Whole mount in situ hybridization of *S. mansoni*

By Jim Collins and Julie "*in situ* robot" Collins

Modified from:

King and Newmark, In situ hybridization protocols for enhanced detection of gene expression in the planarian *S. mediterranea*. BMC Dev. Bio. 2013 13:8.
Pearson BJ, et al. Formaldehyde-based whole-mount in situ hybridization method for planarians. Dev Dyn. 2009 Feb;238(2):443-50.
Cogswell AA, et al Whole mount in situ hybridization methodology for *Schistosoma mansoni*. Molecular & Biochemical Parasitology 178 (2011) 46–50.

***Unless otherwise noted all incubations, washes, and fixation steps should be performed on a rocker with moderate agitation.**

1) Collect mixed sexed *S. mansoni* in DMEM + 5% FBS. Initial steps are performed in 15ml conical tubes with 5-10ml of each solution.
2) Separate male and female parasites by incubation in a 0.25% solution of the anesthetic ethyl 3-aminobenzoate methanesulfonate (Sigma-Aldrich, St. Louis, MO) dissolved in DMEM+FBS. Alternatively, add 1/10 volume of a 2.5% solution. Rock samples by hand gently for 1-2 minutes or until parasites are relaxed and separated.
3) Kill the parasites in 1ml of 0.6 M $MgCl_2$ for ~1 min
4) Replace $MgCl_2$ with 4% Formaldehyde in PBSTx, incubate 4 hours at RT
5) Rinse 1X with PBSTx.
6) Dehydrate in Methanol and store at -20°C. Samples can be stored for weeks, if not months or years, at -20°C.
7) Rehydrate samples in 50% Methanol solution in PBSTx, 5-10 minutes, RT
8) Incubate in PBSTx, 5-10 minutes, RT
9) Add bleaching solution (9ml H_2O, 500µL Formamide, 250µL 20x SSC, 400µL 30% H_2O_2), incubate 1hr at RT under bright light.
10) Rinse 2x in PBSTx and then incubate in 5ug/ml Proteinase K (Invitrogen) in 1x PBSTx for 45 minutes at RT. **Note: ProK potency appears to vary greatly depending on the source and age of the enzyme. Thus, we suggest empirically determining appropriate enzyme concentration.**
11) Post-fix in 10 ml 4% Formaldehyde in PBSTx, 10 min at RT.
12) Wash in 1:1 (PBSTx:PreHyb), 10 minutes, RT
13) Move samples to small Petri dish, place speciemens in small sized baskets (Intavis Bioanalytical Instruments) in 48-well plate
14) Replace with Prehybe, at least 2hrs, 52°C
15) Replace with Hybe Solution + riboprobe (~200ng/ml), and hybridize overnight (>16hrs) at 52°C. Before adding heat probe and hybe solution to 72°C for 5 minutes and allow to cool to 52°C.
16) Remove Hybe solution and wash at 52°C in preheated solutions:
    2 x 30min Wash hyb
    2 x 30min 2xSSC + 0.1% Triton-X
    2 x 30min 0.2xSSC + 0.1% Triton-X
17) Wash with TNT 2X 10min RT, proceed to either **Colorimetric** or **FISH** detection steps

For Colorimetric Detection of Transcripts

18_Color) Add **Colorimetric Block Solution**, 2 hrs. at RT
19_Color) Add Anti-DIG-AP (1:2000, Roche) in **Colorimetric Block Solution**, incubate O/N at 4°C.
20_Color) Wash 5min, 10 min, then 6x 20 min with TNT.
21_Color) Develop in **AP Buffer** with 4.5µl/ml NBT (Roche) and 3.5µl/ml BCIP (Roche). Develop until desired signal to noise is reached.
22_Color) Development can be stopped by removing development solution and adding PBSTx. Rinse 2x times in PBSTx.
23_Color) Incubate in 100 % Ethanol for 10-20min at RT
24_Color) Remove ethanol and add a few drops of PBSTx ~5 min.
25_Color) Remove PBSTx and add 80% Glycerol in 1x PBS, at least 1 hour.
26_Color) Mount on slides at your leisure.

For Fluorescent Detection of Transcripts

18_FISH) Add **FISH Block Solution**, 2 hrs. at RT.
19_FISH) Add Anti-DIG-POD (1:1000), Anti-FITC-POD (1:1000), or Anti-DNP-HRP (1:300) in **FISH Block Solution**. Incubate O/N at 4°C.
20_FISH) Wash 5min, 10 min, then 6x 20 min with TNT.
21_FISH) Incubate in fresh **Tyramide Solution** for 10 min at RT in dark.
22_FISH) Wash 2x for 5 min each in TNT.
23_FISH) For one color FISH wash overnight in TNT at 4°C with DAPI at 1ug/ml, and proceed to step 28. For multi-color FISH, quench HRP activity with 100mM Sodium Azide in TNT for 45 min at RT.
24_FISH) Wash 4x for 10 min each in TNT.
25_FISH) Add **FISH Block solution**, 1 hr. at RT.
26_FISH) Incubate in Second Antibody solution O/N at 4°C.
27_FISH) Repeat steps 20-26 as needed.
28_FISH) Wash 4x for 10 min each in TNT.
29_FISH) Wash overnight in TNT at 4°C with DAPI at 1ug/ml.
30_FISH) Clear in 80% Glycerol brought to volume with PBS.
31_FISH) When clear move samples to slides and mount in Vectashield (Vector Laboratories).

Solutions

PBSTx: 1X PBS + 0.3% Triton-X 100
Prehybe: 50% De-ionized Formamide (Roche), 5x SSC, 1mg/ml yeast RNA (Sigma), 1% Tween-20 (Sigma, from 10% stock)
Hyb: 50% De-ionized Formamide (Roche), 10% Dextran Sulfate (Sigma), 5x SSC, 1mg/ml yeast RNA (Sigma), 1% Tween-20
Wash Hyb: 25% Standard Formamide (Roche), 3.5x SSC, 0.5% Tween-20, 0.05% Triton X100
Colorimetric Block Solution: 7.5% Horse serum in TNT
FISH Block Solution: 5.0% Horse serum, 0.5% Roche Western Blocking Reagent in TNT
AP Buffer: 100mM Tris, pH 9.5; 100mM NaCl; 50mM $MgCl_2$; 0.1 % Tween-20 brought up to volume with 10% polyvinylalcohol solution (PVA; Sigma).
TNT: 0.1 M Tris pH 7.5, 0.15 M NaCl, and 0.1% Tween-20.
TSA Buffer: 2 M NaCl, 0.1 M Boric acid, pH 8.5; filter sterilized and stored at 4°C.
Tyramide Solution: 1:500 flour-conjugated tyrimide, 1:1000 4-IPBA, 0.003% H_2O_2 in **TSA Buffer**. For 0.003% H_2O_2 make a 1:10 dilution of 30% H_2O_2 in TSA buffer; add 1 µL of this dilution per 1ml of **Tyramide Solution. Bring TSA Buffer to RT before reaction.**