Hybridize Oligo Pool

1. Add 5 µl ACD1 to 1 well of the HYP plate.
2. Add 5 µl gDNA to each remaining well.
3. Add 5 µl ACP1 to the well containing ACD1.
4. Add 5 µl AFP1 to each well containing gDNA.
5. Centrifuge at 1000 × g for 1 minute.
6. Add 40 µl OHS1. Pipette to mix.
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 2 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 2 minutes.

Extend and Ligate Bound Oligos

1. Add 45 µl ELM3 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 plate on a TruSeq Index Plate Fixture.
☐ 4. Add 4 µl of each Index 1 (i7) adapter down each column.
☐ 5. Add 4 µl of each Index 2 (i5) adapter across each row.
☐ 6. Remove the FPU plate from the incubator and do the following:
   ☐ a. Replace the aluminum foil seal with the filter plate lid.
   ☐ b. Centrifuge at 2400 × g for 2 minutes.
   ☐ c. Add 25 µl 50 mM NaOH. Pipette to mix.
   ☐ d. Incubate at room temperature for 5 minutes.
☐ 7. Add 56 µl TDP1 to a full tube (2.8 ml) of PMM2. Invert to mix.
☐ 8. Transfer 22 µl PMM2/TDP1 mixture to the IAP plate.
☐ 9. Transfer eluted samples from the FPU plate to the IAP plate.
☐ 10. Centrifuge at 1000 × g for 1 minute.
☐ 11. Transfer the IAP plate to the post-amplification area.
☐ 12. Place on the preprogrammed thermal cycler and run the PCR program.

SAFE STOPPING POINT
If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Libraries

☐ 1. Centrifuge the IAP plate at 1000 × g for 1 minute.
☐ 2. Run an aliquot of library and control 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. Transfer 800 µl LNB1 to the tube of LNA1.
☐ 4. Add 45 µl LNA1/LNB1 to the LNP plate.
☐ 5. Shake at 1800 rpm for 30 minutes.
☐ 6. Place on a magnetic stand until liquid is clear.
☐ 7. Remove and discard all supernatant.
☐ 8. Remove from the magnetic stand.
☐ 9. Wash 2 times with 45 µl LNW1.
☐ 10. Use a 20 µl pipette to remove residual LNW1.
☐ 11. Remove from the magnetic stand.
☐ 12. Add 30 µl fresh 0.1 N NaOH.
☐ 13. Shake at 1800 rpm for 5 minutes.
☐ 14. Place the LNP plate on a magnetic stand until liquid is clear.
☐ 15. Add 30 µl LNS1 to the SGP plate.
☐ 16. Transfer 30 µl supernatant from the LNP plate to the SGP plate.
☐ 17. Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT
If you are stopping, seal the plate and store at -25°C to -15°C for up to 30 days.
Pool Libraries

☐ 1 Centrifuge at 1000 × g for 1 minute.
☐ 2 Transfer 5 µl of each library to an 8-tube strip.
☐ 3 Transfer the contents of the 8-tube strip to the PAL tube. Pipette to mix.
☐ 4 Denature and dilute pooled libraries to the loading concentration for the sequencing 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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<td>AFP1</td>
<td>Amplicon Fixed Panel 1</td>
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<tr>
<td>CLP</td>
<td>Clean-up Plate</td>
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<tr>
<td>EBT</td>
<td>Elution Buffer with Tris</td>
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<td>ELM3</td>
<td>Extension Ligation Mix 3</td>
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<td>FPU</td>
<td>Filter Plate Unit</td>
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<td>HYP</td>
<td>Hybridization Plate</td>
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<td>IAP</td>
<td>Indexed Amplification Plate</td>
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<td>LNA1</td>
<td>Library Normalization Additives 1</td>
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<td>PCR Master Mix 2</td>
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<tr>
<td>TDP1</td>
<td>TruSeq DNA Polymerase 1</td>
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<tr>
<td>UB1</td>
<td>Universal Buffer 1</td>
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