Assay Guide

BioQule™ CLARETBIO SRSLY PicoPlus™ NGS LIBRARY PREP METHOD

PN: PR-00009 Rev B
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1 Introduction

1.1 Overview

The BioQule™ NGS system is an innovative automated platform that simplifies most commercially available Next Generation Sequencing workflows with the push of a button. It employs simplified micro and macro-scale geometries to efficiently perform DNA extraction, library preparation, and library quantification. DNA libraries prepared using the platform meet high-quality standards regarding coverage bias, yield, and fragment size. It is an open system that can integrate and automate different NGS workflows, including Illumina, Element Biosciences, and Oxford Nanopore Technologies NGS workflows.

BioQule™ opens the door for NGS library preparation automation to low throughput customers, eliminating the need for automation expertise! Effortlessly load your samples onto the pre-plated reagent kit, insert the kit and cartridge into the instrument, close the door, and kick-start the run. Experience an 80% reduction in hands-on time, elevating your lab's efficiency, and say goodbye to human errors caused by pipetting mishaps. BioQule™ brings seamless automation to your fingertips, transforming your workflow with precision and ease.

Features

- Low input requirement for oligos down to 250 pg/sample.
- Complete library prep solution, including size selection beads and fluorescence measurements for quantifying libraries.
- Robust genome coverage and reliable performance with sequencing bias mitigation.
- Functionally tested with Illumina™ sequencing platform.

Specifications

Input Type:	Oligos
Input Amount:	250 pg - 10 ng
Number of Reactions: Sample	8
Indexes Available:	384
Sequencing Platforms:	Illumina NGS

1.2 Storage and Stability

- The Reagent Plate can be stored at room temperature upon arrival to laboratory.
- Store the Optics Buffer, Optics Standard and Pretreatment at -20 °C upon arrival to laboratory.
- Store the ClaretBio SRSLY NGS Library Preparation Base Kit, UDI PCR Primers at -20 °C. maximum freeze-thaw cycles is 12.
- Store the Clarefy beads at 4 °C upon arrival. Do not freeze the the beads.

1.3 Product Use

- Do not use the pretreatment buffer and optics reagents past their expiration date.
- BioQule™ Cartridges cannot be re-used. Take care not to damage or misalign the pipette tips or cartridge tubing. Damaged cartridges or tips may result in assay failure.
- BioQule™ assays are intended for research use only.
- This manual is a property of Revvity™ Inc.

1.4 Warnings and Precautions

We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and assay manuals. Therefore, it is important to follow the current protocol, which can be found on the Revvity website. If you need further assistance, you may contact your local distributor, or contact us at L3BioQule@Revvity.com.

- Do not use the kit past the expiration date. Use kit within 18 months from product shipment when stored correctly.
- Do not use unlabeled or wrongly stored input oligo (ssDNA)
- Do not store reagents or pipettes inside the BioQule™ box.
- Wear gloves and eye protection while setting up the reagent plate for the run.
- Do not place any appendages inside the BioQule™ box while it is running.
- Do not heat Primers above room temperature.
- Do not freeze the beads. Always vortex the beads to achieve a uniform suspension before pipetting.
- To enable multiplexing, please use the appropriate combination of Unique Dual Index Primers.
- Always prepare aliquot reagents since the maximum freeze-thaw cycles is 12.

1.5 Prior to Starting

- Register your BioQule™ by sending email to L3BioQule@Revvity.com and get access to training videos, training material, community assay development and software up-dates.
- Ensure a laboratory temperature of 20 ° - 25 °C (68 ° - 77 °F).
- Identify all reagents and equipment needed before beginning assay preparation.

2 Contents

2.1 Kit Contents

ⓘ The BioQule™ ClaretBio SRSLY PicoPlus™ NGS Library Accessory Kit, P/N 900-000017, contains sufficient materials to prepare 32 ssDNA-Seq libraries. The kit has the following components:

- 4 x BioQule™ ClaretBio SRSLY PicoPlus™ Accessory Plate, P/N 810-000018). Each plate comes with a 384 Deep Well Plate, a plate map and a plate loading template insert.
- 4 x BioQule™ Optics Standard (P/N 820-000057)
- 8 x BioQule™ Optics Solution (P/N 820-000058)
- 4 x Pretreatment Solution (P/N 820-000056)

2.2 Additional Equipment, Reagents and Labware

- Equipment
  - BioQule™ cartridge (Revvity, PN. CLS157064)
  - BioQule™ NGS Library Prep Instrument (Revvity, PN. CLS155700)
  - Micropipettes: 0.5-10 µl, 2-20 µl, 20-200 µl, 200-1000 µl
  - Microcentrifuge for 0.2 ml tubes
  - Vortexer
  - Plate Centrifuge for SBS Footprint Deep Well Plates
  - Qubit® 2.0, 3.0 or 4.0 Fluoremeter (ThermoFisher Scientific) or other appropriate fluorometer and accessories for quantification of input DNA and final libraries.
  - LabChip GXII Touch (Revvity, PN. CLS137032), or equivalent for electrophoretic analysis of nucleic acids.
- Reagents
  - Isopropyl Alcohol (IPA)
  - Nuclease Free Water
  - 1X TE
  - 0.5 % Tween 20
  - 10 % Tween 20
  - ClaretBio SRSLY PicoPlus™ NGS Library kit (CBS-K250B-24 or CBS-K250B-24)
  - Clarefy Beads (CBS-BD-24 or CBS-BD-96)
  - Claret Bio Unique Dual Indexing PCR Primers
- Supplies and Labware
  - Filtered Pipette Tips, Nuclease Free
  - 0.2 ml PCR Strip Tubes
  - 10 ml centrifuge tubes

To Order:

- Revvity, www.revvity.com
- Fisher Scientific, www.fishersci.com

3 Planning the Run

3.1 Workflow and Time Required

ClaretBio SRSLY PicoPlus™ NGS Library Prep for BioQule™ Library Prep is a completely automated DNA-Seq library preparation workflow. Each run takes approximately 6 hours with only 30 minutes of hands on time. Figure 1. demonstrates the difference between manual and automated library preparation workflows.

