BD CLiC™ Whole Genome-KHP

Instructions For Use
For use with kits: REF 30297 and REF 30298

Doc ID: 42510 Rev. 3.0
For Research Use Only. Not for Diagnostic or Therapeutic Procedures
Required equipment and materials

BD CLiC Kit contents

**BD CLiC™ Whole Genome-KHP NGS Library Preparation Kit components REF 36303 and REF 36304**

### Components/consumables

**Protocol Activation Barcode**
The protocol activation barcode, located inside each BD CLiC kit, is required to run the BD CLiC instrument and is kit specific. Keep the barcode in a safe place until the run is completed.

- 96-Well Sample Plate (1) or 384-Well Sample Plate (1)
- 384-Well Bead Plate (1) (designated as Shaker Plate in software)
- 384-Well Barcode Adapter Plate (1)
- 384-Well Library Collection Plate (1)
- GC2 Oil
- 5 mL Tube for Library Pool Collection one (1) for KAPA 96 and four (4) for KAPA 384
- 200 µL Reagent Dispenser Pipette Tips* (96)
- 1,000 µL Reagent Dispenser Pipette Tips* (96)

**Product insert**

*Use only the pipette tips provided in the BD CLiC Kit for loading reagents into the Reagent Dispenser. Use of other pipette tip types may lead to clogging of the Reagent Dispenser/Reagent Chip and resultant loss of reagent addition during key dispensing steps.

User-supplied equipment

The following equipment is required to run a KAPA HyperPlus protocol:

<table>
<thead>
<tr>
<th>Description</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 mL tubes</td>
<td>Major laboratory supplier (MLS)</td>
</tr>
<tr>
<td>0.5 mL tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>0.1–2.5 µL pipette</td>
<td>MLS</td>
</tr>
<tr>
<td>0.5–10 µL pipette</td>
<td>MLS</td>
</tr>
<tr>
<td>2–20 µL pipette</td>
<td>MLS</td>
</tr>
<tr>
<td>10–100 µL pipette</td>
<td>MLS</td>
</tr>
<tr>
<td>20–200 µL pipette</td>
<td>MLS</td>
</tr>
<tr>
<td>100–1000 µL pipette</td>
<td>MLS</td>
</tr>
<tr>
<td>20–200 µL 8-channel pipette</td>
<td>MLS</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Plate centrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Bench vortex</td>
<td>MLS</td>
</tr>
<tr>
<td>NanoDrop</td>
<td>Life Technologies</td>
</tr>
<tr>
<td>2100 Bioanalyzer or equivalent</td>
<td>Agilent Technologies</td>
</tr>
<tr>
<td>Qubit™ fluorometer or equivalent</td>
<td>Life Technologies</td>
</tr>
<tr>
<td>384-well plate pipetting guides</td>
<td>QIAGEN (338125)</td>
</tr>
</tbody>
</table>

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## Required equipment and materials

### User-supplied reagents and materials

The following reagents and materials are required to run a KAPA HyperPlus protocol:

<table>
<thead>
<tr>
<th>Components/consumables</th>
<th>Supplier</th>
<th>Part number(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAPA HyperPlus Library Preparation Kit</td>
<td>KAPA Biosystems</td>
<td>KK8514 (96rxn), KK8512 (384rxn), KK8510 (TBD)</td>
</tr>
<tr>
<td>Dual-indexed pre-duplexed adapters modified for use with the KAPA HyperPlus protocol</td>
<td>Illumina® TruSeq® dual-indexed adapters</td>
<td>Custom</td>
</tr>
<tr>
<td>10 mM Tris-HCl, pH 8.0 Solution</td>
<td>TEKNOVA or equivalent</td>
<td>T1173 (1L)</td>
</tr>
<tr>
<td>DNA Suspension Buffer</td>
<td>TEKNOVA</td>
<td>T0223</td>
</tr>
<tr>
<td>Quant-iT™ dsDNA Assay kit - high sensitivity or KAPA Library Quantification Kits KAPA Biosystems (KK4824)</td>
<td>Thermo Fisher Scientific</td>
<td>Q33120</td>
</tr>
<tr>
<td>Qubit Assay Tubes</td>
<td>Thermo Fisher Scientific</td>
<td>Q32856</td>
</tr>
<tr>
<td>High Sensitivity Bioanalyzer Kit</td>
<td>Agilent Technologies</td>
<td>5067-4626</td>
</tr>
<tr>
<td>PCR Foil Seal</td>
<td>4titude®</td>
<td>4ti-0550</td>
</tr>
<tr>
<td>TipOne® 200 µL Graduated Filter Tips Sterile</td>
<td>Starlab/USA Scientific®</td>
<td>S1120-8810</td>
</tr>
<tr>
<td>TipOne 1000 µL Graduated Filter Tips Sterile</td>
<td>Starlab/USA Scientific</td>
<td>S1126-7810</td>
</tr>
</tbody>
</table>

**Use only the pipette tips provided in the BD CLiC kit to load reagents into the Reagent Dispenser. Use of other pipette tip types may lead to clogging of the Reagent Dispenser/Reagent Chip and result in loss of reagent addition during key dispensing steps.**

