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

96-Well Format

User Guide

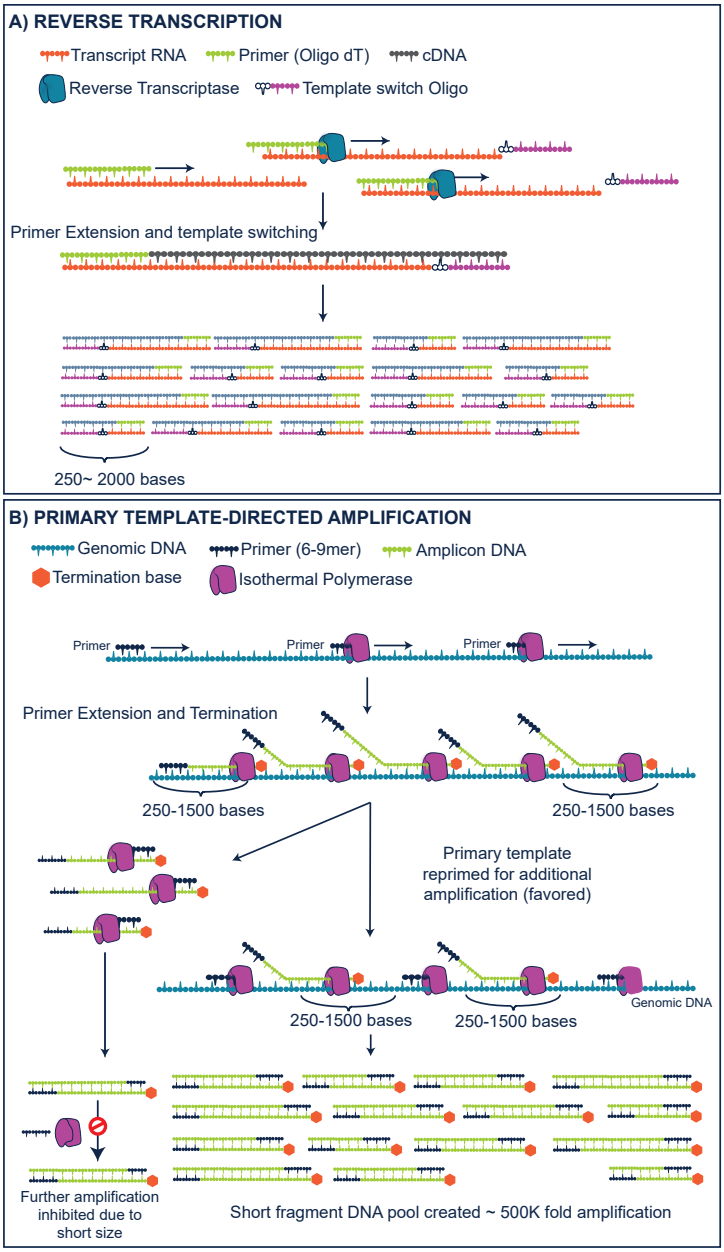
TAS-082.1 | 11/2025

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).

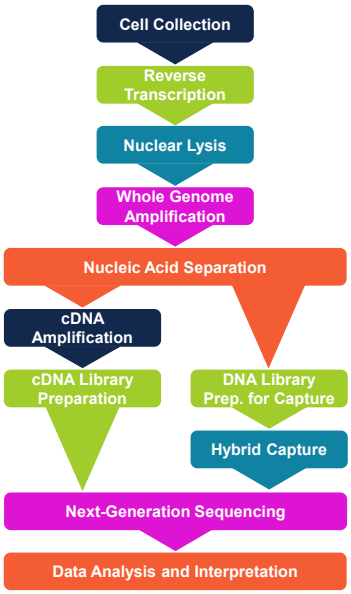


Figure 2. The ResolveOME Workflow With Hybrid Capture First strand cDNA synthesis and genome amplification occur sequentially in one tube followed by separation and library preparation for hybrid capture and multiomic NGS analysis.