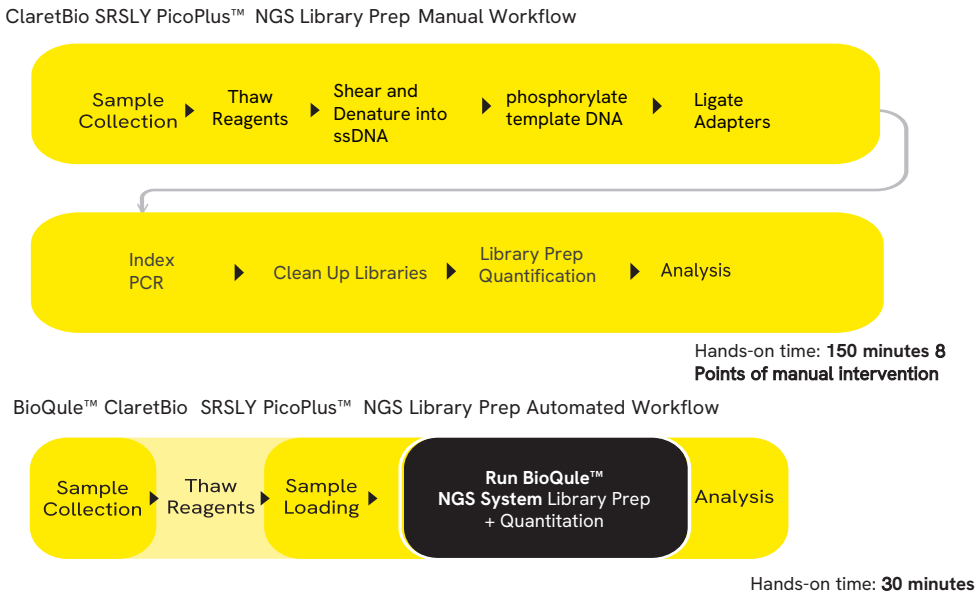

Figure 1. BioQule™ vs Manual Workflow of ClaretBio SRSLY PicoPlus™ NGS Library Prep

3.2 Input DNA Requirements

DNA Quantity

This kit is compatible with a total ssDNA input of between 250 pg ng to 10 ng. Accurate quantification of ssDNA is required to ensure the minimum input is met. We recommend single-stranded DNA be quantified using the Qubit ssDNA assay kit and the manufacturer's instructions. Oligos are usually quite concentrated and therefore usually require dilution before quantification. Each set of 8 samples should be normalized to the same input amount to ensure equal amplification for each sample. Oligo length is determined during oligo design and synthesis. Therefore, size visualization of oligos before NGS library preparation is not necessary.

DNA Integrity

BioQule™ Library Prep will generate the best library when ssDNA samples of high molecular weight and low evidence of degradation are utilized. The BioQule™ has not been tested with degraded samples. ssDNA Integrity can be determined by utilizing a LabChip GXII Touch or equivalent equipment.

3.3 Sequencing Recommendations and Guidelines

BioQule ClaretBio SRSLY PicoPlus™ NGS Library Prep protocol produces ssDNA-seq libraries which are compatible with Illumina™ Sequencing platforms and should follow Illumina's sequencer specific recommendations.

Index Read Recommendations

ClaretBio SRSLY PicoPlus™ NGS Library Prep uses 10 base Unique Dual Indexes (UDI) for sample multiplexing. Both Index 1 (i7) and Index 2 (i5) should be sequenced. These indexes can be found in Appendix 6.1.

3.4 Data Analysis

Once Sequencing data has been generated and parsed, data analysis may be employed according to the requirements of the experiment. If the user requires assistance in this pursuit please contact L3BioQule@Revvity.com.

3.5 Library Storage

Libraries prepared by the BioQule™ should be stored at a -20°C Freezer without a defrost cycle.

4 Procedure

4.1 Reagent Plate Setup

The steps described below detail how to set up the provided reagent plate for a run on the BioQule™. The Plate Map helps identify which wells will have volume added. The loading template insert confirms volumes added into each column.

Step 1. Remove the following materials from -20°C storage and thaw for 30 minutes on ice:

a. ClaretBio SRSLY PicoPlus™ NGS Library kit
b. i5/i7 UDI Index Primers plate
c. BioQule™ Optics Standard
d. BioQule™ Optics Buffer
e. BioQule™ Pretreatment Solution
f. BioQule™ Resuspension Buffer

Step 2. Concurrently, remove the Clarefy beads 4°C storage and thaw for 30 minutes.