### Components/consumables

<table>
<thead>
<tr>
<th>Components/consumables</th>
<th>Supplier</th>
<th>Part number(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agencourt® AMPure® XP</td>
<td>Beckman Coulter</td>
<td>A63880, A63881, A63882</td>
</tr>
<tr>
<td>Sodium hypochlorite solution</td>
<td>Sigma Aldrich or equivalent</td>
<td>239305 Note: this stock solution must be diluted 1:10 for use</td>
</tr>
<tr>
<td>Molecular biology–grade water</td>
<td>Life Technologies, Integrated DNA Technologies, or equivalent</td>
<td>AM9932 (1 L), 11-05-01-04, various</td>
</tr>
<tr>
<td>Ethanol (200 proof, molecular grade)</td>
<td>MLS</td>
<td>Note: this stock solution must be diluted to 80% ethanol for use</td>
</tr>
</tbody>
</table>

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General guidelines

The BD CLiC Whole Genome-KHP protocol should be used for Next Generation Sequencing (NGS) library preparation on the BD CLiC instrument using the KAPA HyperPlus library preparation kits. This protocol can generate both pooled libraries and individual libraries. BD CLiC Whole Genome-KHP NGS Library Preparation Kits are available in both 96- and 384-sample kit sizes. Differences associated with kit sizes, pooling, or plating options are specified in this document.

The Activation Barcode provided with your kit is specific for the library preparation method and the sample number being run. Keep Activation Barcodes for use with the specific kits received. They are consumed once the BD CLiC instrument setup is complete and the START RUN button is selected. Keep the Activation Barcode for use with the specific kits received.

Always remember:

- Isolate DNA sample and pipetting steps in an amplicon-free room
- Set up pre-PCR reagents in an amplicon-free room
- Pipet viscous solutions slowly to ensure volume accuracy
- Mix reagent solutions by vortexing
- Minimize freeze-thaws of all frozen reagents
- Do not take amplified libraries into amplicon-free laboratory areas or rooms
- When pipetting barcoded adapters, use good laboratory practices to avoid well-to-well cross-contamination of barcodes (indexes). For BD CLiC protocols that utilize barcode adapters in a plate format, this is critical.
- Wear latex-free gloves when extracting composite liquid cells (CLCs) from a pooled library run. Static from latex gloves can make CLCs difficult to handle.
- Only use plates supplied with BD CLiC kits with the BD CLiC instrument, as plate positions on the instrument require specific plate types.
- Only use pipette tips provided in BD CLiC kits to load reagents into the Reagent Dispenser. Use of other pipette tip types may lead to clogging of the Reagent Dispenser/Reagent Chip and result in reagent loss during key dispensing steps.
Preparing DNA Sample Plates for a run

- DNA samples must be plated into the 96-well Sample Plate or 384-well Sample Plate provided in BD CLiC Whole Genome-KHP NGS Library Preparation Kit. Only use the plate provided, since the Capillary Metering (CM) head is programmed specifically for this plate.
- DNA concentrations in the range of 33.3–66.7 ng/µL (CLiC delivers 0.3 µL, so 10–20 ng of DNA goes into fragmentation) are recommended, quantified by Qubit.
- Dilute DNA in 10 mM Tris-HCl pH 8.0, because KAPA HyperPlus requires DNA in non-EDTA solutions. If EDTA is present, follow the KAPA technical data sheet for information on the addition of conditioning buffer to samples. To accommodate CLiC volumes, adjust the dilutions of the KAPA Frag Conditioning Solution to the appropriate working concentration as listed in the table on the next page.

EDTA concentration must be adjusted to the final CLiC fragmentation volume of 1 µL. Due to the low volume capacity of the CLiC instrument, the maximum final EDTA concentration (per 1 µL reaction) that can be supported by the KAPA Frag Conditioning Solution is 0.5 mM. The complete removal of EDTA from a sample is highly recommended. Adjust the concentration offline prior to starting the BD CLiC instrument.

DNA samples diluted in water should be prepared at time of use.

- The sample volume range for the DNA plates is 5–20 µL/well.

The sample volume range for the 96-well DNA plates is 5–20 µL. The sample volume range for the 384-well DNA plate is 5–10 µL.

- Seal the plate with a plate seal after all DNA samples have been added to the plate.

