Quick Start Protocol
SureSelect XT HS2 DNA with Post-capture Pooling

G9983-90500 Rev A0
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DNA Fragmentation and Library Preparation Workflow

- Fragment DNA: Enzymatic or Covaris
  - 20 min (Enzymatic fragmentation)
- End repair and dA-tail fragments
  - 40 min
- Ligate MBC-tagged adaptors
  - 40 min
- Clean-up ligation
  - 30 min
- Amplify and dual-index
  - 35 min
- Clean-up library
  - 30 min
- Quality control
  - Varies

Hybridization/Capture Workflow

- Hybridize libraries to probe
  - 1.5–2.5 hrs
    Optional overnight hold
- Prepare streptavidin beads
  - 30 min (Concurrent)
- Capture hybridized libraries
  - 70 min
- Amplify enriched libraries
  - 30 min
- Clean-up final libraries
  - 30 min
- Quality control
  - Varies
  - Stopping point
- Pool libraries for multiplex NGS
  - 30 min

Duration estimates are provided as guidelines for 16 reaction runs using 200 ng high-quality input DNA and probe design >5 Mb. Your results may vary.
DNA Fragmentation

Option 1: Enzymatic fragmentation

1. Prepare DNA samples in wells: 7 µl each containing 10–200 ng DNA. Keep on ice.
2. Set up thermal cycler program (Table 1). Start and pause program.

**Table 1: Thermal cycler program for enzymatic fragmentation (vol 10 µl)**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>37 °C</td>
<td>See Table 2</td>
</tr>
<tr>
<td>Step 2</td>
<td>65 °C</td>
<td>5 min</td>
</tr>
<tr>
<td>Step 3</td>
<td>4 °C</td>
<td>Hold</td>
</tr>
</tbody>
</table>

3. Prepare Fragmentation master mix (Frag MM, see Table 3). Mix > spin > keep on ice.
4. Add 3 µl Frag MM to DNA samples. Mix > brief spin.
5. Place in thermal cycler and press play/continue.
6. At 4 °C Hold step, remove from thermal cycler > add 40 µl nuclease-free water > keep on ice.

**Table 2: Duration of fragmentation at 37 °C (Step 1 in Table 1)**

<table>
<thead>
<tr>
<th>NGS Read Length</th>
<th>High-quality DNA</th>
<th>FFPE DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 X 100</td>
<td>15 min</td>
<td>15 min</td>
</tr>
<tr>
<td>2 X 150</td>
<td>10 min</td>
<td>15 min</td>
</tr>
</tbody>
</table>

**Table 3: Frag MM reagent volumes (including excess for supported run sizes)**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Per Rxn</th>
<th>16 Rxn Kits</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X SureSelect Fragmentation Buffer</td>
<td>2 µl</td>
<td>18 µl</td>
<td>36 µl</td>
</tr>
<tr>
<td></td>
<td>52 µl</td>
<td>200 µl</td>
<td></td>
</tr>
<tr>
<td>SureSelect Fragmentation Enzyme</td>
<td>1 µl</td>
<td>9 µl</td>
<td>18 µl</td>
</tr>
<tr>
<td></td>
<td>26 µl</td>
<td>100 µl</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3 µl</td>
<td>27 µl</td>
<td>54 µl</td>
</tr>
<tr>
<td></td>
<td>78 µl</td>
<td>300 µl</td>
<td></td>
</tr>
</tbody>
</table>

Proceed directly to Library Preparation.

Option 2: Covaris shearing

1. Prep Ahead: Set up the Covaris E220 instrument. Refer to the instrument user guide.
2. Prepare DNA samples: 50 µl each containing 10–200 ng DNA. Keep on ice.
3. Transfer DNA sample to the Covaris microTUBE > spin > verify all bubbles released.

**Note:** Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions.

4. Shear DNA using following settings:
   - Duty factor = 10%
   - Peak incident power = 175
   - Cycles per burst = 200
   - Bath temp = 2–8 °C

5. Use shearing duration based on NGS read length and sample type (Table 4).

**Table 4: Shearing duration**

<table>
<thead>
<tr>
<th>NGS Read Length</th>
<th>High-quality DNA</th>
<th>FFPE DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 X 100</td>
<td>2 X 120 sec</td>
<td>240 sec</td>
</tr>
<tr>
<td>2 X 150</td>
<td>2 X 60 sec</td>
<td>240 sec</td>
</tr>
</tbody>
</table>

6. Transfer 50 µl sheared DNA sample to PCR plate or strip well > keep on ice.
7. Spin Covaris microTUBE > transfer residual liquid to same well.

