Institute for Systems Biology Dual-Color Hybridization Protocol

- Take 5 ug of total RNA or 0.5-1 ug of polyA alexa labeled probe per dye color and combine in an eppendorf tube.

- Speed-vac the samples down to less than 5 ul (try not to dry them completely)

- Add 40 ul of hybridization buffer to probe and mix well by pipeting.

**Hybridization Buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIG Easy Hyb</td>
<td>36.2 ul</td>
</tr>
<tr>
<td>SS DNA</td>
<td>1.8 ul</td>
</tr>
<tr>
<td>tRNA</td>
<td>1.8 ul</td>
</tr>
<tr>
<td>dT(50)</td>
<td>0.13 ul</td>
</tr>
</tbody>
</table>

- Heat probe to 95 degrees C for 3 minutes and put immediately on ice for 2 minutes.

- Pipet probe onto slide and cover with a clean coverslip.

- Hybridize in a humid chamber at 42 degrees C for 15 hours.

- Remove coverslip by placing in a shaking petri-dish with 30 mls of 42 degree 1X SSC, 0.2% SDS for 5 minutes.

- Remove slide, wipe the back, and place in Molecular Dynamics Automated Slide Processor for further washing.

**Washes:**

- 2 x 5 minute wash in 1X SSC, 0.2% SDS (3ml) at 42 degrees C
- 2 x 5 minute wash in 0.1X SSC, 0.2% SDS (3ml) at 25 degrees C
- 2 x 5 minute wash in 0.1X SSC (3.5ml) at 25 degrees C

Remove slide from processor, dip in water for 2 seconds and blow dry with compressed air.