- Centrifuge at 1,000 x g for 30 seconds to collect the contents at bottom of wells.
- Store the sealed plate at room temperature until ready to load into the BD CLiC instrument.
Preparing DNA Sample Plates for a run

KAPA Frag Conditioning Solution dilutions for DNA samples containing EDTA (normalized for CLiC volumes)

<table>
<thead>
<tr>
<th>Final EDTA concentration in 1 µL reaction (mM)</th>
<th>Dilution factor</th>
<th>Volume of conditioning solution (per 100 µL)</th>
<th>Volume of molecular biology grade water (per 100 µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02–0.05</td>
<td>13.7</td>
<td>7.3</td>
<td>92.7</td>
</tr>
<tr>
<td>0.1</td>
<td>6.6</td>
<td>15.2</td>
<td>84.8</td>
</tr>
<tr>
<td>0.2</td>
<td>3.2</td>
<td>31.6</td>
<td>68.4</td>
</tr>
<tr>
<td>0.3</td>
<td>2.1</td>
<td>48.8</td>
<td>51.3</td>
</tr>
<tr>
<td>0.4</td>
<td>1.4</td>
<td>70.9</td>
<td>29.1</td>
</tr>
<tr>
<td>0.5</td>
<td>1.1</td>
<td>90.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>
Preparing Barcode Adapter Plate for a run

**IMPORTANT** Be careful to avoid cross-contamination between the plate wells when handling barcoded adapters.

1. Dilute each barcode adapter to a working concentration of 7.5 µM with DNA Suspension Buffer.
2. Transfer 7.0 µL of each diluted barcode adapter to the designated well of the 384-well Barcode Adapter Plate provided in the BD CLiC kit. See the layouts for 96-sample kit and 384-sample kit.

   Only pipet barcode adapters into the 384-well barcode adapter plate provided in the BD CLiC kit. The CM head is programmed specifically for these 384-well plates.

3. Seal the plate with a plate seal after the barcoded adapters have been transferred.
4. Centrifuge the plate at full speed for 30 seconds.
5. Store the sealed plate at room temperature until you are ready to load into the BD CLiC instrument.
Preparing solutions for the EFM

Both the ethanol and wash solutions may be prepared up to 48 hours in advance if bottled, sealed, and stored at 4°C.

1. Prepare 80% ethanol solution:

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Absolute ethanol (mL)</th>
<th>Molecular biology grade water (mL)</th>
<th>Total (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96x</td>
<td>160</td>
<td>40</td>
<td>200</td>
</tr>
<tr>
<td>384x</td>
<td>400</td>
<td>100</td>
<td>500</td>
</tr>
</tbody>
</table>

2. Prepare Wash Solution:

<table>
<thead>
<tr>
<th>Sample size</th>
<th>5% sodium hypochlorite (mL)</th>
<th>Molecular biology grade water (mL)</th>
<th>Total (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96x</td>
<td>20</td>
<td>180</td>
<td>200</td>
</tr>
<tr>
<td>384x</td>
<td>30</td>
<td>270</td>
<td>300</td>
</tr>
</tbody>
</table>

3. Obtain 200 mL of molecular biology grade water for the 96x sample size or 400 mL of molecular biology grade water for the 384x sample size.

4. Prepare additional fluids needed for the run:

<table>
<thead>
<tr>
<th>Additional fluids</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular biology grade water</td>
<td>4</td>
</tr>
<tr>
<td>80% ethanol*</td>
<td>4</td>
</tr>
<tr>
<td>Molecular biology grade water (for pipetting to mantis chips)</td>
<td>4</td>
</tr>
<tr>
<td>80% Ethanol* (for chip cleaning and rinsing)</td>
<td>4</td>
</tr>
</tbody>
</table>

*80% Ethanol should be prepared using absolute ethanol and molecular biology grade water. Do not use denatured ethanol.
Thawing KAPA HyperPlus reagents

There are nine (9) components in the KAPA HyperPlus Library Preparation Kit, but only eight (8) are required for the BD CLiC library protocol. The KAPA Frag Conditioning Solution must be added to samples only.

1. Completely thaw all the contents of the six (6) required KAPA HyperPlus Kit™ components at 4°C:
   - KAPA Frag Enzyme (5X) – red cap
   - KAPA Frag Buffer (10X) – blue cap
   - End repair & A-Tailing Enzyme – purple cap
   - DNA Ligase – yellow cap
   - Library Amplification Primer Mix (10X) – green cap
   - KAPA HiFi Hotstart ReadyMix (2X) – clear cap, large bottle

2. Thaw the End Repair & A-Tailing Buffer and the Ligation Buffer at room temperature for at least 30 minutes.
   - End repair & A-Tailing Buffer – purple cap
   - Ligation Buffer – clear cap, small bottle

3. Vortex each reagent tube and centrifuge briefly
4. Ensure each reagent is thoroughly mixed.
5. Place reagent tubes on ice until they are ready for use.

The KAPA HyperPlus End Repair and A-Tailing Buffer may contain precipitate when thawed. Vortex these buffers thoroughly to ensure they are fully homogenized before use.
Preparing EFM instrument fluids: GC1, GC2, and Waste

1. Fill GC1 bottle with GC1 solution.  
2. Fill GC2 bottle in the EFM with the entire contents of the GC2 bottle supplied with the BD CLiC Whole Genome-KHP NGS Library Preparation Kit. This will provide enough GC2 for a single run.  
3. Ensure that the Waste Bottle is empty.
Preparation EFM instrument fluids: ethanol, wash, and water

1. Fill the reagents into their designated bottles:

<table>
<thead>
<tr>
<th>Sample size</th>
<th>80% ethanol (mL)</th>
<th>Wash solution (mL)</th>
<th>Molecular biology grade water (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96x</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>384x</td>
<td>500</td>
<td>300</td>
<td>400</td>
</tr>
</tbody>
</table>

