BioSkryb GENOMICS

ResolveOME™ Whole Genome and Transcriptome Single-Cell Core Kit

Protocol to Prepare DNA Library Pool for Exome Hybrid Capture and cDNA (RNA) Libraries for Transcriptome Sequencing:

User Guide
384-Well Format
General Automated Liquid Handler Protocol

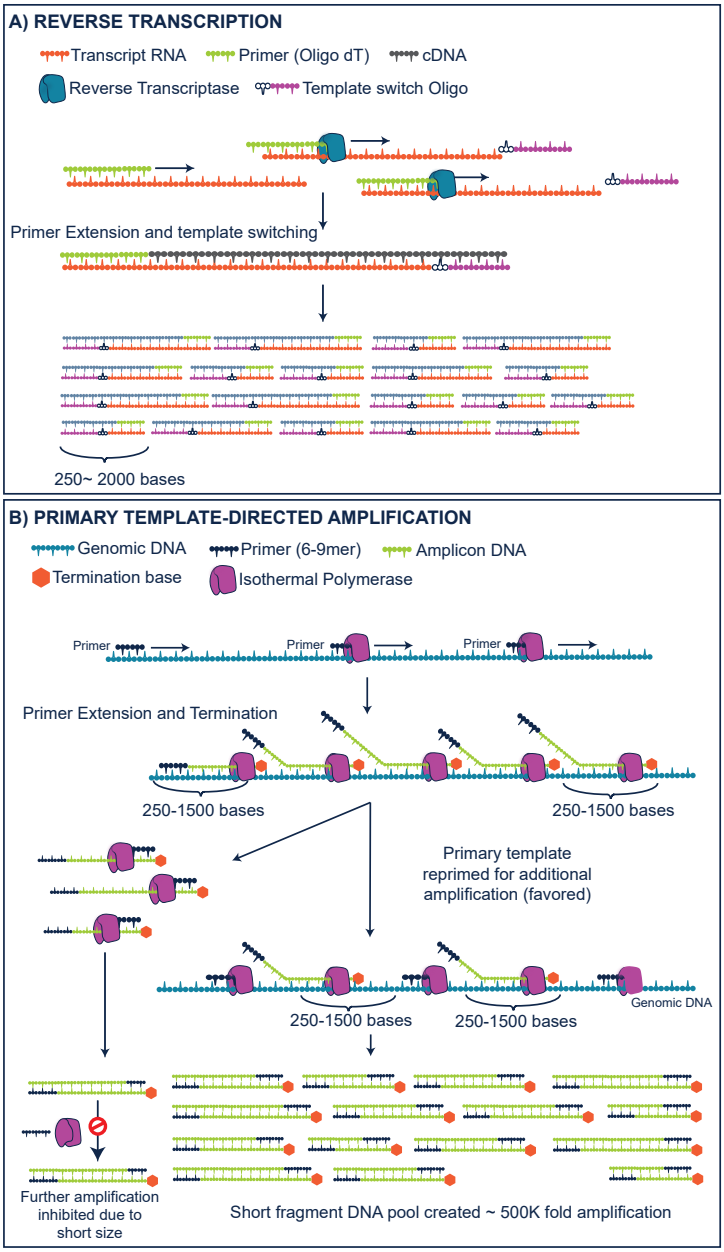
TAS-092.1 | 01/2026

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:

- **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.
- **A unified workflow** for the interrogation of DNA and RNA from the same cell obviates the need for splitting source material or interpreting across data sets.
- **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 With Hybrid Capture

After single cell isolation, the BioSkryb 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 for the genomic and transcriptomic fractions. Whole exomes or gene panels are enriched from the genomic arm of the workflow using third party hybrid capture reagents and protocols. Sequencing and data analysis of all libraries follows (Figure 2).

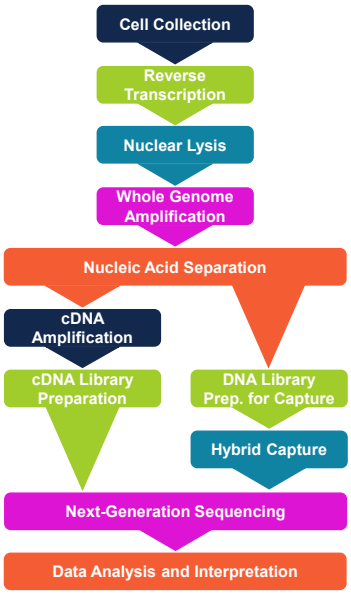


Figure 2. The ResolveOME Workflow With Hybrid Capture First strand cDNA synthesis and genome amplification occur sequentially in one tube followed by separation and library preparation for hybrid capture and multiomic NGS analysis.

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 BioSkryb Genomics Application Support Team (TechSupport@BioSkryb.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.

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	-20°C
	PAC Reagent	100788	Green	-20°C
	LP0B Reagent*	100828	Natural	-20°C
	LP0E 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

*LP0B and LP0E Reagents are NOT REQUIRED for preparing libraries compatible with hybrid capture.

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

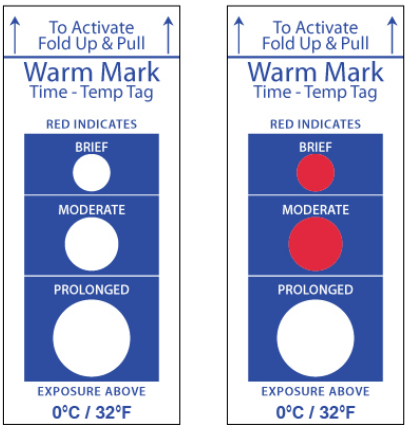
This kit contains components with various shipping and storage requirements. Upon receipt, please carefully check each label and store each kit box as noted on its label.

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.

- ⓘ **Important:** This protocol requires an automated liquid handler capable of high accuracy delivery of sub-microliter volumes into a 384-well plate.
- ⓘ **Important:** Users should ensure thermal cycler compatibility with the skirted plates required for the digital dispensers before beginning the protocol.

Products from Third-Party Suppliers		
Product Name	**Company**	**Catalog Number**
384-well Low Elution Post Magnet Plate with Integrated Cushion Base	Permagen	P384LE
VP 741I6G Heat Transfer Plate	V & P Scientific, Inc	—
PCR Plate Sealing Film	ThermoFisher Scientific	AB0558
twin.tec 384-well PCR Plate	Eppendorf	0030128508
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 Screentape	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 µL 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 (techsupport@bioskryb.com) should

you have any questions on sample compatibility.

