Hybridization Mix:

- Thaw hybridization mix in 65°C water bath for 15 minutes. Ensure that all components are mixed thoroughly by inversion.
- Add appropriate reagents for 125ul hybridization mixture.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrated cDNA and H₂O</td>
<td>23ul</td>
</tr>
<tr>
<td>Ambion Hyb Buffer #3 (Cat#AM8863)</td>
<td>100ul</td>
</tr>
<tr>
<td>LNA dT blocker (Genisphere Cat#CW3910 or CW3920)</td>
<td>2ul</td>
</tr>
</tbody>
</table>

- Incubate reaction mixture at 80°C for 10 minutes and then hold at 65°C until sample injection.
- Inject the entire volume, 125ul, into the injection port.

Tecan 4800 HS Pro Program Tree: *(Caveat: This is to be used as a guideline and each lab may have to optimize to suite the researchers needs.)*

PROGRAM START Name: Kooplab Tecan Buffer 3, Comment:

1 WASH    Temp.°C: 23.0, 23.0, 23.0, First: Yes, Ch.: 2, Runs: 3, Wash time: 0:00:30, Soak time: 0:00:30

2 WASH    Temp.°C: 23.0, 23.0, 23.0, First: No, Ch.: 6, Runs: 2, Wash time: 0:01:00, Soak time: 0:00:30

3 WASH    Temp.°C: 49.0, 49.0, 49.0, First: No, Ch.: 1, Runs: 1, Wash time: 0:01:00, Soak time: 0:00:00

4 HYBRIDIZATION Temp.°C: 49.0, 49.0, 49.0, Agitation Frequency: Medium, High Viscosity Mode: No, Time: 1:00:00

5 WASH    Temp.°C: 49.0, 49.0, 49.0, First: No, Ch.: 4, Runs: 2, Wash time: 0:01:00, Soak time: 0:00:30

6 SAMPLE INJECTION Temp.°C: 49.0, 49.0, 49.0, Agitation: Yes, BCR: No
7 HYBRIDIZATION    Temp.°C: 49.0, 49.0, 49.0, Agitation Frequency: Medium, High Viscosity Mode: Yes, Time: 1:00:00

8 HYBRIDIZATION    Temp.°C: 53.0, 53.0, 53.0, Agitation Frequency: Medium, High Viscosity Mode: Yes, Time: 1:00:00

9 HYBRIDIZATION    Temp.°C: 49.0, 49.0, 49.0, Agitation Frequency: Medium, High Viscosity Mode: Yes, Time: 4:00:00

10 HYBRIDIZATION   Temp.°C: 53.0, 53.0, 53.0, Agitation Frequency: Medium, High Viscosity Mode: Yes, Time: 1:00:00

11 HYBRIDIZATION   Temp.°C: 49.0, 49.0, 49.0, Agitation Frequency: Medium, High Viscosity Mode: Yes, Time: 4:00:00

12 HYBRIDIZATION   Temp.°C: 53.0, 53.0, 53.0, Agitation Frequency: Medium, High Viscosity Mode: Yes, Time: 1:00:00

13 HYBRIDIZATION   Temp.°C: 49.0, 49.0, 49.0, Agitation Frequency: Medium, High Viscosity Mode: Yes, Time: 4:00:00

14 WASH            Temp.°C: 49.0, 49.0, 49.0, First: No, Ch.: 4, Runs: 1, Wash time: 0:01:00, Soak time: 0:00:00

15 HYBRIDIZATION   Temp.°C: 49.0, 49.0, 49.0, Agitation Frequency: High, High Viscosity Mode: No, Time: 0:03:00

16 WASH            Temp.°C: 49.0, 49.0, 49.0, First: No, Ch.: 4, Runs: 1, Wash time: 0:00:30, Soak time: 0:00:00

17 WASH            Temp.°C: 39.0, 39.0, 39.0, First: No, Ch.: 4, Runs: 1, Wash time: 0:00:20, Soak time: 0:00:00

18 WASH            Temp.°C: 30.0, 30.0, 30.0, First: No, Ch.: 4, Runs: 1, Wash time: 0:00:20, Soak time: 0:00:00

19 WASH            Temp.°C: 23.0, 23.0, 23.0, First: No, Ch.: 3, Runs: 1, Wash time: 0:00:20, Soak time: 0:00:00

20 WASH            Temp.°C: 23.0, 23.0, 23.0, First: No, Ch.: 6, Runs: 3, Wash time: 0:00:30, Soak time: 0:00:00

21 SLIDE DRYING    Temp.°C: 23.0, 23.0, 23.0, Time: 0:02:30, Final Manifold Cleaning: Yes, Ch.: 5
**Liquid Definition:**

Liquid Channel 1: 5xSSC, 0.01% SDS, 0.2% BSA
Liquid Channel 2: 0.1xSSC, 0.1% SDS
Liquid Channel 3: 1xSSC
Liquid Channel 4: 2xSSC, 0.014% SDS
Liquid Channel 5: Water
Liquid Channel 6: 0.2xSSC