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 100818)	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	100785	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	100718	Red	-20°C
Box 2: Post-PCR (PN 100820)	PAP Reagent	100728	Orange	-20°C
	PAC Reagent	100788	Green	-20°C
	LP0B Reagent*	100833	Natural ○	-20°C
	LP0E Reagent*	100791	Clear ⊗	-20°C
	LP1B Reagent	100740	Teal	-20°C
	LP1E Reagent	100743	Purple	-20°C
	LP2L Reagent	100746	Gold	-20°C
	LP3A Reagent	100749	Clear Bottle ☒	-20°C
	LP3P Reagent	100752	Red	-20°C
Single Use Library Adapter Plates	(2) 96-well Single Use Adapter Plates from adapter set A-H	100940-100947	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.

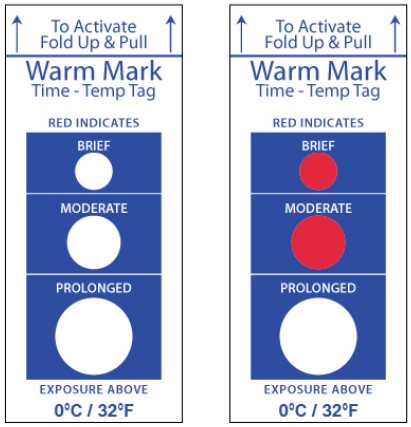


Figure 3. Temperature Indicator Tag (right) Each dry ice shipment includes a temperature tag designed to indicate exposure above 0°C. If the shipment stays below the target temperature, the windows will remain white.

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

I. Equipment and Consumables Available from BioSkryb

The following products have been tested with our workflow to provide optimal results. While these products are not provided with the kit, interested parties can contact the BioSkryb Sales Department (sales@bioskryb.com).

Product Name	Company	Catalog Number
ResolveDNA® Cell Buffer Bottle Kit	BioSkryb	100183
ResolveDNA® PTA-Grade Cell Buffer Pack (12X 500 µL)	BioSkryb	100177

II. 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. 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.

Product Name	Company	Catalog Number
Thermal Cycler	General Lab Supplier (GLS)	—
Magnet PCR Separation Plate	Permagen	MSP750
twin.tec 96-well PCR Plate	Eppendorf	0030128648
PCR Cooler	Eppendorf	022510541
PCR Plate Sealing Film	ThermoFisher Scientific	AB0558
Fluorometer (Qubit)	ThermoFisher Scientific	—
High Sensitivity dsDNA Assay kit	ThermoFisher Scientific	Q32854
Agilent TapeStation	Agilent	—
HS D5000 ScreenTape and Reagent	Agilent	5067-5592 & 5067-5593
HS D1000 ScreenTape and Reagent	Agilent	5067-5584 & 5067-5585
PCR Plate Mixer	GLS	—
PCR Plate Spinner	GLS	—
PCR Strip Tube	GLS	—
Benchtop PCR Strip Tube Centrifuge	GLS	—
RT-PCR Grade Water	GLS	—
Absolute (200 proof) Ethanol	GLS	—
Vortexer	GLS	—
Microcentrifuge Tubes	GLS	—
Microcentrifuge	GLS	—
Ice	GLS	—
Pipettes and Filter Pipette Tips	GLS	—
Magnet Stand for 1.5 mL Microcentrifuge Tubes	GLS	—
Plate Sealing Roller	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 3 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. 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 (techsupport@bioskryb.com).

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

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. 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 96-well plates, PCR strips, or single PCR tubes. 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 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	3 µL Cell Buffer
Positive DNA Control	Correct execution of Whole Genome Amplification and DNA library preparation steps	100 pg Control Genomic DNA in 3 µL Cell Buffer
Positive mRNA Control	Correct execution of Reverse Transcription, RNA Amplification, and RNA library preparation steps	100 pg Control RNA in 3 µL Cell Buffer

Running controls in duplicate is recommended each time the assay is performed to baseline each ResolveOME experiment.

