Assay Guide

BioQule™ Watchmaker™ RNA Library Prep Method

PN: PR-00010 Rev C
Publication Date: October 1, 2024

Table of Contents

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 reagents and samples onto the plate, insert the plate and cartridge into the instrument, close the door, and kickstart 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 RNA down to 20 ng/sample.
- Complete library prep solution, including size selection beads and fluorescence measurements for quantifying libraries.
- Functionally tested with Illumina™ sequencing platform.

Watchmaker RNA Library Prep Specifications:

Input Type:	Total RNA, mRNA, depleted RNA
Input Amount:	20 ng -100 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 Watchmaker RNA Library Prep Kit at -20 °C.

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.
- This protocol is compatible with RNA samples that have gone through mRNA capture or ribosomal depletion, provided that the post-enrichment or depletion RNA is sufficient to meet input amount requirements.

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 most recent protocol, which is 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.
- Do not use unlabeled or wrongly stored input sample.
- Do not store reagents or pipettes inside the BioQule™ box.
- Unless otherwise stated, keep all components and reaction mixes on ice or a cooled reagent block during routine use.
- The 1st Strand Buffer is photosensitive. Keep it in the kit box until thawing is required. Take care to protect it from direct sunlight while thawing and in use.
- The master mixes should be stable for up to 24 hours at 4 ˚C.
- 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.
- Make sure that the adapter plate is on the correct side. Always take note of the adapters that you use

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™ Watchmaker™ RNA Library Accessory Kit, P/N 900-000018, contains sufficient materials to prepare 32 RNA libraries. The kit has the following components:

- 4 x BioQule™ Watchmaker™ RNA Accessory Plate, P/N 810-000019. Each plate comes with a 384 Deep Well Plate, a Plate Map and plate loading template insert.
- 4 x BioQule™ Optics Standard (P/N 820-000060)
- 8 x BioQule™ Optics Solution (P/N 820-000059)
- 4 x Pretreatment Buffer (P/N 820-000056)
- 0.5 % Tween 20 (P/N 820-000093)

2.2 Additional Equipment, Reagents and Labware

- Equipment
  - BioQule™ Cartridge (Revvity, PN. CLS158240)
  - 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 RNA and final libraries.
  - LabChip GXII Touch (Revvity, PN. CLS137032), or equivalent for electrophoretic analysis of nucleic acids.
- Reagents
  - 100% Isopropyl Alcohol (IPA)
  - Nuclease Free Water
  - NEXTFLEX RNA-seq 2.0 UDI barcodes (NOVA-512920/NOVA-512921/NOVA-512922/NOVA-512923)
  - Watchmaker RNA Library Prep Kit (7K0078-024 or 7K0078-096)
  - AmPure XP Beads

- Supplies and Labware
  - Filtered Pipette Tips
  - 0.2 ml PCR Strip Tubes

To Order:

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

3 Planning the Run

3.1 Workflow and Time Required

The Watchmaker™ RNA Library Prep for BioQule™ Library Prep is a completely automated RNA 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.

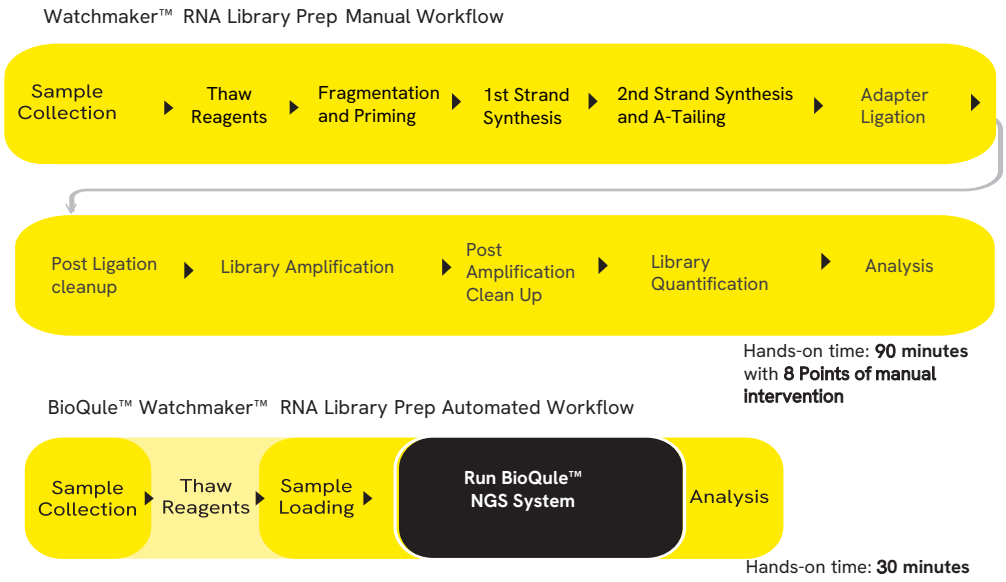

Figure 1. BioQule™ vs Manual Workflow of Watchmaker™ RNA Library Prep

3.2 Input RNA Requirements

Input RNA quality and quantity

RNA should be accurately quantified by Qubit™ Fluorometer or similar before starting. High-quality RNA ranging from 20 – 100 ng has been tested and shown to produce high-performing libraries.

Assess Input RNA Purity

RNA inputs should be free from contaminating DNA that may be carried over from extraction. If the RNA contains DNA, remove the contamination by incubating with DNase I (not supplied with the kit). Residual DNase I may interfere with library preparation, so it is important to ensure no residual enzyme remains in the sample. RNA should be suspended in RNase-free water and be free of salts (e.g., Mg2+, or guanidinium salts), chelating agents (e.g., EDTA or EGTA), and organics (e.g., phenol or ethanol).

RNA Handling

To avoid RNase contamination, work in a laminar flow hood, if available, and keep all sample and reagent tubes closed unless in use. Wear gloves when handling reagents and preparing libraries. Change gloves and pipette tips if they come into contact with non-sterile surfaces. To avoid RNA degradation, store RNA in an RNase-free diluent and limit the number of sample freeze-thaw cycles.

3.3 Sequencing Recommendations and Guidelines

BioQule™ Watchmaker™ RNA Library Prep protocol produces libraries which are compatible with Illumina Sequencing platforms and should follow Illumina™ sequencer specific recommendations.

Adapters

This workflow is also compatible with the NEXTFLEX™ RNA-seq 2.0 UDI barcoded adapters that offer robust multiplexing capabilities for RNA-seq on Illumina ™ platform. The use of these adapters with unique dual indices during sequencing prevents such mis-assigned reads from appearing in final data sets, allowing for the highest assurance of data integrity .

Other compatible adapters that have 3' overhanging T to facilitate adapter orientation during dscDNA ligation are compatible with the workflow.

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. Watchmaker RNA Library Prep Kit
b. Adapters
c. Optics Standard (1 tube)
d. Optics Solution (2 tubes)
e. Optics Buffer (1 tube)
f. Pretreatment Buffer

Step 2. Concurrently, prepare the following materials:

a. New BioQule™ cartridge
b. 8 x 10 µl RNA samples in water. RNA concentration should be at least 20 ng/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.*

Step3. Remove the 0.5 % Tween 20 from 4 °C.