2. Load the 80% ethanol solution \(A\), 0.5% sodium hypochlorite (wash) solution \(B\), and molecular biology grade water \(C\) in the EFM. See the *BD CLiC™ User’s Guide* for detailed instructions.
Preparing and loading Wash Trough instrument fluids

1. Fill the Lookout® DNA Erase™ side of the wash trough with 7.0 mL DNA Erase solution.
2. Fill the water side of the wash trough with 8.0 mL of molecular biology grade water.
3. Insert the filled Wash Trough into instrument deck. [A]

Use the exact volumes of DNA Erase and water specified. The water trough must have a higher volume than the DNA Erase trough to effectively rinse all traces of DNA Erase from the inside and outside of the CM tips.

**IMPORTANT** Carryover of DNA Erase into the water trough will degrade samples and may cause library preparation failure.
Setting up the Reagent Dispenser

1. Install a tube with 4 mL of 80% ethanol (1), and install a tube with 4 mL of water (2). Empty the contents of the waste tube (3). **A**

2. Wipe the nozzles with an 80% ethanol–soaked tissue. **B**

3. Replace the pipette tips with fresh tips containing 50 µL of water on each active Reagent Dispenser Chip. **C**
Setting up the Reagent Dispenser

4. Verify that no Reagent Dispenser Chip is left on the Reagent Dispenser Arm. If there is a Reagent Dispenser Chip on the Reagent Dispenser Arm, manually remove the chip and replace the chip to the Reagent Dispenser Chip position. The Reagent Dispenser Arm will return to the home position during the instrument initialization step.

See the BD CLiC™ User’s Guide for additional detailed instructions about the Reagent Dispenser.
Starting the BD CLiC software and instrument

See the BD CLiC™ User’s Guide for general instructions on how to set up the BD CLiC instrument for a protocol run

1. Start the BD CLiC instrument and software:
   a. Turn on power to the BD CLiC instrument.
   b. Turn on power to the computer.
   c. Launch the BD CLiC application.
   d. Turn on the liquid cooling bath.

2. Confirm the correct sample collection adapter is on the BD CLiC instrument (deck).

Sample collection adapters on the BD CLiC instrument are based on the desired library output type. Change the adapter on the instrument deck between pooling (5 mL tube adapter) or plate collection (384-well collection plate adapter).

3. Select KAPA HyperPlus Kit from the drop-down menu. A

4. Select Standard Run Pool (for pooled libraries) or Standard Run Plate (for individual libraries). B

5. Click START. C
Selecting samples and input run description

1. **At the Select Samples screen:**
   a. Select the number of samples.  
   b. (Optional) Enter comments about the run.

The number of samples can only be selected if it is a standard run.

2. Click **CONTINUE**.
Reviewing the run setup

1. At the Review Run screen, confirm the deck layout.  
   ![Image A]

2. Tab to Purification Settings, and confirm Purification Settings.  
   ![Image B]

3. Confirm Temperature Profile Settings.  
   ![Image C]

4. Scan the BD CLiC kit barcode.  
   ![Image D]

5. If everything is correct, click CONTINUE.  
   ![Image E]

Temperature profile settings may be modified when setting up a Custom Run. For more information, see the BD CLiC™ User’s Guide.
Initializing the BD CLiC instrument

1. At the Initialize Instrument screen, do the following:
   - Switch on the power for the external cooler. Click COMPLETE. A
   - Confirm that no Reagent Dispenser chip is left on the Reagent Dispenser arm. If a Reagent Dispenser chip has been left on the Reagent Dispenser arm, manually remove the chip, and replace the chip to the Reagent Dispenser chip position. See the BD CLiC™ User's Guide for additional information on the Reagent Dispenser. Click COMPLETE. B
   - Close the instrument door. Click COMPLETE. C
   - Click the checkbox to confirm the correct adapter is in place. If the incorrect adapter is in place, see the BD CLiC™ User’s Guide.

2. Click CONTINUE. D

The instrument will go through initialization for approximately 1–2 minutes.
Checking EFM fluids: GC1, GC2, and Waste

1. At the Check Fluids screen, do the following:
   - Confirm the GC1 Bottle is properly installed. Click COMPLETE.  
   - Confirm the GC2 Bottle is properly installed. Click COMPLETE.  
   - Confirm the Waste Bottle is properly installed. Click COMPLETE. 

2. Start Warm-up:
   - Close the instrument door, and click START WARM-UP.

The warm-up process takes approximately 30 minutes. Prepare the KAPA HyperPlus reagents during the instrument warm-up.
Preparing reagents for 96-sample run

This section provides instructions for preparing the reagents required for the Reagent Dispenser. For a 96-sample run, prepare each of the four (4) reagents in 0.5 mL tubes according to the table on this page.

1. Vortex and centrifuge each of the mixes briefly.
2. Store the four (4) tubes of mixed reagents at 4°C or on ice until the Reagent Dispenser is ready to be loaded using the TipOne 200 µl Graduated Filter Tips supplied in the BD CLiC Whole Genome-KHP NGS Library Preparation Kit.

