

Materials List for:

High Resolution Fluorescent *In Situ* Hybridization in *Drosophila* Embryos and Tissues Using Tyramide Signal Amplification

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Materials

| Name | Company | Catalog Number | Comments |
|--|----------------------------|----------------|---|
| 15 ml Polypropylene conical centrifuge tubes | Frogga Bio | TB15-500 | Certified DNase and RNase free |
| 2x PCR Master Mix with dye(Ambiogene) | Biomart (Canada) | 141106-1K | Certified DNase and RNase free |
| 50 ml Polypropylene conical centrifuge tubes | Frogga Bio | TB50-500 | Certified DNase and RNase free |
| 96-well filtration plate (1.2 um, hydrophilic PVDF membrane) | EMD MILLIPORE | MSBVS1210 | Used to remove liquid from bottom of the plate with floating tissues |
| 96-well PCR plate (200 µl) | Denville | C18096-10 | |
| Acrodisc syringe filter (0.2 u) | PALL | PN4612 | |
| Acrodisc syringe filter (0.45 u) | PALL | PN4614 | |
| Agarose | Bioshop | AGA002.500 | |
| XYGEN 96-well assay storage plates | UltiDent Scientific | 24-P96-450V-C | To store bacterial glycerol stocks |
| XYGEN Aluminum Plate Seals | UltiDent Scientific | 24-PCR-AS-200 | To seal plates |
| XYGEN Wide-bore tips (200 ul) | UltiDent Scientific | 24-T205-WB-C | To transfer samples |
| Biotin-SP (long spacer) IgG Fraction Monoclonal Mouse Anti-Digoxin | Jackson ImmunoResearch Lab | 200062156 | Primary antibody for in situ (stock conc. 1 mg/ml, working conc. 2.5 µg/ml or 1/400 dilution. |
| BRAND 8-channel manifold (autoclavable) | BrandTech Scientific Inc. | 704526 | To remove liquids from the top of 96-well PCR plates |
| Chloramphenicol | Sigma-aldrich | C1919 | Stock concentration 34 mg/ml dissolved and 0.2µm filtered in 100 % EtOH (use as 1/1000) |
| Cyanine 3 Tyramide (Cy3-TSA) | Home made | | Could be ordered from Perkin-Elmer Cat# SAT704A001EA |
| DABCO (1,4-Diazabicyclo[2.2.2]octane) | Sigma-aldrich | D2522 | Antifade reagent in mounting media |
| Digoxigenin-11-uridine-5'-triphosphate | Roche | 11-209-256-910 | |
| Embryo collection cage | Home made | N/A | Size: 75 cm X 75 cm X 75 cm made with Polymethyl methacrylate |
| Eppendorf centrifuge 5804 | Eppendorf | Model 5804 | Centrifuge for 96-well plates on A-Z-DWP rotor |
| Formamide | Bioshop | FOR001.500 | |
| Glycerol | Bioshop | GLY002.4 | |
| Glycine | Bioshop | GLN001.500 | |

