



ResolveOME™ Whole Genome and Transcriptome Single-Cell Core Kit

Protocol to Prepare WGS and Transcriptome Sequencing Libraries:

User Guide

384-Well Format

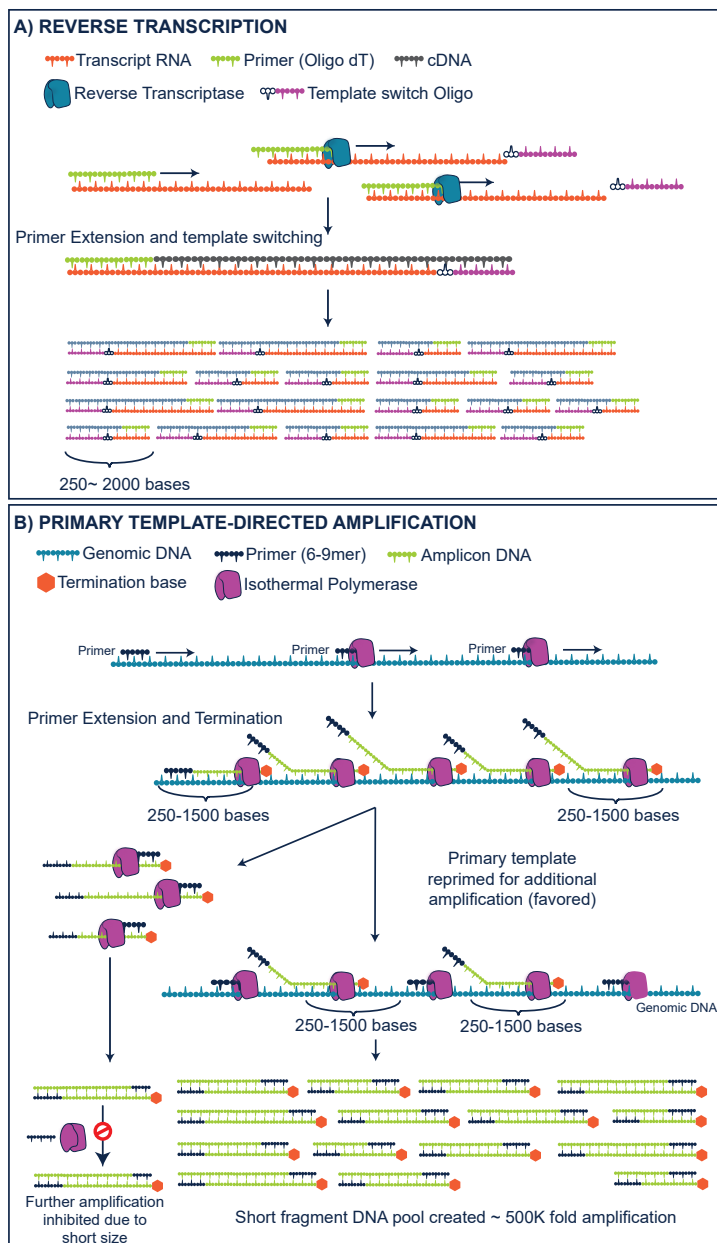
For Use with the ASSIST PLUS from INTEGRA Biosciences

ResolveOME™ Whole Genome and Transcriptome Single-Cell Core Kit

Unified Single-Cell Whole Genome and Transcriptome Amplification

The ResolveOME Whole Genome and Transcriptome Single Cell Core Kit from BioSkryb Genomics combines the breakthrough whole genome amplification (WGA) technology, Primary Template-directed Amplification (PTA), with full-transcript mRNA transcriptome analysis for comprehensive multiomic analysis at single cell resolution.

Capable of unparalleled coverage of both the genome and mRNA transcriptome of a single cell, ResolveOME unifies genomic variation data with transcriptional and translational layers of information to provide a more complete picture of the drivers and consequences of clonal heterogeneity within cell populations.



ResolveOME highlights:

- o **Complete genome and full-length mRNA coverage** reveals the consequence of genomic variation (all major variant classes) on gene expression and transcript structure, and exposes subtle changes in protein sequence that may profoundly impact structure, function, and activity.
- o **A unified, one day workflow** for the interrogation of DNA and RNA from the same cell obviates the need for splitting source material or interpreting across data sets.
- o **Full transcriptome workflow enables enhanced RNA analysis** compared to droplet-based single-cell RNA sequencing, providing full transcript RNA-Seq, splicing and isoform detection, and gene fusion detection.

Figure 1. How ResolveOME Works

(A) Beginning with a single cell, the cytoplasm is lysed to enable reverse transcription (RT) of mRNA into first strand cDNA using an oligo dT primer. (B) Subsequently, the nucleus is lysed to enable whole genome amplification (WGA) through Primary Template-directed Amplification (PTA). PTA utilizes random priming and proprietary termination chemistry to prevent the production of long amplicons, driving primers back to the primary template and resulting in the amplification of a true representation of original sample template. Then, first strand cDNA products are isolated for library preparation apart from the amplified genomic DNA using the BioSkryb ResolveOME library preparation system.

The ResolveOME Process

After single cell isolation, the BioSkrbyb ResolveOME cytosolic lysis and reverse transcription steps are carried out to generate first strand cDNA representing the transcriptome of each cell (Figure 1A, 2). cDNA remains in the sample during nuclear lysis and subsequent whole genome amplification steps, where the genome of each cell is denatured followed by random priming based genome amplification (Figure 1B, 2). PTA utilizes isothermal amplification and proprietary termination chemistry to restrict amplicon size, preferentially directing subsequent priming events back to the primary template (Figure 1B). This critical feature of the ResolveOME chemistry minimizes copying of synthesized genome amplicons and first strand cDNA. First strand cDNA and genome amplicons are then isolated by an affinity purification process and libraries are prepared in parallel for the genomic and transcriptomic fractions, followed by sequencing and data analysis (Figure 2). Appendix A contains time estimates for steps.

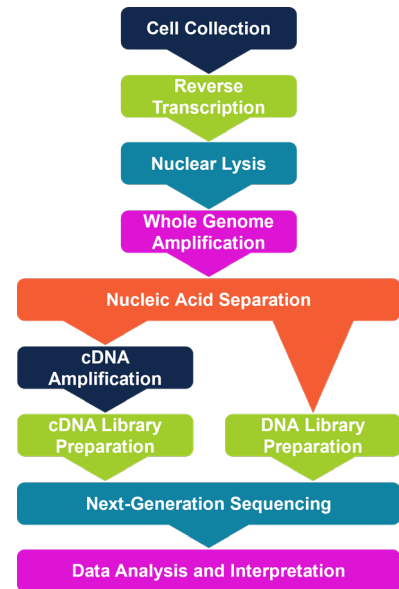


Figure 2. The ResolveOME Workflow First strand cDNA synthesis and genome amplification occur sequentially in one tube followed by separation and library preparation for multiomic NGS analysis.

Safety Precautions and Use of Personal Protective Equipment

I. Biosafety Hazards

Many samples require handling as biohazards under the Universal Precautions doctrine.

Wear appropriate Personal Protective Equipment (PPE) such as lab coats, disposable gloves, and safety goggles.

II. Chemical Hazard.

This kit contains hazardous materials and should be handled only by trained personnel. Always wear appropriate PPE. Users should consult the relevant Safety Data Sheets for more information.

III. Safety Data Sheets

For access to the Safety Data Sheets for this product, please contact the [BioSkrbyb Genomics Application Support Team](mailto:TechSupport@BioSkrbyb.com) (TechSupport@BioSkrbyb.com).

IV. Emergency Response Information

For 24-hour emergency information pertaining to accidents or spills involving ResolveOME products, please contact one of the numbers listed below for information on how to clean up and discard the hazardous waste.

North America: +1-800-535-5053

International: +1-352-323-3500

In the event of a life-threatening emergency, please contact local emergency services.

Intended Use

The ResolveOME Kit is intended for **research use only** and is not intended for prevention, diagnosis, or treatment of disease. The INTEGRA ASSIST PLUS is for **research use only**. Not for use in diagnostic procedures.

Kit Contents and Storage

I. Kit Contents Stored at -20°C

Box	Kit Components	Part Number	Cap Color	Storage
Box 1: Pre-PCR (PN 100952)	Cell Buffer	100641	Clear ☒	-20°C
	Control Genomic DNA 50 ng/μL	101155	Gold ●	-20°C
	Control RNA 50 ng/μL	101156	Orange ●	-20°C
	RB1 Reagent	100697	Teal ●	-20°C
	RTC Reagent	100823	Natural ○	-20°C
	RTP Reagent	100700	Green ●	-20°C
	OL1 Reagent	100703	Purple ●	-20°C
	L2 Reagent	100581	Yellow ●	-20°C
	OL3 Reagent	100706	White ○	-20°C
	OR1 Reagent	100815	Blue ●	-20°C
	OR2 Reagent	100825	Red ●	-20°C
	Box 2: Post-PCR (PN 100975)	PAP Reagent	100728	Orange ●
PAC Reagent		100788	Green ●	-20°C
LPOB Reagent		100828	Natural ○	-20°C
LPOE Reagent		100791	Clear ☒	-20°C
LP1B Reagent		100834	Teal ●	-20°C
LP1E Reagent		100836	Purple ●	-20°C
LP2B Reagent		100838	Blue ●	-20°C
LP2E Reagent		100840	Gold ●	-20°C
LP3A Reagent		100842	Clear Bottle ☒	-20°C
LP3P Reagent		100752	Red ●	-20°C
Single Use Library Adapter Plates	(2) 384-well Single Use Adapter Plates from adapter set W-Z	100948-100951	N/A ☒	-20°C

II. Kit Contents Stored at 4°C

Box	Kit Components	Part Number	Cap Color	Storage
Beads Module (PN 100772)	SEP Reagent	100731	Natural ○	+4°C
	Resolve Beads	100735	Clear Bottle ☒	+4°C
	Elution Buffer	100736	Clear Bottle ☒	+4°C

III. Shipping and Storage

This kit contains components with various shipping and storage requirements. Upon receipt, please

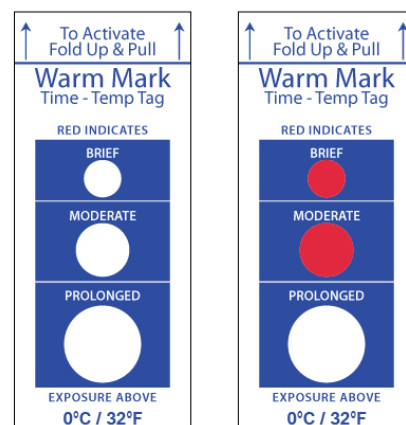
carefully check each label and store each kit box as noted on its label.

The -20°C components are shipped on dry ice, and all reagents and enzymes will be frozen upon arrival. The 4°C components will ship on cold packs or ambient temperature. Boxes should be promptly removed from shipping containers and stored according to the instructions on the box label.

When stored as directed, the kit will perform to specifications for up to the expiration date, 18 months from the date of manufacture (DOM). Do not exceed 5 freeze/thaw cycles for any individual reagent.

Temperature tags are shipped with the kit to ensure frozen materials have not been exposed to elevated temperatures during transit (Figure 3). Please contact the [BioSkryb Genomics Application Support Team](#) if you have any questions about the interpretation of the temperature tags.

Figure 3. Temperature Indicator Tag (right) Each dry ice shipment includes a temperature tag designed to indicate exposure above 0°C. If the shipment stays below the target temperature, the windows will remain white.



Required Equipment, Materials, and Reagents (Not Included in Kit)

I. Necessary Equipment and Consumables Available from Third-Party Vendors

Several additional pieces of laboratory equipment and consumables are required or recommended for execution of the ResolveOME workflow. Where specified, the following products have been tested with our workflow to provide optimal results. The use of any products not included in this list may result in sub-optimal results. Use of the prescribed equipment in BioSkryb’s laboratory setting, with ResolveOME 384, was found to be safe in this workflow, when used in a manner indicated by the equipment manufacturer. Please consult the [BioSkryb Application Support Team](#) (techsupport@bioskryb.com) if you have questions about the suitability of any alternative materials or equipment to be used in conjunction with this protocol.

The ASSIST PLUS instrument and consumables can be purchased from INTEGRA.

Products Sold by INTEGRA		
Product Name	Company	Catalog Number
ASSIST PLUS Pipetting Robot	INTEGRA Biosciences	4505
12ch 12.5ul VIAFLO Electronic Pipette	INTEGRA Biosciences	4631
12 Ch 125ul VOYAGER Electronic Pipette	INTEGRA Biosciences	4732
Communications Modules, 2 Needed	INTEGRA Biosciences	4222
384 Well Cooling Blocks, 2 Needed	INTEGRA Biosciences	6255

96 Well Cooling Block, 2 Needed	INTEGRA Biosciences	6250
MAG Module	INTEGRA Biosciences	4900
384 Adapter for MAG Module	INTEGRA Biosciences	4908
Communications /Charging Stand for VIAFLO, VOYAGER Pipettes	INTEGRA Biosciences	4211
Sterile, Filter, Low Retention 12.5 µL GRIPTIPS	INTEGRA Biosciences	6555
Sterile, Filter, Low Retention 125 µL GRIPTIPS	INTEGRA Biosciences	6565
25 mL Disposable Reservoirs, Sterile	INTEGRA Biosciences	4317

Products from Third-Party Suppliers

Product Name	Company	Catalog Number
384-well Low Elution Post Magnet Plate with Integrated Cushion Base	Permagen	P384LE
PCR Plate Sealing Film	ThermoFisher Scientific	AB0558
MicroAmp EnduraPlate Optical 384-well Clear Reaction Plates	appliedbiosystems	4483285
twin.tec PCR 96 LoBind, Semi Skirted Plates	Eppendorf	E0030129504
8-strip 0.2 mL PCR Tubes	General Lab Supplier (GLS)	—
1.5 mL Microcentrifuge Tubes	GLS	—
15 mL Conical Centrifuge Tubes	GLS	—
Single-channel Pipet Set (P-10, P-20, P200, P1000) and Appropriate Aerosol Barrier Tips	Rainin or GLS	—
8-channel Pipets (P-20, P-200) and Appropriate Aerosol Barrier Tips	Rainin or GLS	—
Agilent TapeStation	Agilent	4200
HS D5000 Screenshot	Agilent	5067-5592
HS D5000 Reagents	Agilent	5067-5593
Fluorometer (Qubit 2-4)	ThermoFisher Scientific	—
High Sensitivity dsDNA Assay Kit	ThermoFisher Scientific	Q32854
PCR Plate Thermal Mixer	GLS	—
Vortexer	GLS	—
PCR Plate Spinner	GLS	—
Microcentrifuge	GLS	—
Ice	GLS	—
Thermal Cycler	GLS	—
Magnet Stand for 1.5 mL Microcentrifuge Tubes	GLS	—
Plate Sealing Roller	GLS	—
Absolute (200 proof) Ethanol	GLS	—
RT-PCR Grade Water	GLS	—



Sample Selection and Preparation

I. Sample Types Supported

This protocol is generally designed to work with single live mammalian cells. Input can be single or multiple cells obtained by common cell isolation methods. No upper limit has been established for multiple cell input. Ensure that cells are viable and placed into 1 μ L of Cell Buffer, then proceed promptly to the ResolveOME protocol or freeze the cells at -80°C for short-term storage.

