

Wholemout *In Situ* Hybridization

Rehydration, Hybridization & Staining

(Rivera Lab)

Experiment#: _____ Date: _____ Probe: _____

Cross: _____ Diss. Date: _____ No. Embryos: _____

<p>DAY 1</p> <p>REHYDRATION</p> <table style="width: 100%; border-collapse: collapse;"> <tr><td><input type="checkbox"/> 75% MetOH/PBT</td><td style="text-align: center;">15 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> 50% MetOH/PBT</td><td style="text-align: center;">15 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> 25% MetOH/PBT</td><td style="text-align: center;">15 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> PBT</td><td style="text-align: center;">20 min</td><td style="text-align: center;">RT</td></tr> </table> <p>PROT K TREATMENT</p> <table style="width: 100%; border-collapse: collapse;"> <tr><td><input type="checkbox"/> 10 µg/ml Prot. K in PBT</td><td style="text-align: center;">5 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> PBT</td><td style="text-align: center;">5 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> PBT</td><td style="text-align: center;">5 min</td><td style="text-align: center;">RT</td></tr> </table> <p>RE-FIXATION</p> <table style="width: 100%; border-collapse: collapse;"> <tr><td><input type="checkbox"/> 4% PFA/0.2% Glutaraldehyde in PBT</td><td style="text-align: center;">20 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> PBT</td><td style="text-align: center;">10 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> PBT</td><td style="text-align: center;">10 min</td><td style="text-align: center;">RT</td></tr> </table> <p>HYBRIDIZATION</p> <table style="width: 100%; border-collapse: collapse;"> <tr><td><input type="checkbox"/> 1:1 PBT/Hyb. Sol.</td><td style="text-align: center;">Let Settle</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> Hyb. Solution</td><td style="text-align: center;">Rinse</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> Hyb. Solution</td><td style="text-align: center;">Rinse</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> Hyb. Solution</td><td style="text-align: center;">2 h</td><td style="text-align: center;">70°C</td></tr> <tr><td><input type="checkbox"/> 0.1 – 1 µg/ml probe in Hyb. Solution</td><td style="text-align: center;">o/n</td><td style="text-align: center;">70°C</td></tr> </table>	<input type="checkbox"/> 75% MetOH/PBT	15 min	RT	<input type="checkbox"/> 50% MetOH/PBT	15 min	RT	<input type="checkbox"/> 25% MetOH/PBT	15 min	RT	<input type="checkbox"/> PBT	20 min	RT	<input type="checkbox"/> 10 µg/ml Prot. K in PBT	5 min	RT	<input type="checkbox"/> PBT	5 min	RT	<input type="checkbox"/> PBT	5 min	RT	<input type="checkbox"/> 4% PFA/0.2% Glutaraldehyde in PBT	20 min	RT	<input type="checkbox"/> PBT	10 min	RT	<input type="checkbox"/> PBT	10 min	RT	<input type="checkbox"/> 1:1 PBT/Hyb. Sol.	Let Settle	RT	<input type="checkbox"/> Hyb. Solution	Rinse	RT	<input type="checkbox"/> Hyb. Solution	Rinse	RT	<input type="checkbox"/> Hyb. Solution	2 h	70°C	<input type="checkbox"/> 0.1 – 1 µg/ml probe in Hyb. Solution	o/n	70°C	<p>BLOCKING</p> <table style="width: 100%; border-collapse: collapse;"> <tr><td><input type="checkbox"/> Blocking Solution 2% BBR in MABT</td><td style="text-align: center;">1 h</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> Blocking Sol. + NGS 2% BBR, 10% NGS in MABT</td><td style="text-align: center;">1 h</td><td style="text-align: center;">RT</td></tr> </table> <p>ANTIBODY</p> <table style="width: 100%; border-collapse: collapse;"> <tr><td><input type="checkbox"/> Antibody Stain 1:2000 α DIG-AP, 2% BBR, 10% NGS in MABT</td><td style="text-align: center;">o/n</td><td style="text-align: center;">4°C</td></tr> </table>	<input type="checkbox"/> Blocking Solution 2% BBR in MABT	1 h	RT	<input type="checkbox"/> Blocking Sol. + NGS 2% BBR, 10% NGS in MABT	1 h	RT	<input type="checkbox"/> Antibody Stain 1:2000 α DIG-AP, 2% BBR, 10% NGS in MABT	o/n	4°C																					
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Sol./MABT</td><td style="text-align: center;">10 min</td><td style="text-align: center;">70°C</td></tr> <tr><td><input type="checkbox"/> MABT</td><td style="text-align: center;">Rinse</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> MABT</td><td style="text-align: center;">Rinse</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> MABT</td><td style="text-align: center;">30 min</td><td style="text-align: center;">RT</td></tr> <tr><td><input type="checkbox"/> MABT</td><td style="text-align: center;">30 min</td><td style="text-align: center;">RT</td></tr> </table>	<input type="checkbox"/> Hyb. Solution	Rinse	70°C	<input type="checkbox"/> Hyb. Solution	Rinse	70°C	<input type="checkbox"/> Hyb. Solution	1 h	70°C	<input type="checkbox"/> Hyb. Solution	1 h	70°C	<input type="checkbox"/> 1:1 Hyb. 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