

***In Situ* Hybridization**  
**(on semi-thin/ultra-thin cryosections)**

1. Prepare probe by diluting DNA/RNA stock to 0.5-1.0 ug/ml in formamide

**Note:** 10ul of tRNA (10mg/ml stock); and 10 ul of salmon sperm DNA (10 mg/ml stock) may be added as well to control for non-specific binding). Generally, I do not do this unless I have significant background problems.

2. Prepare hybridization solution as follows:

250 ul of 20X SSC (final 5X SSC)

750 ul 10%FCS (final 2.5% FCS)

1000 ul total

**Note:** 1:100 dilution of vanadyl riboside complex (BRL 15522-014) to a final concentration of 1 mM; and 25% final of dextran sulfate can be added to control for non-specific binding. Generally, I do not do this unless I have significant background problems.

3. Just before hybridization, heat DNA/RNA probe to 70-80<sup>0</sup>C for 10 min; mix 1:1 with hybridization solution; and add 50-100 ul of mixture to sections (or place grids on 10 ul drops).
4. Slides or grids are incubated at 37<sup>0</sup>C overnight in a humid chamber.
5. Sections/cells are washed 3X in drops/solutions consisting of (50% foramide/2X SSC) and 3X in 2X SSC for a total of 30 min (5 min/ wash).
6. Hybridized DNA/RNA is subsequently detected by Avidin/IgG bound FITC or Au conjugates diluted 1:200 or 1:50 respectively in 10% FCS.
7. Sections or grids are washed with 1X PBS containing 10 uM glycine and 2.5% FCS. (6X, 5 min ea., for a total of 30 min.)
8. Slides for IF are mounted in anti-fade 75% glycerol solution; grids are washed through 10 puddles of ddH<sub>2</sub>O and absorption stained in a solution containing:  
3.2% PVA  
0.2% methyl cellulose  
0.1% uranyl acetate