Step 4 . Prepare **P5/P7 PrimerMix** for loading:

Table 1: P5/P7 Primer mix

	1x (µl)	8.8x (µl)
P5/P7 Primer	5	44
Nuclease Free Water	5	44
Total (µl)	10	88

Note: *Mix Slowly.*

Step5. Prepare **Ligation Master Mix** for loading:

- Prepare Ligation Master Mix (8.8x) according to the table below.
- Slowly mix using a pipette until completely resuspended.
- Keep the Ligation Master Mix on ice until loaded onto the plate. DO NOT VORTEX THE LIGATION MASTER MIX

Table 2: Ligation Master Mix

	1x (µl)	8.8x (µl)
Ligation Buffer	40	352
Ligation Enzyme	5	44
Total (µl)	45	396

Step 6. Depending on the RNA input concentration, pepare Adapter for loading according to the table below:

- Thaw and spin down the adapter plate for 10 seconds.
- Use 10 µL pipette and tips.
- Add the appropriate Index adapters to the adapter wells mentioned in the table below.
- Please immediately put back the adapter plate into -20 °C after use.

Table 3: Adapter Dilution

RNA Input Amount	20 ng	40 ng	100 ng
Adapter (µl)	1	1	2
Nuclease Free Water (µl)	20	15	14
Total (µl)	21	16	16

Note:

- ***Please note that only 10 µL diluted adapter will be used for loading***
- *Always check the bottom of the plate to confirm correct adapters are used. Do not reuse barcodes and take note of barcodes that you have used. Prepare adapter mix separately. DO NOT pool different adapter barcodes.*

Step7. Prepare 10ml of Isopropyl Alcohol 70 % according to the table below:

Table 4: 70 % Isopropyl Alcohol

	1x (µl)	50x (µl)
100% Isopropyl Alcohol	140	7000
Nuclease Free Water	60	3000
Total (µl)	200	10000

Note: *Vortex thoroughly.*

Step8. Prepare the Frag&Prime and RNA Mix according to the table below

Table 5: Frag&Prime and RNA Mix

	Per Sample (µl)
RNA	10
Frag&Prime Buffer	15
Total (µl)	25

Note: *If the RNA volume is less than 10 ul, add nuclease free water to achieve the desired volume. Ensure that total amount of RNA in the 10 ul is between 20 ng and 100 ng.*

Step 9. Prepare the 1st Strand Mix according to the table below

Table 6: 1st Strand Mix

	1x (µl)	8.8x (µl)
1st Strand Buffer	9	79.2
1st Strand Enzyme	1	8.8
Total (µl)	10	88

Step 10. Prepare the 2nd Strand Mix according to the table below

Table 7: 2nd Strand Mix

	1x (µl)	8.8x (µl)
2nd Strand Buffer	14	123.2
2nd Strand Enzyme	1	8.8
Total (µl)	15	132

Step 11. Follow the correct plate loading template to load reagents in the specified order. The plate is loaded in three stage.

- Use the appropriate Loading Template and Plate Map for each stage
- Do not load the Isopropyl Alcohol until Step 13.

Stage 1 Loading : (Wells A, C, E, G, I, K, M, O)

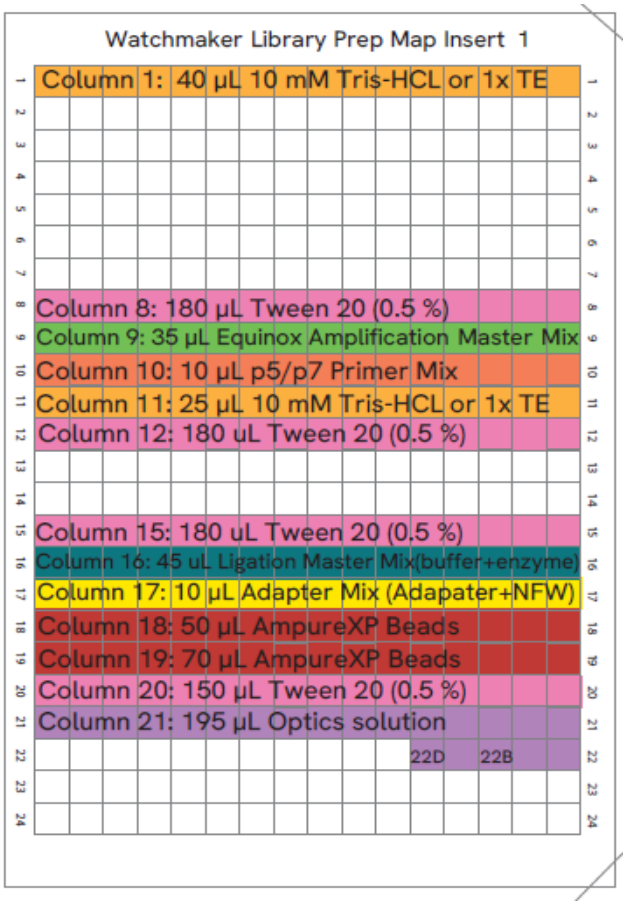

Figure 2. Watchmaker RNA Library Prep Stage 1 Loading Template Insert

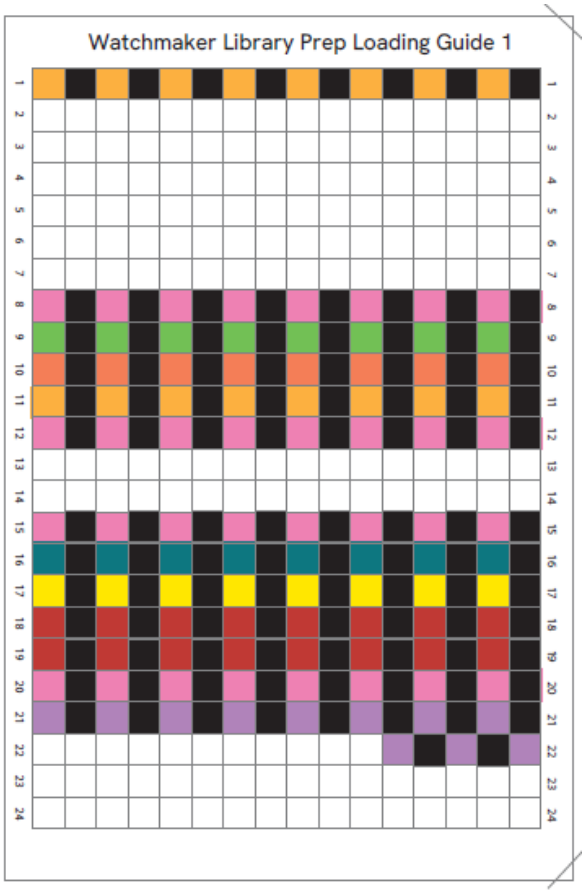

Figure 3. Watchmaker RNA Library Prep Stage 1 Plate Map

a. Column 1: Load 40 µl of 10 mM Tris-HCl or 1X TE.
b. Column 8, 12 and 15: Load 180 ul of the 0.5 % Tween 20 (use multichannel pipette)
c. Columns 20: Load 150 ul of the 0.5% Tween 20 (use multichannel pipette).
d. Columns 9: Load 35 ul of the Equinox Amplification Master Mix.
e. Columns 10: Load 10 ul of the P5/P7 Primer Mix prepared in step 4.
f. Column 11: Load the 25 µl of the 10 mM Tris-HCl or 1X TE.
g. Column 16: Load 45 µl of the Ligation Master Mix prepared in step 5.
h. Column 17: Load 10 µl of the Adapter Diluted Mix (Table 3) prepared in step 6.
i. Column 18: Load 50 µl of the Ampure XP Beads.
j. Column 19: Load 70 µl of the Ampure XP Beads.
k. Well 21: Load 195 µl of the Optics Solution.
l. Well 22B and 22D: Load 190 µl of the Optics Solution.
m. Well 22B: Load 10 µl of RSB and pipette mix.
n. Well 22D: Add 10 µl of Optics Standard and pipette mix slowly.

