Quality control and validation

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# Un-templated background

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>“Stickiness” of probes, circle formation and RCA</th>
</tr>
</thead>
</table>
| **How does it look?** | • Signal in acellular regions  
• Tends be in z planes abutting the glass surface  
• Tends to occur when pooled probe concentration >1000nm per gene. |
| **Problems due to background** | • distinguishing low-expression from no-expression  
• when staining a uniform population (no “internal control”) |
| **Steps to eliminate/reduce background** | • Avoid regions with low Tm  
• Abundant transcripts: design 5 probe sets.  
• Moderate – Rare transcripts: design ~ 10 probe sets (pooled concentration should not exceed 1000nM)  
• Stringent buffer washes after H probe hybridization and ligation of circles.  
• Use truncated H probes or scrambled oligos |
## Templated background

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<th>Possible cause</th>
<th>partial complementarity to non-target transcripts</th>
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</thead>
<tbody>
<tr>
<td>How does it look?</td>
<td>• Looks like true signal in cells</td>
</tr>
</tbody>
</table>
| Problems due to background | • distinguishing low-expression from no-expression.  
• when staining a uniform population (no “internal control”) |
| Steps to eliminate/reduce background | • Avoid regions with low Tm  
• BLAST sequences when designing probes (especially if the transcript has several homologues)  
• Stringent buffer washes after H probe hybridization and ligation of circles. |

**False negatives:**
- H probes with potential dimers / hairpins
ISH in mouse

- Perform ISH in gene knockouts (negative control)

- ISH for transcript along with immunostaining for the corresponding protein.

SPC ISH
SPC IHC
ISH in human

• Perform ISH in cell lines (www.proteinatlas.org) that express your gene of interest (positive control). Use cells with knockdown of the gene as negative control.

• Use previously well characterized expression pattern in certain tissues for validation

• ISH for transcript along with immunostaining for the corresponding protein.

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<th>Control</th>
<th>Scrambled siRNA knockdown</th>
<th>BMPR2 siRNA knockdown</th>
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Adam Andruska
A Hybrid Approach: PL-ISH