Step 3. Prepare the following materials:

a. Prepare 8 ml of 70% Isopropyl alcohol (IPA)
b. New BioQule™ cartridge
c. 8 x 25 µl ssDNA samples in water. Ensure that the DNA concentration is at least 250 pg/µl

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. Prepare Primer mix for loading:

- Thaw and spin down the primer plate for 10 seconds.
- Use 10 ul pipette and tips
- Prepare the primer mix in PCR tubes. Number of tubes should correspond to number of samples.
- Please immediately put back the primer plate into -20C after use.

Table 1: Primer mix

	1x (µl)
Primer	5
Nuclease Free Water	5
Total	10

Note: *Mix Slowly. Do not reuse barcodes and take note of barcodes that you have used. Prepare primer mix separately. DO NOT pool different primer barcodes.*

Step 5. Prepare Adapter A & B Mix (8.8x) according to the tables below.

Table 2: Adapter Mix A

	1x (µl)	8.8x (µl)
Adapter A	2	17.6
Nuclease Free Water	2	17.6
Total	4	35.2

Note: *Mix Slowly.*

Table 3: Adapter Mix B

	1x (µl)	8.8x (µl)
Adapter B	2	17.6
Nuclease Free Water	2	17.6
Total	4	35.2

Step 6. Prepare Clarefy Bead Mix according to the table below:

Table 4: Clarefy Bead Mix

	1x (µl)	8.8x (µl)
Clarefy Beads	65.2	573.76
Nuclease Free Water	48	422.4
100 % IPA	12	105.6
Total	125.2	1101.76

Note: *Mix thoroughly until homogeneous.*

Step 7. Prepare the ssEnhancer Mix according to the table below

Table 5: ssEnhancer and Sample Mix

	1x (µl)	8.8x (µl)
Oligo ssDNA	5	44
Nuclease Free Water	23	202.4
ssEnhancer	2	17.6
Total	30	264

Note: *If the sample volume is less than 5 ul, add nuclease free water to achieve the desired volume.*

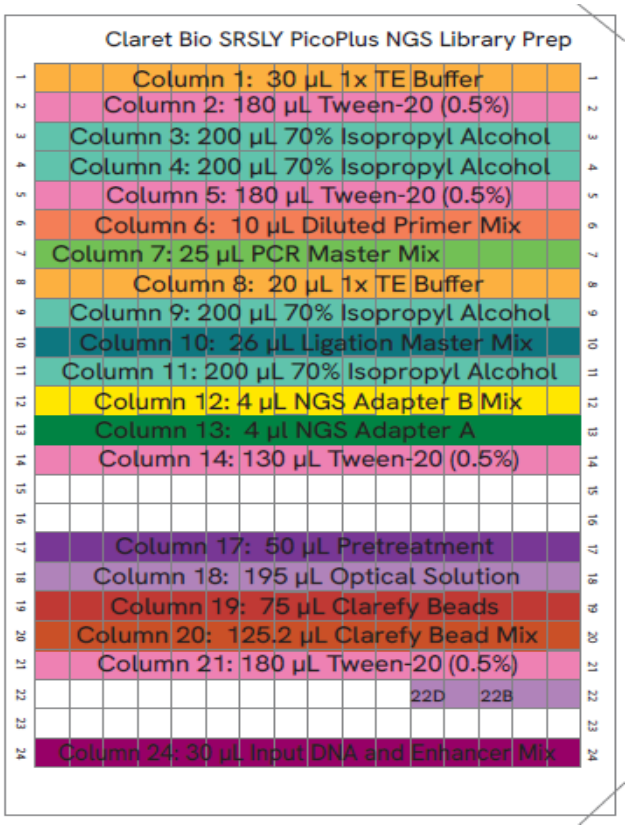

Figure 2. ClaretBio SRSLY PicoPlus™ NGS Library Prep Loading Template Insert

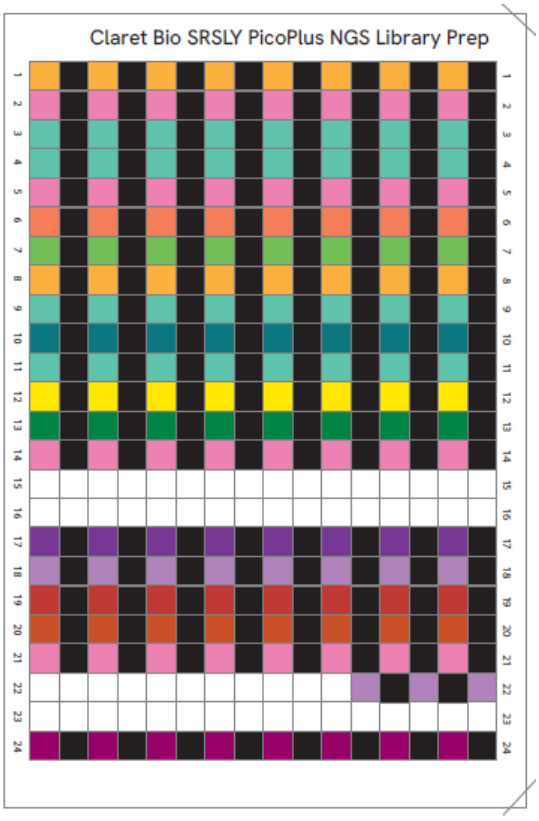

Figure 3. ClaretBio SRSLY PicoPlus™ NGS Library Prep Plate Map

Step 8. Follow the plate loading template. Reagents should be loaded in wells marked on the plate map in the following order:
(Do not load the Isopropyl Alcohol until Step 10.)

