Introduction

Next Generation Sequencing (NGS) DNA library construction kits are essential to various sequencing applications spanning whole genome sequencing (WGS) and whole exome sequencing (WES) to target capture and chromatin immunoprecipitation (ChIP). The BioQule™ automation platform offers a streamlined workflow for the generation of 8 NEXTFLEX® Rapid DNA-Seq 2.0 libraries – with only 15 minutes of hands-on setup and 6 hours of machine-time. The BioQule™ platform guides the user through this quick setup to generate high quality WGS libraries. The Quick Start Guide serves to help users set up and run NEXTFLEX® Rapid DNA-Seq 2.0 on BioQule™ Library Prep System.

Manual Workflow: NEXTFLEX Rapid DNA-Seq Kit 2.0

<table>
<thead>
<tr>
<th>Thaw ERA Reagents</th>
<th>Thermocycle ERA</th>
<th>Thermocycle Ligation</th>
<th>Bead Wash #1</th>
<th>Thermocycle PCR</th>
<th>Bead Wash #2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Thaw Ligation Reagents</td>
<td></td>
<td>Thaw PCR</td>
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</tr>
</tbody>
</table>

BioQule Workflow: NEXTFLEX Rapid DNA-Seq Kit 2.0

<table>
<thead>
<tr>
<th>Thaw Plate</th>
<th>Run BioQule NGS System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load Reagents</td>
<td>Thaw Plate</td>
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</tbody>
</table>

For research use only. Not for use in diagnostic procedures.
Required Materials Not Provided

- 10 ng – 1 μg of fragmented genomic DNA
- If multiplexing: NEXFLEX® Unique Dual Index Barcodes (Cat # 514150-EVAL16, 514150, 514151, 514152, 514153) or 1,536 NEXFLEX® Unique Dual Index Barcodes (Cat # 534100)
- Isopropyl Alcohol
- Cartridge (PerkinElmer, CLS157064)
- Covaris® System (S2, E210) or other method for DNA fragmentation
- 2, 10, 20, 200 and 1000 μL pipettes and pipette tips
- Plate centrifuge

Revision History

<table>
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<tr>
<th>Date</th>
<th>Revision</th>
</tr>
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<tbody>
<tr>
<td>November 2022</td>
<td>Product Launch</td>
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<tr>
<td>March 2023</td>
<td>Quick Guide Update</td>
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Warnings & Precautions

We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at NGS@PerkinElmer.com.

- Do not use the kit past the expiration date
- Do not heat NEXFLEX® Unique Dual Index Barcodes above room temperature
- To enable multiplexing, please use the appropriate combination of NEXFLEX® Unique Dual Index Barcodes
- Maintain a laboratory temperature of 20º–25ºC (68º–77ºF)
- DNA sample quality may vary between preparations. It is the user’s responsibility to utilize high quality DNA. DNA that is heavily nicked or damaged may cause library preparation failure. Absorbance measurements at 260 nm are commonly used to quantify DNA and 260 nm / 280 nm ratios of 1.8 - 2.0 usually indicate relatively pure DNA. Other quantification methods using fluorescent dyes may also be used. The user should be aware that contaminating RNA, nucleotides and single-stranded DNA may affect the amount of usable DNA in a sample preparation
- DNA fragmentation methods that physically break up DNA into pieces of less than 800 bp are compatible with this kit
NEXTFLEX® Rapid DNA-Seq 2.0 Automated Library Preparation

The steps described below detail how to initiate the library preparation module. This workflow includes A-Tailing, adapter ligation, post-ligation cleanup, followed by an on-deck PCR amplification. This protocol can be completed in approximately 6 hours.

Step 1. Remove the following materials from -20°C storage and thaw in the vacuum packaging for 30 minutes:
   a. NEXTFLEX® Rapid DNA-seq 2.0 reagent plate (vacuum sealed)
   b. NEXTFLEX® Ligase Enzyme 2.0
   c. NEXTFLEX® End-Repair & Adenylation Enzyme Mix 2.0
   d. Optics Standard
   e. Adapter Index (refer to materials not provided section)

Step 2. Concurrently, remove the NEXTFLEX® Cleanup Beads 2.0 from 4°C storage and thaw for 30 minutes.

Step 3. Concurrently, prepare the following materials:
   a. Prepare 8 mL of 80% Isopropyl alcohol (IPA)
   b. 8 x 10 µL DNA samples in water or resuspension buffer
   c. New BioQule™ cartridge

Note: BioQule™ cartridges cannot be re-used. Take care not to damage or misalign the pipette tips or cartridge tubing. Assay failure may result.

Step 4. After the Adapter Indexes have thawed take 2 µL and dilute with water based on gDNA input used according to Table 1.