Proceed directly to Library Preparation.
Step 1: Prepare ligation master mix

- Vortex thawed Ligation Buffer at high speed for 15 sec.  
  *Reagent is viscous and must be vortexed.*
- Prepare Ligation master mix (Lig MM, see Table 5). Mix > spin > keep at RT during end repair/A-tailing steps.

Table 5: Lig MM reagent volumes (including excess for supported run sizes)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>16 Rxn Kits</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per Rxn</td>
<td>8 Rxn</td>
</tr>
<tr>
<td>Ligation Buffer</td>
<td>23 μl</td>
<td>207 μl</td>
</tr>
<tr>
<td>T4 DNA Ligase</td>
<td>2 μl</td>
<td>18 μl</td>
</tr>
<tr>
<td>Total</td>
<td>25 μl</td>
<td>225 μl</td>
</tr>
</tbody>
</table>

Step 2: End repair and dA-tail fragments

- Set up thermal cycler program (Table 6). Start and pause program.
- Vortex thawed End Repair-A Tailing Buffer at high speed for 15 sec.  
  *Reagent is viscous and must be vortexed.*
- Prepare End repair/A-tailing master mix (ER-AT MM, see Table 7). Mix > spin > keep on ice.

Table 7: ER-AT MM reagent volumes (including excess for supported run sizes)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>16 Rxn Kits</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per Rxn</td>
<td>8 Rxn</td>
</tr>
<tr>
<td>End Repair-A Tailing Buffer</td>
<td>16 μl</td>
<td>144 μl</td>
</tr>
<tr>
<td>End Repair-A Tailing Enzyme Mix</td>
<td>4 μl</td>
<td>36 μl</td>
</tr>
<tr>
<td>Total</td>
<td>20 μl</td>
<td>180 μl</td>
</tr>
</tbody>
</table>

Step 3: Ligate MBC-tagged adaptors

- Get 70 μl DNA samples from ice. Add 25 μl Lig MM from Step 1. Mix > spin.
- Add 5 μl SureSelect XT HS2 Adaptor Oligo Mix. Mix > spin.
- Place in thermal cycler and press play/continue.
- At 4 °C Hold step, remove from cycler > keep on ice.

Step 4: Clean-up ligation

- Get AMPure XP beads held at RT, vortex until homogeneous.
- Add 80 μl of AMPure XP beads to each DNA sample well. Mix > incubate at RT 5 min.
- Collect beads using magnet (5–10 min) > remove and discard supernatant.
- Wash beads 2X with 200 μl freshly-prepared 70% ethanol per wash. Washes completed: 1 2
- After second wash solution removed, spin briefly > remove residual ethanol.
- Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- Elute by adding 35 μl nuclease-free water to each sample well. Seal wells > vortex > brief spin. Incubate at RT 2 min.
- Collect beads using magnet (~5 min).
- Transfer 34 μl cleared supernatant to fresh well > keep on ice.
### Step 5: Amplify and dual index (Pre-capture PCR)

**35 min**

Set up thermal cycler program (Table 9). Start and pause program.

**Table 9: Thermal cycler program for pre-capture PCR (vol 50 µl)**

<table>
<thead>
<tr>
<th>Step</th>
<th>Number of Cycles</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>1</td>
<td>98 °C</td>
<td>2 min</td>
</tr>
<tr>
<td>Step 2</td>
<td>8 for 100–200 ng high-quality input library</td>
<td>98 °C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>9 for 50 ng high-quality input library</td>
<td>60 °C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>11 for 10 ng high-quality input library</td>
<td>72 °C</td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td>11 for 100–200 ng FFPE input library</td>
<td>72 °C</td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td>12 for 50 ng FFPE input library</td>
<td>72 °C</td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td>14 for 10 ng FFPE input library</td>
<td>72 °C</td>
<td>1 min</td>
</tr>
<tr>
<td>Step 3</td>
<td>1</td>
<td>72 °C</td>
<td>5 min</td>
</tr>
<tr>
<td>Step 4</td>
<td>1</td>
<td>4 °C</td>
<td>Hold</td>
</tr>
</tbody>
</table>

Prepare Pre-capture PCR master mix (Pre-PCR MM; Table 10). Mix ▶ spin ▶ keep on ice.