a. Column 1 : Load 30 µl of 1X TE .
b. Column 8: Load 20 ul of 1X TE.
c. Vortex the 0.5% Tween 20 and load 180 ul (use multichannel pipette) in Columns 2, 5 and 21.
d. Vortex the 0.5% Tween 20 and load 130 ul (use multichannel pipette) in Columns 14
e. Column 6: Load the 10 µl of the primer.
f. Column 7: Load 25 µl of the PCR master mix.
g. Column 10: Load 26 µl of the ligation reaction Mix .
h. Column 12: Load 4 µl of the NGS Adapter B mix (prepared in step 5).
i. Column 13: Load 4 µl of the NGS Adapter A mix (prepared in step 5).
j. Column 17: Load 50 µl of the pretreatment mix.
k. Column 19: Vortex the Clarefy beads until resuspended and load 75 µL.
l. Well 20: Load 125.2 µl of the bead mix prepared in step 6.
m. Well 24: Load 30 µl of the ssEnhancer and sample mix (prepared in step 7).
n. Well 18: Load 195 µl of the optics solution.
o. Well 22B and 22D: Load 190 µl of the optics solutuion
p. Well 22B: Load 10 µl of Resuspension Buffer and pipette mix.
q. Well 22D: Add 10 µl of Optics Standard and pipette mix slowly.

Note: *If large bubbles are present in any column, use a 10 µl pipette tip to pop them gently. Make sure all the wells have the loading reagent.*

Step 9. Cover the plate with a 384-well pierceable plate seal. Ensure that the wells are aligned with the grid on the plate seal. Centrifuge the plate for 10 seconds (~1000 rpm)

Step 10. Load 200 µl of 70% IPA into columns 3, 4, 9 and 11. Take extreme care to not spill IPA into adjacent wells, and try not to leave droplets on the plate seal.

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

The Reagent Plate is now ready to run. It should look like Figure 4.

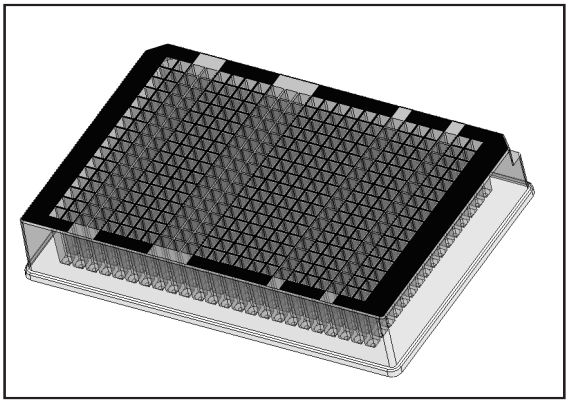

Figure 4. Removing the plate seal from the plate

4.2 BioQule™ Run Setup

The steps described below assists users with BioQule setup and run kickoff procedures .

Step 1. Turn on the BioQule Library Prep System and associated computer, connect the two machines using the provided USB cable. Launch the BioQule User Interface on the Computer. Press the Refresh Devices Button to update the Box Connections.

Step 2. 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 and identify the selected machine to ensure the correct one is selected. Press Connect to Device to continue.

Step 3. On the following screen, select the BioQule ClaretBio SRSLY PicoPlus™ NGS Library Prep Assay from the list of available assays on the BioQule™ User Interface.

Step 4. Insert the Reagent plate onto BioQule™ Plate, as shown in Figure 5.

a. Make sure the reagent plate is in the correct orientation – the barcode should be facing forwards toward the user, the black 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 flat spring on the left side of the x-plate, and then back to depress the flat spring on the back of the x-plate.

c. Ensure the plate is loaded correctly and is flat against the x-plate

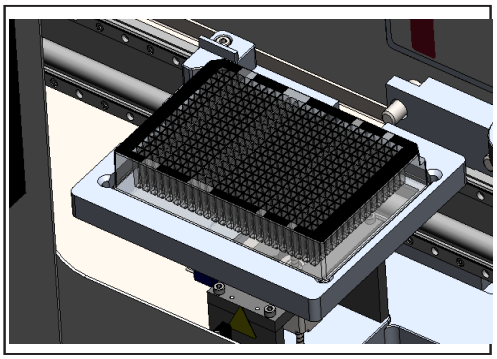

Figure 5. Placing Reagent Plate onto the X-plate

Step 5. 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.

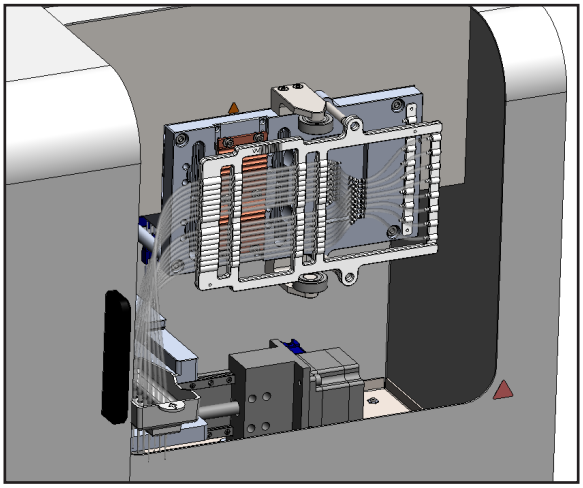

Figure 6. Cartridge insertion into BioQule™

Step 6. Once the cartridge and reagent plate are placed, scan the barcode (using a barcode reader) on each consumable into the correct field on the BioQule™ UI. Press Enter to confirm Barcodes and then press Next.