Stage2Loading: (Wells B, D, F, H, J, L, N, P)

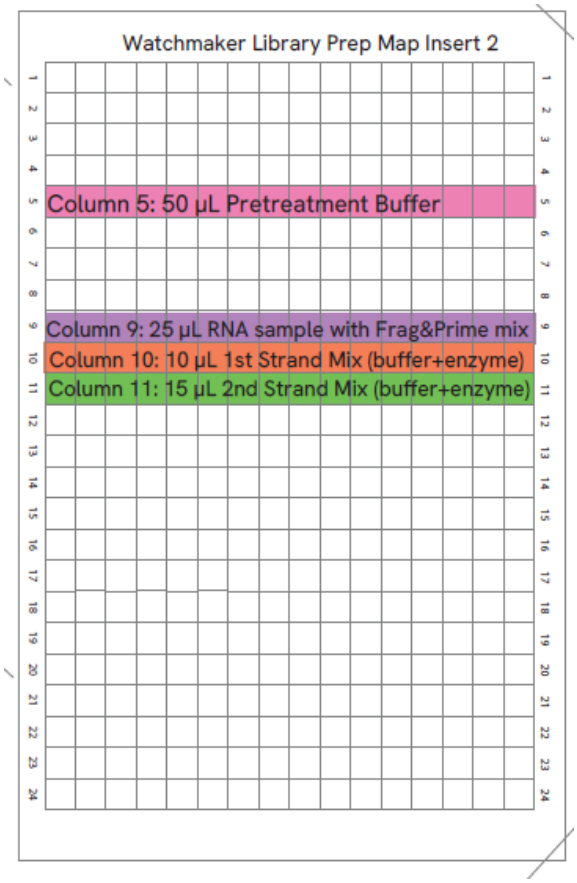

Figure 4. Watchmaker RNA Library Prep Stage 2 Loading Template Insert

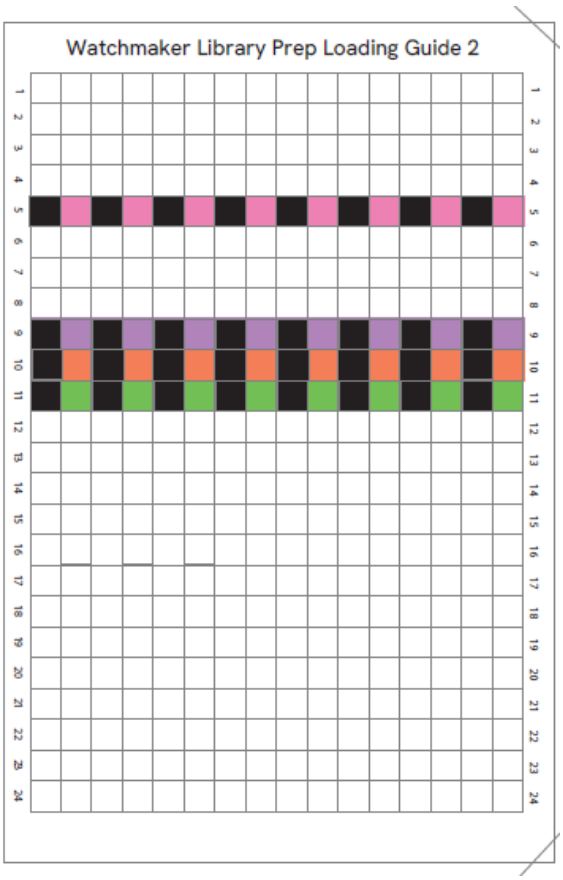

Figure 5. Watchmaker RNA Library Prep Stage 2 Plate Map

a. Column 5: Load 50 µl of the Pretreatment Buffer
b. Column 9: Load 25 ul of the RNA sample with Frag&Prime and RNA Mix prepared in step 8.
c. Column 10: Load 10 µl of the 1st Strand Mix prepared in step 9.
d. Column 11: Load 15 µl of the 2nd Strand Mix prepared in step 10.

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 12. 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 13. Load 220 µl of 70% IPA into columns 6, 7, and load 200 µl of 70% IPA into columns 13 and 14. Take extreme care to not spill IPA into adjacent wells, and try not to leave droplets on the plate seal.

Stage 3 Loading: (Wells A, C, E, G, I, K, M, O)

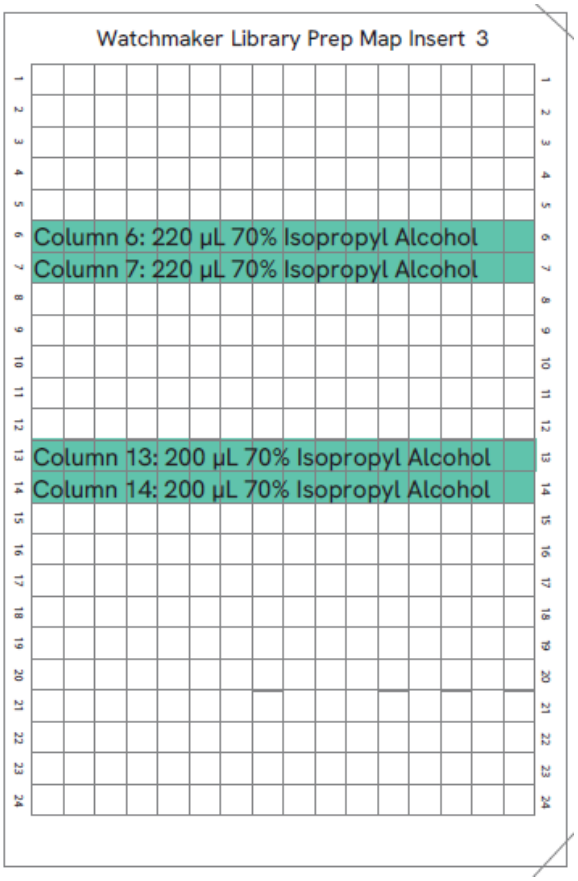

Figure 6. Watchmaker RNA Library Prep Stage 3 Loading Template Insert

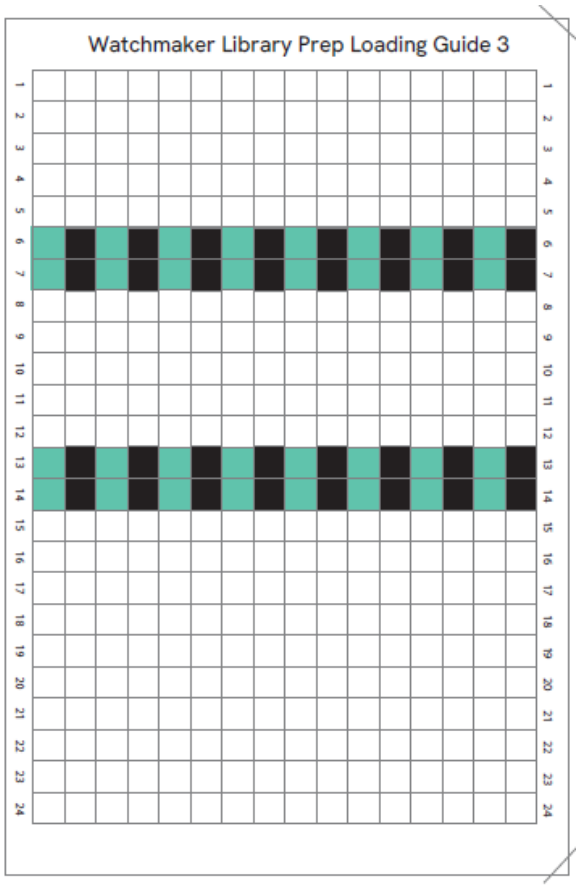

Figure 7 Watchmaker RNA Library Prep Stage 3 Plate Map

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 8.