**Table 10: Pre-PCR MM reagent volumes (including excess for supported run sizes)**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>16 Rxn Kits</th>
<th>8 Rxn</th>
<th>16 Rxn</th>
<th>24 Rxn</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X Herculase II Buffer with dNTPs</td>
<td>10 µl</td>
<td>90 µl</td>
<td>180 µl</td>
<td>260 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Herculase II Fusion DNA Polymerase</td>
<td>1 µl</td>
<td>9 µl</td>
<td>18 µl</td>
<td>26 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Total</td>
<td>11 µl</td>
<td>99 µl</td>
<td>198 µl</td>
<td>286 µl</td>
<td>1100 µl</td>
</tr>
</tbody>
</table>

Prepare Pre-capture PCR master mix (Pre-PCR MM; Table 10). Mix ▶ spin ▶ keep on ice.

- Get 34 µl purified library samples from ice. Add 11 µl Pre-PCR MM.
- Add 5 µl of the appropriate SureSelect XT HS2 Index Primer Pair to each sample well. Vortex ▶ spin.
- Resume thermal cycler program without adding samples. Once cycler reaches 98 °C, add sample plate/strip ▶ close lid ▶ press play/continue.

### Step 6: Clean-up library

**30 min**

Get AMPure XP beads held at RT, vortex until homogeneous.

- Remove samples from cycler ▶ add 50 µl of AMPure XP beads to each amplified DNA well. Mix ▶ incubate at RT 5 min.
- Collect beads using magnet (5 min) ▶ remove and discard supernatant.
- Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash. Washes completed: □ 1 □ 2
- After second wash solution removed, spin briefly ▶ remove residual ethanol.
- Dry samples unsealed on the thermal cycler at 37 °C (1−2 min).
- Elute by adding 15 µl nuclease-free water to each sample well. Seal wells ▶ vortex ▶ brief spin. Incubate at RT 2 min.
- Collect beads using magnet (2−3 min).
- Transfer cleared supernatant to fresh well ▶ keep on ice.

### Step 7: Quality control

Assess quality and quantity using one of these platforms:

- Agilent 2100 Bioanalyzer System with DNA 1000 Assay
- Agilent 4200 or 4150 TapeStation System with D1000 Assay
- Agilent 5200, 5300 or 5400 Fragment Analyzer System with NGS Fragment Kit (1−6000 bp)

**Stopping point**
Hybridization/Capture

Step 1: Hybridize libraries to probe

1.5–2.5 hrs/overnight

Set up thermal cycler program (Table 11). Start and pause program.

Table 11: Thermal cycler program for hybridization (vol 30 µl; heated lid ON)

<table>
<thead>
<tr>
<th>Step</th>
<th>Probe</th>
<th>Number of Cycles</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>All probes</td>
<td>1</td>
<td>95 °C</td>
<td>5 min</td>
</tr>
<tr>
<td>Step 2</td>
<td>All probes</td>
<td>1</td>
<td>65 °C</td>
<td>10 min</td>
</tr>
<tr>
<td>Step 3</td>
<td>All probes</td>
<td>1</td>
<td>65 °C</td>
<td>1 min</td>
</tr>
</tbody>
</table>

Hybridization program variation footnotes:
1. Hybridization at 65 °C (steps 4 and 5) is optimal for XT HS designs. Optimal temperature may be lower for some legacy XT probe designs.
2. For overnight workflow, replace the final brief hold at 65 °C with hold at 21 °C for up to 16 hours.

Prepare 25% RNase Block solution (see Table 12). Mix > keep on ice.

Table 12: 25% RNase Block prep volumes (including excess for supported run sizes)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>16 Rxn Kits</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per Rxn</td>
<td>8 Rxn</td>
<td>16 Rxn</td>
</tr>
<tr>
<td>SureSelect RNase Block</td>
<td>0.5 µl</td>
<td>4.5 µl</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>1.5 µl</td>
<td>13.5 µl</td>
</tr>
<tr>
<td>Total</td>
<td>2 µl</td>
<td>18 µl</td>
</tr>
</tbody>
</table>

Place prepared DNA libraries in wells: 500–1000 ng DNA brought to 12 µl with nuclease-free water for each sample.

Add 5 µl SureSelect XT HS2 Blocker Mix to sample wells. Seal wells > vortex 5 sec > brief spin > verify absence of bubbles in wells.

Place in thermal cycler and press play/continue. Run through Step 2, pausing cycler at Step 3. During cycler Steps 1–2, do reagent prep tasks below.

Prepare 25% RNase Block solution (see Table 12). Mix > keep on ice.