This protocol is not optimized for use with fixed cells, nuclei, or intact tissues.

Please contact the [BioSkryb Genomics Application Support Team](mailto:techsupport@bioskryb.com) (techsupport@bioskryb.com) should you have any questions on sample compatibility.

II. FACS

Fluorescence-activated cell sorting (FACS) is currently the most common method used to enrich cell populations of interest. Cells can be sorted based on surface markers, fluorescent staining and light scattering properties. In preparation for the ResolveOME protocol, cells should be sorted into the ResolveOME Cell Buffer in tube or plate format. Additional information on FACS (TAS-062) can be obtained from the BioSkryb Application Support Team.

III. Spatial Cell Picking Technology

A number of systems enable fully-automated cell picking. Refer to the BioSkryb [“Integrated Workflow for Spatial Single Cell Genome Analysis”](https://bioskryb.com/eap-cellselector/) for one example (bioskryb.com/eap-cellselector/).

IV. Other Methods of Single Cell Singulation

Most methods of live cell isolation are compatible with ResolveOME.

ResolveOME Whole Genome and Transcriptome Single-Cell Core Kit Protocol

I. Before You Begin

1. The protocol describes execution of the ResolveOME Whole Genome and Transcriptome Single-Cell Core Kit workflow, which includes ResolveOME v2.0, and is different than ResolveOME v1.0 workflow. The two versions of ResolveOME are not interchangeable. Similarly, the 96-well format kit is not interchangeable with the 384-well format kit. Please, contact BioSkryb Application Support Team (techsupport@bioskryb.com) for more information.
2. Read through the entire protocol and ensure all required equipment (see Required Equipment, Materials, and Reagents on page 5), reagents, and consumables are on hand.
3. This protocol is compatible with low-bind skirted 384-well plates. Ensure that the tube format chosen is compatible with your thermal cycler, thermal mixer and magnet before beginning the protocol.

II. Best Practices

1. **Location** - The reverse transcription, lysis, and whole genome amplification (WGA) setup steps must be executed in an RNase- and DNA-free, pre-amplification workspace or PCR hood enclosure to avoid the possible introduction of exogenous DNA from the operator or the lab environment. The amplification incubation itself, and subsequent steps, may be executed under general laboratory conditions.
2. **Use of Controls** - The control set provided may be used to interpret the appropriate execution of the ResolveOME chemistry.

Control	Purpose	Formulation
Negative Control or No template control (NTC)	Detection of nucleic acid contamination across wells, reagents or in the lab environment	1 μ L Cell Buffer
Positive DNA Control	Correct execution of Whole Genome Amplification and DNA library preparation steps	100 pg Control Genomic DNA in 1 μ L Cell Buffer
Positive mRNA Control	Correct execution of Reverse Transcription, RNA Amplification, and RNA library preparation steps	100 pg Control RNA in 1 μ L Cell Buffer

BioSkryb control material is comprised of bulk-isolated human nucleic acids (DNA or mRNA) from the NIST benchmark HG002 (<https://www.nist.gov/programs-projects/genome-bottle>). Use of this material as indicated herein enables customers to both confirm proper execution of the workflow as well as analytically confirm the genomic and transcriptomic performance of the assay. In addition to benchmarked genomic and transcriptomic values provided by NIST, BioSkryb has extensively tested the material. It is strongly recommended to include these controls, and a negative NTC well, with each experimental run for troubleshooting.

3. **Master Mix Preparation** - Use a vortex mixer to thoroughly mix all reagents and master mixes after thawing unless otherwise instructed.
 - Always keep reactions and reagents chilled on ice unless otherwise instructed.
 - Lab cooling blocks designed to keep reactions chilled during handling are recommended.
 - Necessary overages are accounted for in master mix tables. These may vary from those requested by the software.
 - When instructed to “briefly spin down,” the intent is to ensure any droplets dispersed

within a tube are collected. A quick pulse (10 seconds) on a benchtop microcentrifuge is usually sufficient.

- It is not recommended to use vortex mixers on isolated cells, cells, lysates, and other reaction intermediaries during the protocol as this can lead to variable performance (See “Gentle and Thorough Mixing” below).

4. Pipetting Technique - To avoid material loss in the reaction, it is important to avoid direct contact between pipet tips and cell suspension, lysate, or other reaction intermediaries during manual reagent additions. Loss of a small amount of liquid is unavoidable whenever the pipet tip is allowed to come into contact with the reaction mix. All reagent additions should be dispensed onto the wall of the tube as shown in Figure 4.

5. Multichannel Pipetting - For the manual addition of PreAmp and Library Amplification master mixes, throughput can be facilitated by distributing the master mixes in 8-well strip tubes and use of a multichannel pipet to dispense reagents into each well. It is recommended to use a new tip for each well to prevent cross-sample contamination.

6. Gentle and Thorough Mixing - Once the reagent has been added to the tube, it is vital to ensure gentle and thorough mixing of the reaction components. Any non-homogeneity within the reaction will lead to inefficiency and diminish the performance of the kit. To ensure each reagent addition is mixed into the reaction thoroughly, first seal the plate/tubes and briefly spin down in a centrifuge (10 seconds at ~750 X g is sufficient). Then place the reaction plate/tubes in a programmable thermal mixer and gently mix according to the instructions in this protocol, e.g. 1 minute at 1400 rpm. After mixing, briefly spin down the reactions again to ensure any droplets generated during the mixing process are recombined in the bottom of the plate/tubes. In summary, for best results ALWAYS pipet reagent additions on the side of the tube, avoiding any contact with the material in the bottom of the tube, then SEAL-SPIN-MIX-SPIN. After these steps, proceed with any incubation or move on to the next reagent addition per the protocol.

7. Thermal Cycler Usage - Pre-program a thermal cycler to run the various programs outlined within this protocol prior to beginning (Table 1, Table 4, Table 8, Table 9, Table 11, Table 13, and Table 15).

- Unless otherwise noted, when using PCR thermal cyclers for isothermal incubation at temperatures below 55°C, it is recommended to set the temperature of the heated lid to 70°C.
- To maximize time efficiency during the protocol, it will be necessary to have two programmed thermal cyclers available.

8. Quantification - Use a fluorimetric method of quantification (such as Qubit) with the amplification products and sequencing libraries produced with ResolveOME. The use of spectrophotometric quantification methods (such as Nanodrop) is not recommended.

9. ASSIST PLUS Best Practices

- The scripts provided are suitable for a full 384-well plate. If running less than a full 384-well run, modifications can be made to wells that do not contain cells (non-active wells) within the VIALAB software. It is recommended to save a copy of the full script before modification.

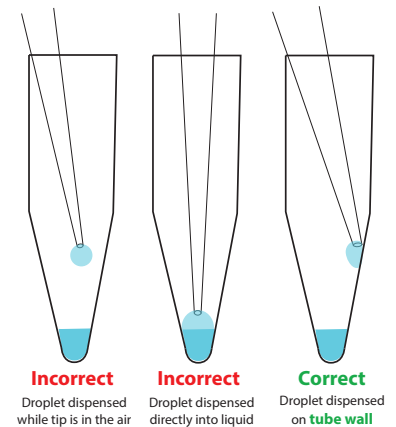


Figure 4. Pipetting Technique. All reagent additions should be carried out by dispensing the added reagent onto the wall of the tube as shown

- For further information on script modification, contact your INTEGRA Account Manager or [INTEGRA Customer Support](#) at orders-us@integra-biosciences.com or contact [BioSkryb Genomics Application Support Team](#) (techsupport@bioskryb.com).
- The scripts provided are optimized for MicroAmp EnduraPlate Optical 384-well Clear reaction plates from appliedbiosystems and twin.tec PCR 96 LoBind, semi skirted plates from Eppendorf.
 - If additional plate types are used, scripts will need to be further optimized.
 - For further information on script modification, refer to INTEGRA's user guide.
 - For further information on script modification, contact your INTEGRA Account Manager or [INTEGRA Customer Support](#) at orders-us@integra-biosciences.com or contact [BioSkryb Genomics Application Support Team](#) (techsupport@bioskryb.com).
- A single-channel pipette, or electronic pipette, is recommended for aliquoting reagents across the designated row of the 96-well Master Mix plates.
- It is recommended to label each row of the 96-well Master Mix Plates with the corresponding master mixes throughout the protocol.
 - It is recommended to cover the used rows of the 96-well Master Mix Plates after each program is run to avoid errors.
- This protocol requires the use of the VIAFLO 12-channel 12.5 µL pipette and the VOYAGER 12-channel 125 µL pipette.
 - For more information on changing the pipette on the ASSIST PLUS, refer to the manufacturer's operating instructions.
- When not in use, make sure to keep the 96-well and 384-well PCR cooling blocks on ice or stored at 4°C to stay cool. The only exception is the SEP bead protocol, Section 04.
- Before starting the protocol, ensure there are enough tips for the entire workflow.
- At the beginning of the protocol, ensure all scripts are uploaded to the corresponding INTEGRA pipettes.

Please contact the [BioSkryb Application Support Team](#) (techsupport@bioskryb.com) with any questions about these recommendations.

III. Reagent Retrieval and Control Setup

1. Retrieve **Box 1: Pre-PCR** from -20°C storage and place the following reagents on ice for 30 to 60 minutes to thaw before use:
 - Cell Buffer ⊗
 - Control Genomic DNA ●
 - Control RNA ●
 - RTP Reagent ●
 - RBI Reagent ●

- **RTC Reagent** ○




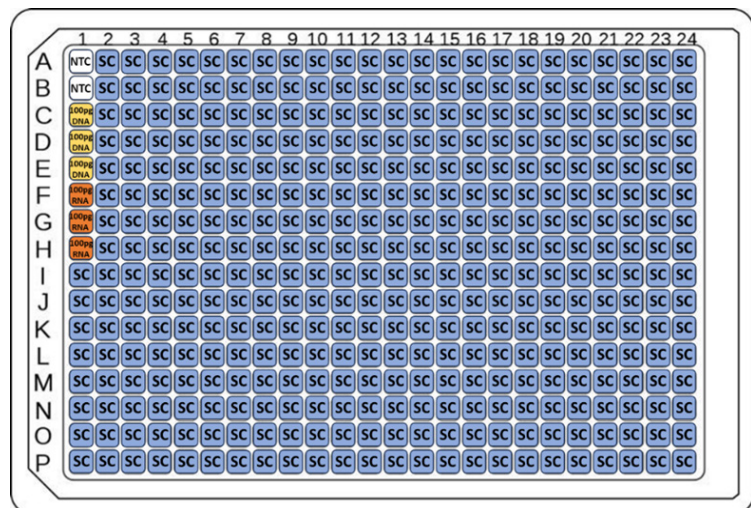
- The remaining reagents should be kept in -20°C storage until required.
- After thawing, vortex each reagent, except **RTC Reagent** ○, spin briefly, and place back on ice.
- Prepare INTEGRA 384-well and 96-well cooling blocks by placing them on ice for at least 10 minutes before use.
- Place the plate containing samples on ice. Input samples should be suspended in $1\ \mu\text{L}$ of **Cell Buffer** ⊗. **Cell Buffer** ⊗ can remain on ice throughout the rest of the protocol.
 -  **For cells stored at -80°C** , thaw the cells on ice for 10 minutes, spin for 10 seconds, and place on ice.
 -  **For freshly isolated cells**, maintain on ice and proceed with reverse transcription immediately.
- Prepare a $1\ \text{ng}/\mu\text{L}$ stock of gDNA by diluting $1\ \mu\text{L}$ **Control Genomic DNA** ● in $49\ \mu\text{L}$ **Cell Buffer** ⊗.
- Add $5\ \mu\text{L}$ of the $1\ \text{ng}/\mu\text{L}$ gDNA stock solution to $45\ \mu\text{L}$ **Cell Buffer** ⊗ to produce working concentration of $100\ \text{pg}/\mu\text{L}$ and place on ice.
- Prepare a $1\ \text{ng}/\mu\text{L}$ stock of RNA by diluting $1\ \mu\text{L}$ **Control RNA** ● in $49\ \mu\text{L}$ **Cell Buffer** ⊗.
- Add $5\ \mu\text{L}$ of the $1\ \text{ng}/\mu\text{L}$ RNA stock solution to $45\ \mu\text{L}$ **Cell Buffer** ⊗ to produce a working concentration of $100\ \text{pg}/\mu\text{L}$ and place on ice.
 -  **(Optional):** Verify the concentration of the **Control Genomic DNA** ● and **Control RNA** ● samples using a fluorometric method such as Qubit.
- To each negative control (NTC) well add $1\ \mu\text{L}$ of **Cell Buffer** ⊗ only, using a single-channel pipet. An example layout for controls and cell samples is shown in Figure 5.
- Using a single-channel pipet, add $1\ \mu\text{L}$ of the prepared $100\ \text{pg}/\mu\text{L}$ DNA and RNA controls ($100\ \text{pg}/\text{well}$) to the appropriate wells.

Figure 5. Example 384-well Plate Experimental Layout. The plate map illustrates a typical reaction setup, including replicate NTC, genomic DNA or RNA controls. Prior to processing with the ResolveOME Kit, add $1\ \mu\text{L}$ of each of the controls in this format to the thawed plate containing samples in cell buffer.



IV. Reverse Transcription (RT)

1. Initiate the OMEv2-384-RT program on thermal cycler (Table 1). Allow the thermal cycler to warm up to temperature and pause the program.

Table 1. OMEv2-384-RT (lid temperature 70°C, reaction volume 2 µL)

Step	Temperature	Time
Hold 1	42°C	40 minutes
Hold 2	50°C	20 minutes
Hold 3	4°C	∞
Total Time	-	~60 minutes

2. Calculate the volume of each reagent for the **RTX Mix** (Table 2) based on the number of samples with 33% overage using the formula:

$$\text{Number of samples} \times \text{volume per reaction} \times 1.33$$

3. Vortex **RB1 Reagent** ● and **RTP Reagent** ● reagents to mix and centrifuge to bring all liquids to the bottom of the tubes.
4. Mix the **RTC Reagent** ○ by inversion, briefly spin, and place back on ice.
 - ① **Important: Do not vortex the RTC Reagent** ○.
5. Prepare **RTX Mix** in a fresh tube by combining the components in the order listed in Table 2, vortexing for 10 seconds, spin briefly, and place on ice.

Table 2. Volume of Components in RTX Mix.