II. Uno Single Cell Dispenser

The Uno can be used to deposit single cells into individual wells of a 384-well plate. For downstream use of these cells with ResolveOME, it is recommended that investigators optimize their cell dissociation protocols, enrich for cell populations of interest, remove dead cells, count viable cells, and titer the cells prior to loading the cassette for cell depositing. These general steps are recommended to maximize the number of live cells per experiment; however each cell line and cell suspension may perform differently and additional steps and optimizations may be necessary. Live cells of interest should be loaded into the cassette in Cell Buffer, additional bottles which are available for purchase from BioSkryb. Refer to the "Culture Preparation for Dispensing Mammalian Cells" on the Uno Single Cell Dispenser™ product page (https://lifesciences.tecan.com/products/liquid_handling_and_automation/uno-single-cell-dispenser) for best practices.

III. 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.

IV. 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" for one example (bioskryb.com/eap-cellcelector/).

V. 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 plate format chosen is compatible with your thermal cycler, thermal mixer and magnet before beginning the protocol. Sequencing library cleanup steps for the DNA (genomic) Fraction are performed with pooled libraries in 1.5 mL microcentrifuge tubes. Ensure that the tube format chosen is compatible with your 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 µL Cell Buffer
Positive DNA Control	Correct execution of Whole Genome Amplification and DNA library preparation steps	100 pg Control Genomic DNA in 1 µL Cell Buffer
Positive mRNA Control	Correct execution of Reverse Transcription, RNA Amplification, and RNA library preparation steps	100 pg Control RNA in 1 µL 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 (such as the heat transfer plate, VP 741I6G) 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 digital dispenser 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).

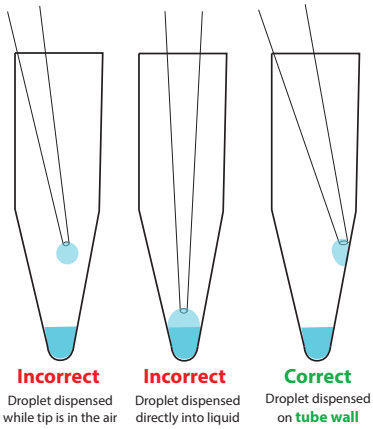


Figure 4. Pipetting Technique. All reagent additions should be carried out by dispensing the added reagent onto the wall of the tube as shown

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 (Tables 1, 4, 8, 9, 11, 13, 15, 17, 19).
    - 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. **General Automated Liquid Handler Best Practices**
    - Follow the automated liquid handler manufacturer's instructions.
    - It is important to keep plates cold during reagent dispensing.
        - For example, use an aluminum cold block that stays cold for the duration of the reagent dispense. This can be done by storing the aluminum cold block at 4°C or subsequently the aluminum cold block can be stored on ice when not in use. If stored on ice, ensure all ice and liquid is removed from the bottom of the aluminum cold block prior to placing on the liquid handler. Moisture on the bottom of the aluminum cold block may cause the plate to shift during dispensing.
        - Once the aluminum cold block is wiped off, add the plate to the aluminum cold block and remove the plate seal before loading onto the instrument. Removal of the plate seal while the plate is on an automated liquid handler may cause misalignment of the instrument if too much pressure is used to remove the seal.
    - The protocol recommends preparing at least 20% overage for each reaction mix. This volume may be different than the volume requested by automated liquid handler program; however, the overage suggested is sufficient to run each of the protocols.
    - Seal and spin plates after liquid dispensing to ensure all liquid is in the bottom of the wells.

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

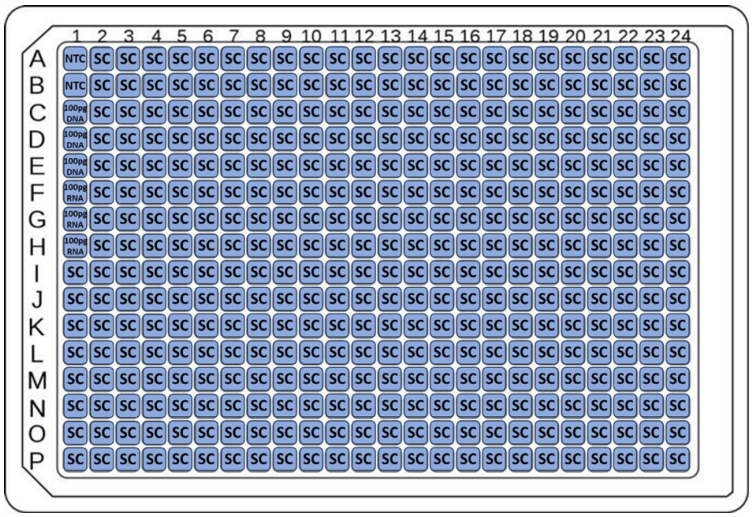
III. Reagent Retrieval and Control Setup

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** 🟢
    - **RB1 Reagent** 🟢
    - **RTC Reagent** ○
2. The remaining reagents should be kept in -20°C storage until required.
3. After thawing, vortex each reagent, except **RTC Reagent** ○, spin briefly, and place back on ice.
4. Prepare an aluminum 384-well cooling block (ex. VP 741I6G heat transfer block) by placing on ice for at least 10 minutes before use.
5. Prepare a 1 ng/µL stock of gDNA by diluting 1 µL **Control Genomic DNA** 🟡 in 49 µL **Cell Buffer** ⊗.
6. Add 5 µL of the 1 ng/µL gDNA stock solution to 45 µL **Cell Buffer** ⊗ to produce working concentration of 100 pg/µL and place on ice.
7. Prepare a 1 ng/µL stock of RNA by diluting 1 µL **Control RNA** 🟠 in 49 µL **Cell Buffer** ⊗.
8. Add 5 µL of the 1 ng/µL RNA stock solution to 45 µL **Cell Buffer** ⊗ to produce a working concentration of 100 pg/µL and place on ice.

- **(Optional):** Verify the concentration of the **Control Genomic DNA** and **Control RNA** samples using a fluorometric method such as Qubit.

