Hybridized Chain Reaction Fluorescent in situ Hybridization (HCR-FISH)

Ruby Lab

Abstract

Hybridized Chain Reaction Fluroescent *In Situ* Hybridization for squid tissue and colonized *V. fischeri*.

Citation: Ruby Lab Hybridized Chain Reaction Fluorescent in situ Hybridization (HCR-FISH). **protocols.io** dx.doi.org/10.17504/protocols.io.dsg6bv
Published: 09 Sep 2015

Protocol

Step 1.
Materials Needed

1) Filtered natural seawater or artificial Instant Ocean (Aquarium Systems, Mentor, OH)
2) Marine PBS (mPBS) – 50 mM phosphate buffer, pH 7.4; 0.45 M NaCl
3) Permeabilization Buffer – mPBS containing 1% (v/v) Tween-20
4) 50% Hyb Buffer – DNA Hybridization Buffer (Molecular Instruments; www.molecularinstruments.org) mixed 1:1 with mPBS
5) Probe Wash Buffer – Provided by Molecular Instruments
6) DNA Amplification Buffer – Provided by Molecular Instruments
7) 5X SSC (750 mM NaCl, 75 mM sodium citrate, pH 7)
8) 5X SSCTw – 5X SSC containing 0.05% Tween-20

Step 2.
Collection and Fixation of *E. scolopes*

[1.1] Collect juvenile squid from hatching table (refer to colonization protocol as needed) and isolate into collection cups. Colonize with desired V. fischeri strain.
[1.2] Anesthetize squid for 2 min by placing into 2% ethanol in filtered seawater.
[1.3] Under dissecting scope, split and peel back mantle on anterior side. Then carefully pull back the funnel to expose the light organ.
[1.4] Place dissected squid into 4% paraformaldehyde (PFA) and incubate overnight at 4 °C on shaker to fix. The best vessels to use are 1.5mL screw-cap vials, to prevent leaking at later wash steps.

Step 3.
Permeabilization by Proteinase K

Use RNase-free equipment and solutions throughout the remainder of the protocol. Perform all treatments, hybridizations, and washes in a 500-µL volume, on a rotator/shaker, unless otherwise noted.
[2.1] Wash each sample five times (5 min per wash) with Permeabilization Buffer at room temperature (RT).
[2.2] Treat with 0.01 mg/mL Proteinase K Permeabilization Buffer at RT for 15-20 minutes. Do not place on shaker or rotator. [2.3] Stop the proteinase K digestion with two washes of 2 mg/mL glycine in Permeabilization Buffer.

[2.4] Post-fix in Permeabilization Buffer with 4% PFA for 1 h at RT on the shaker.
[2.5] Wash with Permeabilization Buffer five times (5 min per wash).
Note: Permeabilized juveniles can be used immediately, or stored for no longer than 1 week at 4 °C in Permeabilization Buffer.

Step 4.
Pre-hybridization for Probes
[3.1] Remove as much of the Permeabilization Buffer as possible from the sample. Incubate sample in 500 µL of 50% Hyb Buffer at 65 °C for 30 min.
[3.2] Change sample into 500 µL of fresh 50% Hyb Buffer, and incubate at 65 °C for 2.5 h.
Note: Prevent drying during prolonged incubations. When using petri plates, place them in a humidified chamber or seal the cover of the plate with a strip of parafilm.

Step 5.
Probe Hybridization
To identify possible artifacts and confounding effects, the following alternate sample preparations should be performed: Autofluorescence (AF) – Follow protocol but do not add probes (step 4) or hairpins (step 7)
Non-Specific Amplification of hairpins (NSA) – Sample incubated without probes (step 4) but with hairpins included.
Non-Specific Detection of targets (NSD) – This control is applicable only for (i) transgenic (non-endogenous) targets, where a wild-type sample missing the target transcript is treated using the same protocol, and with the test probes and hairpins; or (ii) non-ubiquitous endogenous target transcript, where the locus of expression is known beforehand, and for which surrounding tissue can give an estimate of NSD in the same sample after treatment.

[4.1] Mix 1 pmol of each probe in 500 µL of 50% Hyb buffer at 45 °C for 30 min (this step should be coordinated with step [3.2] so that they are completed at the same time).
[4.2] Remove the 50% Hyb buffer from [3.2], and add this probe solution to samples for overnight (16 h) incubation at 45 °C.

Step 6.
Probe Washes
All solutions used here must be pre-warmed to 45 °C. Probe solution is not reused, and hence discarded at the start of the washes.[5.1] Wash samples in 500 µL Probe Wash Buffer for 15 min at 45 °C.
[5.2] Wash samples in 500 µL (75% of Probe Wash Buffer + 25% of 5X SSC) for 15 min at 45 °C.
[5.3] Wash samples in 500 µL (50% of Probe Wash Buffer + 50% of 5X SSC) for 15 min at 45 °C.
[5.4] Wash samples in 500 µL (25% of Probe Wash Buffer + 75% of 5X SSC) for 15 min at 45 °C.
[5.5] Wash samples 2 times, in 500 µL of 5X SSC for 15 min at 45 °C.
[5.6] Wash samples 2 times, in 500 µL of 5X SSC for 30 min at 45 °C.

Step 7.
Pre-hybridization for Hairpins
[6.1] Incubate samples in 500 µL of DNA Amplification Buffer at RT for 30 min.
[6.2] Incubate samples in fresh 500 µL of DNA Amplification Buffer at RT for 30 min.
[6.3] Aliquot 6 pmol (for every 100 µL of DNA Amplification Buffer) of each hairpin in a separate PCR tube.
[6.4] Heat the hairpins to 95 °C for 90 sec (e.g., using a PCR machine/thermal cycler).
[6.5] Store the heated hairpins in the dark for 30 min at RT (keep hairpins unmixed).
[6.6] Prepare 100 µL of fresh DNA Amplification Buffer equilibrated at RT.
Note: (Steps [6.2] through [6.6] should be coordinated so that they are completed at the same time

for all samples)

Step 8.

Hairpin Amplification

[7.1] Mix all hairpins in 100 µL of pre-equilibrated DNA Amplification Buffer at RT. (Final concentration of each hairpin is 60 nM.) **Note**: The volume of this incubation can be scaled up if needed (i.e., for high-abundance squid transcripts); however, the hairpin concentration must be kept constant.
[7.2] Remove final wash solution from [6.2] and add the hairpin solution to the samples for an overnight (16 h) incubation at RT.
[7.3] Wrap the sample tubes (or incubation oven) in aluminum foil to keep light out.

Step 9.

Hairpin Washes

Note: All solutions used here must be pre-equilibrated to RT.
[8.1] Wash samples 4 times, in 500 µL of 5X SSCTw for 5 min each at RT
[8.2] Wash samples 2 times, in 500 µL of 5X SSCTw for 30 min each at RT

Step 10.

Imaging

[9.1] Samples can be imaged directly in 5X SSCTw, or stored in 5X SSCTw at 4 °C.
Note: The processed samples can be counterstained with phalloidin or wheat germ agglutinin following standard protocols.