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|--|---------------------|---------------------------|---|
| Heparin | Sigma-Aldrich | H4784-250MG | Stock: 50 mg/ml. Working concentration 1 mg/ml in Hybridization solution |
| Lab Armor Beads | DiaMed | LAA42370-001 | Fill in heating unit |
| Labnet Gyro M Mini Nutating Mixer | Fisher Scientific | 50-998-347 | Sample mixing |
| Mesh - 100 micons | FlyStuff | 57-103 | For collecting embryos |
| Mesh - 800 microns | SEFAR - Nytex | 06-780/53 | For catching loose tissue / may be used for special tube in figure 4. |
| Micro Cover Glasses | VWR | 48366-067 | Size: 22 X 22 mm |
| Micro slides, frstd | VWR | 48312-003 | Size: 25 X 75 mm |
| Multi-Well Plate Vacuum Manifold | Pall Corporation | P/N 5017 | Remove liquid from bottom of 96-well filter plate |
| Paraformaldehyd (PFA) | Bioshop | PAR070.500 | Fixation |
| PCR machine | Bio-Rad | Model: DNA Engine PTC-200 | 96-well plate PCR and probe denature |
| Picric acid solution | Sigma-aldrich | 80456 | For tissue fixation I solution |
| Potassium Chloride (KCL) | Bioshop | POC308 | |
| PP lid for 96-well plates, 100/case | UltiDent Scientific | 24-P-LID-PP | Lid for storing plates |
| Progene disposable Reagent Reservoirs (50 ml, sterile) | UltiDent Scientific | 825-1-50-5S | Liquid handling |
| Progene Pierceable Aluminum Foil | UltiDent Scientific | 87-CFILM-AL | Seal 96-well plate, DNase and RNase free |
| Proteinase K | Sigma-aldrich | P2308- 5MG | Embryo permeabilization |
| RiboLock Ribonuclease Inhibitor | Thermo Scientific | EO0381 | 40 Unit/ul |
| Ribonucleoside Triphosphate (NTP) set | Roche | 11277057001 | RNA probe sythesis |
| RNase free water | GIBCO | 10977015-500ml | RNA probe sythesis |
| RNaseZap | Ambion | AM9780 | Remove RNase contamination |
| Single stranded DNA from salmon testes | Sigma-aldrich | D9156 | For hybridization solution |
| Skim milk (non fat power) | Bioshop | SKI400.500 | Working concentration is 1 % in 1 X PBT or PBTT as a blocking reagent |
| Sodium Acetate (NaOAc) 3 M, pH=5.2 (RNase free) | Bioshop | SAA333 | Working concentration is 10 % (v/v) for DNA or RNA precipitation |
| SP6 RNA Polymerase | Thermo Scientific | EP0131 | 20 Unit/ul |
| Stainless Steel Shot for Sand Bath | VWR | 13259-274. | To fill the heating unit |
| Stereomicroscope & gooseneck light source | Leica | Model: MZ6 | Tissue dissection and mounting |
| Streptavidin, horseradish peroxidase (HRP) conjugate | Molecular Probes | S911 | Secondary antibody stock: 1 mg/ml, use 1/1000 dilution (1 µg/ml) for in situ. |
| T3 RNA Polymerase | Thermo Scientific | EP0101 | 20 Unit/ul |
| T7 RNA Polymerase | Thermo Scientific | EP0111 | 20 Unit/ul |
| Tip station (10 ul) | Axygen | T-300-STK-S | Certified DNase and RNase free |
| Tip station (200 ul) | Sorbio | 27770T | Certified DNase and RNase free |
| Tips (1ml) | Sorbio | 10200 | Certified DNase and RNase free |
| TRITON X-100 | Bioshop | TRX506 | Working concentration is 0.3% in 1 X PBT for tissue in situ |
| Tween 20 | Bioshop | TWN510 | Working concentration is 0.1% in 1 X PBT for embryo in situ |

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| Whatman qualitative filter paper, Grade 3 | Sigma-aldrich | WHA1003917 | |
| Name | Company | Catalog Number | Comments |
| Solutions | | | Preparation |
| DABCO (1,4-Diazabicyclo[2.2.2]octane) glycerol anti-fade mounting media | | | DABCO (1.25 g), Glycerol (35 ml or v/v 70 %) and 1 X PBS (15 ml, or v/v 30 %). Keep dark and rock in cold room. store at -20 °C |
| 1 X PBT (1L) | | | 1 X PBS with 1 ml Tween 20 (0.1 %) |
| 1 X PBTT (1L) | | | 1 X PBS with 1 ml Tween 20 (0.1 %) and 3 ml of Triton-X-100 (0.3 %) |
| 1 X PBTB (1L) | | | 1 % Skim milk in 1 X PBT |
| 1 X PBTTB (1L) | | | 1 % Skim milk in 1 X PBTT |