9. Place the plate containing samples on ice. Input samples should be suspended in 1 µL 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.
10. To each negative control (NTC) well add 1 µL of Cell Buffer ⊗ only, using a single-channel pipet. An example layout for controls and cell samples is shown in Figure 5.

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 µL of each of the controls in this format to the thawed plate containing samples in cell buffer.



11. Using a single-channel pipet, add 1 µL of the prepared 100 pg/µL DNA and RNA controls (100 pg/well) to the appropriate wells.

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 30% overage using the formula:

Number of samples X volume per reaction X 1.30

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.

   ⓘ **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	250.0	
RTP	0.38	190 .0	
RTC	0.13	65.0	
Total Volume	**1.01**	**505.0**	
			*30% overage included

6. Using an automated liquid handler, add 1 µL of **RTX Mix** to each well.

   ✎ **Note:** Due to the viscosity of the **RTX Mix**, it may be necessary to include an offset in the volume settings of the automated liquid handler software.
7. Once the program is complete, seal and spin down for 10 seconds.
8. In the thermal mixer, mix at room temperature for 1 minute at 1400 rpm.
9. Spin down for 10 seconds.
10. Place on the thermal cycler and run the OMEv2-384-RT program to execute the reverse transcription incubation step.
11. 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**
    - **OR1 Reagent**
12. Thaw L2 Reagent at room temperature for at least 30-60 minutes. Once completely thawed, place on ice.

    ⓘ **Important:** Once **L2 Reagent** has reached room temperature, vortex thoroughly ***until any precipitate is fully dissolved***, briefly spin down, and place on ice.
13. After thawing, vortex each reagent to mix, briefly spin, and place back on ice.
14. After the reverse transcription step is complete, remove the samples from the thermal cycler, briefly spin down, and place at room temperature.
15. Proceed immediately to the Nuclear Lysis step.

V. Nuclear Lysis

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

   Number of samples X volume per reaction X 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. Remove the plate seal.
4. Using an automated liquid handler, add 1 µL of **Lysis Mix** to each well.

   Note: Due to the viscosity of the **Lysis Mix**, it may be necessary to include an offset in the volume settings of the automated liquid handler software.

5. Once the program is complete, seal and spin down for 10 seconds.
6. In the thermal mixer, mix at room temperature for 10 minutes at 1400 rpm.
7. Spin down for 10 seconds and place on ice.
8. 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 45% overage using the formula:

   Number of samples X volume per reaction X 1.45

3. Remove the **OR2 Reagent** 🔴 from the freezer, mix by inversion, briefly spin, and place back on ice.

ⓘ **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.

   ⓘ **Important:** Do not prepare **PTA Mix** more than 30 minutes prior to use. Keep on ice.

   ⓘ **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	1503.4	
OR2 🔴	0.3	167.0	
Total Volume	**3.0**	**1670.4**	
			*45% overage included

5. Remove the plate seal.
6. Using an automated liquid handler, add 3 µL of **PTA Mix** to each well.

   ✎ **Note:** Due to the viscosity of the **PTA Mix**, it may be necessary to include an offset in the volume settings of the automated liquid handler software.

7. Once the program is complete, seal and spin down for 10 seconds.
8. In the thermal mixer, mix at room temperature for 1 minutes at 1000 rpm.
9. Spin down for 10 seconds.
10. 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.

11. 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 1.3 (for 30% overage)

Volume (µL) of Cell Buffer ⊗ = Number of Samples x 0.48 µL x 1.3 (for 30% 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. Retrieve the samples from the thermal cycler after completion of the whole genome amplification step and spin down 10 seconds to collect any condensation.
11. Remove the plate seal.
12. Using an automated liquid handler, add 1 µL of **SEP Reagent Mix** to each well.

✎ **Note:** Due to the viscosity of the SEP Reagent, it may be necessary to include an offset in the volume settings of the automated liquid handler software.

13. Once the program is complete, seal and spin down for 10 seconds.
14. Visually inspect beads are evenly dispersed in the well. If not, select the wells in the script and redispense the wells.
15. In the thermal mixer, mix at room temperature for 10 minutes with constant shaking at 1000 rpm.
16. While incubating, prepare a new 384–well plate by first labeling it "DNA Fraction".
17. Manually dispense 20 µL of **Elution Buffer** ⊠ into each corresponding well of the DNA Fraction plate.
18. Vortex and briefly spin down the RNA Amplification Reagents.
19. Calculate the volume of components for the **RNA Amplification Mix** (Table 7, next page) based on the number of samples with 20% overage using the formula:

Number of samples X volume per reaction X 1.2

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	1152.0	
PAP Reagent 🟠	2.5	1152.0	
Total Volume	**5.0**	**2304.0**	
			*20% overage included

20. Prepare the **RNA Amplification Mix** by combining the reagents in Table 7, vortex for 10 seconds, spin briefly, aliquot evenly across an 0.2 mL 8-strip tube, and place on ice.
21. After the step 17 incubation, remove the samples from the thermal mixer, vortex, and spin down for 20 seconds.
22. Place on magnet for 2 minutes or until the supernatant clears.
23. While on magnet, use a P20 multichannel pipet to transfer supernatant containing the amplified DNA (~ 7 µL) to the DNA fraction plate, being careful not to disturb beads.
24. Seal the DNA Fraction plate and place on ice.
25. Proceed immediately to the RNA Fraction Amplification step. Do not allow the SEP beads to dry.

VIII. RNA Fraction Amplification

1. With the samples at room temperature, dispense 5 µL of **RNA Amplification Mix** (prepared above in Table 7) to each sample using an appropriate pipette or automated liquid handler.

   ⓘ **IMPORTANT:** Do not let the beads dry prior to adding RNA Amplification Mix.

2. Seal and spin down for 10 seconds.
3. Vortex for 10 seconds.
4. Spin down for 10 seconds and place on ice.
5. 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**

6. Place on the thermal cycler and run the OMEv2-384-RNA-AMP program (Table 8).

7. Proceed with DNA Fraction plate to Quantification and Library Prep.
8. While incubating, prepare a new 384–well plate by first labeling it "RNA Fraction".
9. Dispense 12 µL of **Elution Buffer** ⊠ into each corresponding well of the RNA Fraction plate using an appropriate pipette or automated liquid handler.
10. After incubation completes, remove the samples from the thermal cycler, vortex, and spin down for 20 seconds.
11. Place on magnet for 2 minutes or until the supernatant clears.
12. While on magnet, transfer supernatant containing the amplified cDNA (~5 µL) to the RNA fraction plate, being careful not to disturb beads.
13. Seal the RNA Fraction plate and place on ice.
14. Proceed to DNA and cDNA Fraction Quantification Analysis to determine DNA and cDNA yields or proceed directly to Library Preparation.