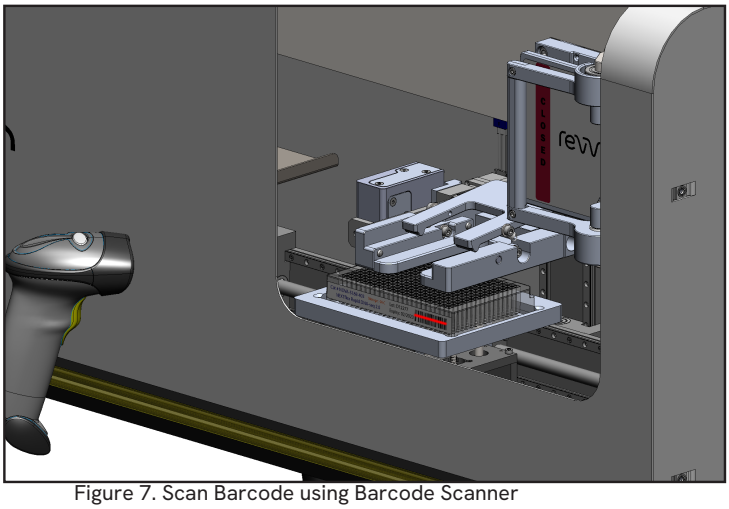

Figure 7. Scan Barcode using Barcode Scanner

Step 8. Place the PCR door with the latch on the left onto BioQule™. Then close the PCR door by turning the latch to the right.

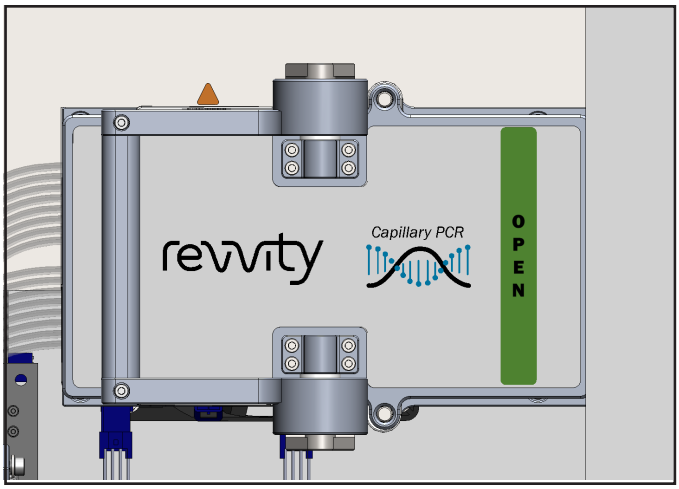

Figure 8. PCR Door Placement

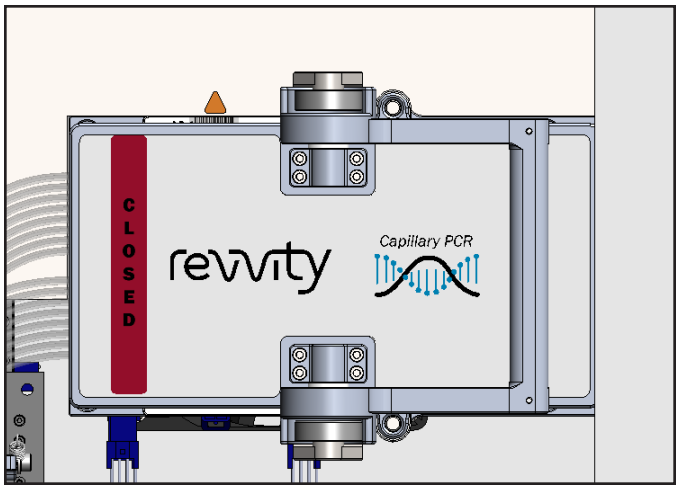

Figure 9. PCR Door Shut

Step 9. Add the names of the Samples and the Sample concentrations to the Spreadsheet. Slide the BioQule™ door shut and press Run. The Assay will not run unless the door is closed.

- The Assay will now run. It will take approximately 6 hours to complete.
- The Finish button will activate upon completion. ssDNA Library will be ready and can be found in column 1.

Step 11. Spin down the reagent plate

Note: 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.3. Samples can be stored at -25°C to -15°C for up to 30 days

It is recommended to perform Library quality and size distribution checks using a LabChip™ GX Nucleic Acid Analyzer prior to sequencing. Assess the quality of the library or pooled libraries using the following method.

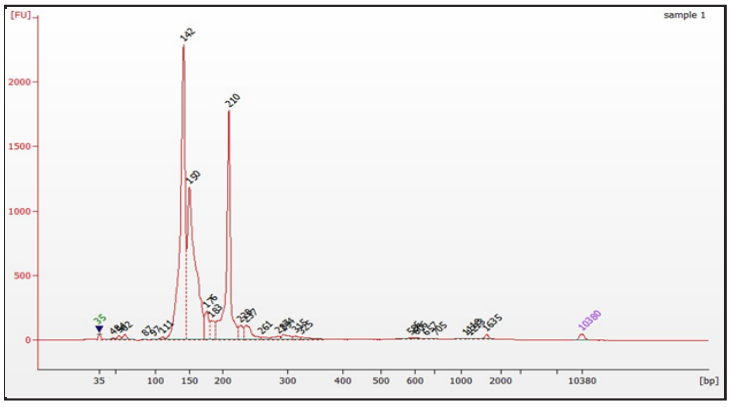

Figure 10. Example Electropherogram Plot

Note: An equivalent electrophoresis platform can be used to analyze the library quality in place of the LabChip™ GX Nucleic Acid Analyzer

5 Troubleshooting

See Training Videos for explanations of any troubleshooting queries.