<table>
<thead>
<tr>
<th>Reagent 1 (Frag Mix)</th>
<th>96-Sample Volume µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular biology grade water</td>
<td>55.4</td>
</tr>
<tr>
<td>KAPA Frag Buffer 10X</td>
<td>13.9</td>
</tr>
<tr>
<td>KAPA Frag Enzyme</td>
<td>27.7</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>97.0</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagent 2 (End Repair/A-Tailing Mix)</th>
<th>96-Sample Volume µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>End Repair &amp; A-Tailing Buffer</td>
<td>28.0</td>
</tr>
<tr>
<td>End Repair &amp; A-Tailing Enzyme Mix</td>
<td>12.0</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>40.0</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagent 3 (Ligation Mix)</th>
<th>96-Sample Volume µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular biology grade water</td>
<td>66.0</td>
</tr>
<tr>
<td>Ligation Buffer</td>
<td>96.4</td>
</tr>
<tr>
<td>DNA Ligase</td>
<td>35.6</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>198.0</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagent 4 (PCR Mix)</th>
<th>96-Sample Volume µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X KAPA HiFi HotStart ReadyMix</td>
<td>165.0</td>
</tr>
<tr>
<td>10X KAPA Library Amplification Primer Mix</td>
<td>33.0</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>198.0</strong></td>
</tr>
</tbody>
</table>
Preparing reagents for 384-sample run

This section provides instructions for preparing the reagents required for the Reagent Dispenser. For a 384 samples run, prepare each of the four (4) reagents in 1.5 mL tubes according to the table on this page.

1. Vortex and centrifuge each of the mixes briefly.
2. Store the four (4) tubes of mixed reagents at 4°C or on ice until the Reagent Dispenser is ready to be loaded using the TipOne 200 µl and 1000 µl Graduated Filter Tips supplied in the BD CLiC Whole Genome-KHP NGS Library Preparation Kit.

### Reagent 1 (Frag Mix)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular biology grade water</td>
<td>198.3</td>
</tr>
<tr>
<td>KAPA Frag Buffer 10X</td>
<td>49.6</td>
</tr>
<tr>
<td>KAPA Frag Enzyme</td>
<td>99.1</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>347.0</strong></td>
</tr>
</tbody>
</table>

### Reagent 2 (End Repair/A-Tailing Mix)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>End Repair &amp; A-Tailing Buffer</td>
<td>81.9</td>
</tr>
<tr>
<td>End Repair &amp; A-Tailing Enzyme Mix</td>
<td>35.1</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>117.0</strong></td>
</tr>
</tbody>
</table>

### Reagent 3 (Ligation Mix)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular biology grade water</td>
<td>242.0</td>
</tr>
<tr>
<td>Ligation Buffer</td>
<td>353.3</td>
</tr>
<tr>
<td>DNA Ligase</td>
<td>130.7</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>726.0</strong></td>
</tr>
</tbody>
</table>

### Reagent 4 (PCR Mix)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X KAPA HiFi HotStart ReadyMix</td>
<td>605.0</td>
</tr>
<tr>
<td>10X KAPA Library Amplification Primer Mix</td>
<td>121.0</td>
</tr>
<tr>
<td><strong>Total Volume (µl)</strong></td>
<td><strong>726.0</strong></td>
</tr>
</tbody>
</table>
Checking instrument fluids: Ethanol, wash, water

1. Confirm the Ethanol Bottle is filled with 200 mL (96 samples) or 500 mL (384 samples) of prepared 80% ethanol and is properly installed. Click COMPLETE. A

2. Confirm the Wash Bottle is filled with 200 mL (96 samples) or 300 mL (384 samples) of 0.5% sodium hypochlorite solution (bleach) and is properly installed. Click COMPLETE. B

3. Confirm the Water Bottle is filled with 200 mL (96 samples) or 400 mL (384 samples) of molecular biology grade water and is properly installed. Click COMPLETE. C

4. Click CONTINUE. D
Performing the Capillary Metering Head test

DNA Erase/Wash Trough

1. At the Test CM Head Dispense screen, do the following:
   a. Open the instrument door.
   b. Confirm the DNA Erase/Wash Trough is filled and properly installed on the BD CLiC instrument deck. A
   c. Click COMPLETE. B

Use the exact volumes of DNA Erase and water specified. The water trough must have a higher volume than the DNA Erase trough to effectively rinse all traces of DNA Erase from the inside and outside of the CM tips.