IX. 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/µL and running 2 µL 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 A 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.

DNA Fraction Library Preparation steps are on pages 18–23

RNA (cDNA) Fraction Library Preparation steps are on pages 24–31

DNA Fraction Library Preparation For Downstream Hybrid Capture and Whole Exome or Targeted Panel Sequencing

- ⓘ 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.
- ⓘ The following library preparation protocol **creates DNA libraries with fragment sizes (~325–400bp) compatible with hybrid capture for downstream whole exome or targeted panel sequencing.** A separate library preparation protocol is available to create libraries with larger fragment sizes for whole genome sequencing (WGS). Contact our Application Support Team (techsupport@bioskryb.com) to obtain the library preparation protocol for WGS.
- ⓘ DNA Libraries **must be eluted in RT-PCR grade water** after cleanup steps using Resolve Beads. Elution using Elution Buffer will compromise subsequent hybrid capture.
- ⓘ The following steps should **only be applied to the DNA Fraction plate**. Library preparation steps for the RNA (cDNA) Fraction plate begin on page 24.

X. DNA Fraction Library Prep: Fragmentation and End Repair

1. Retrieve the following library preparation reagents from -20°C and place on ice to thaw:
   - **LP1B Reagent** 🟢
   - **LP1E Reagent** 🟣
2. Retrieve the following library preparation reagents from 4°C and place at room temperature:
   - **Elution Buffer** ☒
   - **Resolve Beads** ☒
3. Prepare the DNA Library Prep plate by adding 20 ng (i.e., roughly 2 µL for most cell line samples) of DNA sample in each well. Dilute the sample with **Elution Buffer** ☒ from the 4°C box to a total volume of 3 µL in each well.
   - ⓘ The workflow has been designed for 20 ng DNA input but has been demonstrated to be robust for library prep input of 10–40 ng. Calculating and inputing precise DNA input is optional.
4. Initiate OMEv2–384–ERAT-HC program on thermal cycler (Table 9). Allow the thermal cycler to cool to 4°C and pause the program.

   Table 9. OMEv2–384–ERAT-HC (lid temperature 105°C, reaction volume 5 µL)

Step	Temperature	Time
Hold 1	4°C	30 seconds
Hold 2	37°C	10 minutes
Hold 3	65°C	30 minutes
Hold 4	4°C	∞
Total time		**~ 40 minutes**

5. Calculate the volume of reagents for the **Fragmentation Mix** (Table 10) based on the number of

samples with 30% overage using the formula:

Number of samples X volume per reaction X 1.3

6. Assemble the **Fragmentation Mix** on ice by combining the components in the order listed in Table 10.

Table 10. Volume of Components in Fragmentation Mix

Product Name	Volume (µL) per number of reactions (rxn)			
	1 rxn	192 rxn*	384 rxn*	__ rxn
LP1B Reagent 🟢	0.4	99.8	199.7	
LP1E 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

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

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

8. Remove the plate seal.
9. Using an automated liquid handler, add 2 µL of **Fragmentation Master Mix** to each well.

   ✎ **Note:** Due to the viscosity of the **Fragmentation Master Mix**, it may be necessary to include an offset in the volume settings of the automated liquid handler software.

10. 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.
11. Vortex for 10 seconds.
12. Spin down for 20 seconds and place on ice or cooling block.

    ✎ **Note:** Complete mixing and centrifuging is critical to achieve desired fragment lengths.

13. Place on the thermal cycler and run the OMEv2-ERAT-HC program (Table 9).

    ✎ **Note:** Do not load the plate into the thermal cycler until the block has reached 4°C.

14. 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** 🔴
15. When the thermal cycler program has completed, proceed immediately to **Ligation**.

XI. DNA Fraction Library Prep: Ligation

1. Remove plate from thermal cycler, spin briefly and place on ice or on aluminum cooling block.

2. Start the thermal cycler OMEv2–384–Ligation protocol (Table 11) and pause preheated thermal cycler until plate is ready.

Table 11. OMEv2–384–Ligation (lid temperature 50°C, reaction volume 8 µL)

Step	Temperature	Time
Hold 1	20°C	30 minutes
Hold 2	4°C	∞
Total time		**~ 30 minutes**

3. Vortex the **LP2B Reagent** 🔵 for at least 10 seconds and briefly centrifuge to collect liquid at the bottom of the tube.
4. Invert the **LP2E Reagent** 🟡 10 times to homogenize and ensure complete mixing. Briefly centrifuge to collect all liquid in the bottom of the tube.
5. Prepare the **Ligation Mix** in a 1.5 mL Eppendorf tube on ice by adding the following reagents with 30% overage:

Number of samples X volume per reaction X 1.3

Table 12. 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			

6. Vortex the **Ligation Mix** for 10 seconds and briefly centrifuge to collect all liquid in the bottom of the tube and keep on ice.
7. Vortex thawed **Single Use Adapter Plate(s)** and briefly centrifuge.
8. Add 2 µL of **Single Use Adapter(s)** to each well using an appropriate multi-channel pipette or automated liquid handler.

> ✎ **Note:** Be careful not to add the same adapter to different wells and ensure each well receives a unique adapter.

9. Remove the plate seal.
10. Using an automated liquid handler, add 1 µL of **Ligation Mix** to each well.

> ✎ **Note:** Due to the viscosity of the **Ligation Mix**, it may be necessary to include an offset in the volume settings of the automated liquid handler software.

11. 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 back on the cooling block.
12. Place the plate on the preheated thermal cycler and initiate the OMEv2–384–Ligation protocol.

13. While the plate is on the thermal cycler, prepare the **Library Amplification Mix**.
14. Once the OMEv2–384–Ligation protocol is complete, spin down for 10 seconds and proceed with **Library Amplification**.

XII. DNA Fraction Library Prep: Library Amplification

1. Initiate OMEv2–384–LIB-AMP program on thermal cycler (Table 13). Allow the thermal cycler to warm up to temperature and pause the program.