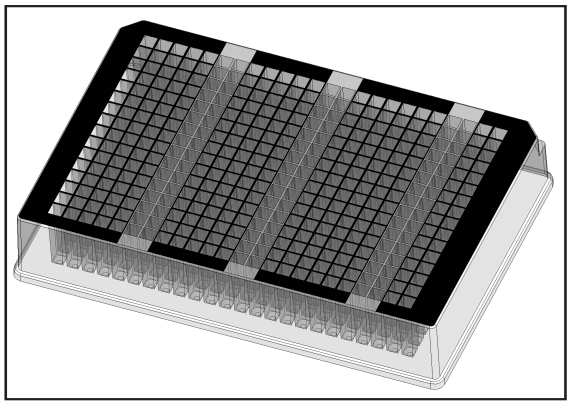

Figure 8. Removing the plate seal from the plate

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 Watchmaker RNA 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 9.

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

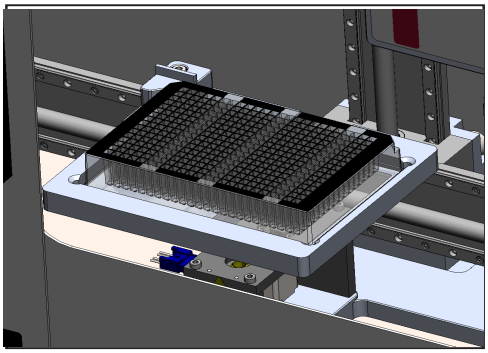

Figure 9. Placing Reagent Plate onto the X-plate

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.

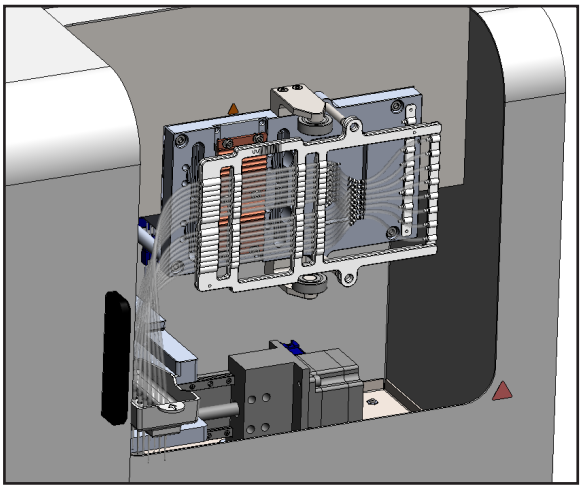

Figure 10. Cartridge insertion into BioQule™

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.

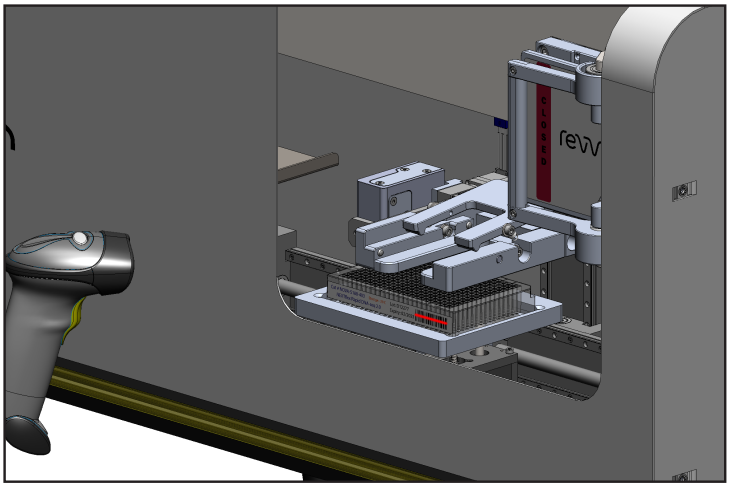

Figure 11. Scan Barcode using Barcode Scanner

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.

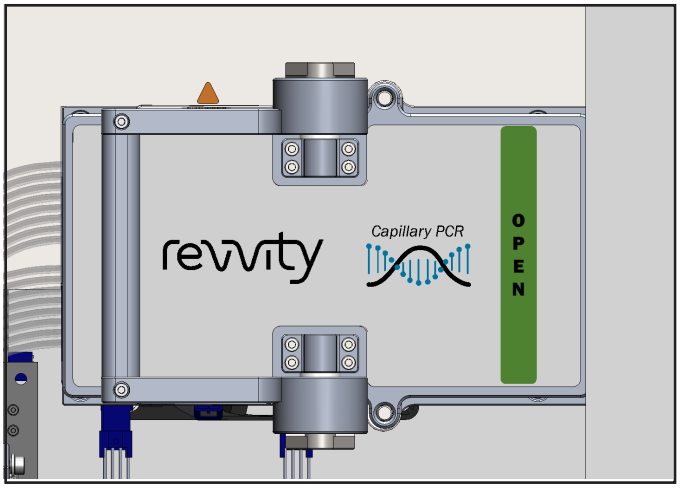

Figure 12. PCR Door Placement

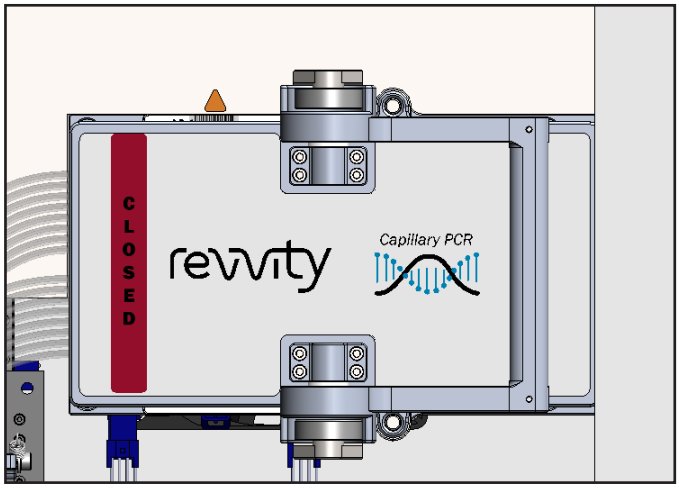

Figure 13. PCR Door Shut

Step 9. Add the names of the Samples and the Sample concentrations to the Spreadsheet. Slide the BioQule™ soor 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. The 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.

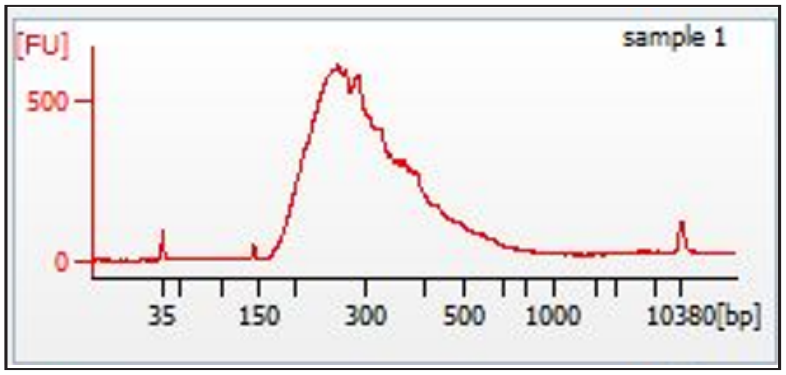

Figure 14. Example Electropherogram Plot

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.