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 Eppendorf PCR Cooler) designed to keep reactions chilled during handling are recommended.
    - Necessary overages are accounted for in master mix tables.
    - 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, 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.

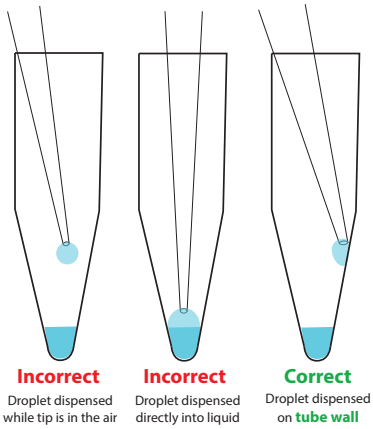


Figure 4. Pipetting Technique. All reagent additions should be carried out by dispensing the added reagent onto the wall of the tube as shown

5. **Multichannel Pipetting** - For each reagent addition step, 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 12, Table 15, and Table 17).
    - 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.

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 to mix, spin briefly, and place back on ice.
4. Prepare a 1 ng/µL stock of gDNA by diluting 1 µL **Control Genomic DNA** 🟡 in 49 µL **Cell Buffer** ⊗.
5. Add 2 µL of the 1 ng/µL gDNA stock solution to 58 µL **Cell Buffer** ⊗ to produce a working concentration of 33 pg/µL (i.e. 100 pg in 3 µL) and place on ice.
6. Prepare a 1 ng/µL stock of RNA by diluting 1 µL **Control RNA** 🟠 in 49 µL **Cell Buffer** ⊗.
7. Add 2 µL of the 1 ng/µL **Control RNA** 🟠 stock solution to 58 µL **Cell Buffer** ⊗ to produce a working concentration of 33 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.
8. Place the plate containing samples on ice. Input samples should be suspended in 3 µL of **Cell Buffer** ⊗.
    - **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 amplification immediately.
9. To each negative control (NTC) well add 3 µL of **Cell Buffer** ⊗ only. An example layout for controls and cell samples is shown in Figure 5.
10. Add 3 µL of the prepared 33 pg/µL DNA and RNA controls (100 pg/well) to the appropriate wells.

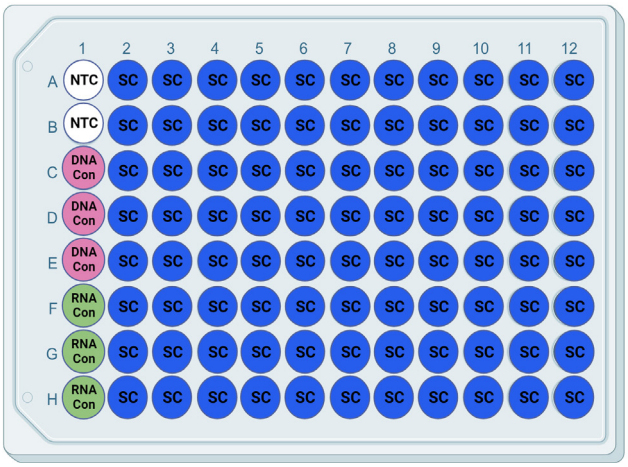


Figure 5. Example 96-well Plate Experimental Layout. The plate map illustrates a typical reaction setup, including replicate NTC, genomic DNA, and RNA controls added into a 96-well plate containing sorted single cells. Prior to processing with the ResolveOME Kit, 3 µL of the samples are added to column 2 through 12 as shown.

IV. Reverse Transcription (RT)

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

   Table 1. OMEv2–RT (lid temperature 70°C, reaction volume 7 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 25% overage using the formula:

   Number of samples X volume per reaction X 1.25

   Note: Use 25% overage when preparing this master mix.

