

Xenopus tropicalis FISH protocols

Biotin labeling of BAC clones

(using Biotin-High Prime *Roche*)

1. To 1.5 ml tube, add 500 ng BAC DNA and ddH₂O to 16 microliters.
2. Heat in boiling water bath 10 minutes, then quick chill on ice.
3. Mix Biotin-High Prime solution and add 4 microliters to tube.
4. Mix gently and briefly centrifuge.
5. Incubate at 37°C overnight
6. Heat to 65°C for 10 minutes to stop reaction
7. Should generate 2000-2500 ng probe. Store undiluted at -20°C.

Pre-Treatment of Slides

1. Incubate chromosomal spreads with 100 microliters of 100µg/ml RNase A (in 2xSSC) 1 hr 37°C.
(2)
2. Wash 3 x 5 minutes with 2x SSC.
3. Dehydrate in ethanol series (70%-90%-100%) 5 minutes each.
4. Incubate with 0.01% Pepsin in 10 mM HCl for 10 minutes at 37°C.
5. Wash 2 x 5 minutes with PBS; then once with PBS with 50 mM MgCl₂.
6. Fix in PBS with 50 mM MgCl₂ plus 1% formaldehyde for 10 minutes.
7. Wash 5 minutes with PBS; then dehydrate in ethanol series as in #3.

Prepare Hybridization Solution

1. Precipitate probe with unlabeled *tropicalis* DNA.
 - For each slide add 10-100 µg *tropicalis* DNA to 100-200 ng probe. (3)
 - Add 1/10 volume 3M NaAcetate (pH 5.6) and 2.5 volumes 100% EtOH.
 - Incubate -20°C for 30 minutes then centrifuge at 4°C (13000 x g) for 15 minutes.
 - Discard supernatant and dry pellet.
2. Resuspend pellet in 10 µl (per slide) of 50% formamide; 2xSSC; 10% Dextran Sulfate; 50mM NaPO₄ (7.0).
3. Denature hybridization solution at 75°C for 5 minutes; then quick chill on ice.
4. Incubate hybridization solution 2 hours at 37°C to reanneal repetitive DNA.

Hybridization

1. Incubate chromosomes on slides with 70% formamide; 2xSSC; 50mM NaPO₄ (pH 7.0); under a coverslip at 80°C for 3 minutes to denature.
2. Dehydrate in ethanol series (70%-90%-100%) 5 minutes each.
3. Air dry on hot plate 37°C.
4. Add 10 µl of prepared hybridization solution to each slide under cover slip overnight at 37°C.
5. Wash 3 x 5 minutes at 45°C in 2x SSC/50% formamide; then 3 x 5 minutes at 60°C in 0.1x SSC.

Fluorescent Detection

1. After washing. Incubate slides at 37°C for 1 hour in 1x ISH blocking solution (diluted from 5x: *Vector Labs*).
2. Prepare fresh Fluorescein Avidin DCS (1 ml) and Biotinylated Anti-Avidin (0.5 ml) solutions (*Vector Labs*) at 5 µg/ml in 1x blocking solution.
3. Drain off blocking solution by tipping slides and add 100 µl of Fluorescein Avidin DCS to each slide and incubate for 30 minutes at room temperature.
4. Wash slides 2 x 3 minutes in blocking solution.
5. Incubate slides in 100 µl each Biotinylated Anti-Avidin solution for 30 minutes at RT
6. Wash slides 2 x 3 minutes in 1x blocking solution.
7. Add 100 microliters of Fluorescein Avidin DCS to each slide and incubate for 30 minutes at RT
8. Wash slides 2 x 5 minutes in 4x SSC; 0.1% Tween 20.

9. Mount on slide using Vectashield mounting medium with propidium iodide (*Vector Labs*).
10. View at 100X.

Notes:

1. The Biotin high prime protocols give a range of how much labeled probe different amounts of template DNA will produce depending on incubation times. For example, if you are in a hurry and don't need much probe DNA you can generate ~200 ng of probe from 50 ng of template with a 1 hour incubation.
2. We use coverslips made from parafilm but glass coverslips work just as well. All 37°C incubations were done in a humidified chamber.
3. The general rule of thumb is to use 500 fold excess total DNA or 50 fold excess CoT1 DNA to block repetitive sequences. This varies with the type of probe DNA you are using, but we have gotten fair results using a 100 fold excess of total DNA against a BAC probe (200 ng probe and 20 µg total DNA).
4. Reagents used were as follows:
 - Biotin-High Prime; Cat #: 1 585 649 Roche.
 - 5x ISH blocking solution; Cat #: MB-1220 Vector Labs
 - Fluorescein Avidin DCS; A-2011 Vector Labs
 - Biotinylated Anti-Avidin; BA-0300 Vector Labs
 - Vectashield mounting medium with propidium iodide; Cat #: H-1300 Vector Labs