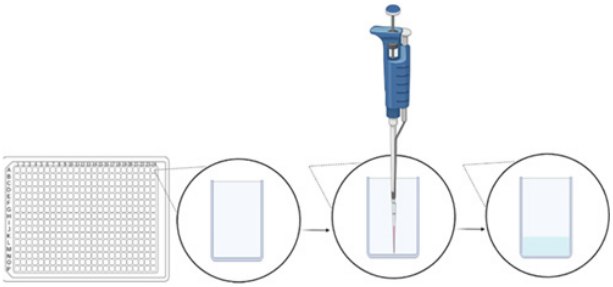

Figure 15. Pipetting directly at bottom of wells

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

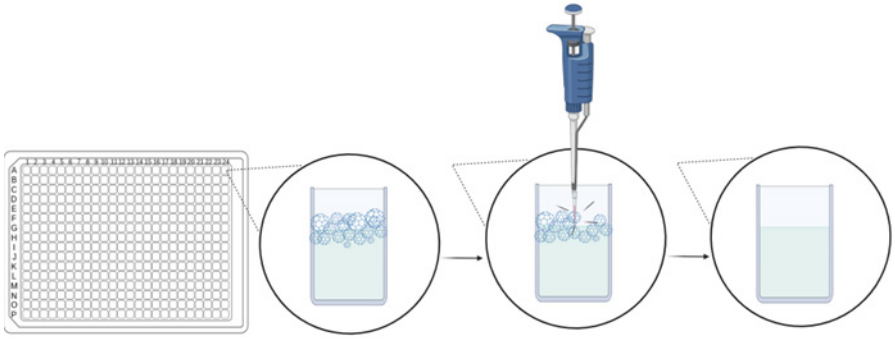

Figure 16. Popping bubbles using a 10 µL Pipette tip

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

5.2 Low Yield Library Generated

Possible causes include:

- Low quality RNA was used. RNA sample quality may vary between preparations.
- Input RNA amount was incorrect. Input RNA 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 dilution was used.
- IPA was not freshly made and had expired.
- The 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

Index or Barcode Sequences to be used on Watchmaker RNA Library prep 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.

Table 10: Nextflex UDI Barcodes

Index_ID	I7 index	I5 Sequence	I5 Sequence Reverse Complemen
UDI0001	AATCGTTA	AATAACGT	ACGTTATT
UDI0002	GTCTACAT	TTCTTGAA	TTCAAGAA
UDI0003	CGCTGCTC	GGCAGATC	GATCTGCC
UDI0004	GATCAACA	CTATGTTA	TAACATAG
UDI0005	CGAAGGAC	GTTGACGC	GCGTCAAC
UDI0006	GATGCCGG	ATCTACGA	TCGTAGAT
UDI0007	CTACGAAG	CTCGACAG	CTGTCGAG
UDI0008	GATGCGTC	GAGGCTGC	GCAGCCTC
UDI0009	CTACGGCA	CCTCGTAG	CTACGAGG
UDI0010	GATTCCTT	CATAGGCA	TGCCTATG
UDI0011	CTACTCGA	AGATGAAC	GTTCATCT
UDI0012	GATTCGAG	CCGAGTAT	ATACTCGG
UDI0013	AATCGGCG	AATATTGA	TCAATATT
UDI0014	TTCGCCGA	GTATACCG	CGGTATAC
UDI0015	CTGGCCTC	GATCCAAC	GTTGGATC
UDI0016	GAACTTAT	AGATACGC	GCGTATCT
UDI0017	CGTATTGG	GGTATCTT	AAGATACC
UDI0018	GAAGCACA	CCTCTGGC	GCCAGAGG
UDI0019	CTTAATAC	CCATTGTG	CACAATGG
UDI0020	GAAGTCTT	ACTACGGT	ACCGTAGT
UDI0021	GAAGAGGC	AAGTGCTA	TAGCACTT
UDI0022	CGGATAAC	GCCGAACG	CGTTCGGC
UDI0023	GAATCTGG	TGTCCACG	CGTGGACA
UDI0024	CTGATTGA	GACACACT	AGTGTGTC
UDI0025	AATCCGTT	AATATGCT	AGCATATT
UDI0026	TGCGTACA	TTCTCATA	TATGAGAA
UDI0027	GAATCAAT	TCTGTGAT	ATCACAGA
UDI0028	TGAGTCAG	CCGAACTT	AAGTTCGG
UDI0029	GAATGCTC	GTCTAACA	TGTTAGAC
UDI0030	GAATATCC	GACGCCAT	ATGGCGTC
UDI0031	CTTATGAA	GCCAATGT	ACATTGGC
UDI0032	TCGGCACC	CCAACGTC	GACGTTGG
UDI0033	AAGAAGCG	GTAGATAA	TTATCTAC
UDI0034	CTCACGAT	CTTACGGC	GCCGTAAG
UDI0035	TCGGTCGA	CCAAGTGC	GCACTTGG
UDI0036	TCGGTAAG	CTAACTCA	TGAGTTAG
UDI0037	AAGATACA	AATATCTG	CAGATATT
UDI0038	GTCGCTGT	TTATATCA	TGATATAA
UDI0039	TCGGATGT	CTGCGGAT	ATCCGCAG
UDI0040	CGAGCCGG	GCGGCTTG	CAAGCCGC
UDI0041	CGATTATC	GAGTTGAT	ATCAACTC
UDI0042	TCGAAGCT	GCACTGAG	CTCAGTGC
UDI0043	CTATCATT	GACCACCT	AGGTGGTC
UDI0044	CGCGCCAA	TGGCTAGG	CCTAGCCA
UDI0045	CGAACGGA	CCTACCGG	CCGGTAGG
UDI0046	CTACTGAC	GGAGGATG	CATCCTCC
UDI0047	TCTTAAGT	CGCTGAAT	ATTCAGCG
UDI0048	TTAGAGTC	TGTGACGA	TCGTCACA
UDI0049	AAGACGAA	AATAGATT	AATCTATT
UDI0050	TTATTATG	TTAGCGCA	TGCGCTAA
UDI0051	CGCTATTA	GCGGCCGT	ACGGCCGC
UDI0052	TCTATCAG	CAGTAACC	GGTTACTG
UDI0053	CGGTGGTA	GCCTAGTA	TACTAGGC
UDI0054	TCACCAAT	CACGGCGC	GCGCCGTG
UDI0055	CTGGAAGC	GGTGCAGA	TCTGCACC
UDI0056	TCCTCGAT	GTAACTGC	GCAGTTAC
UDI0057	AAGAGAGC	CAGCCAGT	ACTGGCTG
UDI0058	TCAACGAG	CGTCAACC	GGTTGACG
UDI0059	TGCGAGAC	GCCGGCGA	TCGCCGGC
UDI0060	CCTGGTGT	GCCTCCGG	CCGGAGGC
UDI0061	AAGTAAGT	AATAGTCC	GGACTATT
UDI0062	TGACTGAA	TTAGACGT	ACGTCTAA
UDI0063	AAGACTGT	GTGGACTA	TAGTCCAC
UDI0064	CAATGATG	CACGGACG	CGTCCGTG
UDI0065	CACAGTAA	CACTAGAG	CTCTAGTG
UDI0066	TGGTCATT	GCAGATGG	CCATCTGC
UDI0067	CAACCGTG	CTCTCACG	CGTGAGAG
UDI0068	TGGTGCAC	GGAATCAC	GTGATTCC
UDI0069	CCACAATG	CGTTGACG	CGTCAACG
UDI0070	TGTGTGCC	CATCAGGT	ACCTGATG
UDI0071	CACCACGG	CGTTGTAA	TTACAACG
UDI0072	TGTGTTAA	GGCACGGT	ACCGTGCC
UDI0073	AAGTTATC	AATAGCAA	TTGCTATT
UDI0074	GTACAGCT	TGATCGGT	ACCGATCA
UDI0075	CAACTGCT	AGTAGTAT	ATACTACT
UDI0076	CATGATGA	GTTAGAGG	CCTCTAAC
UDI0077	TGACTACT	CCTTACAG	CTGTAAGG
UDI0078	CAGAAGAT	GTACATTG	CAATGTAC
UDI0079	TGAGGCGC	GGAGACCA	TGGTCTCC
UDI0080	CAGGTTCC	CGAACACC	GGTGTTCG
UDI0081	TGAACAGG	GAGAACAA	TTGTTCTC
UDI0082	CAGTGTGG	TGTGAATC	GATTCACA
UDI0083	TTCCACCA	GGTTAAGG	CCTTAACC
UDI0084	CCGCTGTT	AGACCGCA	TGCGGTCT
UDI0085	AAGTTGGA	AATACAGG	CCTGTATT
UDI0086	GGACAACG	TGATGGCC	GGCCATCA
UDI0087	TTCGAACC	TGTCACCT	AGGTGACA
UDI0088	CAGACCAC	GCTTCGGC	GCCGAAGC
UDI0089	TTCTGGTG	CCAGTGGT	ACCACTGG
UDI0090	CAATCGAA	GCACACGC	GCGTGTGC
UDI0091	AAGTACAG	GTCACGTC	GACGTGAC
UDI0092	CCGTGCCA	GCAGCTCC	GGAGCTGC
UDI0093	CATTGCAC	CATGCAGC	GCTGCATG
UDI0094	TTACCTGG	ACGATTGC	GCAATCGT
UDI0095	CTGCAACG	GACATTCG	CGAATGTC
UDI0096	TACTGTTA	GCGAATAC	GTATTCGC