Product Name	Volume per Reaction (µL)	Volume per 384 Reactions (µL)*	Volume per _ Reactions (µL)
RB1 ●	0.5	255.4	
RTP ●	0.38	194.1	
RTC ○	0.13	66.4	
Total Volume	1.01	510.7	

*33% overage included

6. Using a single-channel pipette, aliquot the **RTX Mix** evenly across all 12 wells of Row A of the 96-well **Amplification Master Mix Plate** (Figure 6). Seal the plate, briefly spin to collect all liquids at the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.

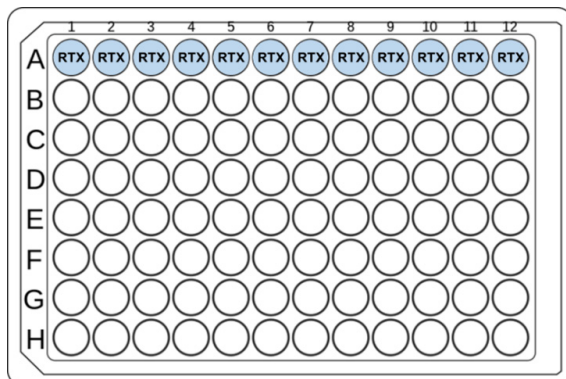


Figure 6: RTX Mix in Amplification Master Mix Plate.

7. Load the VIAFLO 12.5 μ L 12-channel pipette and tips onto the ASSIST PLUS.
8. Place the cold 96-well and 384-well PCR cooling blocks onto the ASSIST PLUS deck at Positions A and B, respectively (Figure 7).
9. Place the **Amplification Master Mix Plate** on the 96-well PCR cooling block in Position A and place the sample plate on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 7.

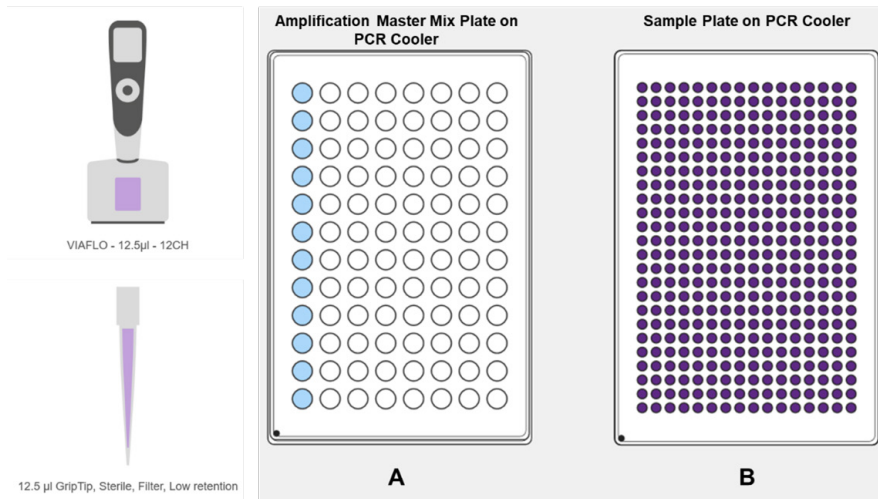


Figure 7: RO384_01_RT.

10. Remove the plate seals.
11. Select the program “RO384_01_RT” on the pipette.
12. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 01 – RT”
 - “RT in Row A” – RT Mix is loaded into Row A of the Amplification Master Mix plate in Position A on the deck.
 - “384 in Pos B” – 384-well sample plate is located in Position B on the deck.
13. Confirm the message on the screen and start the program by following the instructions on the pipette screen.
 - ✎ **Note:** Refer to ASSIST PLUS Best Practices (Section II.9).
14. Once the program is complete, seal and spin plate down for 10 seconds
15. In the thermal mixer, mix at room temperature for 1 minute at 1400 rpm.
16. Spin down for 10 seconds.
17. Place on the thermal cycler and run the OMEv2-384-RT program to execute the reverse transcription incubation step.
18. Remove the PCR cooling blocks from the ASSIST PLUS deck and place back on ice or in the fridge

at 4°C.

19. During the reverse transcription incubation step, retrieve **Box 1: Pre-PCR** from -20°C storage and place the following reagents on ice for 30 – 60 minutes to thaw:
 - **OL1 Reagent** ●
 - **L2 Reagent** ●
 - **OL3 Reagent** ○
 - **ORI Reagent** ●
20. Thaw L2 Reagent ● at room temperature for at least 30–60 minutes. Once completely thawed, place on ice.
 - ① **Important:** Once **L2 Reagent** ● has reached room temperature, vortex thoroughly **until any precipitate is fully dissolved**, briefly spin down, and place on ice.
21. After thawing, vortex each reagent to mix, briefly spin, and place back on ice.
22. After the reverse transcription step is complete, remove the samples from the thermal cycler, briefly spin down, and place at room temperature.
23. Proceed immediately to the Nuclear Lysis step.

V. Nuclear Lysis

1. Calculate the volume of each reagent for the **Lysis Mix** (Table 3, next page) based on the number of samples with 40% overage using the formula:

$$\text{Number of samples} \times \text{volume per reaction} \times 1.4$$

2. Prepare **Lysis Mix** in a new tube by combining the components in the order listed in Table 3, vortex for 10 seconds, spin briefly, and place on ice.

Table 3. Volume of Components in Lysis Mix.

Product Name	Volume per Reaction (µL)	Volume per 384 Reactions (µL)*	Volume per _ Reactions (µL)
OL1 ●	0.63	338.7	
L2 ●	0.06	32.3	
OL3 ○	0.31	166.7	
Total Volume	1.0	537.7	
*40% overage included			

3. Using a single-channel pipette, aliquot the **Lysis Mix** evenly across all 12 wells of Row B of the 96-well **Amplification Master Mix Plate** (Figure 8). Seal the plate, briefly spin to collect all liquids in the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.

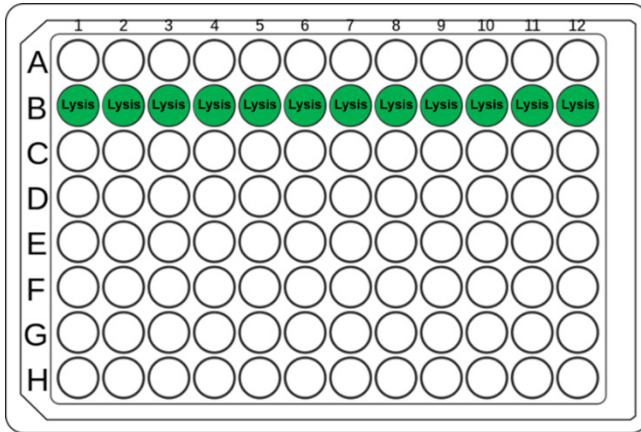


Figure 8: Lysis Mix in Amplification Master Mix Plate.

4. Ensure the VIAFLO 12.5 µL 12-channel pipette and tips are loaded onto the ASSIST PLUS.
5. Place the cold 96-well and 384-well PCR cooling blocks onto the ASSIST PLUS deck at Positions A and B, respectively (Figure 9).
6. Place the Amplification Master Mix plate on the 96-well PCR cooling block in Position A and place the sample plate on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 9.

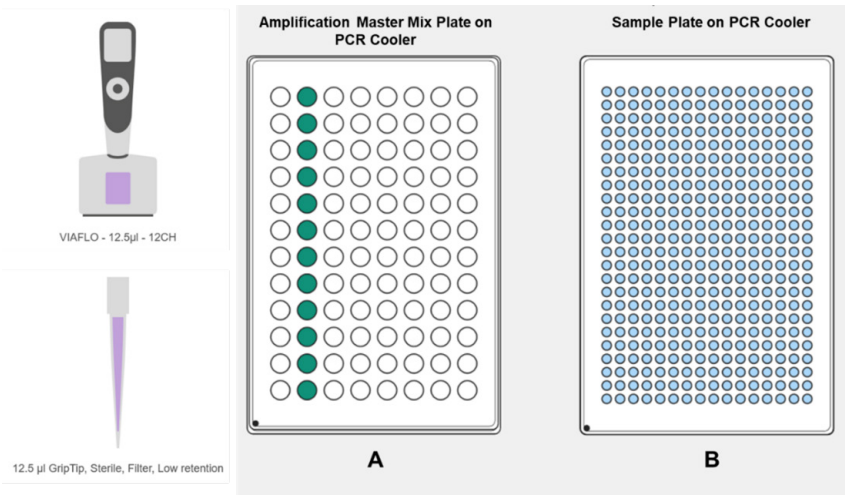


Figure 9: RO384_02_Lysis.

7. Remove the plate seals.
8. Select the program “RO384_02_Lysis” on the pipette.
9. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 02 – Lysis”
 - “Lysis Row B” – Lysis Mix is loaded into Row B of the Amplification Master Mix plate in Position A on the deck.

- **“384 in Pos B”** – 384-well sample plate is located in Position B on the deck.
10. Once confirmed, start the program by following the instructions on the pipette screen.
 - ✎ **Note:** Refer to ASSIST PLUS Best Practices.
 11. Once the program is complete, seal and spin down for 10 seconds.
 12. In the thermal mixer, mix at room temperature for 10 minutes at 1400 rpm.
 13. While the plate is on the thermal mixer, remove the cold blocks from the INTEGRA base and place back on ice.
 14. Once complete, spin down for 10 seconds and place on ice.
 15. Proceed immediately to Whole Genome Amplification.

VI. Whole Genome Amplification

1. Initiate OMEv2-384-DNA-AMP program on thermal cycler (Table 4). Allow the thermal cycler to warm up to temperature and pause the program.

Table 4. OMEv2-384-DNA-AMP (lid temperature 70°C, reaction volume 6 µL)

Step	Temperature	Time
Hold 1	30°C	90 minutes
Hold 2	65°C	4 minutes
Hold 3	4°C	∞
Total Time	-	~95 minutes

2. Calculate the volume of components for the **PTA Mix** (Table 5) based on the number of samples with 50% overage using the formula:

$$\text{Number of samples} \times \text{volume per reaction} \times 1.5$$

3. Remove the **OR2 Reagent** ● from the freezer, mix by inversion, briefly spin, and place back on ice.
 - ① **Important:** Do not vortex the **OR2 Reagent** ●.
4. Prepare **PTA Mix**, by combining the reagents listed in Table 5, vortex, spin briefly, and place on ice.
 - ① **Important:** Do not prepare **PTA Mix** more than 30 minutes prior to use. Keep on ice.
 - ① **Important:** Return the **OR2 Reagent** ● to the freezer once the **PTA Mix** is prepared.

Table 5. Volume of Components in PTA Mix.

Product Name	Volume per Reaction (µL)	Volume per 384 Reactions (µL)*	Volume per _ Reactions (µL)
OR1 ●	2.7	1,555.2	
OR2 ●	0.3	172.8	
Total Volume	3.0	1,728	
*50% overage included			

- Using a single-channel pipette, aliquot the **PTA Mix** evenly across all 12 wells of Row C of the 96-well **Amplification Master Mix Plate** (Figure 10). Seal the plate, briefly spin to collect all liquids in the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.

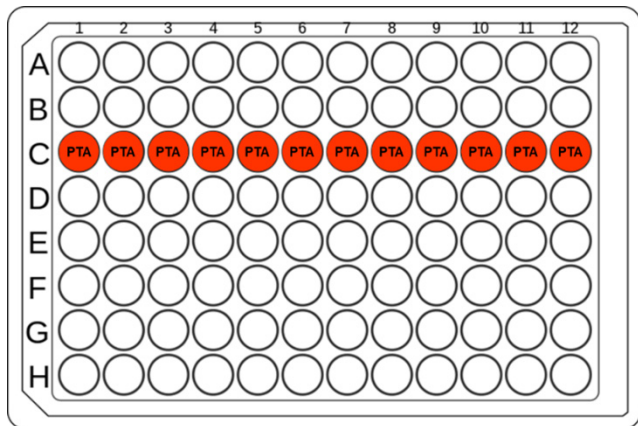


Figure 10: PTA Mix in Amplification Master Mix Plate.

- Place the **Amplification Master Mix Plate** on the 96-well PCR cooling block in Position A and place the sample plate on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 11.

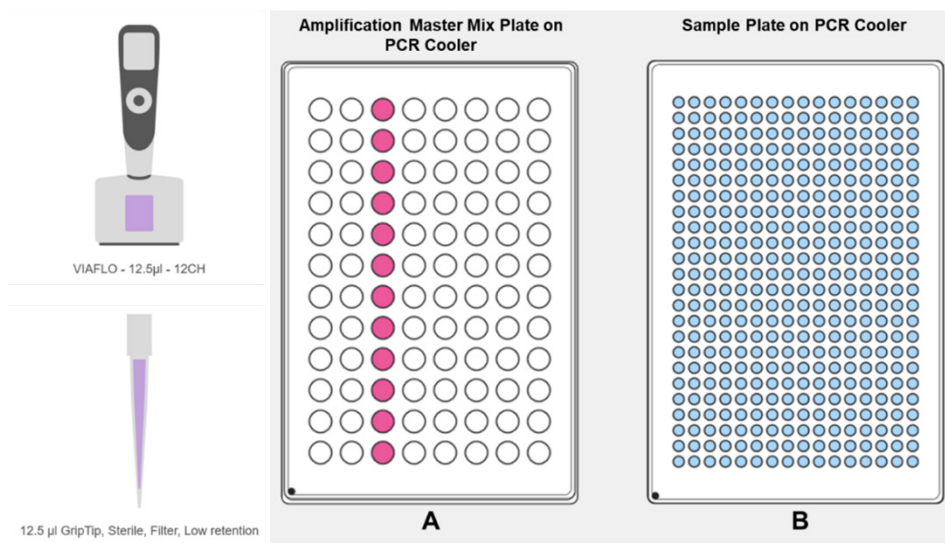



Figure 11: RO384_03_PTA.

- Remove the plate seal.
- Select the program “**RO384_03_PTA**” on the pipette.
- Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.

- “Step 03 – PTA”
 - “PTA in Row C” – PTA Mix is loaded into Row C of the Amplification Master Mix plate in Position A on the deck.
 - “384 in Pos B” – 384-well sample plate is located in Position B on the deck.
10. Once confirmed, start the program by following the instructions on the pipette screen.
-  **Note:** Refer to ASSIST PLUS Best Practices.
11. Once the program is complete, seal and spin down for 10 seconds.
12. In the thermal mixer, mix at room temperature for 1 minutes at 1000 rpm.
13. Spin down for 10 seconds.
14. Place on the pre-heated thermal cycler and run the OMEv2–384–DNA–AMP program.
- ⊖ **Safe Stop:** The whole genome amplification reaction incubation can be held at 4°C overnight after completion.
15. 96-well and 384-well PCR cooling blocks can remain on the deck for the Affinity Purification step.
16. Proceed with Affinity Purification.