3. 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 96 Reactions (µL)*	Volume per _ Reactions (µL)
RB1	2.0	240	
RTP	1.5	180	
RTC	0.5	60	
Total Volume	**4.0**	**480**	
			*25% overage included

4. Add 4 µL of **RTX Mix** to each sample.

   Note: Pipet the **RTX Mix** onto the side wall of the sample, ensuring the pipet tip does not contact the cell suspension at the bottom of the tube (see "Pipetting Technique" in the "Best Practices" section).

5. Seal and spin down for 10 seconds.
6. In the thermal mixer, mix at room temperature for 1 minute at 1400 rpm.
7. Spin down for 10 seconds.
8. Place on the thermal cycler and run the OMEv2–RT program to execute the reverse transcription incubation step.
9. 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** 🔵
- **OR2 Reagent** 🔴

10. After thawing, vortex each reagent to mix, except **OR2 Reagent** 🔴, briefly spin, and place back on ice.

    ⓘ **Important:** Do not vortex the **OR2 Reagent** 🔴.

    ⓘ **Important:** Once **L2 Reagent** 🟡 has reached room temperature, vortex thoroughly ***until any precipitate is fully dissolved***, briefly spin down, and place on ice.

11. After the reverse transcription step is complete, remove the samples from the thermal cycler, briefly spin down, and place at room temperature.
12. 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 20% overage using the formula:

    Number of samples X volume per reaction X 1.2

    ✎ **Note:** Use 20% overage when preparing this master mix.

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 96 Reactions (µL)*	Volume per _ Reactions (µL)
OL1 🟣	2.5	288	
L2 🟡	0.25	28.8	
OL3 ○	1.25	144	
Total Volume	**4.0**	**460.8**	
			*20% overage included

3. Add 4 µL of **Lysis Mix** to each sample, taking care to pipet onto the side wall.
4. Seal and spin down for 10 seconds.
5. In the thermal mixer, mix at room temperature for 10 minutes at 1400 rpm.
6. Spin down for 10 seconds and place on ice.
7. Proceed immediately to Whole Genome Amplification.

VI. Whole Genome Amplification

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

Table 4. OMEv2-DNA-AMP (lid temperature 70°C, reaction volume 23 µL)

Step	Temperature	Time
Hold 1	30°C	90 minutes
Hold 2	65°C	3 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 15% overage using the formula:

   Number of samples X volume per reaction X 1.15

3. 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.

Table 5. Volume of Components in PTA Mix.

Product Name	Volume per Reaction (µL)	Volume per 96 Reactions (µL)*	Volume per _ Reactions (µL)
OR1 🔵	10.8	1192	
OR2 🔴	1.2	132.5	
Total Volume	**12.0**	**1324.5**	
			*15% overage included

4. Add 12 µL of **PTA Mix** to each sample, taking care to pipet onto the side wall.
5. Seal and spin down for 10 seconds.
6. In the thermal mixer, mix at room temperature for 1 minute at 1000 rpm.
7. Spin down for 10 seconds.
8. Place on the pre-heated thermal cycler and run the OMEv2-DNA-AMP program.

   ⦸ **Safe Stop**: The whole genome amplification reaction incubation can be held at 4°C overnight after completion.

9. 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:
   - **PAC Reagent** 🟢
   - **PAP Reagent** 🟠
2. Retrieve the following separation reagents from 4°C and warm to room temperature:
   - **SEP Reagent** ○
   - **Elution 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 **Elution 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. Calculate the volume of **Elution Buffer** ⊠ required.

   Volume of (µL) Elution Buffer ⊠ *to transfer = Number of Samples X 4.8 µL*

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

Number of Samples	Volume of SEP Reagent (µL)	Volume of Elution Buffer (µL)
24	24	115
48	48	230
96	96	461

8. Remove the tube containing the isolated **SEP Reagent** ○ from the magnet and add the appropriate volume of **Elution 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. At room temperature, remove seal and add 4 µL of the prepared **SEP Reagent Mix** to each sample.
12. Seal and spin down for 10 seconds.
13. Lightly vortex and visually inspect beads are evenly dispersed in the well.
14. In the thermal mixer, mix at room temperature for 10 minutes with constant shaking at 1000 rpm.
15. While incubating, prepare a new 96–well plate by first labeling it "DNA Fraction".
16. Dispense 60 µL of **Elution Buffer** ⊠ into each corresponding well of the DNA Fraction plate.