Index_ID	I7 index	I5 Sequence	I5 Sequence Reverse Complemen
UDI0097	AAGTGTAT	AATTAATG	CATTAATT
UDI0098	GTCCACTC	TTAATTCC	GGAATTAA
UDI0099	CTTGCTAT	GAAGGAAC	GTTCCTTC
UDI0100	TACATAGA	CAGGCATA	TATGCCTG
UDI0101	TAGCCGAT	CCAATACT	AGTATTGG
UDI0102	CGATCCAC	TCGCGCAT	ATGCGCGA
UDI0103	TAGCGTTG	GTGTGAAC	GTTCACAC
UDI0104	CTCATCAC	GGTGGCAC	GTGCCACC
UDI0105	TATGCGGT	TAGGTGCT	AGCACCTA
UDI0106	TAACTCGC	GAGCGGTT	AACCGCTC
UDI0107	CGTACGTT	AGGATTAG	CTAATCCT
UDI0108	TAAGTACC	GTGAGCCA	TGGCTCAC
UDI0109	AAGTCGTG	AATTATGC	GCATAATT
UDI0110	TTCAGAAC	TTACGCCG	CGGCGTAA
UDI0111	GTTATATA	GTAGTGAT	ATCACTAC
UDI0112	ACCGCTAT	TGGTTGCA	TGCAACCA
UDI0113	ACCGTCCT	GTCCGACC	GGTCGGAC
UDI0114	TGTCTAAC	GCCAGCCG	CGGCTGGC
UDI0115	ACCGAGGT	CTCGTGTC	GACACGAG
UDI0116	ACCGATTA	GCCGTGGC	GCCACGGC
UDI0117	GTTCTACT	GTAGCCAC	GTGGCTAC
UDI0118	ACCTGACT	GACTTATA	TATAAGTC
UDI0119	GGTAATCG	GAACGTCG	CGACGTTC
UDI0120	ACCTTAGA	ATGCGTAG	CTACGCAT
UDI0121	AAGGAGTT	AATTAGAT	ATCTAATT
UDI0122	TGAAGCCA	TTACGATA	TATCGTAA
UDI0123	GTGTTGTA	CTGTACAA	TTGTACAG
UDI0124	ACCACACG	CGTGGTTG	CAACCACG
UDI0125	ACCAGGAC	TGTAGCGG	CCGCTACA
UDI0126	TTGGCAGG	GTGTCATG	CATGACAC
UDI0127	ACCATTAA	GAACCGGT	ACCGGTTC
UDI0128	TTGTAGAT	CACTCTGA	TCAGAGTG
UDI0129	ACCATATC	TACACGTT	AACGTGTA
UDI0130	TTGGTGGC	GAGTGCCT	AGGCACTC
UDI0131	ACCAACAT	GACGGTCC	GGACCGTC
UDI0132	GTCTGTGC	CTGATGCT	AGCATCAG
UDI0133	AAGGCAAT	AATTACCA	TGGTAATT
UDI0134	TGCATTGC	GTCCTGTT	AACAGGAC
UDI0135	ACGCCACT	GTTAACAG	CTGTTAAC
UDI0136	AAGTCTCC	AGCCTGTA	TACAGGCT
UDI0137	GGATCTCT	ACGTTGCT	AGCAACGT
UDI0138	ACGCGATC	GGTGTTGC	GCAACACC
UDI0139	GGATAATA	TGGTACTA	TAGTACCA
UDI0140	ACGCTTAT	CATCATTG	CAATGATG
UDI0141	TTAGGTTG	GACGATTG	CAATCGTC
UDI0142	ACGCACAA	CGGCCGAA	TTCGGCCG
UDI0143	TGAATATA	CTGCCGCC	GGCGGCAG
UDI0144	TGCTCCGC	ACTAACCG	CGGTTAGT
UDI0145	AAGCAATA	AATTGAGA	TCTCAATT
UDI0146	TTATGTAT	TTCAAGAG	CTCTTGAA
UDI0147	ACGGCAGA	GTCCGCAA	TTGCGGAC
UDI0148	GGACTCTG	CAAGGTAG	CTACCTTG
UDI0149	GTACGTAC	AGCGACTC	GAGTCGCT
UDI0150	GGAAGGTA	GTAGTATC	GATACTAC
UDI0151	ACGTCCAT	GTGGCGAA	TTCGCCAC
UDI0152	ACGTGTTG	ACGTGCGC	GCGCACGT
UDI0153	TGAAGAAT	GAAGACAG	CTGTCTTC
UDI0154	ACGTAGTC	GCGCAGAT	ATCTGCGC
UDI0155	AAGGATAA	AGAGACAT	ATGTCTCT
UDI0156	GGCGAGGA	CACTTGTT	AACAAGTG
UDI0157	AACAAGGC	AATTGGTC	GACCAATT
UDI0158	TTGGTCCG	TTAACACT	AGTGTTAA
UDI0159	ACGAGCCT	GCTCAGCG	CGCTGAGC
UDI0160	GGAGATTC	GTTCAGAC	GTCTGAAC
UDI0161	TGCGCGCT	CAGTTGCG	CGCAACTG
UDI0162	ACGATCTA	GCAATATG	CATATTGC
UDI0163	TGCTGAGG	CGCAACGT	ACGTTGCG
UDI0164	ACTCCTCC	GGTGAGAT	ATCTCACC
UDI0165	ACTCTACG	CCAGCTGA	TCAGCTGG
UDI0166	TTCGTTCT	GTTGAGCA	TGCTCAAC
UDI0167	TGCAGTCG	CTGTAGTG	CACTACAG
UDI0168	TGCCGTAA	GTGCATCC	GGATGCAC
UDI0169	AACAGTTG	AATTCACT	AGTGAATT
UDI0170	TGTTAACA	TTCAGTGA	TCACTGAA
UDI0171	ACTTCCTG	AGTCGCGA	TCGCGACT
UDI0172	TGAATGCG	CCATATCG	CGATATGG
UDI0173	ACTTGTAC	AAGCATGG	CCATGCTT
UDI0174	ACTACTTA	CGTGTCTA	TAGACACG
UDI0175	CTCTTCGT	CCGTCTAA	TTAGACGG
UDI0176	ACTAGCTC	CGAGAGTG	CACTCTCG
UDI0177	TTATCAAC	CGAGTACG	CGTACTCG
UDI0178	TGCTTGTC	GTGTCTCA	TGAGACAC
UDI0179	AAGGTAGG	CCATCGCT	AGCGATGG
UDI0180	TGGTATGG	CTTCGCTC	GAGCGAAG
UDI0181	AACACATA	AATTCTAA	TTAGAATT
UDI0182	TGGTGTCT	TTCATCTG	CAGATGAA
UDI0183	ACACTAAC	ACAGTGAA	TTCACTGT
UDI0184	TTGTGTTC	AGATGTGA	TCACATCT
UDI0185	ACACACCT	GGCAACTG	CAGTTGCC
UDI0186	GGTGTCCG	CAGCGTCT	AGACGCTG
UDI0187	ACACATTC	CTGTCGGT	ACCGACAG
UDI0188	TTGCTTAA	TCGGCGTT	AACGCCGA
UDI0189	ACAGCCTT	CACACGCG	CGCGTGTG
UDI0190	TTGCAGTA	AGCCAACA	TGTTGGCT
UDI0191	ACAGGCAG	AGCAAGTT	AACTTGCT
UDI0192	TTGCCATC	ACGCGTCA	TGACGCGT
UDI0193	ATAATGTA	AATGAACC	GGTTCATT
UDI0194	TCGTACCG	GGAAGTGG	CCACTTCC
UDI0195	GTTATTAT	CGCTAAGC	GCTTAGCG
UDI0196	ACAGAAGC	GTCATAGC	GCTATGAC
UDI0197	TTGATAAT	GTGACCAT	ATGGTCAC
UDI0198	ACATCGGA	GAAGTTGA	TCAACTTC
UDI0199	ACCTCTCA	GCGGTTAA	TTAACCGC
UDI0200	GTGAGTGT	CGCCTCTT	AAGAGGCG
UDI0201	ACATCACC	GTTCTGGA	TCCAGAAC
UDI0202	CTCGCTTG	ACTGCATC	GATGCAGT
UDI0203	ACATGGAT	GGTTAGCC	GGCTAACC
UDI0204	TCAGGATC	TCCACAGC	GCTGTGGA
UDI0205	ATAAGGCT	AATGTGGC	GCCACATT
UDI0206	TCTTACTC	GTCACAAG	CTTGTGAC
UDI0207	ACATGATA	CGTAGATA	TATCTACG
UDI0208	TTGCCTCT	TGCGGCTA	TAGCCGCA
UDI0209	ACATTCTC	ACAACTCT	AGAGTTGT
UDI0210	ACATATGT	GATCGCAG	CTGCGATC
UDI0211	GTTGGTAG	TGGAACAT	ATGTTCCA
UDI0212	ACAACCGC	CACATACC	GGTATGTG
UDI0213	AAGCAGAC	GCTAAGAA	TTCTTAGC
UDI0214	ACAAGGTG	CTTAACCA	TGGTTAAG
UDI0215	ACAAGAGT	GCCTTCAA	TTGAAGGC
UDI0216	TTGGAATT	AACTGGAA	TTCCAGTT
UDI0217	ATAACAGA	AATGGAAG	CTTCCATT
UDI0218	TATTGTCG	TTCAATTC	GAATTGAA
UDI0219	ACAATGCC	CGTCCTTA	TAAGGACG
UDI0220	TATGGTTC	CACCGGTA	TACCGGTG
UDI0221	AGCCACAG	AGGAACCA	TGGTTCCT
UDI0222	GCTATCGA	GATCTTCC	GGAAGATC
UDI0223	AGCGCTCG	CGGCTTGT	ACAAGCCG
UDI0224	TGCGCTTC	GCCGACAC	GTGTCGGC
UDI0225	TCTACGCC	CGGCGTAC	GTACGCCG
UDI0226	TATCACAA	GCCTCATT	AATGAGGC
UDI0227	AGCTTGGT	CGGCACGA	TCGTGCCG
UDI0228	AGCTTATG	GCCTGTAG	CTACAGGC
UDI0229	ATAACGAC	AATGGCCT	AGGCCATT
UDI0230	GATGGAGT	TTCCAGGC	GCCTGGAA
UDI0231	TGATCTAA	TTAACCTC	GAGGTTAA
UDI0232	AGCTATAT	CGTTAGGA	TCCTAACG
UDI0233	AGCAGAGA	GCCATAAT	ATTATGGC
UDI0234	GACGTAAG	GCCAAGCC	GGCTTGGC
UDI0235	AGGCCTGA	CTCGGTCA	TGACCGAG
UDI0236	CCGCCTCG	ACGTAACA	TGTTACGT
UDI0237	AGGCGAAT	GCGTCCTC	GAGGACGC
UDI0238	TTGTTCTT	CTACAAGT	ACTTGTAG
UDI0239	TACTCCTG	GCGTGTCC	GGACACGC
UDI0240	GACTGGCG	GCGACTGA	TCAGTCGC
UDI0241	ATATATAC	AATGCTTG	CAAGCATT
UDI0242	TAGATCGG	TTAATCAA	TTGATTAA
UDI0243	AGGCAAGC	CTCTGGAC	GTCCAGAG
UDI0244	TCCAGGTA	GCTCCAGT	ACTGGAGC
UDI0245	AGGTCAAG	CGCGTTAT	ATAACGCG
UDI0246	TACACTGG	GCTCAATC	GATTGAGC
UDI0247	TCCTTGCG	CGCATGTG	CACATGCG
UDI0248	AGGACCTC	GCTGCCAA	TTGGCAGC
UDI0249	TAAGCATT	CGAACTAT	ATAGTTCG
UDI0250	AGGATATT	GCTGTCTC	GAGACAGC
UDI0251	TCATTCGG	CGACGGTT	AACCGTCG
UDI0252	AGTCCGAC	GCTTCACA	TGTGAAGC
UDI0253	ATATTACA	AATGCGCA	TGCGCATT
UDI0254	TCTCCGTG	TGAATCGT	ACGATTCA
UDI0255	TAATAGGA	CTAACCAG	CTGGTTAG
UDI0256	TTGTAAGA	GCTTGTGA	TCACAAGC
UDI0257	GAATGTAA	CTGGCACT	AGTGCCAG
UDI0258	AGTCAATT	GCACGGTC	GACCGTGC
UDI0259	GAACAATT	CTTGGATT	AATCCAAG
UDI0260	AGTGGTCA	GCACATCA	TGATGTGC
UDI0261	TACAATAT	CTTCAATA	TATTGAAG
UDI0262	AAGCGCGT	TTACTGGT	ACCAGTAA
UDI0263	GAACCTTA	CTGAGTCC	GGACTCAG
UDI0264	AGTGTACT	GCATACTT	AAGTATGC
UDI0265	TATACCAT	AATGCCAC	GTGGCATT
UDI0266	ATCGTGGA	GGCCGTTG	CAACGGCC
UDI0267	GCAAGACA	GCATAGAC	GTCTATGC
UDI0268	TACAGGCC	CATTCGCC	GGCGAATG
UDI0269	AGTTCCGT	GGCCGCGT	ACGCGGCC
UDI0270	TCAATTAT	CCTGAAGG	CCTTCAGG
UDI0271	AGTTCATA	GGCCTTCA	TGAAGGCC
UDI0272	TCACCGGC	GGCTTACT	AGTAAGCC