VII. Affinity Purification to Separate DNA and RNA Fractions

1. Retrieve the following RNA amplification reagents from -20°C and place on ice to thaw for 30 - 60 minutes:
 - PAC Reagent ●
 - PAP Reagent ●
2. Retrieve the following separation reagents from -20°C or 4°C and warm to room temperature for 30 - 60 minutes before use:
 - SEP Reagent ○
 - Cell Buffer ⊗


 **Note:** Separation reagents and samples should be maintained at room temperature during the Nucleic Acid Separation process unless otherwise instructed.
3. Vortex the **SEP Reagent** ○, a slurry of beads and storage buffer, for 10 seconds to fully resuspend.
4. Calculate the volume of **SEP Reagent** ○ needed and transfer it to a 0.2 or 1.5 mL microcentrifuge tube. Table 6 shows **SEP Reagent** ○ and **Cell Buffer** ⊗ volumes for several common run sizes.
5. Place the tube on a magnet stand for 2 minutes or until the supernatant clears.
6. While on the magnet, remove and discard the SEP storage buffer supernatant, taking care not to disturb the beads.
7. Determine the volume of **Cell Buffer** ⊗ required from Table 6.

Table 6. SEP Reagent and Cell Buffer volumes for common run sizes.

Number of Reactions	Volume of SEP Reagent (μL)	Volume of Cell Buffer (μL)
96	25.0	60.0
192	50.0	120.0
384	100.0	240.0

 **Note:** If running a different number of reactions than suggested in Table 6, calculate the volume of **SEP Reagent** ○ and **Cell Buffer** ⊗ required per reaction.”

Volume (μL) of SEP Reagent ○ to transfer = Number of Samples x 0.2 μL x 1.31 (for 31% overage)

Volume (μL) of Cell Buffer ⊗ = Number of Samples x 0.48 μL x 1.31 (for 31% overage)

8. Remove the tube containing the isolated **SEP Reagent** ○ from the magnet and add the appropriate volume of **Cell Buffer** ⊗. This mixture is the **SEP Reagent Mix**.
9. Mix by vortexing or pipetting up and down to fully resuspend the **SEP Reagent** ○, using the pipet tip to dislodge any **SEP Reagent** ○ adhering to the tube of the **SEP Reagent Mix**.
10. Using a single-channel pipette, aliquot the **SEP Reagent Mix** evenly across all 12 wells of Row D of the 96-well **Amplification Master Mix Plate** (Figure 12). Seal the plate and briefly spin to collect all liquid in the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.

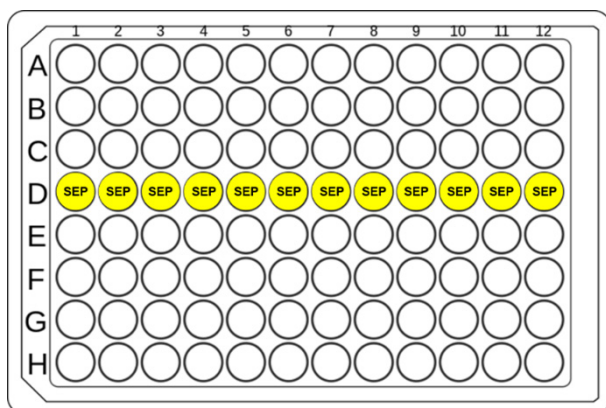


Figure 12: SEP Reagent Mix in Amplification Master Mix Plate.

11. Retrieve the samples from the thermal cycler after completion of the whole genome amplification step and spin down 10 seconds to collect any condensation.
12. Place the **Amplification Master Mix Plate** on the 96-well PCR cooling block in Position A and place the sample plate on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 13.

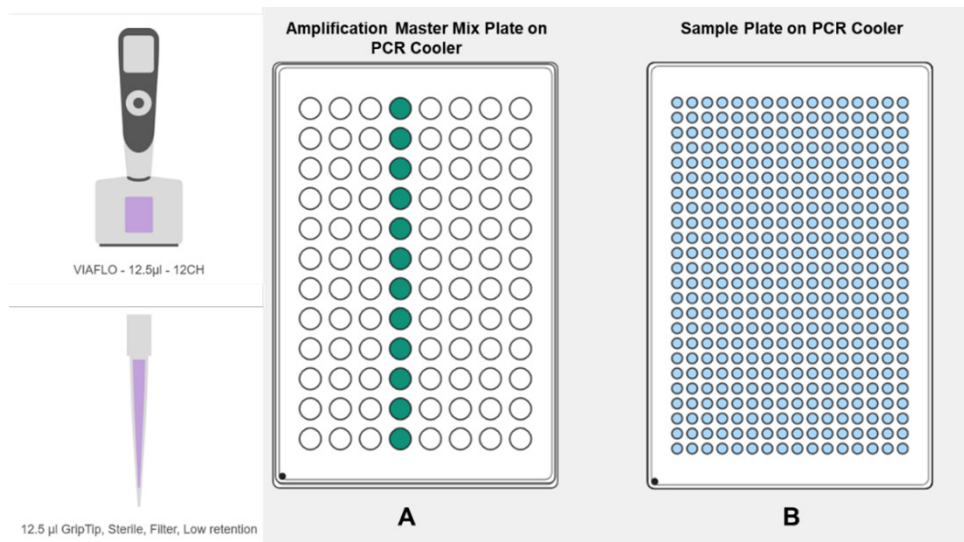


Figure 13: RO384_04A_SEP.

13. Remove the plate seal.
14. Select the program “RO384_04A_SEP” on the pipette.
15. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - **“Step 04A – SEP”**
 - **“SEP in Row C”** – SEP Reagent is loaded into Row D of the Amplification Master Mix plate in Position A on the deck.
 - **“384 in Pos B”** – 384-well sample plate is located in Position B on the deck.
16. Once confirmed, start the program by following the instructions on the pipette screen.
 - ✎ **Note:** Refer to ASSIST PLUS Best Practices.
17. Once the program is complete, seal and spin down for 10 seconds.
18. Visually inspect beads are evenly dispersed in the well. If not, select the wells in the VIALAB software and re-dispense the wells.
19. In the thermal mixer, mix at room temperature for 10 minutes with constant shaking at 1000 rpm.
20. Return the 96-well and 384-well PCR cooling blocks to ice
21. While incubating, prepare new 384-well plates by labeling them “DNA Fraction” and “RNA Fraction”.
22. Load the VOYAGER 125 µL 12-channel pipette and tips onto the ASSIST PLUS.
 - ✎ **Note:** Refer to ASSIST PLUS Best Practices.
23. Place a 25 mL reservoir in the reservoir base and place in Position A on the deck. Place two cold 384-well PCR cooling blocks in Positions B and C on the deck (Figure 14).
24. Place the “DNA Fraction” and “RNA Fraction” plates on the 384-well PCR cooling block in Positions B and C, respectively, with position A1 of both plates in the bottom left, as indicated by the dot in

Figure 14.

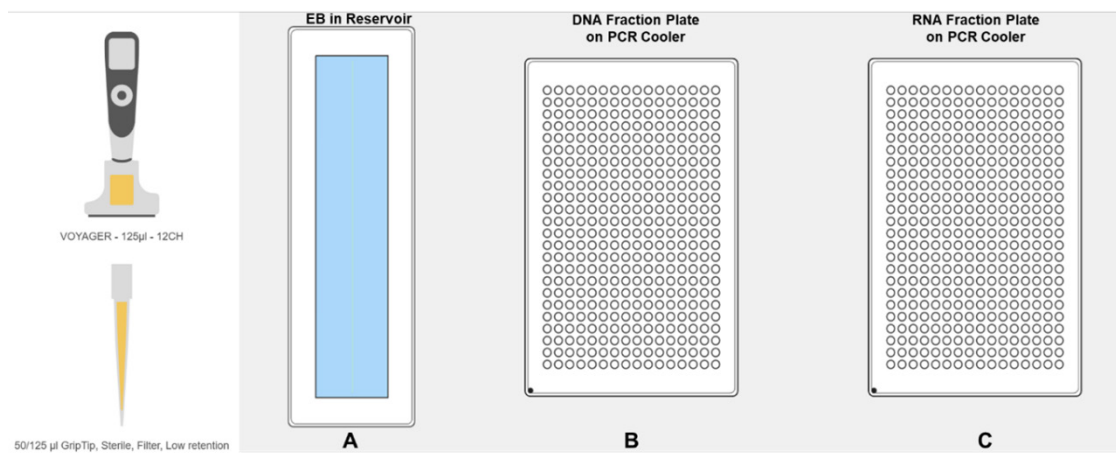


Figure 14: RO384_04B_EB.

25. Fill the reservoir with **Elution Buffer** ☒ up to the 10–15 mL mark.
26. Select the program “RO384_04B_EB” on the pipette
27. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 04B – EB”
 - “EB in Res” – Elution Buffer (EB) is in Reservoir in Position A on the deck.
 - “384 Pos B&C” – Empty 384–well plates are located in Position B and C on the deck.
28. Once confirmed, start the program by following the instructions on the pipette screen.
 - ✎ **Note:** Refer to ASSIST PLUS Best Practices.
29. Once the program is complete, seal and spin down for 10 seconds, and place on ice.
30. Proceed immediately to the RNA Fraction Amplification step.

VIII. RNA Fraction Amplification

1. Load the VIAFLO 12.5 µL 12–channel pipette and tips onto the ASSIST PLUS.
2. Load and connect the MAG Module with 384–well adapter in Position C on the deck.
3. Vortex and briefly spin down the RNA Amplification Reagents.
4. Calculate the volume of components for the **RNA Amplification Mix** (Table 7) based on the number of samples with 30% overage using the formula:

$$\text{Number of samples} \times \text{volume per reaction} \times 1.3$$

Table 7. Volume of Components in RNA Amplification Mix

Product Name	Volume per Reaction (µL)	Volume per 384 Reactions (µL)*	Volume per _ Reactions (µL)
PAC Reagent ●	2.5	1248.0	
PAP Reagent ●	2.5	1248.0	
Total Volume	5.0	2496.0	

*30% overage included

- Prepare the **RNA Amplification Mix** by combining the reagents in Table 7, vortex for 10 seconds, spin briefly, and place on ice.
- Using a single-channel pipette, aliquot the **RNA Amplification Mix** evenly across all 24 wells of Rows E and F of the 96-well **Amplification Master Mix Plate** (Figure 15). Seal the plate and briefly spin to collect all liquid in the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.

 **Note:** If running half a 384-well plate or less, load **RNA Amplification Mix** into Row E only.

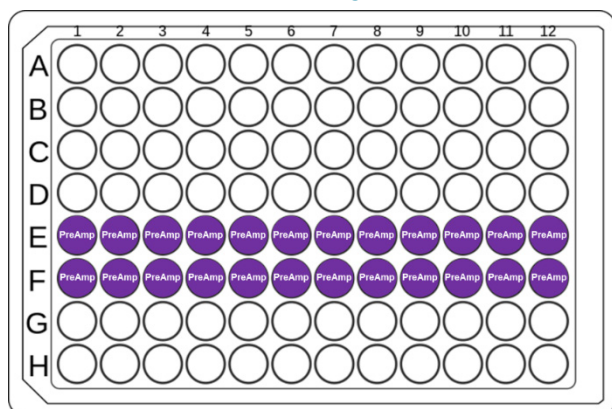


Figure 15: RNA Amplification Mix (PreAmp) in Amplification Master Mix Plate.

- After the step VII.19 incubation, remove the samples from the thermal mixer, vortex, and spin down for 20 seconds.
- Place on the MAG Module in Position C on the ASSIST PLUS deck (Figure 16). Ensure the MAG Module is connected and turned on.
- Remove the plate seals.
- Select the program “RO384_05_TRF-PA” on the pipette.
- Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “**Step 05-TRF-PA**”
 - “**DNA in Pos B**” – 384-well DNA Fraction plate containing EB is located in Position B on the deck.

- “384 in Pos C” – 384-well sample plate is located on the MAG Module in Position C on the deck.

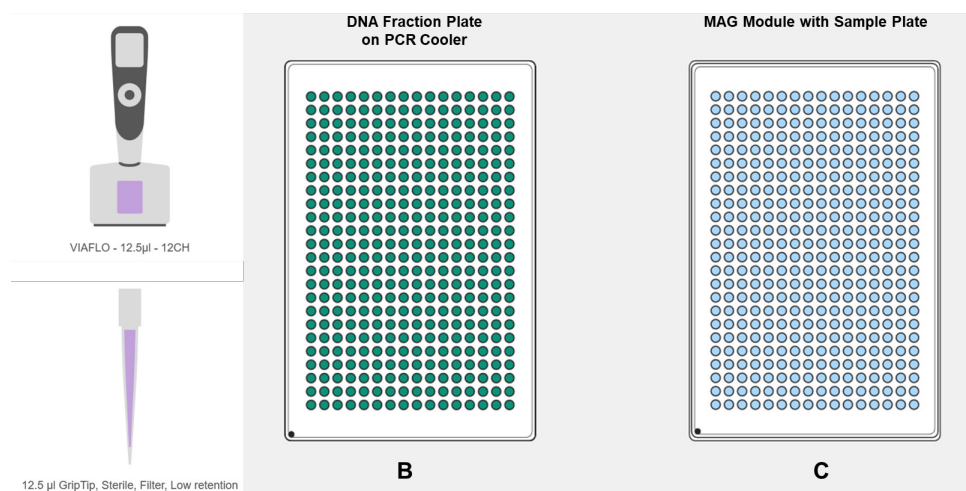


Figure 16: RO384_05_TRF-PA.

12. Once confirmed, start the program by following the instructions on the pipette screen.

 **Note:** Refer to ASSIST PLUS Best Practices.

13. While the script is running, initiate OMEv2-384-RNA-AMP program on thermal cycler (Table 8). Allow the thermal cycler to warm up to temperature and pause the program.

Table 8. OMEv2-384-RNA-AMP (lid temperature 105°C, reaction volume 5 µL)

Step	Temperature	Time
Hold 1	37°C	15 minutes
Hold 2	98°C	10 seconds
27 Cycles	98°C	10 seconds
	55°C	20 seconds
	68°C	45 seconds
Hold 3	72°C	1 minute
Hold 4	4°C	∞
Total time	-	~1 hour

14. Once the program is complete, seal both the DNA Fraction and Sample plate and spin down for 10 seconds.

15. Vortex for 10 seconds.

16. Spin down for 10 seconds and place on ice.

17. Place Sample plate on preheated thermal cycler and run the OMEv2-384-RNA-AMP program (Table 8).

18. Proceed with DNA Fraction plate to Quantification and Library Prep.

IX. RNA Transfer

1. After incubation completes, remove the samples from the thermal cycler, vortex, and spin down for 20 seconds.
2. Load the RNA Fraction plate on Position B of the ASSIST PLUS deck and the Sample plate on the MAG Module in Position C (Figure 17). Ensure the MAG Module is connected and turned on.