Table 13. 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

2. Calculate the volume of reagents needed for the **Library Amplification Reaction Mix** (Table 14) based on the number of samples with 20% overage using the formula:

Number of samples X volume per reaction X 1.2

3. Invert **LP3A Reagent** ☒ several times to mix.

Note: Do not vortex the **LP3A Reagent** ☒.

4. Vortex **LP3P Reagent** 🔴 and briefly spin down.
5. 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 14.

Table 14. 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			

6. Vortex the **Library Amplification Master Mix** for 10 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
7. Split the **Library Amplification Master Mix** evenly across each tube of one 0.2 mL 8–strip tube.
8. Dispense 7 µL of **Library Amplification Mix** to each well of the plate using a multi-channel pipette or automated liquid handler.
9. Seal, vortex for 10 seconds, spin down for 10 seconds, and place on ice.

10. Place on the thermal cycler and run the OMEv2-384-LIB-AMP program (Table 13).
11. During this incubation, allow **Resolve Beads** ☒ and RT-PCR Grade Water to equilibrate to room temperature.
12. Proceed immediately to **Post Library Amplification Cleanup**.

> ⊘ **Safe Stop:** Store DNA Fraction Plate at -20°C up to one week or proceed immediately to the Post Library Amplification Cleanup step.

XIII. DNA Fraction Library Prep: Post Library Amplification Cleanup

> ✎ **Note:** The following steps pool equal volumes of 96 individual libraries for a total of four 96-plex enrichment pools per set of 384 individually prepped libraries. Pooling is followed by two sequential cleanups using 1X the sample volume of Resolve Beads. Alternatively, each library can be cleaned up individually (without pooling) also using two sequential cleanups with 1X the sample volume of Resolve Beads (e.g. 40 µL of library volume plus 40 µL of bead volume, performed twice).

> ⓘ DNA libraries **must be eluted in RT-PCR grade water** after cleanup steps using Resolve Beads. Elution using Elution Buffer will compromise subsequent hybrid capture.

1. Pool 96 DNA samples together by adding 7.5 µL of each DNA sample well into a 1.5 mL Eppendorf tube. Spin tube down for 10 seconds.

> ✎ **Note:** The Post Library Amplification Cleanup calls for pooling equal volumes of 96 individual libraries for a total of four 96-plex pools per 384 individually prepped libraries.

2. Vortex the **Resolve Beads** ☒ until fully suspended.
3. Add 1X the sample liquid volume of **Resolve Beads** ☒ (i.e. 720 µL beads per each 720 µL pooled libraries from 96 DNA sample wells) to each tube. Vortex for 10 seconds, and spin down for 3 seconds.
4. Incubate at room temperature for 5 minutes.
5. Place the tubes on a tube magnet for 3 minutes or until the supernatant clears.
6. While the supernatant clears, make 5 mL of 80% ethanol.
7. 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.

8. While on the magnet, wash the beads by adding 200 µL 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).
9. Repeat the wash step in step 8 a second time (2nd wash).
10. Remove the tubes from the magnet and centrifuge to collect all remaining ethanol at the bottom of the tubes.
11. Place the tubes back on the magnet until the supernatant clears.
12. Remove any remaining ethanol using a P20 pipet.
13. Dry the beads at room temperature for 3 minutes.

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

14. Remove the tubes from the magnet and add a volume of 410 µL **RT-PCR Grade Water**. **Libraries must be eluted in water for successful downstream hybrid capture.**
15. Vortex for 10 seconds and spin down for 3 seconds.
16. Incubate for 5 minutes.
17. Place the tubes on the magnet for 3 minutes, or until the supernatant clears.
18. Transfer 400 µL of the eluted DNA to a new tube.
19. Perform a second bead clean-up of water-eluted DNA from step 18. Add 400 µL of Resolve Beads and vortex for 10 seconds and spin down.
20. Incubate at room temperature for 5 minutes.
21. Repeat steps 5–13.
22. Resuspend the beads in 110 µl of **RT-PCR grade water. Libraries must be eluted in water for successful downstream hybrid capture.** Pipette multiple times to mix well and then vortex the tube.
23. Incubate the tube at room temperature for 2 minutes to elute DNA off the beads.
24. Briefly spin the tube and place on the magnet for 2 minutes or until the liquid is clear.
25. Transfer 100 µL of the eluted DNA in water to a new tube.
26. Proceed to Post Library Amplification Quantification and Sizing.

XIV. DNA Fraction Library Prep: Post Library Amplification Quantification and Sizing

1. To assess library pool yield, add 2 µL of amplified library pool 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 small aliquot of library pool diluted to 2 ng/ µL in a fresh tube by diluting with **Elution Buffer**, seal the tube, vortex briefly, and spin down.
3. Determine fragment size distribution by running 2 µL of each 2ng/µL diluted library pool using a TapeStation HS D1000 ScreenTape or other fragment analysis instrument using manufacturer's instructions.
4. Refer to Appendix B for example quality control data.
5. Refer to Appendix C for recommendations for hybrid capture.
6. Refer to Appendix D for more information on sequencing and analysis.

RNA Fraction Library Preparation For Downstream Transcriptome Sequencing

ⓘ 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.

ⓘ The following steps should **only be applied to the RNA (cDNA) Fraction plate**. Library preparation steps for the DNA Fraction plate begin on page 18.

XV. RNA (cDNA) Fraction Library Prep: Fragmentation and End Repair

1. Retrieve the following library preparation reagents from -20°C and place on ice to thaw (if not previously thawed for DNA Fraction Library Preparation):
   - **LP1B Reagent** 🟢
   - **LP1E Reagent** 🟣
2. Retrieve the following library preparation reagents from 4°C and place at room temperature (if not previously at room temperature from DNA Fraction Library Preparation):
   - **Elution Buffer** ☒
   - **Resolve Beads** ☒
3. Prepare the RNA Library Prep plate by adding 20 ng (i.e., roughly 2 µL for most cell line samples) of RNA (cDNA) sample in each well. Dilute the sample with **Elution Buffer** ☒ from the 4°C box to a total volume of 3 µL in each well.