UDI0284	ATATAAGG	GGTCATGT	ACATGACC
UDI0285	ATAGAATA	CAACGACC	GGTCGTTG
UDI0286	TCTAGAGA	GGTTCTTG	CAAGAACC
UDI0287	ATAGGCCA	CAACATAC	GTATGTTG
UDI0288	ATAGCGGT	GGTTGATT	AATCAACC
UDI0289	ATATCCTA	AATAATAG	CTATTATT
UDI0290	GATAGGAT	GTCGGATA	TATCCGAC
UDI0291	ATACTGCG	CACATTGT	ACAATGTG
UDI0292	TCTTCCGA	GGTTACAA	TTGTAACC
UDI0293	ATACCTAT	CACGAATT	AATTCGTG
UDI0294	TCGTTATA	GGTATGCA	TGCATACC
UDI0295	TAATTAGT	CAGGACCT	AGGTCCTG
UDI0296	ATTATTCG	GGACTGGC	GCCAGTCC
UDI0297	TAATAATC	CAGAGCGA	TCGCTCTG
UDI0298	ATTATCGC	GGAGCTAG	CTAGCTCC
UDI0299	TAATATAG	CAGCTTAG	CTAAGCTG
UDI0300	ATTAGACA	CCGCCAAC	GTTGGCGG
UDI0301	ATAGATCT	CCTATGCG	CGCATAGG
UDI0302	TAGAGCTC	GGATCCGA	TCGGATCC
UDI0303	ATTACAAT	GGATTGTT	AACAATCC
UDI0304	ATTGAAGT	CAGGTGAA	TTCACCTG
UDI0305	ATTGATTC	GGAACATT	AATGTTCC
UDI0306	TAACTAAG	CAAGACTC	GAGTCTTG
UDI0307	ATTGACAA	GTGCGGCG	CGCCGCAC
UDI0308	ATTGTGTT	CAATCTTC	GAAGATTG
UDI0309	ATTGCTGA	GTGCTCAG	CTGAGCAC
UDI0310	TACATCCT	CAACGCAT	ATGCGTTG
UDI0311	ATTCACGG	GTGTAGGC	GCCTACAC
UDI0312	TAATTGAC	CAACTATA	TATAGTTG
UDI0313	ATAGTCTG	GCATTGCA	TGCAATGC
UDI0314	TATAAGAC	CTTACATG	CATGTAAG
UDI0315	ATTCTTAC	GTGTATAG	CTATACAC
UDI0316	TCGATTCA	AACAATCA	TGATTGTT
UDI0317	ATTCTCTA	CACTATCT	AGATAGTG
UDI0318	ATTCGATG	GTGATCTC	GAGATCAC
UDI0319	TAAGCTAC	CAATCCGT	ACGGATTG
UDI0320	ATTCGTGT	GTTCGTCA	TGACGAAC
UDI0321	ATGAATAT	CCACTTAA	TTAAGTGG
UDI0322	ATGAAGGA	GTTGAATG	CATTCAAC
UDI0323	ATGAACTG	CCAGCAAG	CTTGCTGG
UDI0324	ATGAGCAC	GTTAGTTC	GAACTAAC
UDI0325	ATAGGAAT	CCAGGCTA	TAGCCTGG
UDI0326	TAGACGGC	GGTCCGCT	AGCGGACC
UDI0327	GAATAGTG	CACTTCCA	TGGAAGTG
UDI0328	ATGACACC	GTTAAGTT	AACTTAAC
UDI0329	ATGACGTT	AATCTAGT	ACTAGATT
UDI0330	ATGTATTA	GTTAATGA	TCATTAAC