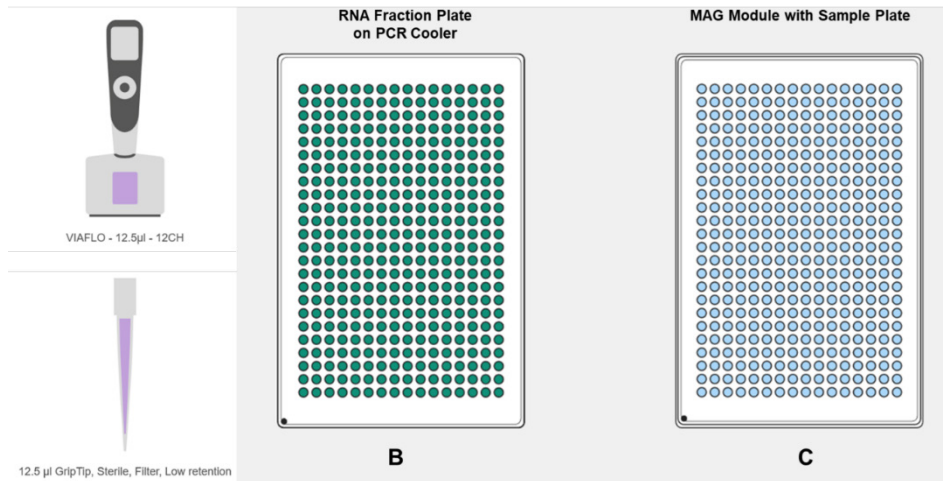



Figure 17: RO384_06_RNATRF.

3. Remove the plate seals.
4. Select the program “RO384_06_RNATRF” on the pipette.
5. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 06–RNATRF”
 - “RNA in Pos B” – 384–well RNA Fraction plate containing EB is located in Position B on the deck.
 - “384 in Pos C” – 384–well sample plate is located on the MAG Module in Position C on the deck.
6. Once confirmed, start the program by following the instructions on the pipette screen.
 - ✎ **Note:** Refer to ASSIST PLUS Best Practices.
7. Once the program is complete, seal the RNA Fraction plate and spin down for 10 seconds.
8. Vortex for 10 seconds.
9. Spin down for 10 seconds and place on ice.
10. Proceed to DNA and cDNA Fraction Quantification Analysis to determine DNA and cDNA yields or proceed directly to Library Preparation

X. DNA and cDNA Fraction Quantification

1. To assess DNA or RNA (cDNA) yield, add 2 µL of amplified product to 198 µL Qubit reagent and



measure the concentration using the High Sensitivity dsDNA Assay kit, per the manufacturer's instructions.

2. **(Optional):** Determine fragment size distribution by diluting an aliquot of the sample to 2 ng/ μ L and running 2 μ L of each sample of amplified product using a TapeStation HS D5000 ScreenTape or other fragment analysis instrument, per the manufacturer's instructions.
3. Refer to Appendix B for more information on interpreting the QC data.
 - ⊗ **Safe Stop:** Store DNA and cDNA Fraction Plates at -20°C up to one week or proceed immediately to the Library Preparation step.

Library Preparation

- ① The ResolveOME v2.0 workflow requires the use of the included ResolveOME library preparation kit reagents. The use of third-party library preparation kits will fail to produce libraries.
- ① Each sample requires the preparation of two sequencing libraries, one for DNA from the DNA Fraction plate, and one from RNA (cDNA) from the RNA Fraction plate.
- ① The DNA Fraction requires an extra step, XII. DNA Preparation. All other steps are performed for both DNA and RNA Fractions.
- ① The steps listed below are outlined for one library prep plate. **The protocol will need to be repeated for the second library prep plate.**

XI. Library Sample Plate Preparation

1. To create the DNA Library Prep Plate, place an empty 384-well plate labeled “DNA Library Prep Plate” in a PCR cooler on Position B on the deck. Place the DNA Fraction plate on the 384-well MAG Module in Position C (Figure 18). Ensure the MAG Module is turned on.

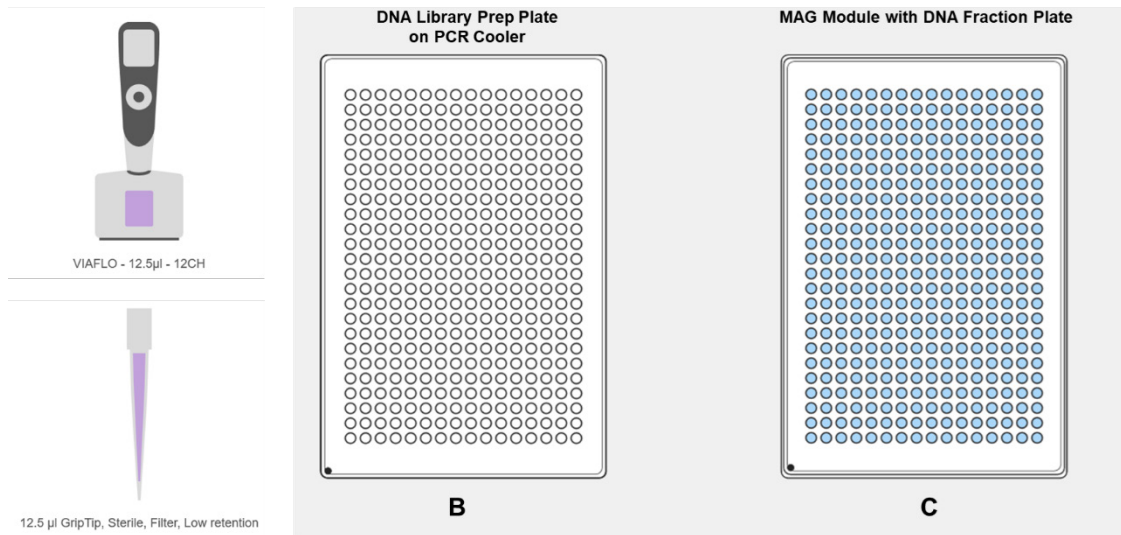



Figure 18: RO384_08D_LPDNA.

2. Remove the plate seals.
3. Select the program “RO384_08D_LPDNA” on the pipette.
4. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 08D-LPDNA”
 - “DNALP in B” – empty 384-well plate located in Position B on the deck.
 - “DNAFrac in C” – 384-well DNA Fraction plate located on the MAG Module in Position C on the deck.

5. Once confirmed, start the program by following the instructions on the pipette screen.
 -  **Note:** Refer to ASSIST PLUS Best Practices.
6. Once the program is complete, seal both plates and return the DNA Fraction place to storage at -20°C. Spin down the DNA Library Prep Plate for 10 seconds.
7. Vortex for 10 seconds.
8. Spin down for 10 seconds and place on ice.
9. To create the RNA Library Prep Plate, place an empty 384-well plate labeled “RNA Library Prep Plate” in a PCR cooler on Position B on the deck. Place the RNA Fraction plate on the 384-well MAG Module in Position C (Figure 19).

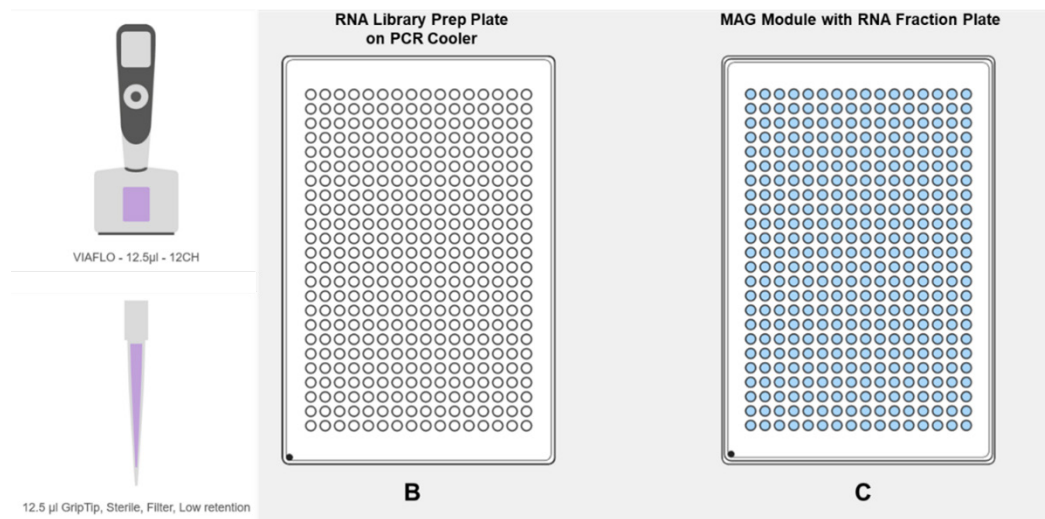



Figure 19: RO384_08R_LPRNA.

10. Remove the plate seals.
11. Select the program “RO384_08R_LPRNA” on the pipette.
12. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 08R-LPRNA”
 - “RNALP in B” – empty 384-well plate located in Position B on the deck.
 - “RNAFrac in C” – 384-well RNA Fraction plate located on the MAG Module in Position C on the deck.
13. Once confirmed, start the program by following the instructions on the pipette screen.
 -  **Note:** Refer to ASSIST PLUS Best Practices.
14. Once the program is complete, seal both plates and return the RNA Fraction place to storage at -20°C. Spin down the RNA Library Prep Plate for 10 seconds.
15. Vortex for 10 seconds.
16. Spin down for 10 seconds and place on ice.

XII. DNA Preparation (DNA Fraction Only)

- Retrieve the following library preparation reagents from -20°C and place on ice to thaw:
 - **LPOB Reagent** ○
 - **LPOE Reagent** ⊗
 - **LP1B Reagent** ●
 - **LP1E Reagent** ●
- Retrieve the following library preparation reagents from 4°C and place at room temperature:
 - **Elution Buffer** ☒
 - **Resolve Beads** ☒
- Initiate DNAPREP program on thermal cycler (Table 9). Allow the thermal cycler to warm up to temperature and pause the program.

Table 9. DNAPREP Program (lid temperature 105°C, reaction volume 3 µL)

Step	Temperature	Time
Hold 1	37°C	10 minutes
Hold 2	4°C	∞
Total Time	-	~10 minutes

- Vortex **LPOB Reagent** ○ to mix and centrifuge to bring all liquids to the bottom of the tube.
- Mix the **LPOE Reagent** ⊗ by inversion, briefly spin, and place back on ice.
 - ⓘ **Important: Do not vortex the LPOE Reagent** ⊗.
- Prepare the **DNA Prep Master Mix** in a 1.5 mL Eppendorf tube on ice by adding the components in Table 10.

Table 10. DNA Prep Master Mix

Reagent Name	Number of Samples		
	<96 Reactions	192 Reactions*	384 Reactions*
LPOB Reagent ○	192.0 µL	384.0 µL	768.0 µL
LPOE Reagent ⊗	1.0 µL	2.0 µL	4.0 µL
Total Volume	193.0 µL	386.0 µL	772.0 µL
*30% overage included			

- Using a single-channel pipette, aliquot the **DNA Prep Master Mix** evenly across all 12 wells of ROW A of the 96-well **Library Prep Master Mix plate** (Figure 20). Seal the plate and briefly spin to collect all liquid in the bottom of the wells and place on ice. Ensure there are no bubbles present in the bottom of the wells.

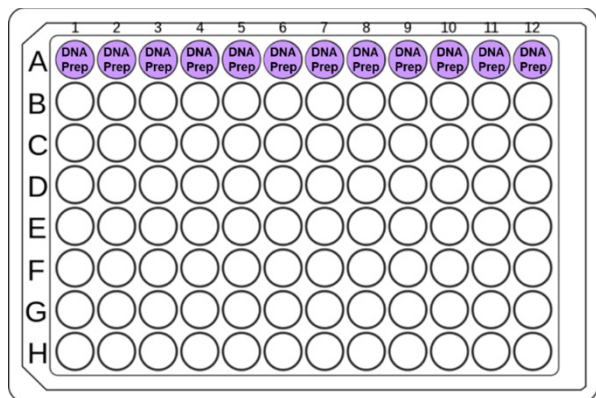


Figure 20: DNA Prep Mix in Library Prep Master Mix Plate.

8. Place the cold 96-well and 384-well PCR cooling blocks onto the ASSIST PLUS deck at Positions A and B, respectively (Figure 21).
9. Place the **Library Prep Master Mix Plate** on the 96-well PCR cooling block in Position A and place the **DNA Library Prep Plate** on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 21.

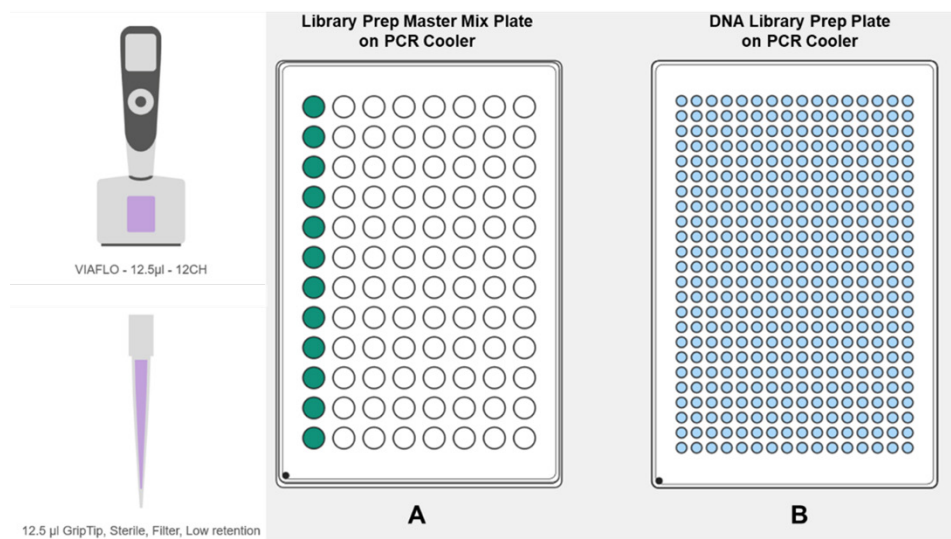




Figure 21: RO384_09_DNRPrp.

10. Remove the plate seals.
11. Select the program “RO384_09_DNAPrp” on the pipette.
12. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 09-DNAPrp”
 - “LPO in RowA” – DNA Prep Master Mix (LPO) is in Row A of the 96-well Library Prep Master Mix Plate that is in the PCR cooling block located in Position A on the deck.

- “384 in PosB” – 384-well DNA Library Prep plate located in Position B on the deck.
- Once confirmed, start the program by following the instructions on the pipette screen.
 -  **Note:** Refer to ASSIST PLUS Best Practices.
 - Once the program is complete, seal the plate with a sealing film and briefly centrifuge the plate to collect all liquids at the bottom of the wells.
 - Vortex the plate for 5–10 seconds to mix the samples.
 -  **Note:** Complete mixing and centrifuging is critical to reduce chimera percentage.
 - Spin the plate for 20 seconds and place back on the cooling block.
 - Place the plate on the preheated thermal cycler and run the DNAPREP protocol (Table 9).
 - While the plate is on the thermal cycler, prepare the RNA Library Prep plate and **Fragmentation Mix** for the next step.