   ⓘ The workflow has been designed for 20 ng cDNA input but has been demonstrated to be robust for library prep input of 10–40 ng. Calculating and inputing precise cDNA input is optional.
4. Initiate OMEv2–384–ERAT program on thermal cycler (Table 15). Allow the thermal cycler to cool to 4°C and pause the program.

   Table 15. 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**

5. Calculate the volume of reagents for the **Fragmentation Mix** (Table 16) based on the number of samples with 30% overage using the formula:

   Number of samples X volume per reaction X 1.3
6. Assemble the **Fragmentation Mix** on ice by combining the components in the order listed in Table 16.

Table 16. Volume of Components in Fragmentation Mix

Product Name	Volume (µL) per number of reactions (rxn)			
	1 rxn	192 rxn*	384 rxn*	__ rxn
LP1B Reagent 🟢	0.4	99.8	199.7	
LP1E 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				

7. Vortex for 10 seconds, spin briefly, and place on ice.
   - **Note:** This mixture is stable on ice for up to 4 hours.
8. Remove the plate seal.
9. Using an automated liquid handler, add 2 µL of **Fragmentation Master Mix** to each well.
   - **Note:** Due to the viscosity of the **Fragmentation Master Mix**, it may be necessary to include an offset in the volume settings of the automated liquid handler software.
10. 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.
11. Vortex for 10 seconds.
12. Spin down for 20 seconds and place on ice or cooling block.
    - **Note:** Complete mixing and centrifuging is critical to achieve desired fragment lengths.
13. Place on the thermal cycler and run the OMEv2–ERAT program (Table 15).
    - **Note:** Do not load the plate into the thermal cycler until the block has reached 4°C.
14. 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** 🔴
15. When the thermal cycler program has completed, proceed immediately to **Ligation**.

XVI. RNA (cDNA) Fraction Library Prep: Ligation

1. Remove plate from thermal cycler, spin briefly and place on ice or on aluminum cooling block.
2. Start the thermal cycler OMEv2–384–Ligation protocol (Table 17) and pause preheated thermal cycler until plate is ready.

Table 17. OMEv2–384–Ligation (lid temperature 50°C, reaction volume 8 µL)

Step	Temperature	Time
Hold 1	20°C	30 minutes
Hold 2	4°C	∞
Total time		**~ 30 minutes**

3. Vortex the **LP2B Reagent** 🔵 for at least 10 seconds and briefly centrifuge to collect liquid at the bottom of the tube.
4. Invert the **LP2E Reagent** 🟡 10 times to homogenize and ensure complete mixing. Briefly centrifuge to collect all liquid in the bottom of the tube.
5. Prepare the **Ligation Mix** in a 1.5 mL Eppendorf tube on ice by adding the following reagents with 30% overage:

Number of samples X volume per reaction X 1.3

Table 18. 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			

6. Vortex the **Ligation Mix** for 10 seconds and briefly centrifuge to collect all liquid in the bottom of the tube and keep on ice.
7. Vortex thawed **Single Use Adapter Plate(s)** and briefly centrifuge.
8. Add 2 µL of **Single Use Adapter(s)** to each well using an appropriate multi-channel pipette or automated liquid handler.

   ✎ **Note:** Be careful not to add the same adapter to different wells and ensure each well receives a unique adapter.
9. Remove the plate seal.
10. Using an automated liquid handler, add 1 µL of **Ligation Mix** to each well.

    ✎ **Note:** Due to the viscosity of the **Ligation Mix**, it may be necessary to include an offset in the volume settings of the automated liquid handler software.
11. 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 back on the cooling block.
12. Place the plate on the preheated thermal cycler and initiate the OMEv2–384–Ligation protocol.
13. While the plate is on the thermal cycler, prepare the **Library Amplification Mix**.

14. Once the OMEv2–384–Ligation protocol is complete, spin down for 10 seconds and proceed with **Library Amplification**.

XVII. RNA (cDNA) Fraction Library Prep: Library Amplification

1. Initiate OMEv2–384–LIB-AMP program on thermal cycler (Table 19). Allow the thermal cycler to warm up to temperature and pause the program.

Table 19. 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

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

Number of samples X volume per reaction X 1.2

3. Invert **LP3A Reagent** ☒ several times to mix.

Note: Do not vortex the **LP3A Reagent** ☒.

4. Vortex **LP3P Reagent** 🔴 and briefly spin down.
5. 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 20.

Table 20. 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			

6. Vortex the **Library Amplification Master Mix** for 10 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
7. Split the **Library Amplification Master Mix** evenly across each tube of one 0.2 mL 8–strip tube.
8. Dispense 7 µL of **Library Amplification Mix** to each well of the plate using a multi-channel pipette or automated liquid handler.
9. Seal, vortex for 10 seconds, spin down for 10 seconds, and place on ice.
10. Place on the thermal cycler and run the OMEv2–384–LIB-AMP program (Table 19).

11. During this incubation, allow **Resolve Beads** ☒ and **Elution Buffer** ☒ to equilibrate to room temperature.
12. Proceed immediately to **Post Library Amplification Cleanup**.

    ⦸ **Safe Stop:** Store cDNA Fraction Plate at -20°C up to one week or proceed immediately to the Post Library Amplification Cleanup step.

XVIII. RNA (cDNA) Fraction Library Prep: Post Library Amplification Cleanup (Pooled)

ⓘ For greatest downstream flexibility, RNA (cDNA) samples may be cleaned up individually, balanced and pooled for sequencing. If RNA (cDNA) samples are cleaned individually, maintain a 0.75X volume ratio of Resolve Beads to total Amplification Reaction product (i.e., add 11.3 µL of Resolve Beads per individual sample well), refer to section XVI. Post Library Amplification Cleanup (Optional, Individual)

ⓘ Pooling strategies will vary depending on sequencer, read length and target depth. Consider this before deciding how many samples to pool.

1. Add 1–2 µL of each RNA (cDNA) sample wells into a 1.5 mL Eppendorf tube and spin tubes down for 10 seconds.
2. Vortex the **Resolve Beads** ☒ until fully suspended.
3. Add 0.75X the sample liquid volume of **Resolve Beads** ☒ (i.e. 75 µL beads per each 100 µL pooled amplified library) to each tube. Vortex for 10 seconds, and spin down for 3 seconds.
4. Incubate at room temperature for 5 minutes.
5. Place the tubes on a tube magnet for 3 minutes or until the supernatant clears.
6. While the supernatant clears, make 5 mL of 80% ethanol.
7. 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.
8. While on the magnet, wash the beads by adding 200 µL 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).
9. Repeat the wash step in step 8 a second time (2nd wash).
10. Remove the tubes from the magnet and centrifuge to collect all remaining ethanol at the bottom of the tubes.
11. Place the tubes back on the magnet until the supernatant clears.
12. Remove any remaining ethanol using a P20 pipet.
13. Dry the beads at room temperature for 3 minutes.