Table 13: Probe Hyb Mix for probes ≥3 Mb (includes excess for supported run sizes)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>16 Rxn Kits</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per Rxn</td>
<td>8 Rxn</td>
<td>16 Rxn</td>
</tr>
<tr>
<td>25% RNase Block (table 12)</td>
<td>2 µl</td>
<td>18 µl</td>
</tr>
<tr>
<td>Probe (&lt;3 Mb design)</td>
<td>5 µl</td>
<td>45 µl</td>
</tr>
<tr>
<td>SureSelect Fast Hybridization Buffer</td>
<td>6 µl</td>
<td>54 µl</td>
</tr>
<tr>
<td>Total</td>
<td>13 µl</td>
<td>117 µl</td>
</tr>
</tbody>
</table>

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions.

Resuspend washed beads in 200 µl SureSelect Binding Buffer > keep at RT.

With thermal cycler paused at Step 3, add 13 µl Probe Hyb Mix to each sample well. Pipette 8–10X to mix > cap wells with domed caps. Wells must be fully sealed to prevent evaporation.

Brief vortex > brief spin > verify absence of bubbles in wells. Immediately return samples to cycler > press play/continue to run hybridization.

Step 2: Prepare streptavidin beads

30 min

Get SureSelect Streptavidin Beads or Dynabeads MyOne Streptavidin T1 Beads from storage at 4 °C, vortex until homogeneous.

Place 50 µl of streptavidin beads in each sample well of fresh plate/strip.

Prepare the Probe Hyb Mix needed for your probe design. Use Table 13 for designs ≥3 Mb or use Table 14 for designs <3 Mb. Vortex > spin > use immediately (keep briefly at RT while adding to hyb).

Table 14: Probe Hyb Mix for probes <3 Mb (includes excess for supported run sizes)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>16 Rxn Kits</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per Rxn</td>
<td>8 Rxn</td>
<td>16 Rxn</td>
</tr>
<tr>
<td>25% RNase Block (table 12)</td>
<td>2 µl</td>
<td>18 µl</td>
</tr>
<tr>
<td>Probe (&lt;3 Mb design)</td>
<td>5 µl</td>
<td>45 µl</td>
</tr>
<tr>
<td>SureSelect Fast Hybridization Buffer</td>
<td>6 µl</td>
<td>54 µl</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>3 µl</td>
<td>27 µl</td>
</tr>
<tr>
<td>Total</td>
<td>13 µl</td>
<td>117 µl</td>
</tr>
</tbody>
</table>

Collect beads using magnet (5 min) > remove and discard supernatant. Washes completed:

1. Add 200 µl Binding Buffer per well of beads > mix well > spin briefly.
2. Collect beads using magnet (5 min) > remove and discard supernatant.
3. Resuspend washed beads in 200 µl SureSelect Binding Buffer > keep at RT.
Step 3: Capture hybridized libraries

- Once streptavidin beads prepared and hybridization program reaches final hold, transfer samples briefly to RT.
- Immediately transfer hyb reactions (~30 µl) to wells containing 200 µl washed beads. Pipette 8X to mix > seal wells with fresh domed caps.
- Incubate on a 96-well plate mixer at 1400–1900 rpm for 30 min at RT.
- During 30 min hyb capture, prep pre-warmed SureSelect Wash Buffer 2 (6 aliquots per library) for post-capture washes:
  - Place 200 µl Wash Buffer 2 in fresh plate/strip wells.
  - Seal wells > place in thermal cycler held at 70 °C.
- After 30 min hyb capture, spin samples briefly > collect beads using magnet (until solution clear) > remove and discard supernatant.
- Resuspend capture beads at RT in 200 µl SureSelect Wash Buffer 1 by pipetting up and down 15–20X or until beads fully resuspended.
- Collect capture beads using magnet (~1 min) > remove and discard supernatant.
- Remove from magnet > keep at RT. Wash capture beads 6X using the pre-warmed SureSelect Wash Buffer 2 aliquots held at 70 °C. For each wash:
  - Add 200 µl Wash Buffer 2 at 70 °C to beads in wells.
  - Pipette up and down 15–20X.
  - Seal wells > vortex 8 sec > spin briefly (do not pellet).
  - Incubate 5 min at 70 °C in thermal cycler.
  - Collect beads using magnet (1 min) at RT > remove and discard supernatant.
  - Washes completed: 1 2 3 4 5 6
- After all wash buffer removed from final wash, add 25 µl nuclease-free water per well > pipette up and down 8X > keep on ice.
- Captured DNA remains on the streptavidin beads for post-capture amplification.

Step 4: Amplify enriched libraries (Post-capture PCR)

- Set up thermal cycler program (Table 15). Start and pause program.