    Note: Customers may adapt dilution volumes to their own needs or preferences.
17. Vortex and briefly spin down the RNA Amplification Reagents.
18. Calculate the volume of components for the **RNA Amplification Mix** (Table 7) based on the number of samples with 10% overage using the formula:

    Number of samples X volume per reaction X 1.1

Table 7. Volume of Components in RNA Amplification Mix

Product Name	Volume per Reaction (µL)	Volume per 96 Reactions (µL)*	Volume per _ Reactions (µL)
PAC Reagent	10	1056	
PAP Reagent	10	1056	
Total Volume	**20**	**2112**	
			*10% overage included

19. Prepare the **RNA Amplification Mix** by combining the reagents in Table 7, vortex for 10 seconds, spin briefly, and place on ice.
20. After the step 14 incubation, remove the samples from the thermal mixer, vortex, and spin down for 20 seconds.
21. Place on magnet for 2 minutes or until the supernatant clears.
22. While on magnet, use a P200 multichannel pipet to transfer supernatant containing the amplified DNA (~ 27 µL) to the DNA Fraction plate, being careful not to disturb beads.
23. Place the DNA Fraction plate on ice.
24. Proceed immediately to the RNA Fraction Amplification step. Do not allow the SEP beads in the RNA Fraction plate to dry.

VIII. RNA Fraction Amplification

1. Keeping the samples at room temperature, add 20 µL of **RNA Amplification Mix** (prepared above) to each sample.

   ⓘ **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. Visually inspect that beads are fully resuspended, and if bead pellets remain, continue to vortex.
4. Spin down for 10 seconds and place on ice.
5. Initiate OMEv2–RNA-AMP program on thermal cycler (Table 8). Allow the thermal cycler to warm up to temperature and pause the program.

Table 8. OMEv2–RNA-AMP (lid temperature 105°C, reaction volume 20 µL)

Step	Temperature	Time
Hold 1	37°C	10 minutes
Hold 2	98°C	10 seconds
27 Cycles	98°C	10 seconds
	55°C	5 seconds
	68°C	30 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–RNA-AMP program (Table 8).
7. Proceed with DNA Fraction plate to Quantification and Library Prep
8. While incubating, prepare a new 96–well plate by first labeling it "RNA Fraction".
9. Dispense 20 µL of **Elution Buffer** ☒ into each corresponding well of the RNA Fraction plate.

   Note: Customers may adapt dilution volumes to their own needs or preferences.
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 (~20 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 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 steps.

DNA Fraction Library Preparation steps are on pages 17–22

RNA (cDNA) Fraction Library Preparation steps are on pages 23–26

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 (~325–375bp) 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.
- ⓘ 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 23.

X. DNA Fraction Library Prep: Enzymatic Fragmentation, End Repair, and A-Tailing (FERAT)

1. Prepare a PCR Cooler per the manufacturer's instructions (e.g. place at -20°C for 2 hours followed by 10 minutes at room temperature).
2. Thaw all library preparation kit reagents (except LP0B and LP0E) on ice and maintain the reagents on ice. Always keep reactions and reagents on ice unless otherwise instructed.
   - ⓘ **Important: DO NOT VORTEX** reagents **LP1E, LP2L, and LP3A**. These reagents should be mixed by inversion and briefly spun down after thawing. All other reagents should be vortexed for 10 seconds and briefly spun down after thawing.
3. Add 20 ng of each product from the DNA Fraction plate to a fresh 96-well plate on a PCR cooler. Add Elution Buffer to bring the total volume to 6 µL.
   - ✎ The workflow has been designed for 20 ng DNA input calculated using Qubit concentration values for each ResolveOME DNA Fraction well. Alternatively, an average concentration can be calculated by obtaining Qubit measurements for a random sampling of ResolveOME DNA Fraction wells. Post-capture heterogeneity may increase when using an averaged concentration.
4. Vortex **LP1B Reagent** for 5 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
5. Invert **LP1E Reagent** 10 times to homogenize and flick several times to ensure complete mixing. Briefly centrifuge to collect all liquid in the bottom of the tube.
6. Program a thermal cycler to run the FERAT-HC Program (Table 9). Initiate the run to cool the block to 4°C and pause the program once cooled.