XIII. Fragmentation and End Repair

- Remove DNA Library Prep plate from the thermal cycler, briefly centrifuge to collect all the liquids at the bottom of the wells and place on ice.
- Initiate OMEv2–384–ERAT program on thermal cycler (Table 11). Allow the thermal cycler to cool to 4°C and pause the program.

Table 11. OMEv2–384–ERAT (lid temperature 105°C, reaction volume 5 µL)




Step	Temperature	Time
Hold 1	4°C	30 seconds
Hold 2	30°C	5 minutes
Hold 3	65°C	30 minutes
Hold 4	4°C	∞
Total time		~ 35 minutes

- Calculate the volume of reagents for the **Fragmentation Mix** (Table 12) based on the number of samples with 30% overage using the formula:

$$\text{Number of samples} \times \text{volume per reaction} \times 1.3$$

- Assemble the **Fragmentation Mix** on ice by combining the components in order listed in Table 12.

Table 12. Volume of Components in Fragmentation Mix

Product Name	Volume (µL) per number of reactions (rxn)			
	1 rxn	192 rxn*	384 rxn*	__ rxn
LPIB Reagent 	0.4	99.8	199.7	
LPIE Reagent 	0.6	149.8	299.5	
Elution Buffer 	1.0	249.9	499.2	
Total Volume	2.0	499.5	998.4	
*30% overage included				

5. Vortex for 10 seconds, spin briefly, and place on ice.

 **Note:** This mixture is stable on ice for up to 4 hours.

6. Using a single-channel pipette, aliquot the **Fragmentation Master Mix (ERAT)** evenly across all 12 wells of Row B of the 96-well **Library Prep Master Mix Plate** (Figure 22). Seal the plate and briefly spin to collect all liquid in the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.

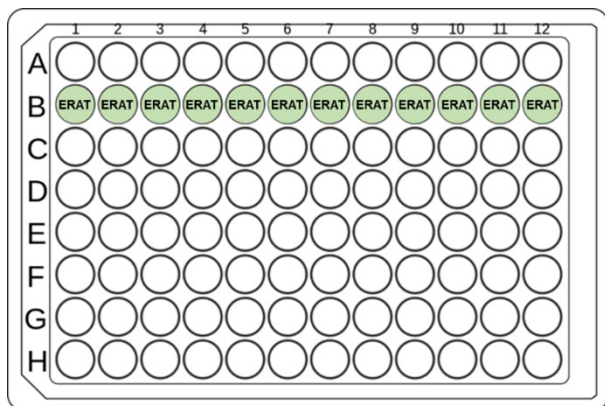


Figure 22: Fragmentation Master Mix (ERAT) in Library Prep Master Mix Plate.

7. Place the Library Prep Master Mix Plate on the 96-well PCR cooling block in Position A and place the Library Prep plate on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 23.

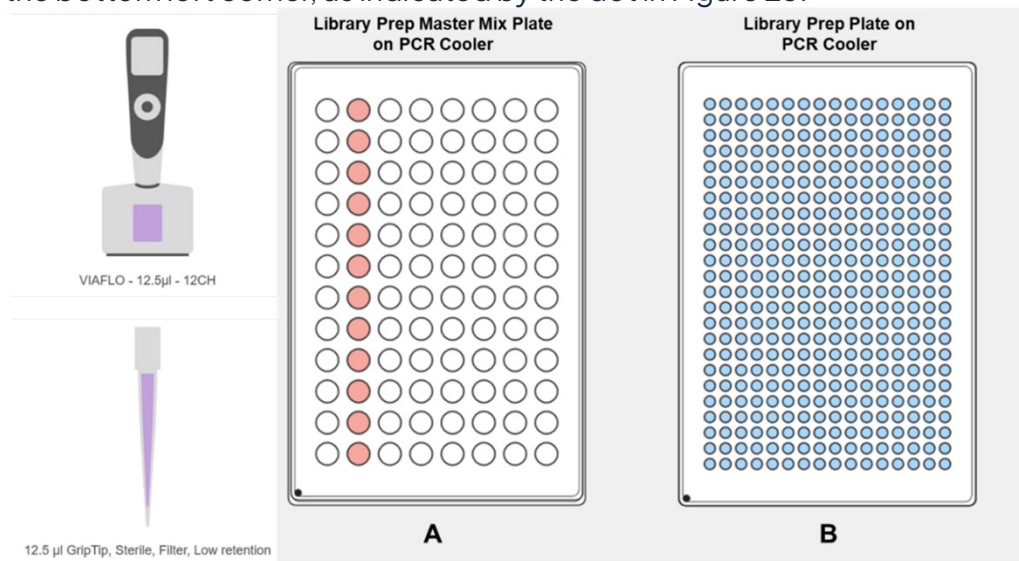





Figure 23: RO384_10_ERAT.

8. Remove the plate seal.

9. Select the program “RO384_10_ERAT” on the pipette.

10. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.

- “Step 10–ERAT”
 - “ERAT in RowB” – Fragmentation Master Mix (ERAT) is in Row B of the 96–well Library Prep Master Mix Plate that is in the PCR cooling block located in Position A on the deck.
 - “384 in PosB” – 384–well DNA Library Prep plate located in Position B on the deck.
- Once confirmed, start the program by following the instructions on the pipette screen.
 -  **Note:** Refer to ASSIST PLUS Best Practices.
 - Once the program is complete, seal the plate with a sealing film and briefly centrifuge the plate to collect all liquids at the bottom of the wells.
 - Vortex for 10 seconds.
 - Spin down for 20 seconds and place on ice or cooling block.
 -  **Note:** Complete mixing and centrifuging is critical to achieve desired fragment lengths.
 - Place on the thermal cycler and run the OMEv2–ERAT program (Table 11).
 -  **Note:** Do not load the plate into the thermal cycler until the block has reached 4°C.
 - While incubating, retrieve the following reagents from Box 2 in –20°C storage and place them on ice for 30 minutes to thaw:
 - LP2E Reagent ●
 - LP2B Reagent ●
 - Single Use Adapter Plate(s)
 - LP3A Reagent ☒
 - LP3P Reagent ●
 - When the thermal cycler program has completed, proceed immediately to Ligation.
 - Remove the 96–well and 384–well PCR cooling blocks from the ASSIST PLUS deck and place on ice.

XIV. Ligation

- Remove plate from thermal cycler, spin briefly and place on ice.
- Start the thermal cycler OMEv2–384–Ligation protocol (Table 13) and pause preheated thermal cycler until plate is ready.

Table 13. OMEv2–384–Ligation (lid temperature 50°C, reaction volume 8 µL)

Step	Temperature	Time
Hold1	20°C	30 minutes
Hold2	4°C	∞
Total time		~ 30 minutes

- Vortex the LP2B Reagent ● for at least 10 seconds and briefly centrifuge to collect liquid at the bottom of the tube.
- Invert the LP2E Reagent ● 10 times to homogenize and ensure complete mixing. Briefly centrifuge

to collect all liquid in the bottom of the tube.

- Prepare the **Ligation Mix** in a 1.5 mL Eppendorf tube on ice by adding the following reagents with 30% overage:

$$\text{Number of samples} \times \text{volume per reaction} \times 1.3$$

Table 14. Volume of Components in Ligation Mix

Product Name	Volume (µL) per number of reactions (rxn)		
	1 rxn	384 rxn*	__rxn
LP2B Reagent ●	0.9	449.3	
LP2E Reagent ●	0.1	49.9	
Total Volume	1.0	499.2	
*30% overage included			

- Vortex the **Ligation Mix** for 10 seconds and briefly centrifuge to collect all liquid in the bottom of the tube and keep on ice.
- Using a single-channel pipette, aliquot the **Ligation Mix** evenly across all 12 wells of Row C in the 96-well **Library Prep Master Mix** plate (Figure 24). Seal the plate and briefly spin to collect all liquid in the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.

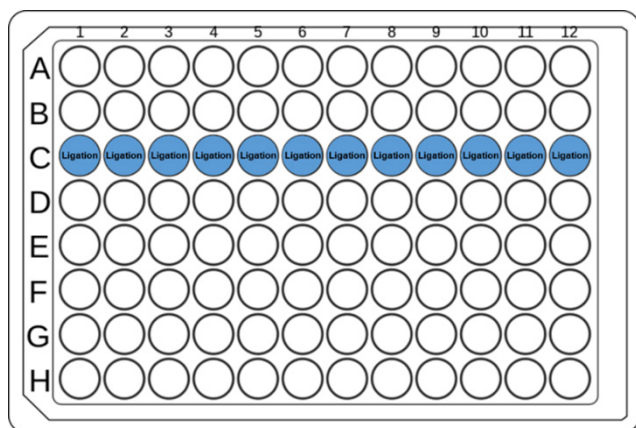


Figure 24: Ligation Mix in Library Prep Master Mix Plate.

- Vortex thawed **Single Use Adapter Plate(s)** and briefly centrifuge.
- Place the cold 96-well and 384-well PCR cooling blocks onto the ASSIST PLUS deck at Positions A, B, and C (Figure 25).
- Place the Library Prep Master Mix Plate on the 96-well PCR cooling block in Position A, the Single Use Adapter Plate on the 384-well PCR cooling block in Position B, and the Library Prep plate on the 384-well PCR cooling block in Position C with position A1 of all three plates in the bottom left corner, as indicated by the dot in Figure 25.

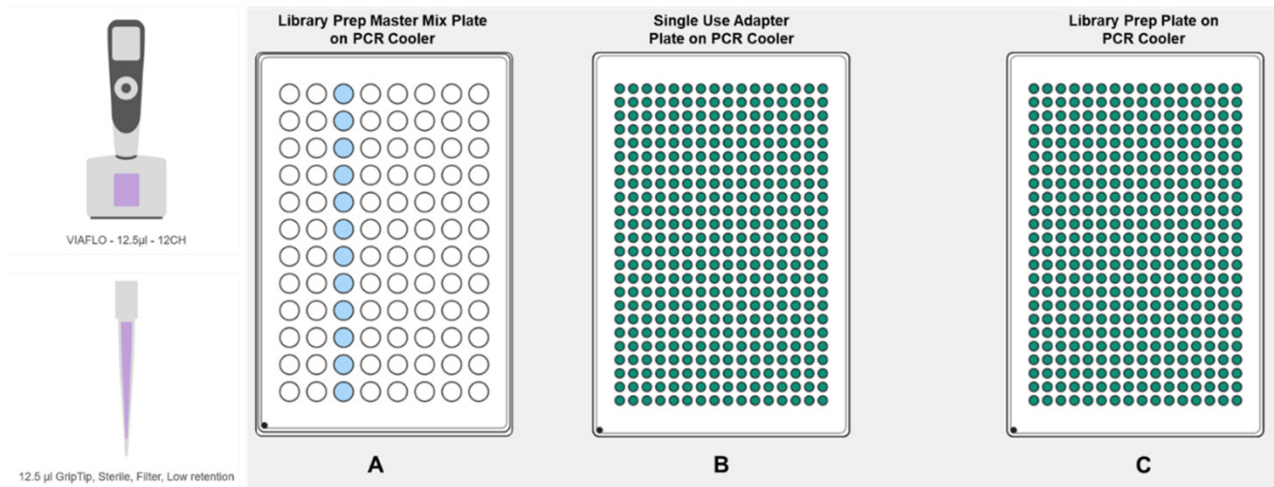



Figure 25: Figure 28. RO384_11_AdpLig.

11. Remove the plate seal.
12. Select the program “RO384_11_AdpLig” on the pipette.
13. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 11-AdpLig”
 - “Adp in Pos B” – 384-well adapter plate is located in Position B on the deck.
 - “384 in Pos C” – 384-well DNA Library Prep plate located in Position C on the deck.
14. Once confirmed, start the program by following the instructions on the pipette screen.
 -  **Note:** Refer to ASSIST PLUS Best Practices.
15. Once the program is complete, seal the plate with a sealing film and briefly centrifuge the plate to collect all liquids at the bottom of the wells. Vortex the plate for 5–10 seconds to mix the samples. Spin the plate for 20 seconds and place on ice.
16. Place the plate on the preheated thermal cycler and initiate the OMEv2–384–Ligation protocol.
17. While the plate is on the thermal cycler, prepare the **Library Amplification Mix**.
18. Remove the 96-well and 384-well PCR cooling blocks from the ASSIST PLUS deck and place on ice.
19. Once the OMEv2–384–Ligation protocol is complete, spin down for 10 seconds and proceed with step 7, XV. Library Amplification.

XV. Library Amplification

1. Initiate OMEv2–384–LIB-AMP program on thermal cycler (Table 15). Allow the thermal cycler to warm up to temperature and pause the program.

Table 15. OMEv2–384–LIB–AMP (Lid 105°C, Reaction volume 15 µL)

Step	Temperature	Time	Cycles
Initial Denaturation	98°C	45 seconds	1
Denaturation	98°C	15 seconds	8
Annealing	60°C	30 seconds	
Extension	72°C	90 seconds	
Final extension	72°C	1 minute	1
Hold	4°C	∞	1

- Calculate the volume of reagents needed for the **Library Amplification Reaction Mix** (Table 16) based on the number of samples with 20% overage using the formula:

$$\text{Number of samples} \times \text{volume per reaction} \times 1.2$$








- Invert **LP3A Reagent**  several times to mix.
 -  **Note:** Do not vortex the **LP3A Reagent** .
- Vortex **LP3P Reagent**  and briefly spin down.
- Assemble each **Library Amplification Mix** in a 15 mL Falcon tube on ice by adding the following reagents with 20% overage with reagents listed in Table 16.

Table 16. Volume of Components of Library Amplification Mix

Product Name	Volume (µL) per number of reactions (rxn)		
	1 rxn	384 rxn*	-- rxn
LP3A Reagent 	6.5	2,995.2	
LP3P Reagent 	0.5	230.4	
Total Volume	7.0	3,225.6	
*20% overage included			

- Vortex the **Library Amplification Master Mix** for 10 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
- Using a single-channel pipette, aliquot the **Library Amplification Master Mix (Lib Amp)** evenly across all 24 wells of Rows D and E of the 96–well **Library Prep Master Mix Plate** (Figure 26). Seal the plate and briefly spin to collect all liquid in the bottom of the wells, and place on ice. Ensure there are no bubbles present in the bottom of the wells.
 -  **Note:** If running half a 384–well plate or less, load **RNA Amplification Mix** into Row E only.
- Place the cold 96–well and 384–well PCR cooling blocks onto the ASSIST PLUS deck at Positions A and B, respectively (Figure 27).
- Place the Library Prep Master Mix Plate on the 96–well PCR cooling block in Position A and place the Library Prep plate on the 384–well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 27.