5.1 Low Volume of Library Generated

Possible causes include:

- Evaporation. Please only leave the plate in the instrument for up to 24 hours. Check the humidity (desire range is 30-50%) and temperature (20 - 25C) of the lab. The instrument should be far from any devices that vent hot air into the atmosphere.
- Incorrect script was used.
- There may have be bubbles injected into manually loaded wells during the reagent plate loading. Pipetting slowly and into the bottom of the wells is recommended.

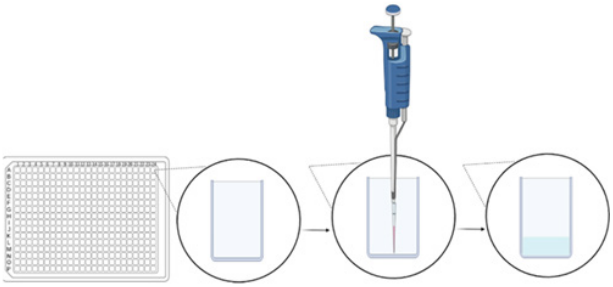

Figure 11. Pipetting directly at bottom of wells

- Large bubbles may have generated in pre-loaded wells upon pipette mixing. Use a 10 µL pipette tip to pop them gently.

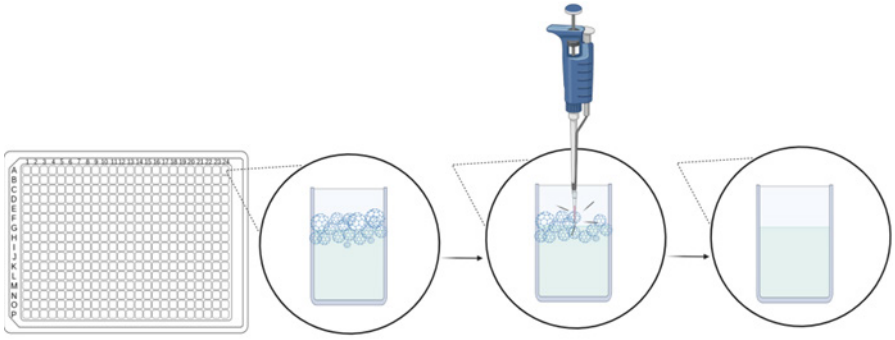

Figure 12. Popping bubbles using a 10 µL Pipette tip

- BioQule™ motor stages may have skewed. Please contact L3BioQule@revvity.com.

5.2 Low Yield Library Generated

Possible causes include:

- Low quality ssDNA was used. ssDNA sample quality may vary between preparations.
- Input ssDNA amount was incorrect. Input ssDNA amount should be measured by Qubit or another device.
- Incorrect pipetting may result in low yield and high adapters.

5.3 High Adapter Dimer

Possible causes include:

- Incorrect adapter or primer dilution was used.
- IPA was not freshly made and had expired.
- The Clarefy beads were accidentally frozen by mistake or due to a shipment error.
- The beads were not well resuspended prior to loading onto the BioQule™.

6 Appendix

6.1 Index (UDI) Sequences

Appendix A: UDI Index PCR without UMIs – sequences and concentrations (UD Kits: CBS-UD-24 or CBS-UD-96).

Index or Barcode Sequences to be used on ClaretBio SRSLY Picoplus kit are given below. The actual adapter sequence and what goes onto the sequencing sample sheet is different. The I5 index on the Sample Sheet also differs based on which Sequencer is being used. Use Column 5 if using a NovaSeq, MiSeq, HiSeq 2000/2500. Use Column 6 if using a iSeq, MiniSeq, NextSeq, HiSeq 3000/4000.