**IMPORTANT** Carryover of DNA Erase into the water trough will degrade samples and may cause library preparation failure.
Performing the Capillary Metering Head test

Capillary Metering Head 1 dispense test

2. With the DNA Erase/Wash Trough ready, do the following:
   - CM1 Head Test: Close the instrument door, and click RUN TEST. 🔄
   - When prompted, open the instrument door, and check for a satisfactory dispense (see the image, or see the *BD CLiC™ User’s Guide* for more information).
   - Repeat the test if necessary. Click COMPLETE. 🔄
Performing the Capillary Metering Head test

Capillary Metering Head 2 dispense test

3. Once the CM Head 1 test is complete:
   a. CM2 Head Test: Close the instrument door, and click **RUN TEST**. A
   b. When prompted, open the instrument door, and check for a satisfactory dispense (see the image, or see the *BD CLiC™ User’s Guide* for more information).
   c. Repeat the test if necessary. Click **COMPLETE**. B

4. Click **CONTINUE**. C

Proper CM Head dispense pattern

Dispense area on deck
Setting up the Reagent Dispenser

1. When prompted, load reagents onto the Reagent Dispenser following the process outlined below: (See the BD CLiC™ User’s Guide for detailed instructions):

   a. Confirm the tube with 4 mL of 80% Ethanol and the tube with 4 mL of water are installed. Click COMPLETE. A
   
   b. Confirm the nozzles have been cleaned with Ethanol. Click COMPLETE. B
   
   c. Confirm the pipette tips containing 50 µL of water on each active Reagent Dispenser Chip are installed. Click RINSE. C
   
   d. Confirm Rinse: Check the fluid level in the pipette tips. Are they all empty? Click YES or NO.
Loading reagents on the Reagent Dispenser

Use the provided Reagent Dispenser pipette tips in the BD CLiC kit, since the instrument has been programmed specifically for these dimensions. Not using these tips can result in Reagent Dispenser/Reagent Chips clogging and may result in loss of reagents during key dispensing steps.

Load the entire contents of each prepared reagent into the pipette tips designated in the table. Load the pipette tips on the Mantis Reagent Dispenser according to the figure.

**Pipette tip size**

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Reagent 1 (position 1)</th>
<th>Reagent 2 (position 2)</th>
<th>Reagent 3 (position 3)</th>
<th>Reagent 4 (position 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96x</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>384x</td>
<td>1000</td>
<td>200</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Do not use prepared reagents that have been stored on ice for longer than 4 hours. If storage of reagents was longer than 4 hours, it may be best to prepare fresh reagents.

When loading the pipette tips, ensure that there are no air bubbles in the pipette tips and that each pipette tip is fully loaded (has no air gap at the pipette tip end), as in the graphic below.

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Loading reagents on the Reagent Dispenser

1. When finished adding all filled tips to the Reagent Dispenser, click COMPLETE A

2. Click CONTINUE B
Preparing Bead Plate (Shaker Plate) for the run

- Ensure that the AMPure XP beads are equilibrated to room temperature. Thoroughly vortex to disperse the AMPure XP beads prior to dispensing.
- Ensure that no AMPure XP beads are pipetted into the wells that will contain the DNA Suspension Buffer (eluent). If this occurs, discard plate, and start again.
- To ensure that there is no air pocket in each water well, centrifuge the plate after the addition of DNA Suspension Buffer.

1. Pour the DNA Suspension Buffer into a sterile trough.
2. Use an 8-channel, manual pipette (8 channels are required) to dispense 80.0 μL of DNA Suspension Buffer (eluent) into the Bead Plate provided in the BD CLiC Whole Genome-KHP NGS Library Preparation Kit (see the plate layout for a 96- and 384-sample run). Discard pipette tips after use.
3. Seal the plate with a plate seal, and centrifuge the plate at full speed for 30 seconds.
4. Pour AMPure XP beads into a sterile trough.
5. Unseal the Bead Plate containing the DNA Suspension Buffer. Use an 8-channel manual pipette (8 channels are required) to dispense 80.0 μL of resuspended AMPure XP beads into the Bead Plate (see the plate layout for a 96- and 384-sample run). Discard the pipette tips after use.

Helpful Hint: 384-Well Plate Pipetting Guides (Qiagen PN 338125) can aid in pipetting samples into specific 96-well quadrants of a 384-well plate. Use the COLOR guide to pipet AMPure XP beads into Quadrant 1. Use the COLOR guide to pipet eluant (molecular biology grade water or DNA Suspension Buffer) into Quadrant 2.

**IMPORTANT** Failure to pipet AMPure XP beads and eluant into the correct wells at the correct volumes per well can result in failed libraries.
## Loading prepared plates on the BD CLiC instrument

1. Load the prepared 96-well DNA Plate in position 1 on the instrument deck, as shown. Position A1 of the DNA Plate will be at the front left.

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>DNA Plate</th>
<th>Barcode Adapter Plate</th>
<th>Bead Plate</th>
<th>Collection Plate or Pool Vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>96x</td>
<td>Position 1</td>
<td>Position 3</td>
<td>Position 6</td>
<td>Position 5</td>
</tr>
<tr>
<td>384x</td>
<td>Position 2</td>
<td>Position 3</td>
<td>Position 6</td>
<td>Position 5</td>
</tr>
</tbody>
</table>

2. Load the prepared 384-well Barcode Adapter Plate in position 3. Position A1 of the Barcode Adapter Plate will be at the front left.