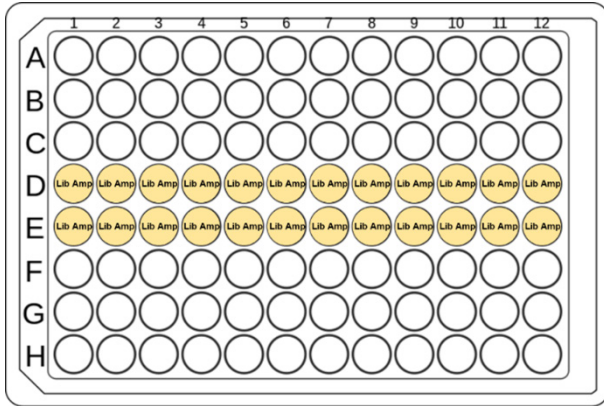


Figure 26: Library Amplification Master Mix (Lib Amp) in the Library Prep Master Mix Plate.

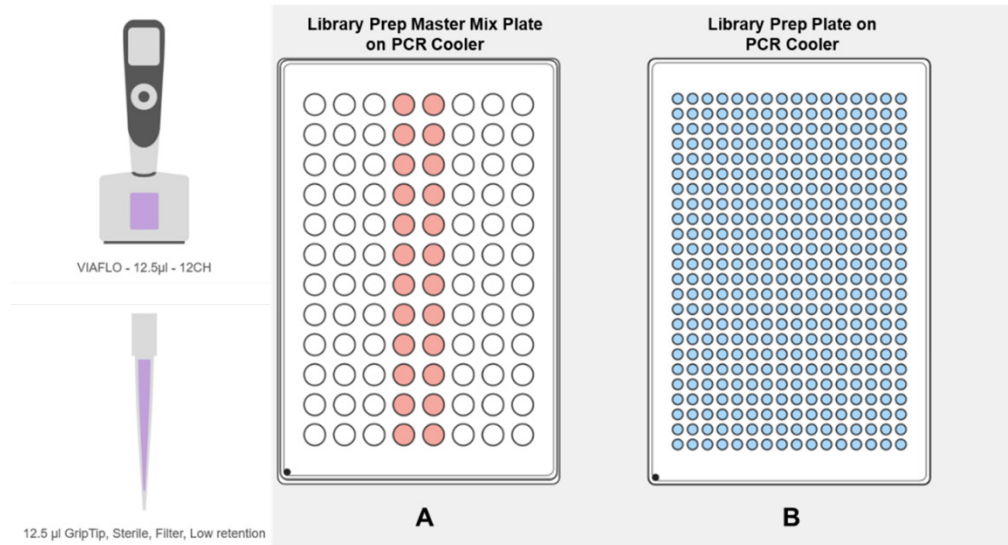


Figure 27: RO384_12_LibAmp.

10. Remove the plate seal.
11. Select the program “RO384_12_LibAmp” on the pipette.
12. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “**Step 12-LibAmp**”
 - “**Amp in Row DE**” – Library Amplification Master Mix is in Rows D and E of the 96-well Library Prep Master Mix Plate on Position B on the deck.
 - “**384 in Pos B**” – 384-well DNA Library Prep plate located in Position B on the deck.
13. Once confirmed, start the program by following the instructions on the pipette screen.
 - ✎ **Note:** Refer to ASSIST PLUS Best Practices.
14. Once the program is complete, seal the plate, vortex for 10 seconds, spin down for 10 seconds and place on ice.
15. Place on the thermal cycler and run the OMEv2-384-LIB-AMP program (Table 15).

16. Remove the 96-well and 384-well PCR cooling blocks from the ASSIST PLUS deck and place on ice.
17. During this incubation, allow Resolve Beads and Elution Buffer to equilibrate to room temperature.
18. Proceed to Library Pooling of DNA/RNA plates.
 - ⊖ **Safe Stop: Store DNA and cDNA Fraction Plates at -20°C.**

XVI. Pooling of DNA Library Prep Plate

1. Place the cold 96-well and 384-well PCR cooling blocks onto the ASSIST PLUS deck at Positions A and B, respectively (Figure 28).
 - ① For greatest downstream flexibility, samples may be cleaned up individually, balanced and pooled for sequencing. If samples are cleaned individually, maintain a 0.75X volume ratio of Resolve Beads to total Amplification Reaction product (i.e., add 11.3 μ L of Resolve Beads per individual sample well), refer to section XXI. Post Library Amplification Cleanup (Optional, Individual)

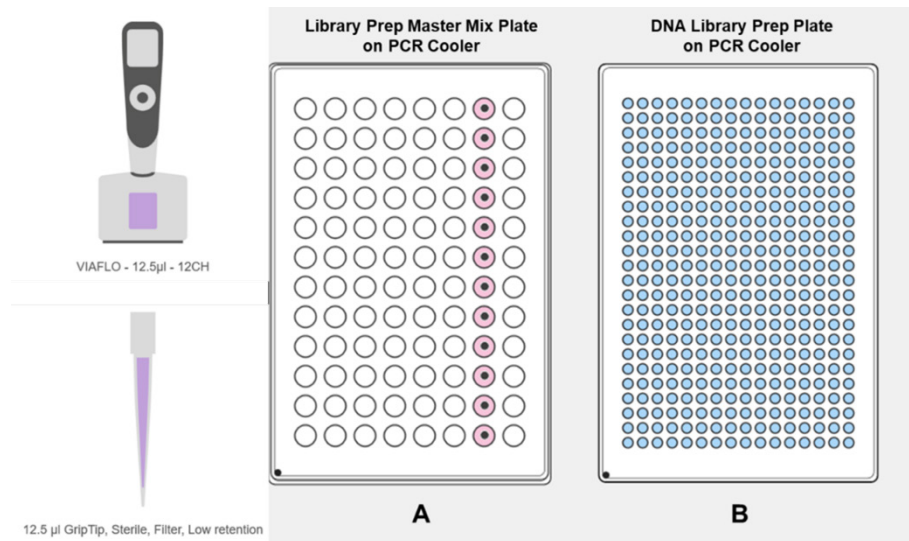



Figure 28: RO384_13D_Dpool.

2. Place the Library Prep Master Mix Plate on the 96-well PCR cooling block in Position A and place the DNA Library Prep plate on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 28.
3. Remove the plate seals.
4. Select the program “**RO384_13D_Dpool**” on the pipette.
5. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “**Step 13D-Dpool**”
 - “**DNA in Row G**” – DNA from DNA Library Prep plate will be pooled into all 12 wells of Row G of the 96-well Library Prep Master Mix Plate on Position B on the deck.

- “384 in Pos B” – 384-well DNA Library Prep plate located in Position B on the deck.
6. Once confirmed, start the program by following the instructions on the pipette screen.
 -  **Note: Refer to ASSIST PLUS Best Practices.**
 7. Once the program is complete, seal both plates and place on ice.

XVII. Pooling of RNA Library Prep Plate

1. Place the cold 96-well and 384-well PCR cooling blocks onto the ASSIST PLUS deck at Positions A and B, respectively (Figure 29).
2. Place the Library Prep Master Mix Plate on the 96-well PCR cooling block in Position A and place the RNA Library Prep plate on the 384-well PCR cooling block in Position B with position A1 of both plates in the bottom left corner, as indicated by the dot in Figure 29.

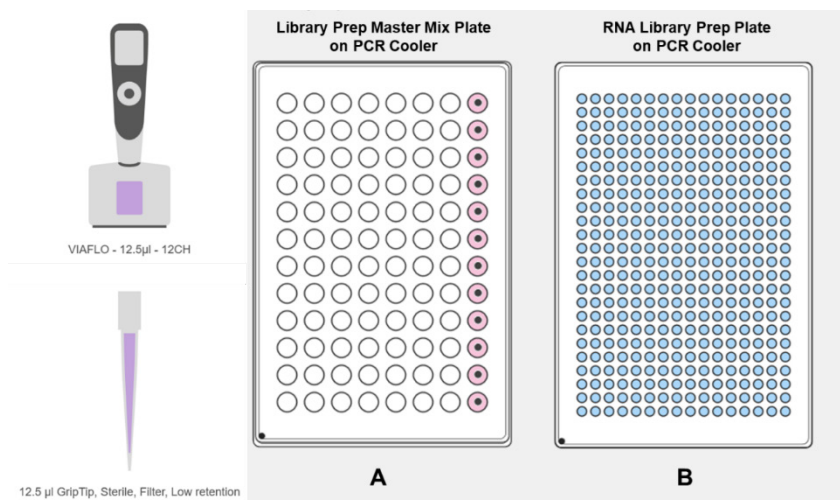



Figure 29: RO384_13R_Rpool.

3. Remove the plate seals.
4. Select the program “RO384_13R_Rpool” on the pipette.
5. Once selected, read the message on the pipette screen to ensure everything is correct before initiating the program.
 - “Step 13R-Rpool”
 - “RNA in Row H” – RNA from RNA Library Prep plate will be pooled into all 12 wells of Row H of the 96-well Library Prep Master Mix Plate on Position B on the deck.
 - “384 in Pos B” – 384-well DNA Library Prep plate located in Position B on the deck.
6. Once confirmed, start the program by following the instructions on the pipette screen.
 -  **Note: Refer to ASSIST PLUS Best Practices.**
7. Once the program is complete, seal both plates and place on ice.



XVIII. Final Pooling of DNA and RNA Library Prep Plates

1. Once samples have been pooled, seal the 96-well plate and spin down to collect all liquids in the bottom of the wells.
2. With a single-channel pipette, collect liquid from all wells of Row G, DNA, into one 1.5 mL Eppendorf tube and liquid from all wells of Row H, RNA, into a second 1.5 mL Eppendorf tube and spin tubes down for 10 seconds.
3. Proceed with Post Library Amplification Cleanup (Pooled).


XIX. Post Library Amplification Cleanup (Pooled)

 **Note:** The following steps are written for performing the cleanup as separate DNA and RNA pools. This may result in increased variability in sequencing depth of samples.

- ① It is recommended to keep DNA Fraction and RNA Fraction libraries in separate pools for cleanup and characterization prior to final pooling for sequencing.
- ① Pooling strategies will vary depending on sequencer, read length and target depth. Consider this before deciding how many samples to pool and make changes to the pooling scripts to select the appropriate number of samples.

1. Vortex the **Resolve Beads**  until fully suspended.
2. Add 0.75X the sample liquid volume of **Resolve Beads**  (i.e. 75 μ L beads per each 100 μ L pooled amplified library) to each tube. Vortex for 10 seconds, and spin down for 3 seconds.
3. Incubate at room temperature for 5 minutes.
4. Place the tubes on a tube magnet for 3 minutes or until the supernatant clears.
5. While the supernatant clears, make 5 mL of 80% ethanol.
6. While on the magnet, remove and discard the supernatant using a pipet.

 **Note:** Take care not to disturb the beads here and in the upcoming wash steps.


7. While on the magnet, wash the beads by adding 200 μ L of 80% ethanol to each tube, incubate for 30 seconds at room temperature, then remove and discard the wash solution (1st wash, first pool clean-up).
8. Repeat the wash step in step 8 a second time (2nd wash).
9. Remove the tubes from the magnet and centrifuge to collect all remaining ethanol at the bottom of the tubes.
10. Place the tubes back on the magnet until the supernatant clears.
11. Remove any remaining ethanol using a P20 pipet.
12. Dry the beads at room temperature for 3 minutes.
 - ① **Important:** Over-drying Resolve Beads may result in reduced yield.
13. Remove the tubes from the magnet and add a volume of 100 μ L **Elution Buffer** .
14. Vortex for 10 seconds and spin down for 3 seconds.
15. Incubate for 5 minutes.

16. Place the tubes on the magnet for 3 minutes, or until the supernatant clears.
17. Transfer the eluted DNA and RNA to new tubes.
18. To perform a second bead clean-up for sequencing pools, add 75 μL of Resolve Beads and vortex for 10 seconds and spin down.
19. Incubate at room temperature for 5 minutes.
20. Repeat steps 5–18 (sequencing pool clean-up).
21. Proceed to Post Library Amplification Quantification and Sizing.

XX. Post Library Amplification Quantification and Sizing

1. To assess library yield, add 2 μL of amplified library to 198 μL Qubit reagent and measure the concentration using the High Sensitivity dsDNA Assay kit, as per the manufacturer's instructions.
2. Prepare a 2 ng/ μL dilution in a fresh PCR plate by diluting libraries with **Elution Buffer** , seal the plate, vortex briefly, and spin down.
3. Determine fragment size distribution by running 2 μL of each 2ng/ μL diluted library using a TapeStation HS D1000 ScreenTape or other fragment analysis instrument using manufacturer's instructions.
4. Refer to Appendix C for more information on interpreting quantification results.
5. Refer to Appendix D for more information on pooling DNA and RNA fractions together for simultaneous sequencing.





XXI. Post Library Amplification Cleanup (Optional, Individual)

 **Note:** The following steps are written for performing the cleanup as individual samples, which may provide better distribution of sequencing reads per individual library undergoing deep sequencing.

1. Vortex the **Resolve Beads** until fully suspended.
2. Spin down samples for 10 seconds.
3. Add 0.75X the sample liquid volume of Resolve Beads (11.3 μL per individual well, e.g. 75 μL beads per **each** 100 μL pooled amplified library) to each reaction. Seal, vortex for 10 seconds, and spin down for 3 seconds.
4. Incubate at room temperature for 5 minutes.
5. Place on the magnet for 3 minutes or until the supernatant clears.
6. While the supernatant clears, make 50 mL of 80% ethanol.
7. While on the magnet, remove and discard the supernatant using a multichannel pipet.

 **Note:** Take care not to disturb the beads here and in the upcoming wash steps.

8. Wash by adding 20 μL per well (if pooling, use 200 μL per tube) of 80% ethanol to each tube or well, incubate for 30 seconds at room temperature, then remove and discard the wash solution (1st wash).