Table 6: Primer UDI Appendix A

Sample Name	I7 Bases in Adapter	I7 Bases on Sample Sheet	I5 Bases on Sample Sheet (1)	I5 Bases on Sample Sheet (2)
i5/i7 UDI 1	AACCGCGG	CCGCGGTT	AGCGCTAG	CTAGCGCT
i5/i7 UDI 2	GGTTATAA	TTATAACC	GATATCGA	TCGATATC
i5/i7 UDI 3	CCAAGTCC	GGACTTGG	CGCAGACG	CGTCTGCG
i5/i7 UDI 4	TTGGACTT	AAGTCCAA	TATGAGTA	TACTCATA
i5/i7 UDI 5	CAGTGGAT	ATCCACTG	AGGTGCGT	ACGCACCT
i5/i7 UDI 6	TGACAAGC	GCTTGTCA	GAACATAC	GTATGTTC
i5/i7 UDI 7	CTAGCTTG	CAAGCTAG	ACATAGCG	CGCTATGT
i5/i7 UDI 8	GTGCGATA	TATCGCAC	TCGATCCA	TGGATCGA
i5/i7 UDI 9	CCAACAGA	TCTGTTGG	CCTGAACT	AGTTCAGG
i5/i7 UDI 10	TTCAGGTC	GACCTGAA	TTGGTGAG	CTCACCAA
i5/i7 UDI 11	AGTAGAGA	TCTCTACT	CGCGGTTC	GAACCGCG
i5/i7 UDI 12	GACGAGAG	CTCTCGTC	TATAACCT	AGGTTATA
i5/i7 UDI 13	AGACTTGG	CCAAGTCT	AAGGATGA	TCATCCTT
i5/i7 UDI 14	GAGTCCAA	TTGGACTC	GGAAGCAG	CTGCTTCC
i5/i7 UDI 15	CTTAAGCC	GGCTTAAG	TCGTGACC	GGTCACGA
i5/i7 UDI16	TCCGGATT	AATCCGGA	CTACAGTT	AACTGTAG
i5/i7 UDI 17	CTGTATTA	TAATACAG	ATATTCAC	GTGAATAT
i5/i7 UDI 18	TCACGCCG	CGGCGTGA	GCGCCTGT	ACAGGCGC
i5/i7 UDI 19	ACTTACAT	ATGTAAGT	ACTCTATG	CATAGAGT
i5/i7 UDI 20	GTCCGTGC	GCACGGAC	GTCTCGCA	TGCGAGAC
i5/i7 UDI 21	AAGGTACC	GGTACCTT	AAGACGTC	GACGTCTT
i5/i7 UDI 22	GGAACGTT	AACGTTCC	GGAGTACT	AGTACTCC
i5/i7 UDI 23	ACCGGCCA	TGGCCGGT	AATTCTGC	GCAGAATT
i5/i7 UDI 24	GGCCTCAT	ATGAGGCC	GTTAATTG	CAATTAAC
i5/i7 UDI 25	ATCTTAGT	ACTAAGAT	AACCGCGG	CCGCGGTT
i5/i7 UDI 26	GCTCCGAC	GTCGGAGC	GGTTATAA	TTATAACC
i5/i7 UDI 27	CCAAGTCC	GGACTTGG	ATACCAAG	CTTGGTAT
i5/i7 UDI 28	GCGTTGGA	TCCAACGC	TTGGACTT	AAGTCCAA
i5/i7 UDI 29	CTTCACGG	CCGTGAAG	CAGTGGAT	ATCCACTG
i5/i7 UDI 30	TCCTGTAA	TTACAGGA	TGACAAGC	GCTTGTCA
i5/i7 UDI 31	AGAATGCC	GGCATTCT	CTAGCTTG	CAAGCTAG
i5/i7 UDI 32	GAGGCATT	AATGCCTC	TCGATCCA	TGGATCGA
i5/i7 UDI 33	CCTCGGTA	TACCGAGG	CCTGAACT	AGTTCAGG

Sample Name	I7 Bases in Adapter	I7 Bases on Sample Sheet	I5 Bases on Sample Sheet (1)	I5 Bases on Sample Sheet (2)
i5/i7 UDI 34	TTCTAACG	CGTTAGAA	TTCAGGTC	GACCTGAA
i5/i7 UDI 35	ATGAGGCT	AGCCTCAT	AGTAGAGA	TCTCTACT
i5/i7 UDI 36	GCAGAATC	GATTCTGC	GACGAGAG	CTCTCGTC
i5/i7 UDI 37	CACTACGA	TCGTAGTG	AGACTTGG	CCAAGTCT
i5/i7 UDI 38	TGTCGTAG	CTACGACA	GAGTCCAA	TTGGACTC
i5/i7 UDI 39	CTTAAGCC	GGCTTAAG	ACCACTTA	TAAGTGGT
i5/i7 UDI 40	TCCGGATT	AATCCGGA	GTTGTCCG	CGGACAAC
i5/i7 UDI 41	CTGTATTA	TAATACAG	ATCCATAT	ATATGGAT
i5/i7 UDI 42	GCTTGCGC	GCGCAAGC	TCACGCCG	CGGCGTGA
i5/i7 UDI 43	AGTATCTT	AAGATACT	ACTTACAT	ATGTAAGT
i5/i7 UDI 44	GACGCTCC	GGAGCGTC	GTCCGTGC	GCACGGAC
i5/i7 UDI 45	CATGCCAT	ATGGCATG	AAGGTACC	GGTACCTT
i5/i7 UDI 46	TGCATTGC	GCAATGCA	GGAACGTT	AACGTTCC
i5/i7 UDI 47	ATTGGAAC	GTTCCAAT	AATTCTGC	GCAGAATT
i5/i7 UDI 48	GCCAAGGT	ACCTTGGC	GGCCTCAT	ATGAGGCC
i5/i7 UDI 49	CGAGATAT	ATATCTCG	ATCTTAGT	ACTAAGAT
i5/i7 UDI50	TAGAGCGC	GCGCTCTA	GCTCCGAC	GTCGGAGC
i5/i7 UDI 51	AACCTGTT	AACAGGTT	ATACCAAG	CTTGGTAT
i5/i7 UDI 52	GGTTCACC	GGTGAACC	GCGTTGGA	TCCAACGC
i5/i7 UDI 53	CATTGTTG	CAACAATG	CTTCACGG	CCGTGAAG
i5/i7 UDI 54	TGCCACCA	TGGTGGCA	TCCTGTAA	TTACAGGA
i5/i7 UDI 55	CTCTGCCT	AGGCAGAG	AGAATGCC	GGCATTCT
i5/i7 UDI 56	TCTCATTC	GAATGAGA	GAGGCATT	AATGCCTC
i5/i7 UDI 57	ACGCCGCA	TGCGGCGT	CCTCGGTA	TACCGAGG
i5/i7 UDI 58	GTATTATG	CATAATAC	TTCTAACG	CGTTAGAA
i5/i7 UDI 59	GATAGATC	GATCTATC	ATGAGGCT	AGCCTCAT
i5/i7 UDI 60	AGCGAGCT	AGCTCGCT	GCAGAATC	GATTCTGC
i5/i7 UDI 61	CAGTTCCG	CGGAACTG	CACTACGA	TCGTAGTG
i5/i7 UDI 62	TGACCTTA	TAAGGTCA	TGTCGTAG	CTACGACA
i5/i7 UDI 63	CTAGGCAA	TTGCCTAG	ACCACTTA	TAAGTGGT
i5/i7 UDI 64	TCGAATGG	CCATTCGA	GTTGTCCG	CGGACAAC
i5/i7 UDI 65	CTTAGTGT	ACACTAAG	ATCCATAT	ATATGGAT
i5/i7 UDI 66	TCCGACAC	GTGTCGGA	GCTTGCGC	GCGCAAGC

