### Hybridize Oligo Pool

1. Add 5 µl ACD1 and 5 µl TE or water to 1 well of the HYP plate.
2. Add 10 µl gDNA to each remaining well.
3. Add 5 µl TSO to each well containing gDNA.
4. Add 5 µl TSO to ACD1.
5. Centrifuge at 1000 × g for 1 minute.
7. Centrifuge at 1000 × g for 1 minute.
8. Place on the preheated heat block and incubate for 1 minute.
9. Reset the temperature to 40°C and incubate for 80 minutes.

### Remove Unbound Oligos

1. Make sure that the heat block has cooled to 40°C.
2. Remove from the heat block.
3. Centrifuge at 1000 × g for 1 minute.
4. Transfer each sample to the FPU plate.
5. Cover and centrifuge at 2400 × g for 5 minutes.
6. Wash 2 times with 45 µl SW1.
7. Reassemble the FPU plate.
8. Add 45 µl UB1.
9. Cover and centrifuge at 2400 × g for 5 minutes.

### Extend and Ligate Bound Oligos

1. Add 45 µl ELM4 to the FPU plate.
2. Incubate at 37°C for 45 minutes.
**Amplify Libraries**

- □ 1. Arrange the Index 1 (i7) adapters in columns 1–12.
- □ 2. Arrange the Index 2 (i5) adapters in rows A–H.
- □ 3. Place the IAP plate on a TruSeq Index Plate Fixture.
- □ 4. Use a multichannel pipette to add 4 μl of each Index 1 (i7) adapter to each row.
- □ 5. Use a multichannel pipette to add 4 μl of each Index 2 (i5) adapter to each column.
- □ 6. Add 56 μl TDPI to 2.8 ml PMM2.
- □ 7. Invert to mix.
- □ 8. When incubation is complete, remove the FPU plate from the incubator and remove the seal.
- □ 9. Cover and centrifuge at 2400 × g for 5 minutes.
- □ 10. Use a multichannel pipette to add 25 μl 50 mM NaOH to the filter plate.
- □ 11. Incubate at room temperature for 5 minutes.
- □ 12. Transfer 22 μl PMM2/TDPI master mix to the IAP plate.
- □ 13. Transfer samples eluted from the FPU plate to the IAP plate.
- □ 14. Centrifuge at 1000 × g for 1 minute.
- □ 15. Transfer to the post-amplification area.
- □ 16. Perform PCR on a thermal cycler.

**Clean Up Libraries**

- □ 1. Centrifuge the IAP plate at 1000 × g for 1 minute.
- □ 2. Run an aliquot of libraries on 4% agarose gel (5 μl) or Bioanalyzer (1 μl).
- □ 3. Add 45 μl AMPure XP beads to the CLP plate.
- □ 4. Transfer all the supernatant from the IAP plate to the CLP plate.
- □ 5. Shake at 1800 rpm for 2 minutes.
- □ 6. Incubate at room temperature for 10 minutes.
- □ 7. Place on a magnetic stand until liquid is clear.
- □ 8. Remove and discard all supernatant.
- □ 9. Wash 2 times with 200 μl 80% EtOH.
- □ 10. Use a 20 μl pipette to remove residual EtOH.
- □ 11. Remove from the magnetic stand and air-dry for 10 minutes.
- □ 12. Add 30 μl EBT.
- □ 13. Shake at 1800 rpm for 2 minutes.
- □ 14. Incubate at room temperature for 2 minutes.
- □ 15. Place on a magnetic stand until liquid is clear.
- □ 16. Transfer 20 μl supernatant from the CLP plate to the LNP plate.
- □ 17. Centrifuge at 1000 × g for 1 minute.

**Normalize Libraries**

- □ 1. For 96 samples, add 4.4 ml LNA1 to a new 15 ml conical tube.
- □ 2. Use a P1000 pipette to resuspend LNB1.
- □ 3. For 96 samples, transfer 800 μl LNB1 to the tube of LNA1.
- □ 4. Add the LNA1/LNB1 mix to a trough.
- □ 5. Add 45 μl LNA1/LNB1 to the LNP plate.
- □ 6. Shake at 1800 rpm for 30 minutes.
- □ 7. Place on a magnetic stand until liquid is clear.
- □ 8. Remove and discard all supernatant.
- □ 9. Remove from the magnetic stand.
- □ 10. Wash 2 times with 45 μl LNW1.
- □ 12. Remove from the magnetic stand.
- □ 13. Add 30 μl fresh 0.1 N NaOH.
- □ 14. Shake at 1800 rpm for 5 minutes.
- □ 15. Place the LNP plate on a magnetic stand until liquid is clear.
- □ 16. Add 30 μl LNS2 to the SGP plate.
- □ 17. Transfer 30 μl supernatant from the LNP plate to the SGP plate.
- □ 18. Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days. Alternatively, store at -25°C to -15°C for up to 7 days.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days. Alternatively, store at -25°C to -15°C for up to 30 days.
Pool Libraries

☐ 1 Transfer 5 µl to an 8-tube strip.
☐ 2 Seal the plate and store at -25°C to -15°C.
☐ 3 Transfer the contents of the 8-tube strip to the PAL tube.
☐ 4 Denature and dilute pooled libraries to the loading concentration for the instrument you are using. See the denature and dilute libraries guide for your instrument.

Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACD1</td>
<td>Amplicon Control DNA 1</td>
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<tr>
<td>ACPI</td>
<td>Amplicon Control Oligo Pool 1</td>
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<tr>
<td>TSO</td>
<td>TruSight Oligos</td>
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<tr>
<td>CLP</td>
<td>Clean-up Plate</td>
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<td>EBT</td>
<td>Elution Buffer with Tris</td>
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<td>Extension Ligation Mix 4</td>
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<td>FPU</td>
<td>Filter Plate Unit</td>
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<td>HYP</td>
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<td>IAP</td>
<td>Index Amplification Plate</td>
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<td>LNA1</td>
<td>Library Normalization Additives 1</td>
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<td>Library Normalization Storage Buffer 2</td>
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<td>Library Normalization Wash 1</td>
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<td>PAL</td>
<td>Pooled Amplicon Library</td>
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