<table>
<thead>
<tr>
<th>Step</th>
<th>Number of Cycles</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>1</td>
<td>98 °C</td>
<td>2 min</td>
</tr>
<tr>
<td>Step 2</td>
<td>10–11 for probe designs &gt;5 Mb</td>
<td>98 °C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>11–12 for probe designs 3–5 Mb</td>
<td>60 °C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>12–16 for probe designs 0.2–3 Mb</td>
<td>72 °C</td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td>16 for probe designs &lt;0.2 Mb</td>
<td>72 °C</td>
<td>5 min</td>
</tr>
<tr>
<td>Step 3</td>
<td>1</td>
<td>4 °C Hold</td>
<td></td>
</tr>
<tr>
<td>Step 4</td>
<td>4</td>
<td>4 °C Hold</td>
<td></td>
</tr>
</tbody>
</table>

- Prepare Post-capture PCR master mix (Post-PCR MM; Table 16). Mix > spin > keep on ice.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Per Rxn</th>
<th>8 Rxn Kits</th>
<th>16 Rxn Kits</th>
<th>24 Rxn Kits</th>
<th>96 Rxn Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free water</td>
<td>13 µl</td>
<td>117 µl</td>
<td>234 µl</td>
<td>338 µl</td>
<td>1300 µl</td>
</tr>
<tr>
<td>5X Herculase II Buffer with dNTPs</td>
<td>10 µl</td>
<td>90 µl</td>
<td>180 µl</td>
<td>260 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Herculase II Fusion DNA Polymerase</td>
<td>1 µl</td>
<td>9 µl</td>
<td>18 µl</td>
<td>26 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>SureSelect Post-Capture Primer Mix</td>
<td>1 µl</td>
<td>9 µl</td>
<td>18 µl</td>
<td>26 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Total</td>
<td>25 µl</td>
<td>225 µl</td>
<td>450 µl</td>
<td>650 µl</td>
<td>2500 µl</td>
</tr>
</tbody>
</table>

- Get 25 µl captured library bead suspensions from ice. Add 25 µl Post-PCR MM.
- Mix by pipetting until beads are in homogeneous suspension (Do not spin).
- Place samples in cycler > close lid > press play/continue.
- At 4 °C Hold step, remove from cycler > brief spin.
- Collect streptavidin beads using magnet (2 min).
- Transfer supernatant (~50 µl) to well of fresh plate or strip > keep on ice.

Prep Ahead: Put AMPure XP beads at RT for use in next step (30 min equilibration).

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions. Clear cap is indicated by “c”. 
**Step 5: Clean-up final libraries**

- Get AMPure XP beads held at RT, vortex until homogeneous.
- Add 50 µl of AMPure XP beads to each amplified DNA well > mix until beads in homogeneous suspension. Incubate at RT 5 min.
- Collect beads using magnet (3–5 min) > remove and discard supernatant.
- Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash.
  - Washes completed: □ 1 □ 2
- After second wash solution removed, spin briefly > remove residual ethanol.
- Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- Elute by adding 25 µl 1X Low TE to each sample well.
  - Seal wells > vortex > brief spin. Incubate at RT 2 min.
- Collect beads using magnet (~2 min).
- Transfer cleared supernatant to fresh well > keep on ice.

**Step 6: Quality control**

Assess quality and quantity using one of these platforms:

- Agilent 2100 Bioanalyzer System with High Sensitivity DNA Assay
- Agilent 4200 or 4150 TapeStation System with High Sensitivity D1000 Assay
- Agilent 5200, 5300 or 5400 Fragment Analyzer System with HS NGS Fragment Kit (1–6000 bp)

**Step 7: Pool libraries for multiplex NGS**

- Calculate the number of indexes that can be combined per lane, according to sequencer capacity and amount of sequencing data required per sample.
- Combine the libraries such that each index-tagged sample is present in equimolar amounts in the pool using one of the following methods:
  - Dilute each sample to be pooled to the concentration of the most dilute sample using Low TE > combine equal volumes of all samples in final pool.
  - Combine the appropriate volume of each sample to achieve equimolar concentration in the pool, then adjust the pool to the desired final volume using Low TE. See the assay user guide for more information on this method.

**Run notes**

- Run Date
- Operator
- Probe Name or Design ID
- Index Pairs

- Reagent Lot Information
  - Library Prep Kit:
  - Target Enrichment Kit:
  - Probe:
  - Enzymatic Frag Kit:
  - Other Materials:

- Sample Information
- Library Pooling Information
- Comments

This information is subject to change without notice.