Sample Name	I7 Bases in Adapter	I7 Bases on Sample Sheet	I5 Bases on Sample Sheet (1)	I5 Bases on Sample Sheet (2)
i5/i7 UDI 67	AACAGGAA	TTCCTGTT	AGTATCTT	AAGATACT
i5/i7 UDI 68	GGTGAAGG	CCTTCACC	GACGCTCC	GGAGCGTC
i5/i7 UDI 69	CCTGTGGC	GCCACAGG	CATGCCAT	ATGGCATG
i5/i7 UDI 70	TTCACAAT	ATTGTGAA	TGCATTGC	GCAATGCA
i5/i7 UDI 71	ACACGAGT	ACTCGTGT	ATTGGAAC	GTTCCAAT
i5/i7 UDI 72	GTGTAGAC	GTCTACAC	GCCAAGGT	ACCTTGGC
i5/i7 UDI 73	GTTAATTG	CAATTAAC	CGAGATAT	ATATCTCG
i5/i7 UDI 74	ACCGGCCA	TGGCCGGT	TAGAGCGC	GCGCTCTA
i5/i7 UDI 75	GGAGTACT	AGTACTCC	AACCTGTT	AACAGGTT
i5/i7 UDI 76	AAGACGTC	GACGTCTT	GGTTCACC	GGTGAACC
i5/i7 UDI 77	GTCTCGCA	TGCGAGAC	CATTGTTG	CAACAATG
i5/i7 UDI 78	ACTCTATG	CATAGAGT	TGCCACCA	TGGTGGCA
i5/i7 UDI 79	GCGCCTGT	ACAGGCGC	CTCTGCCT	AGGCAGAG
i5/i7 UDI 80	ATATTCAC	GTGAATAT	TCTCATTC	GAATGAGA
i5/i7 UDI 81	CTACAGTT	AACTGTAG	ACGCCGCA	TGCGGCGT
i5/i7 UDI 82	TCGTGACC	GGTCACGA	GTATTATG	CATAATAC
i5/i7 UDI 83	GGAAGCAG	CTGCTTCC	GATAGATC	GATCTATC
i5/i7 UDI 84	AAGGATGA	TCATCCTT	AGCGAGCT	AGCTCGCT
i5/i7 UDI 85	TATAACCT	AGGTTATA	CAGTTCCG	CGGAACTG
i5/i7 UDI 86	CGCGGTTC	GAACCGCG	TGACCTTA	TAAGGTCA
i5/i7 UDI 87	CTAGGCAA	TTGCCTAG	TTGGTGAG	CTCACCAA
i5/i7 UDI 88	CCAACAGA	TCTGTTGG	TCGAATGG	CCATTCGA
i5/i7 UDI 89	GTGCGATA	TATCGCAC	CTTAGTGT	ACACTAAG
i5/i7 UDI 90	ACATAGCG	CGCTATGT	TCCGACAC	GTGTCGGA
i5/i7 UDI 91	GAACATAC	GTATGTTC	AACAGGAA	TTCCTGTT
i5/i7 UDI 92	AGGTGCGT	ACGCACCT	GGTGAAGG	CCTTCACC
i5/i7 UDI 93	TATGAGTA	TACTCATA	CCTGTGGC	GCCACAGG
i5/i7 UDI 94	CGCAGACG	CGTCTGCG	TTCACAAT	ATTGTGAA
i5/i7 UDI 95	GATATCGA	TCGATATC	ACACGAGT	ACTCGTGT
i5/i7 UDI 96	AGCGCTAG	CTAGCGCT	GTGTAGAC	GTCTACAC

7 Technical Assistance

For help with any of our products, please contact Revvity Technical Support at +1 203-925-4602 (direct) or 800.762.4000 (toll-free, U.S. only) or email L3BioQule@Revvity.com, or fill out a Customer Support form on our website www.revvity.com/customersupport.

8 Revision History

Date	Revision	Revision
October 2nd 2024	B	Reformatting tables

Revvity
940 Winter Street
Waltham, MA, 02451 USA

(800) 762-4000
www.revvity.com