UDI0331	ATGTACCT	CATACCTA	TAGGTATG
UDI0332	TCAATGGA	GTATGAGT	ACTCATAC
UDI0333	GAGGTCAC	TAGATTAC	GTAATCTA
UDI0334	TAACCAGA	GACCGAAT	ATTCGGTC
UDI0335	ATGTGATT	CGTTACTT	AAGTAACG
UDI0336	TAACAGCC	GACCTGAC	GTCAGGTC
UDI0337	TATCTGTC	TTCGGCAT	ATGCCGAA
UDI0338	AGATAACT	CCGAAGGC	GCCTTCGG
UDI0339	ATGTGGCA	GACCATGA	TCATGGTC
UDI0340	TAACACTG	GACGTGCA	TGCACGTC
UDI0341	ATGTCTGG	CGGCGACA	TGTCGCCG
UDI0342	TCAAGCAC	GACTAGGT	ACCTAGTC
UDI0343	ATGGTAAC	CGGTAGAC	GTCTACCG
UDI0344	ATGGTTCA	GACAGCTT	AAGCTGTC
UDI0345	TCCAACGG	CGCTCGCA	TGCGAGCG
UDI0346	GAACAGAA	GAGATAGT	ACTATCTC
UDI0347	ATGGCCAG	AACACTAC	GTAGTGTT
UDI0348	TCACGTGA	GTGTGCGG	CCGCACAC
UDI0349	ATACAACC	ACCGTGTG	CACACGGT
UDI0350	TATGTTGG	TGGACACA	TGTGTCCA
UDI0351	ATGCTAGA	AACTACTT	AAGTAGTT
UDI0352	ATGCTGTC	TTGAGGAA	TTCCTCAA
UDI0353	GATTGACC	AACTTAAT	ATTAAGTT
UDI0354	ATCATACT	TTGACTTA	TAAGTCAA
UDI0355	ATCAGCTA	AACTTGCC	GGCAAGTT
UDI0356	ATCACGCA	GTGCACGT	ACGTGCAC
UDI0357	GAGTTCTG	AACTTCGG	CCGAAGTT
UDI0358	ATCACCGT	TGTACGAA	TTCGTACA
UDI0359	TCTTGGAA	AACTCAGC	GCTGAGTT
UDI0360	ATCTAATC	TGTATTCT	AGAATACA
UDI0361	ATACATGA	GTCGTCGG	CCGACGAC
UDI0362	TATTGAAT	CCGACGCA	TGCGTCGG
UDI0363	GCTAGTCT	AACGAAGA	TCTTCGTT
UDI0364	ATCTAGCG	TGTCGGAT	ATCCGACA
UDI0365	TCTCGCTA	AACGACCG	CGGTCGTT
UDI0366	ATCTGAAG	TGTCGTGC	GCACGACA
UDI0367	TCTAATGC	AACGTCAA	TTGACGTT
UDI0368	GATAGCGC	TGTAAGGT	ACCTTACA
UDI0369	CTCCTACA	ACTGAGAC	GTCTCAGT
UDI0370	TATCAAGC	TTGAATCG	CGATTCAA
UDI0371	ATCGCATT	AACGGTGT	ACACCGTT
UDI0372	ATCCAGAA	AACCAGTG	CACTGGTT
UDI0373	ATACTATT	AATATAAC	GTTATATT
UDI0374	GATGATAC	GGCGCGTG	CACGCGCC
UDI0375	GCGGTATT	TTGTTACC	GGTAACAA
UDI0376	ATCCGTTC	AACCTTGC	GCAAGGTT
UDI0377	AGAATTCA	TGTGCAAT	ATTGCACA
UDI0378	TATGACTT	AACCGAGG	CCTCGGTT
UDI0379	GAGTCAGA	TTGGATAC	GTATCCAA
UDI0380	AGAAGACG	AACCGCAC	GTGCGGTT
UDI0381	AGAAGCTT	TGTTAGTG	CACTAACA
UDI0382	GAGTAGCA	TATTCGTA	TACGAATA
UDI0383	AGAACCAA	ATAATTGT	ACAATTAT
UDI0384	GCTTGGTG	TAGTCCAA	TTGGACTA

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	Notes
May 10th 2024	B	Expanding input samples types.
October 1st 2024	C	Clarifying sample dilutions

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