.

During pre-hybridization, add 200 µL of hybridization buffer with dextran sulfate into a tube

Step 9.

Add 2 µl of the appropriate probe into each tube with dextran sulfate

Step 10.

Remove the hybridization buffer without dextran sulfate from the embryo

Step 11.

Add hybridization buffer with dextran sulfate to embryos

Step 12.

Incubate at 55C by shaking at 125 r.p.m. on the Gyrotory shaker overnight.

Day 2: Hybridization

Step 13.

Add 100 µl of wash buffer at room temperature.

Step 14.
Remove the hybridization buffer–wash buffer mix.

Step 15.

Rinse 2x with wash buffer.

Step 16.

Incubate 8x in the wash buffer at 55C by shaking for 45 min

Step 17.

Incubate in the wash buffer at 55C by shaking overnight.

Day 3: Color Reaction

Step 18.
Rinse in PBT.

Step 19.

Add PBT, goat serum (5% (vol/vol)) and anti-digoxigenin-AP Fab fragments (1:2,000 dilution), and

incubate at room temperature by shaking for 2 h.

Step 20.

Incubate in PBT at room temperature by shaking for 30 min.

Step 21.

Rinse 2x with PBT.

Step 22.

Incubate 9x in PBT at room temperature by shaking for 10 min each.

Step 23.

Rinse 2x with the AP buffer.

Day 3: Color reaction

Step 24.
Wash in the AP buffer at room temperature for 5 min.

Step 25.

Add the AP developing solution

Step 26.

Incubate by shaking in the dark at room temperature until desired color development is achieved (75 min).

Step 27.

Rinse 3 in PBT to stop the color reaction.

Step 28.

Rinse 6x in ethanol.

Step 29.

Rinse 2x in PBT.

Step 30.

Add 70% vol/vol glycerol

PAUSE POINT Stained embryos can be stored at 4C for at least 1 year.

Imaging

Step 31.

Check tubes under a low-power magnification microscope. Embryos are ready to be photographed.