Supplementary Material for:

Fluorescent two-color whole mount in situ hybridization in Platynereis dumerilii (Polychaeta, Annelida), an emerging marine molecular model for evolution and development

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RNA Labeling Protocol

Antisense RNA probes were synthesized by in vitro transcription incubating 1 μg linearized plasmid, 2 μL 100 mM dithiothreitol (DTT), 1.3 μL NTP mixture (15.4 mM each ATP, CTP, GTP, and 10 mM UTP), 0.7 μL 10 mM digoxigenin-UTP or fluorescein-UTP (Roche Applied Science, Indianapolis, IN, USA), and 0.5 μL RNaGuard™ (Amersham Biosciences, Piscataway, NJ, USA), 2 μL 10× transcription buffer, 1 μL RNA polymerase (20 U/μL; Roche Applied Science) in a final volume of 20 μL for 2–4 h at 37°C. After the addition of 1 μL DNase I (Roche Applied Science), the reaction was incubated for additional 15–30 min. RNA probes were cleaned using the RNeasy® mini kit (Qiagen, Valencia, CA, USA) and eluted in 50 μL double-distilled water, of which 2 μL were controlled by gel electrophoresis. The remainder was mixed with 75 μL hybridization mixture and stored at -20°C.

Hybridization Mixture

The hybridization mixture was 50% formamide, 5× standard saline citrate (SSC), 50 μg/mL heparin, 0.1% Tween® 20, and 5 mg/mL Torula RNA (Sigma, St. Louis, MO, USA).