Table 9. FERAT-HC Program (lid temperature 105°C)

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**

7. Prepare the **FERAT Master Mix** in a 1.5 mL Eppendorf tube on ice by adding the components in Table 10.

Table 10. FERAT Master Mix

Reagent Name	Volume (µL) per number of reactions (rxn)		
	1 rxn	96 rxn*	__ rxn
LP1B Reagent	0.8	92.2	
LP1E Reagent	1.2	138.2	
Elution Buffer	2.0	230.4	
Total Volume	**4.0**	**460.8**	
			*20% overage included

8. Vortex the **FERAT Master Mix** on low speed for 5 seconds to ensure equal mixing and briefly spin to collect all liquid in the bottom of the tube.
   - **Note:** this mixture is stable on ice for up to 4 hours.
9. Add 4 µL of the **FERAT Master Mix** to each well while the plate is on a PCR Cooler.
10. Seal the plate and briefly spin to get the liquid to the bottoms of the wells.
11. Vortex the plate at medium speed to homogenize the reaction.
12. Spin for 30 seconds to collect the samples at the bottoms of the wells then place the plate back on a PCR Cooler or ice.
    - Note: Complete mixing is critical to achieve desired fragment lengths.
13. Place the plate into the preheated thermal cycler and initiate the FERAT-HC Program (Table 9).
14. While the thermal cycler is running, remove **Resolve Beads** from storage and allow to equilibrate to room temperature for at least 30 minutes.
15. While the thermal cycler is running, thaw the **Single Use Library Adapter Set** plate on ice.

XI. DNA Fraction Library Prep: Ligation

1. Once the FERAT-HC program is complete, remove the plate from the thermal cycler, briefly spin to collect all liquids at the bottom of the wells, and place plate on a PCR Cooler.
2. Program a thermal cycler to run the LIG Program (Table 11).

Table 11. LIG Program (lid temperature: OFF)

Step	Temperature	Time
Hold 1	20°C	15 minutes
Total Time	-	**~15 minutes**

3. Invert the **LP2L Reagent** 🟡 ten times to homogenize **(DO NOT VORTEX)** and place on ice.
4. Vortex thawed **Single Use Library Adapter Set** plate briefly and centrifuge.
5. Add 5 µL of Single Use Library Adapters to each sample in the plate.

   ✎ **Note:** Ensure each sample well receives a unique adapter. If fewer than 96 samples are being prepared, unused wells on the adapter plate can be refrozen. Adapter index information is available by contacting the BioSkryb Genomics Application Support Team.
6. Add 5 µL of **LP2L Reagent** 🟡 to each sample in the plate.

   ✎ **Note: LP2L Reagent** 🟡 is viscous. Pipette carefully.
7. Seal the plate and briefly spin. Then mix by vortexing at medium speed, and spin for 30 seconds to collect all liquid at the bottom of the wells.
8. Place the plate in the thermal cylcer and initiate the LIG Program (Table 11).
9. After completion of the LIG Program, proceed immediately to Library Amplification.

XII. DNA Fraction Library Prep: Library Amplification

1. Initiate the OMEv2–LIB-AMP program on a thermal cycler (Table 12). Allow the thermal cycler to warm up to temperature and pause the program.