<table>
<thead>
<tr>
<th>Input</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-100 ng</td>
<td>1:40</td>
</tr>
<tr>
<td>&gt;100 ng</td>
<td>1:5</td>
</tr>
</tbody>
</table>

Table 1: Adapter Dilution Volumes

Step 5. Once the reagent plate has thawed, remove the vacuum packaging, and briefly centrifuge the plate (~200rpm). The Plate will have a reagent loading template attached to it like below. The black squares represent the holes in which users should pipette reagents into.

![Figure 1: NEXTFLEX® Rapid-DNA-Seq Kit 2.0 Plate Layout](image-url)
Step 6. Load the following reagents in order: (follow the plate loading template, load everything except IPA)

a. 10 μL of diluted NEXTFLEX® Adapter Mix into Column 11 (Orange)
b. 3 μL NEXTFLEX® Ligase Enzyme 2.0 into Column 12 (Purple) - Pipette Mix
c. 45 μL NEXTFLEX® Cleanup Beads into Column 17 (Dark Brown). Vortex beads thoroughly prior to loading onto reagent plate.
d. 35 μL NEXTFLEX® Cleanup Beads into Column 18 (Light Brown). Vortex beads thoroughly prior to loading onto reagent plate.
e. 10 μL of the Optics Standard into well 22D (Yellow)
f. 10 μL sample DNA into Column 24 (Pink) - Pipette Mix
g. 3 μL NEXTFLEX® End-Repair and Adenylation Enzyme into Column 24 (Pink) - Pipette Mix

Step 7. Centrifuge the plate for 15 seconds (~200rpm)

Note: Take care not to jostle the plate when loading and unloading, to prevent the spillage of any reagents

Step 8. After carefully removing the plate from the centrifuge, place it back on the lab bench and load 198 μL IPA into columns 4, 5, 8, and 9 (Blue). Take extreme care to not spill IPA into the adjacent wells, and try not to leave droplets on the plate seal

Note: Do not invert or tilt the plate after loading the IPA

Step 9. Remove the plate loading template from the reagent plate

Step 10. Use a clean pipette tip to peel away the precut red plate seal sections from the following sections:

a. columns 3-10
b. columns 21-22

Step 11. The plate is now ready to run. Turn on the BioQule™ Library Prep System and associated computer, connect the two machines using provided USB cable. Launch the BioQule™ User Interface on the Computer. Press the Refresh Devices Button to update the Box Connections.

Figure 2: Removing Plate Seal from reagent plate

Figure 3: BioQule™ Library Prep System user interface.
Step 12. Select the BioQule™ machine from the list of options displayed. One computer can run multiple BioQule™ Boxes. Use the Flash Light button to flash the lights of the selected machine to ensure the correct one is selected. Press Connect to Device to continue.

Step 13. On the following screen, select the NEXTFLEX® 2.0 Assay from the list of available assays on the BioQule™ User Interface. Press Next.

![Select Script](image)

Step 14. Insert Reagent plate onto BioQule™ X-Plate as shown below in Figure 6.

a. Make sure the reagent plate is in the correct orientation – the barcode should be facing forwards towards user, the red seal is up and the blunt vertex of the 384 well plate should be oriented to the top left
b. Press the plate to the left to depress the pogo pin on the left side of the x-plate, and then back to depress the 2 pogo pins on the back of the x-plate
c. Ensure the plate is loaded correctly and is flat against the x-plate

![Figure 6](image)
Step 15. The cartridge may now be loaded onto the BioQule™ instrument.

a. Begin by removing the PCR door from the instrument
b. Hold the cartridge with 2 hands, the cannula array in your left, and the tubing scaffold in your right, make sure the barcode on the tubing scaffold is facing you
c. Push the cannula array into the holder, there is an arrow on the pull-tab indication orientation
d. Align the eyelets of the cartridge with the 2 posts on the heating element, with one hand on the cartridge at each eyelet, push the cartridge towards the back of the instrument, onto the heating element.

Step 16. Once the cartridge and reagent plate are placed, scan the barcode on each consumable into the correct field on the BioQule™ UI. Press Enter to confirm Barcodes and then press Next.
Step 17. Upon entering the barcode place the PCR Door with the latch on the left onto BioQule™. Then close the PCR door by turning the latch to the right.

*Figure 11: PCR Door placement*  
*Figure 12: Shut PCR Door*

Step 18. Add the names of the Samples and the Sample concentrations into the Spreadsheet. Slide the BioQule™ Door shut and press Run. The Assay will not run unless the Door is shut.

*Figure 13: Enter Assay Information Page*  
*Figure 14: Shut BioQule door*
Step 19. The Assay will now run. It will take approximately 6 hours to complete. The Finish button will activate upon completion. The Assay is now complete, DNA Library has been generated and can be found in Well 1. Final volume of the library obtained is 28 μL. Spin down the reagent plate and perform Quality Checks using a LabChip® GXI Nucleic Acid Analyzer prior to sequencing. Final libraries are stored in column 1. Libraries can be stored in the plate at room temperature for 24 hours. It is recommended to move the libraries to -20°C as soon as reasonably possible but within 24 hours after completion of the protocol.

Figure 15: Assay Run Page