9. Repeat the wash step in step 7 a second time.
10. Remove any remaining ethanol using a P20 pipet.
11. Dry the beads at room temperature for ~1 minute.
 -  **Note:** **Resolve Beads** dry quickly and are often dried within 1–2 minutes of removing the ethanol from individual wells. If working with >6 columns, add Elution Buffer (step 12) immediately to wells after removal of ethanol from 6 columns then proceed with the remainder of the columns to prevent over drying.
 -  **Important:** Over-drying **Resolve Beads** may result in reduced yield.
12. Remove from the magnet and add 22 μ L of **Elution Buffer** to each well (if pooling, use a volume equal to the input sample volume of the original pool).
13. Seal, vortex for 10 seconds, and spin down for 3 seconds.
14. Incubate for 5 minutes.
15. Place on the magnet for 3 minutes, or until the supernatant clears.
16. Transfer the eluted DNA to a new plate.
 -  **Note:** Attempting to recover the entire elution volume can result in the bead pellet collapsing and beads carrying over into the eluate, so it is recommended to leave a few μ L behind.
17. Quantify individual samples using Qubit prior to normalization for pooling. Refer to XV. Post Library Amplification Quantification and Sizing for assessing library size and yield.
18. To clean the pools for sequencing, add the appropriate volume to normalize input into library pools of each DNA and RNA sample into two 1.5 mL Eppendorf tubes, one DNA and one RNA pool and spin tubes down for 10 seconds.
19. Vortex the **Resolve Beads** until fully suspended.
20. Add 0.75X the sample liquid volume of **Resolve Beads** (i.e. 75 μ L beads per each 100 μ L pooled amplified library) to each tube. Vortex for 10 seconds, and spin down for 3 seconds.
21. Incubate at room temperature for 5 minutes.
22. Place the tubes on a tube magnet for 3 minutes or until the supernatant clears.
23. While on the magnet, remove and discard the supernatant using a pipet.
 -  **Note:** Take care not to disturb the beads here and in the upcoming wash steps.
24. While on the magnet, wash the beads by adding 200 μ L of 80% ethanol to each tube, incubate for 30 seconds at room temperature, then remove and discard the wash solution (1st wash, first pool clean-up).
25. Repeat the wash step in step 8 a second time (2nd wash).
26. Remove the tubes from the magnet and centrifuge to collect all remaining ethanol at the bottom of the tubes.
27. Place the tubes back on the magnet until the supernatant clears.
28. Remove any remaining ethanol using a P20 pipet.
29. Dry the beads at room temperature for 3 minutes.

① **Important:** Over-drying Resolve Beads may result in reduced yield.

30. Remove the tubes from the magnet and add a volume of 100 μ L **Elution Buffer** ☒.
31. Vortex for 10 seconds and spin down for 3 seconds.
32. Incubate for 5 minutes.
33. Place the tubes on the magnet for 3 minutes, or until the supernatant clears.
34. Transfer the eluted DNA and RNA to new tubes.
35. Refer to XX. Post Library Amplification Quantification and Sizing for assessing library size and yield prior to sequencing.

Appendix A: Estimated Workflow Times and Safe Stops

Most individuals divide the amplification and library preparation steps between two days, with DNA and cDNA libraries being prepared sequentially. Below are the times required for an expert user using two pre-programmed thermal cyclers to complete the workflow. New users using two pre-programmed thermal cyclers should anticipate working approximately 25% slower.

Step	From Start of Step Through	Time (New User Estimate)
RNA and DNA Amplification	Step III - V1. Whole Genome Amplification (Safe Stop #1)	~3.5 hours (~4.5 hours)
	Step III - X. DNA and cDNA Fraction Quantification (Safe Stop #2)	~6.25 hours (~8.0 hours)
DNA Library Preparation	Step XI - XVIII. Final Pooling of DNA and RNA Library Prep Plates	~3.5 hours (~4.5 hours)
cDNA Library Preparation	Step XI - XVIII. Final Pooling of DNA and RNA Library Prep Plates	~3.0 hours (~3.75 hours)

Appendix B: Post-Amplification QC Analysis

Amplified DNA from human cells typically results in yields from 200 ng to over 400 ng and amplified RNA from 150 ng to over 300 ng (Figure 30). Different cell types or nuclei may produce lower yields. Lower yields may be sufficient for successful library preparation and higher yields do not necessarily correlate with better sequencing outcomes.

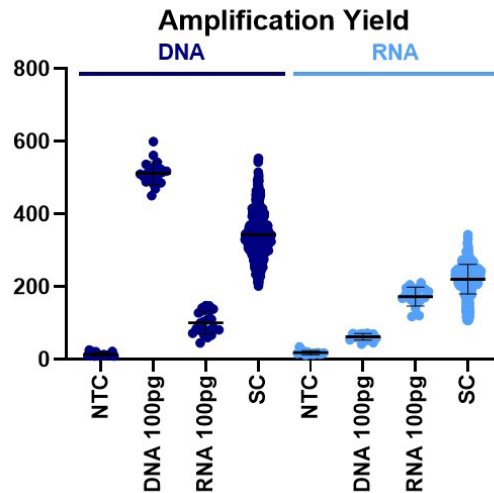


Figure 30. Example of Post Amplification DNA and RNA Fraction Yield.

Amplified DNA should yield a relatively normal distribution of fragment sizes with a peak between 1000 – 1500 bp (Figure 31A). Amplified RNA should yield a moderately uneven normal distribution with a peak between 1500 – 2000 bp (Figure 31B).

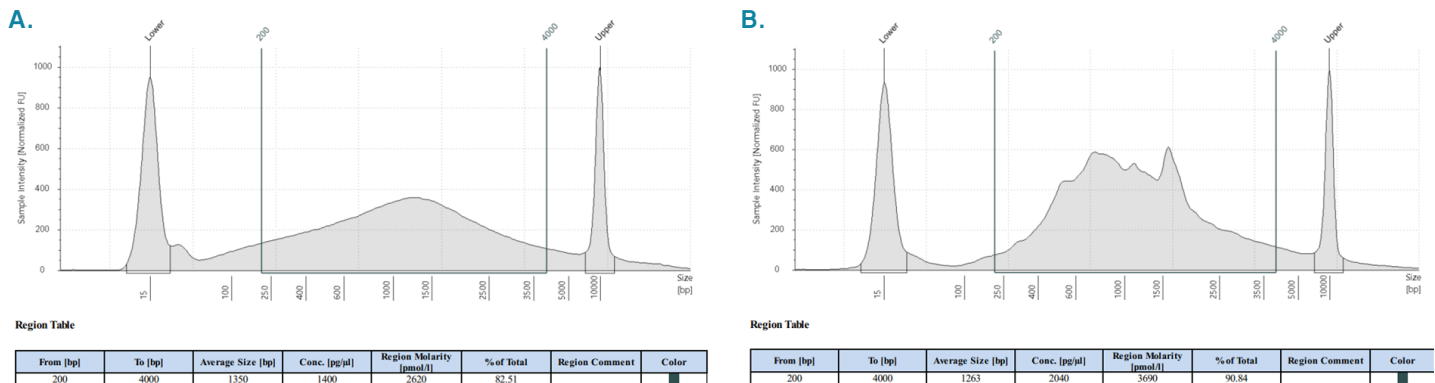


Figure 31. Example of Post Amplification DNA (A) and RNA (B) Fraction Size Distribution. The green lines define the fraction between 0.2 kb – 4 kb. Samples analyzed on the Agilent TapeStation, using a HS D5000 Tape.

All DNA positive controls should show positive amplification in the DNA fraction and limited or no amplification in the RNA fraction. Conversely, all RNA positive control samples should show positive amplification in the RNA fraction and limited or low amplification in DNA fraction.

Typically, only samples with promising yields and size distribution proceed to Library Preparation.

Appendix C: Library QC Analysis

Final sequencing libraries from amplified DNA and RNA of human cells are typically between 150-500 ng, but lower and higher yielding libraries are highly likely to sequence successfully (Figure 32). Libraries should have a relatively normal distribution of fragment sizes, with a peak between 400-550 bp (Figure 33). Different cell types, including nuclei, may produce lower yields.

Typically, only samples with promising yields and size distribution proceed to Sequencing.

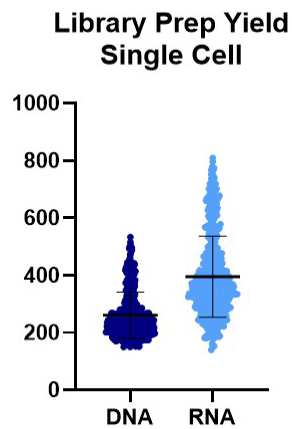
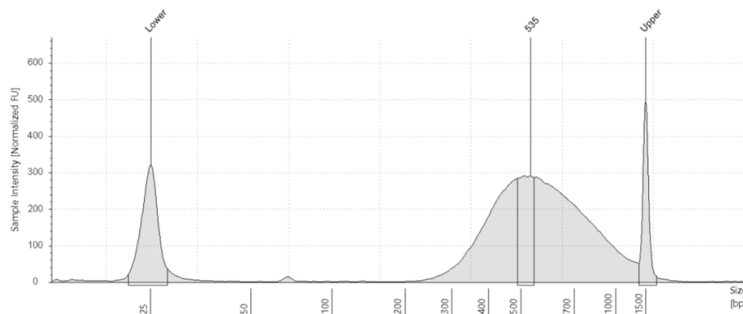


Figure 32. Example of DNA and RNA Fraction Library Yield.

A.



B.

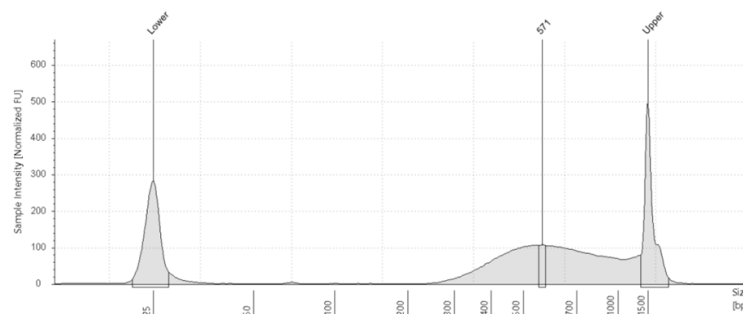


Figure 33. Example of Library DNA (A) and RNA (B) Fraction Size Distribution. Samples analyzed on the Agilent TapeStation, using a HS Uno0 Tape.

Appendix D: Sequencing and Analysis using BaseJumper®

Sequencing Library Preparation

The ResolveOME Whole Genome and Transcriptome Single-Cell Core Kit adds sequencing adapters and barcodes required for multiplex sequencing on Illumina® sequencing platforms.

Sequencing and Preliminary Sample Assessment

When preparing samples for sequencing, follow manufacturer's instructions for loading samples. When aiming to pool together DNA and RNA fractions for sequencing on the same flow cell, normalize each respective fraction to the same concentration and pool them in a 4:1 DNA-to-RNA fraction ratio.

Evaluating performance of DNA fraction sequencing can be performed with 'low-pass sequencing' (50 base paired-end, 2 million reads per cell) to evaluate uniformity and serves as a quality check prior to performing deeper sequencing. Users are recommended to leverage BaseJumper and the pipeline BJ-DNA-QC to evaluate low-pass sequencing data to predict genome coverage at higher depth sequencing levels and for visualizing other general performance metrics. In extension, copy number variation (CNV) analysis can be toggled in this pipeline to survey coverage evenness as well as evaluate large aneuploidies.

In addition, reviewing the expression of the RNA fraction can be performed, usually with 200,000 reads using the BaseJumper BJ-Expression pipeline. This provides users with gene and isoform level counting, along with cellular phenotypic label predictions such as: cell cycle, progenitor, tissue and tumor. This can be leveraged, along with QC from the DNA arm to select specific cells for high quality and phenotype(s) matching your study and progress to deeper sequencing.

For single nucleotide variant analysis (SNV), analysis of other genomic structural variation (such as indels), as well as transcriptomic analysis (RNA-Seq), deep sequencing (20X to 30X genomic coverage) is required. Users may alternatively adopt their own QC pipelines and bioinformatics tools for evaluation.

Performing analyses at each evaluation step can be performed with the BioSkryb BaseJumper Bioinformatics platform.

Data Analysis using BioSkryb BaseJumper Bioinformatics Platform

ResolveOME™ users can choose from pre-defined analytic processes (i.e. pipelines) that uncover genomic variability among samples using the [BaseJumper Bioinformatics platform](https://www.bioskryb.com/basejumper/) (https://www.bioskryb.com/basejumper/). This program includes the following pipelines for multiomic analysis:

- **BJ-DNA-QC** – Based on a low-pass sequencing run (50 base paired-end, 2 million reads per cell), this pipeline estimates library complexity, error rates, chromosomal coverage, and read count metrics. ([Docs](#)) ([Code](#))
- **BJ-WGS** – The whole genome sequencing (WGS) pipeline analyzes single nucleotide variants (SNVs) and small insertions and deletions (indels), providing single cell alignment and variant calling. Coverage statistics along with variant metrics are provided. This pipeline serves as input into useful tertiary pipelines. ([Docs](#)) ([Code](#))
 - **BJ-CNV**. Copy number calling, using full complement of reads (vs. subsampled set as in BJ-DNA-QC).
 - **BJ-SV**. Structural Variant identification. Reports back evidenced structural events such as transversions, inversions, tandem repeats, etc.

- **BJ-VariantAnnotation.** This pipeline provides extended variant annotation such as variant prediction tools and pathogenicity calls from ClinVar.
- **BJ-Expression** – The transcriptomic pipeline makes use of isoform and gene-level counting and normalized counting. In addition there is an end-tagging module which can be used provide external RNA-Seq data from other technologies into the same ResolveOME projects. Additional cell phenotyping output tables (e.g. progenitor, tissue type, cell cycle, etc.) are also provided. ([Docs](#)) ([Code](#))
- **BJ-RNAVariantCalling** performs variant calling on detected genes and isoforms which can be used independently or integrated with those identified in the DNA arm.

Users can create accounts directly on the [BaseJumper Platform](https://basejumper.bioskryb.com) (<https://basejumper.bioskryb.com>) under the “Create Account” tab. To link your account to your Workgroup/Organization, fill out the form “[Add New BaseJumper Organization](#)” (used even if your Organization already exists on the platform). Any additional details about the platform and help with data import can be found in the [BaseJumper Manual](https://docs.basejumper.bioskryb.com/) (<https://docs.basejumper.bioskryb.com/>).

For additional details, please see the BaseJumper ResolveOME DNA Analysis Guide (TAS-071) and RNA Analysis Guide (TAS-072) available from the [BioSkryb Application Support Team](mailto:techsupport@bioskryb.com) (techsupport@bioskryb.com) or in the [BaseJumper Manual](https://docs.basejumper.bioskryb.com/) (<https://docs.basejumper.bioskryb.com/>). Additional technical support for platform or analysis questions can be directed to our bioinformatics support team at basejumper@bioskryb.com.

Appendix E: Library Prep Adapter Sequences

For a complete list of BioSkryb Library Prep Adapter Sequences, please contact our technical support team.

Appendix F: Contact Information for INTEGRA

For further information, contact your INTEGRA Account Manager or [INTEGRA Customer Support](mailto:orders-us@integra-biosciences.com) at orders-us@integra-biosciences.com. Customers can also contact INTEGRA via mail, phone, or by visiting the INTEGRA websites found below:

INTEGRA Biosciences Corporation
22 Friars Drive
Hudson NH 03051
United States

Tel. +1(603) 578 5800 (main)

www.integra-biosciences.com
shop.integra-biosciences.com



BioSkryb

GENOMICS

For more information please contact:

BioSkryb, Inc
2810 Meridian Pkwy, Suite 110
Durham, NC 27713

www.bioskryb.com
techsupport@bioskryb.com
orders@bioskryb.com
(P) +919-370-0841

24 Hr Emergency Response
North America: +1-800-535-5053
International: +1-352-323-3500

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