Larval RNA in situs (from Peter Follette)

-Fix 15'-20' in 4% formaldehyde in PBS
-Fix 15'-20' in 4% formaldehyde in PBT
-Wash 3 x 5' in PBT
-Proteinase K (10microg / ml) 3'-5'
-Wash 2 x 5' in 2mg / ml glycine in PBT
-Fix 15' in 4% formaldehyde in PBS
-Wash 5 x 5' in PBT
-Wash 10' in hybe solution (same solution as for embryos; see below)
-Prehybe at least 1hr @ 70
-Hybe (40ng riboprobe / 100microliter) 24-36hrs @ 70
-Wash 20' @ 70 in hybe sol'n
-Wash 20' @ 70 in 1:1 hybe:PBT
-Wash 10-12hr in PBT @ 70, changing solutions 5 times
-Add 1:2000 anti-DIG-AP 1hr
-Wash 4 x 30' in PBT
-Wash 20' in staining buffer (same as for embryos, but no Triton X-100)
-Add 4.5 microliters NBT, 3.5 microliters X-phosphate per 1ml staining buffer

Hybe solution:

50% deionized formamide
5X SSC
100 ug / ml sonicated, denatured salmon sperm DNA
100 ug / ml E. coli tRNA
50 ug / ml heparin
0.1% Tween 20
pH to 4.5 with Citric Acid

Staining Buffer:

100 mM Tris pH 9.5
100 mM NaCl
50 mM $MgCl_2$