    ⓘ **Important:** Over-drying Resolve Beads may result in reduced yield.
14. Remove the tubes from the magnet and add a volume of 100 µL **Elution Buffer** ☒.
15. Vortex for 10 seconds and spin down for 3 seconds.
16. Incubate for 5 minutes.
17. Place the tubes on the manget for 3 minutes, or until the supernatant clears.

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

XIX. RNA (cDNA) Fraction Library Prep: 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 B for more information on interpreting quantification results.
5. Refer to Appendix D for more information on sequencing and analysis.

XX. RNA (cDNA) Fraction Library Prep: 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 8 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 µL 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 RNA (cDNA) 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 µL behind.

17. Quantify individual samples using Qubit prior to normalization for pooling. Refer to XIX. Post Library Amplification Quantification and Sizing for assessing library size and yield.
18. To clean the RNA pool for sequencing, add the appropriate volume to normalize input into the RNA library pool into one 1.5mL Eppendorf tube 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 µL beads per each 100 µL 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 µL 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 24 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.

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

30. Remove the tubes from the magnet and add a volume of 100 µL **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 RNA (cDNA) to a new tube.
35. Refer to XIX. Post Library Amplification Quantification and Sizing for assessing library size and yield prior to sequencing.

Appendix A: 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 6). 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.

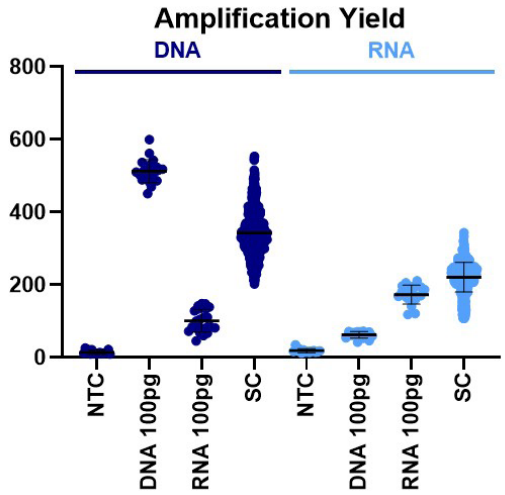
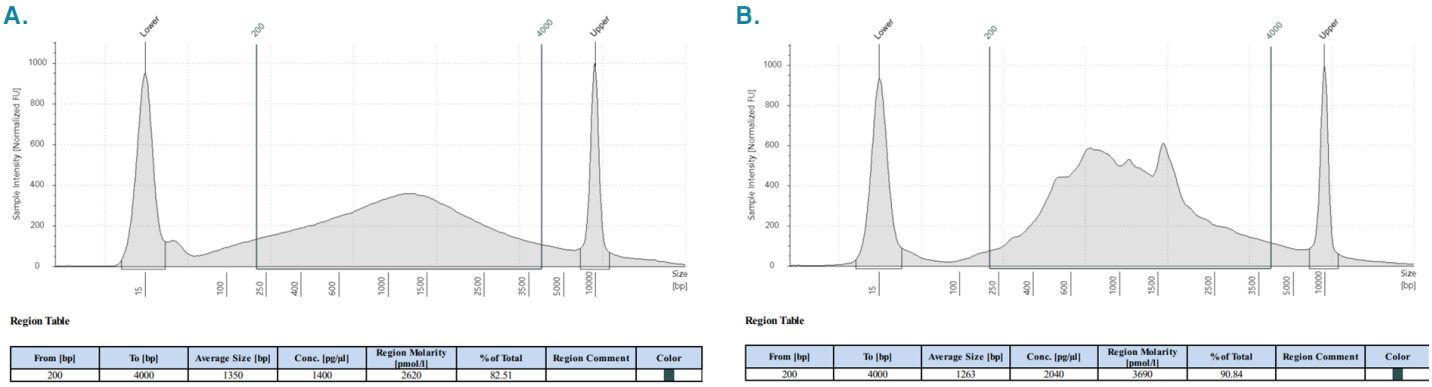


Figure 6. Example of Post Amplification DNA and RNA Fraction Yield.

Amplified DNA should yield a relatively normal distribution of fragment sizes with a peak between 1000 – 1500 bp (Figure 7A). Amplified RNA should yield a moderately uneven normal distribution with a peak between 1500 – 2000 bp (Figure 7B).



From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
200	4000	1350	1400	2620	82.51		

From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
200	4000	1263	2040	3690	90.84		

Figure 7. 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 B: Library QC Analysis

Pre-hybrid capture DNA library pools should have a yield of 13 µg (13,000 ng) or greater to ensure sufficient input for 96–plex hybrid captures (see Appendix C). Typical yields for individual RNA libraries from human cells range from 150-500 ng, but lower and higher yielding libraries are highly likely to sequence successfully (Figure 8). Libraries should have a relatively normal distribution of fragment sizes. Pooled DNA libraries should have an average fragment size of 325-400 bp. RNA libraries should have a peak between 400–550 bp (Figure 9). Different cell types, including nuclei, may produce lower yields. Typically, only samples with promising yields and size distribution proceed to Sequencing.

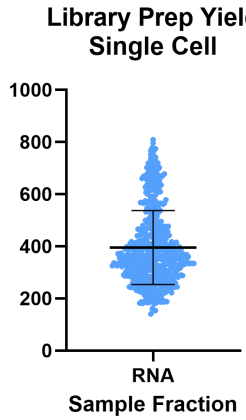
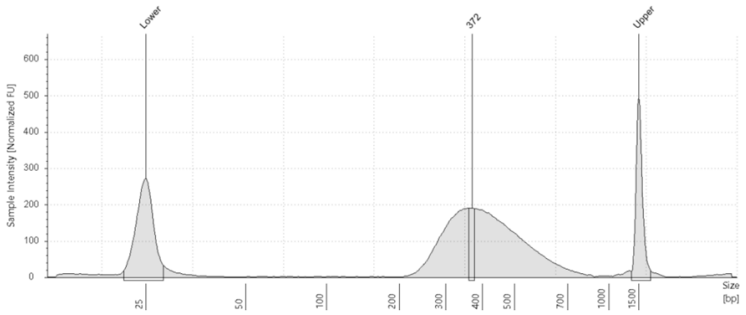


Figure 8. Example of DNA and RNA Fraction Library Yield.