3. Load the prepared AMPure bead and Elution Buffer Plate in position 6. The Bead Plate will click into place.

   - For the Pooled Library Protocol: Load an empty 5 mL tube, provided in the BD CLiC Kit, on the tube adapter in position 5. Fold the cap of the tube back, and tuck it into place.
   - For Individual Library Protocol: Load an empty 384-well Collection Plate from BD CLiC Kit in position 5.

The Bead Plate will immediately begin shaking in order to keep beads in suspension.
Loading prepared plates on the BD CLiC instrument

At the Load Plates & Vessels screen, confirm prepared plates are loaded correctly on the instrument deck.

1. Load and confirm the prepared 96-well DNA plate is in position 1 on the instrument deck or load and confirm that the prepared 384-well DNA plate is in position 2. Position A1 of the DNA plate will be at the front left.
   • (Optional) Provide a Plate ID in the software.
2. Confirm the prepared 384-well barcoded adapter plate is in position 3. Position A1 of the barcoded adapter plate will be at the front left.
   • (Optional) Provide a Plate ID in the software.
3. Confirm the prepared AMPure bead and elution buffer plate in position 6. The Bead Plate will click into place.
   • (Optional) Provide a Plate ID in the software.
4. Confirm the correct collection vessel is installed.
   • For Pooled Library Protocol: Use the empty 5 mL tube provided in the BD CLiC Whole Genome-KHP NGS Library Preparation Kit on the tube adapter in position 5. Fold the cap of the tube back, and tuck it into place.
     • (Optional) Provide a Vessel ID in the software.
   • For Individual Library Protocol: Use the empty 384-well Library Collection plate from the BD CLiC Whole Genome-KHP NGS Library Preparation Kit in position 5.
     • (Optional) Provide a Plate ID in the software.
   • If prompted: Confirm the correct collection vessel adapter is installed. Click to check the box.
5. Remove the plate seals from each plate. Click COMPLETE. A
6. Fold the cap, and tuck it into the holder. Click COMPLETE. B
7. Click CONTINUE. C
Starting the run

At the instrument's prompt, close the instrument door, and click **START RUN.**

![Setup Complete](image-url)
Choosing to clean or unload plates

When the run has completed, the operator has two options regarding the removal of plates:

- Click **START CLEANING NOW** to begin the chip clean shutdown. This pushes the collection of libraries and removal of plates to the end of this process.  

- Click **UNLOAD PLATES NOW** to collect libraries at this point, and remove the plates from deck.

The operator has 30 minutes to make this decision before the system will automatically **START CLEANING NOW**. The time remaining for this decision is displayed on the countdown timer.
Unloading plates

If **UNLOAD PLATES NOW** is selected, the following will apply:

- Remove the library collection plate or 5 mL Eppendorf tube, and click **COMPLETE.** A
- Remove used DNA, Barcode, and Bead Plates, and click **COMPLETE.** B
- Remove CM Wash Trough, and click **COMPLETE.** C
- When complete, click **CONTINUE.** D

At the Ready for Cleaning Thermal Block screen, close the instrument door. Click **START CLEAN** to begin the cleaning procedure. E
Cleaning the instrument

If **START CLEANING NOW** was selected or the 30 minutes expired, once the instrument has been through the cleaning procedure, the operator will be prompted to remove the plates. There is also a system clean completed status displayed on this screen.

1. Remove the library collection plate or 5 mL Eppendorf tube, and click **COMPLETE**. B
2. Remove the used DNA, Barcode, and Bead Plates, and click **COMPLETE**. C
3. Remove the CM wash trough, and click **COMPLETE**. D
4. When complete, click **CONTINUE**. E
Performing Wash Solution system flush

1. The operator will be prompted to fill the wash solution bottle with 300 mL of deionized water. **A**
2. When the Wash Solution bottle has been reinstalled to the EFM, click COMPLETE. **B**
3. Click CONTINUE. **C**
Cleaning the reagent dispenser

At the Clean Reagent Dispenser screen, do the following:

1. Remove the four pipette tips from the reagent dispensing chips.

2. Load each of the chips with 100 µL of 80% ethanol, as prompted, using the same 200 µL pipette tips. Click CLEAN. A

3. Once the clean is complete, remove the four pipette tips again.

4. Load each of the chips with 100 µL of nuclease-free water, as prompted, using the same 200 µL pipette tips. Click RINSE. B

5. Once the rinse is complete, empty the ethanol and water tubes. Click COMPLETE C

6. Click CONTINUE. D
Finishing the run

1. At the Run successfully completed screen, the operator should empty the Waste Bottle from the EFM.  
2. Click **FINISH** to complete the run.
Preparing the pooled library extraction

In a BD CLiC Whole Genome-KHP NGS library preparation run where samples are pooled, all samples are pooled by volume into a single 5.0 mL tube by the BD CLiC Purification Head.

