

## Whole mount hybridization, washing, and detection of probe (method 2)

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### Equipment and reagents

- ◆ Shaking platform and heater block, or shaking platform in 55-67 °C incubator.
- ◆ Petri dishes or multiwell culture dishes
- ◆ Washing solution: 50% formamide, 1 x SSC, 0.1% Tween 20
- ◆ Sheep serum.

It may be beneficial to heat inactivate the serum at 56 °C for 30 min. To avoid high backgrounds, it is important that it is free of endogenous phosphatases. Once a good batch has been identified, store in aliquots at -20 °C.

- ◆ MABT: 100 mM maleic acid pH 7.5, 150 mM NaCl, 0.1% Tween 20 (pH is adjusted using NaOH)
- ◆ NTMT: 100 mM Tris-HCl pH 9.5, 50 mM MgCl<sub>2</sub>, 100 mM NaCl, 0.1% Triton X-100
- ◆ Paraformaldehyde fixative (see [Fixation and pre-treatment of embryos for whole mount hybridization](#))
- ◆ NBT and BCIP stock solutions (Boehringer) or BM Purple (Boehringer)
- ◆ Blocking powder (Boehringer): make 10% stock by dissolving in MABT at 65 °C
- ◆ AP-conjugated anti-DIG or anti-fluorescein antibody (Boehringer)

### Method

- 1 Incubate embryos with hybridization mix containing probe overnight at 55–67 °C on shaking platform.
- 2 Wash with washing solution, twice for 30 min, at 55–67 °C on shaking platform.
- 3 Wash with 1:1 washing solution:MABT for 10 min at 55–67 °C on shaking platform.
- 4 Wash with MABT three times for 5 min, then twice for 15 min, at room temperature on shaking platform.
- 5 Pre-block the embryos with 2% blocking powder, 20% sheep serum in MABT, 2–4 h at room temperature on shaking platform.

- 6 Incubate with 1/2000 dilution of anti-DIG or anti-fluorescein antibody (optional: can be pre-absorbed with embryo powder as described in [Pre-absorption of antibody](#)) in 10% sheep serum in MABT, and rock overnight at 4 °C. The antibody solution can be recovered, stored at 4 °C and used up to three times in total.
- 7 Wash the embryos for 1 h with MABT at room temperature, five or more times, then overnight at 4 °C on shaking platform. The overnight wash is optional, but gives lower backgrounds, and it is more convenient to monitor a slow colour reaction if it is started on the following morning.
- 8 Wash twice in NTMT for 15 min on shaking platform and transfer to a Petri dish or culture dish for easier observation. The NTMT can contain 1 mM levamisol, which inhibits many endogenous alkaline phosphatases, but for many systems this is not needed.
- 9 Incubate in the dark with NTMT containing 4.5 µl/ml NBT stock solution, 3.5 µl/ml BCIP stock solution. Alternatively, use BM Purple solution; this reagent gives a stronger signal and low background but may not stain deeper tissues. Periodically monitor the reaction and when a strong signal is produced and/or any background is observed, stop by washing several times with PBT. Fix in 4% paraformaldehyde in PBS for 1–2 h at room temperature, and store in PBS at 4 °C.