A.



B.

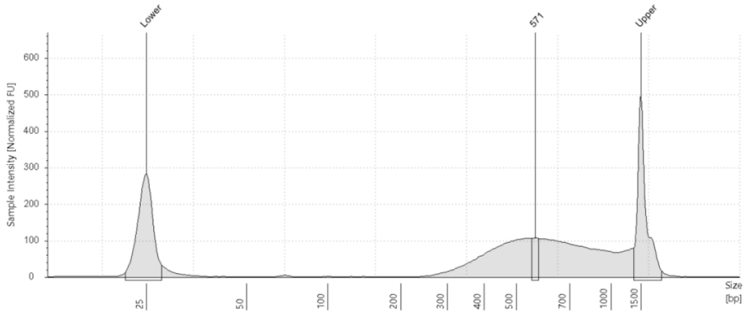


Figure 9. Example of Library DNA (A) and RNA (B) Fraction Size Distribution. Samples analyzed on the Agilent TapeStation, using a HS Uno0 Tape.

Appendix C: Hybrid Capture Recommendations for ResolveOME DNA Libraries

BioSkryb Genomics scientists have performed hybrid capture experiments using the commercially available full exome or focused gene panels described below and single-cell, whole genome libraries produced using the protocols in this document. The following recommendations were developed by our Research and Development team as a guide for ResolveOME users wishing to perform similar hybrid capture experiments. All other parameters and steps should be followed as described in the respective vendor protocols listed below.

Vendor	Panel	Vendor Protocol Version Used	Hybridization Time (Hours)	Number of Post-Capture Amplification Cycles	Plexity Used for Hybrid Capture	Total Pooled Library Input	Sequencing Parameters (Illumina)
Twist Bioscience	Exome 2.0 + Comp. spike-in	Twist Target Enrichment Fast Hybridization Protocol (version: DOC-001066 REV 4.0)	4	8	96	8 µg (2x vendor recommended)	20M paired-end reads (10M clusters) per library X 96 libraries = **1920M paired-end reads (960M clusters) per pool**
IDT™	xGen™ Exome Hyb Panel v2*	xGen Hybridization Capture of DNA Libraries Protocol "Tube Protocol" (version:8)	4	6	96	12 µg (2x vendor recommended)	20M paired-end reads (10M clusters) per library X 96 libraries = **1920M paired-end reads (960M clusters) per pool**
Twist Bioscience	Alliance CNTG Hereditary Oncology Panel (0.2 MB)	Twist Target Enrichment Fast Hybridization Protocol (version: DOC-001066 REV 4.0)	4	13	96	8 µg (2x vendor recommended)	1M paired-end reads (500K clusters) per library X 96 libraries = **96M paired-end reads (48M clusters) per pool**
IDT™	xGen™ Pan-Cancer Hybridization Panel (0.8 MB)	xGen Hybridization Capture of DNA Libraries Protocol "Tube Protocol" (version:8)	16	10	96	12 µg (2x vendor recommended)	1M paired-end reads (500K clusters) per library X 96 libraries = **96M paired-end reads (48M clusters) per pool**

*Order the following blockers from IDT for compatibility with BioSkryb Single Use Library Adapter Sets:

IDT catalogue number 1081100 xGen™ Universal Blockers 10bp TS, 16 rxn

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.

DNA (Genomic) Library Sequencing

Pre-hybrid capture sequencing: The default workflow recommended in this document is for DNA (genomic) libraries to be pooled and subjected to cleanups together in preparation for hybrid capture. This workflow does not include pre-sequencing the individual libraries at low-depth prior to hybrid capture, and thus ResolveOME genomic amplification reactions that drop out completely or are of subpar quality due to cell integrity or technical errors will be included in the hybrid capture. This option is the most streamlined from a workflow perspective, and the user can filter data as appropriate during post-capture analysis to remove libraries that represent poor genomic amplification.

Alternatively, users have the option to pre-screen individual ResolveOME libraries by sequencing to ensure library diversity (or other metric of choice) and then use only libraries of choice to create a pool for hybrid capture. The BJ-DNA-QC pipeline enables pre-screening with 2x50 sequencing of 2 million total reads per cell. While this option requires more sequencing, some users may desire to maximize the output of their enrichment by ensuring only high-performing cells are included in the hybrid capture.

Post-hybrid capture sequencing: Sequencing depth recommendations as a function of plexity are presented in Appendix C. The BaseJumper whole exome sequencing pipeline (BJ-WES) provides commonly sought-after capture QC metrics including on/off target and Fold80 base penalty. In addition, exome variant sensitivity and precision are provided, with variant filtering tools to assist the user with interpretation by functional classification of the variant. BJ-WES is populated with .bed files for IDT™ xGen™ v2 and Twist Exome 2.0 + Comprehensive Spike-In, and can be populated with user-specific .bed files as needed.

RNA (Transcriptomic) Library Sequencing

Reviewing the expression of the RNA fraction can be performed, usually with 200,000 reads per cell 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.

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/). 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.

- **BJ-WES** – The whole exome sequencing (WES) pipeline analyzes single nucleotide variants (SNVs) and small insertions and deletions (indels), providing single cell alignment and target enrichment methods.
- **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 to provide external RNA-Seq data from other technologies into the same ResolveOME projects.
- **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 for online cloud processing (Account Setup Instructions, https://docs.basejumper.bioskryb.com/getting-started/account-setup/account-setup/). Account setup is not required to download code from the BaseJumper local repository (https://github.com/orgs/BioSkryb/repositories?q=visibility%3Apublic+archived%3Afalse).

Users may alternatively adopt their own QC pipelines and bioinformatics tools for evaluation.

Appendix E: Library Prep Adapter Sequences

For a complete list of BioSkryb Library Prep Adapter Sequences, please contact our Application Support Team (techsupport@bioskryb.com).

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