To remove the aqueous sample from the GC2 solution:
1. Using a 1 mL pipette, remove approximately 900 µL of the aqueous phase from the GC2 solution phase.
   - Press the pipette tip against the aqueous sample until only one liquid phase is observed in the pipette tip. Do not vortex the aqueous pool when in oil.
2. Transfer the aqueous phase (sample) into a new 1.5 mL LoBind tube.
3. Inspect the contents of the 1.5 mL tube — No additional GC2 layer should be visible on top of the aqueous layer.
4. The purified library can be stored at 4°C for up to 1 week. For samples stored for longer than 1 week, store at -20°C.
Preparing the plated library extraction

In a BD CLiC Whole Genome-KHP NGS Library preparation run with plate dispense, all samples (384 or 96) are individually eluted to a 384-well dispense plate.

1. Remove the dispense plate from the deck after elution.
2. Centrifuge the dispense plate at 1,000 x g for 1 minute.
3. Ensure that all wells with libraries are dried down.

Due to drying variations among the wells of the collection plate, BD recommends drying down the libraries. The collection plate can be left uncovered on the laboratory bench at room temperature or on the BD CLiC deck for additional hours as necessary. To accelerate drying, dry the libraries at 40°C.

4. Pipet 10 µL of molecular biology grade water into each well to resuspend the libraries.
5. Seal the collection plate, and briefly vortex it. Centrifuge the plate at 1,000 x g for 1 minute.
6. The purified library can be stored at 4°C for up to 1 week. For samples stored longer than 1 week, store at –20°C.
Plated library harvesting

Plate configuration guide for a 96-sample plated library

The mapping of the location of the DNA samples in the CLC nodes of the thermal block is shown here. The DNA samples are added to the thermal block nodes in sets of 12 samples by CM Head 1 as shown in the plate map. The barcoded adapters are added to the thermal block nodes in sets of 12 samples by CM Head 2 as shown in the plate map. For effective troubleshooting of within-run differences in sequencing performance between samples in a pool, it is beneficial to understand these plate maps.
Plated library harvesting

Plate configuration guide for a 96-sample plated library

The mapping of the location of the DNA samples in the CLC nodes of the thermal block is shown here. The DNA samples are added to the thermal block nodes in sets of 12 samples by CM Head 1 as shown in the plate map. The barcoded adapters are added to the thermal block nodes in sets of 12 samples by CM Head 2 as shown in the plate map. For effective troubleshooting of within-run differences in sequencing performance between samples in a pool, it is beneficial to understand these plate maps.

Thermal block and collection plate configuration

1st pick-up
2nd pick-up
3rd pick-up
4th pick-up
5th pick-up
6th pick-up
7th pick-up
8th pick-up
Plated library harvesting

Plate configuration guide for a 384-sample plated library

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DNA Sample Plate

Barcode Adapter Plate

Bead Plate

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Pooled/plated library

1. Library size analysis
   - Run 1 µl of undiluted sample library/pool on a high-sensitivity bioanalyzer chip or equivalent. See the Agilent high sensitivity DNA kit quick start guide (PN G2938-90322) for instructions.
   - In the Agilent Bioanalyzer 2100 Expert software, set the region thresholds to 200 bp and 900 bp, and record the average fragment size as shown.

2. Library quantitation analysis
   - Measure the concentration of the sample library/pool using Quant-iT™ dsDNA High-Sensitivity Assay Kit (PN: Q33120) or equivalent. See the Quant-iT™ dsDNA High-Sensitivity Assay Kit User Guide for instructions.
   - Use the Bioanalyzer (or equivalent) fragment mode size and Quant-iT (or equivalent) concentration to calculate the molarity of each library or pool.

Typical range of Qubit values for a pool is 2–4 ng/µL.
Legal information

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WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

NOTICE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. BD is not responsible for any radio or television interference caused by unauthorized changes or modifications to this equipment. Unauthorized changes or modifications could void the user’s authority to operate the equipment. This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations. Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada.
Contact information

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### REVISION HISTORY

**Version 01 Effective on 15-Mar-2017 by Jay Martin**
KAPA IFU without watermark and updated to "Rev. 02." No other changes.

**Version 02 Effective on 09-May-2017 by Jay Martin**
--KAPA IFU without watermark --GLB0005 changed to 42510 Rev. 02 to match Zen --Rev. 01 changed to Rev. 02 for revision

**Version 03 Effective on 23-May-2017 by Jay Martin**
Changing To • Rev. 3.0 • Added content on conditioning solution on p. 5 and new p. 6 • Minor typographical copyedits

### DOCUMENT ELECTRONIC SIGNATURES

#### DOCUMENT APPROVAL WORKFLOW

**Author Approval**

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature Details</th>
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<tr>
<td>Jay Martin</td>
<td>I am the author of this document. Signed 4:39:44 PM UTC 16-May-2017</td>
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**Required Workflow Steps for this Category**

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<tr>
<td>Anne Devereux</td>
<td>I have reviewed and approve this document. Signed 8:47:41 AM UTC 17-May-2017</td>
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<tr>
<td>Shahid Mustafa</td>
<td>I have reviewed and approve this document. Signed 8:58:27 AM UTC 19-May-2017</td>
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**Additional Steps Added**

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<td>Courteney Swenson</td>
<td>I have reviewed and approve this document. Signed 6:05:12 PM UTC 22-May-2017</td>
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