   Table 12. OMEv2–LIB-AMP program (lid temperature 105°C)

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	45 seconds	
Final extension	72°C	1 minute	1
Hold	4°C	∞	1

2. Invert **LP3A Reagent** several times to mix **(DO NOT VORTEX)**.
3. Vortex **LP3P Reagent** 🔴 and briefly spin down.
4. Prepare the **Amplification Master Mix** by assembling the components in Table 13 in a new tube.

Table 13. Amplification Master Mix

Reagent Name	Volume (µL) per number of reactions (rxn)		
	1 rxn	96 rxn	__rxn
LP3A Reagent ☒	18	1901	
LP3P Reagent 🔴	2	211	
Total Volume	**20**	**2112**	
*10% overage included			

5. Add 20 µL of the **Amplification Master Mix** to each well of the plate containing adapter-ligated DNA for a total reaction volume of 40 µL per well.
6. Seal the plate with a film and briefly spin. Then mix thoroughly by vortexing followed by spinning for 30 seconds.
7. Load the plate into the preheated thermal cycler and initiate the OMEv2–LIB-AMP program in Table 12.
8. After completion of the OMEv2–LIB-AMP program, place the plate on a PCR Cooler.
9. Ensure successful amplification has occurred by obtaining Qubit readings for 10 randomly selected wells and determine the median yield.

   ✎ **Note:** Expected yield for successful amplification is >1000 ng per library.
10. Proceed to post-amplification cleanup.

XIII. DNA Fraction Library Prep: Post Amplification Cleanup

✎ **Note:** The following steps pool equal volumes of individual libraries 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. Make sure **Resolve Beads** are equilibrated to room temperature before use.
2. Pool together equal volumes of individual libraries into a 1.5 mL microcentrifuge tube per instructions below.

   When pooling 96 libraries: Pool 7.5 µL of each library for a total volume of 720 µL.

   When pooing 48 libraries: Pool 15 µL of each library for a total volume of 720 µL.
3. Vortex **Resolve Beads** thoroughly immediately before use to ensure even distribution of beads.
4. Add 720 µL of **Resolve Beads** to each amplified library pool (containing 48 or 96 individual libraries, Table 14).

   ✎ **Note:** Use caution to avoid spills.

Table 14. Resolve Bead Cleanup Mix

Component	Volume per Reaction
Pooled libraries	720 µL
Resolve Beads	720 µL
Total Volume	**1440 µL**

5. Seal the tube and vortex for 10 seconds.
6. Incubate the tube at room temperature for 5 minutes.
7. Briefly spin the tube for 10 seconds.
8. Place tube on a magnet for 5 minutes or until the liquid is clear.
9. While on the magnet, remove and discard the supernatant.
   - **Note:** Take care not to disturb the beads here and in the upcoming wash steps.
10. Keep the tube on the magnet and add 200 µL of freshly prepared 80% ethanol to the tube, being careful not to disturb the beads.
11. Incubate the tube on the magnet at room temperature for 30 seconds.
12. With the tube on the magnet, carefully remove and discard the ethanol using a P200 pipette.
13. Perform a second ethanol bead wash. With the tube on the magnet, add another 200 µL of freshly prepared 80% ethanol to the tube, being careful not to disturb the beads.
14. Incubate the tube on the magnet at room temperature for 30 seconds.
15. With the tube on the magnet, carefully remove and discard the ethanol.
16. Close the tube cap, spin briefly, return to the magnet, and carefully remove the tube cap.
17. Incubate the tube on the magnet at room temperature for 5 minutes or until the supernatant clears.
18. Remove any remaining ethanol using a P20 pipette set to 20 µL.
19. Allow the beads to dry for 3 minutes.
    - **Note: DO NOT** over dry the beads, this will result in reduced yields.
20. Remove the tube from the magnet.
21. Resuspend the beads in 410 µL of **RT-PCR grade water**. **Libraries must be eluted in water for successful downstream hybrid capture.** Pipette multiple times to mix well.
    - **Note:** pipetting carefully will minimize bubbling and allow for greater library recovery.
22. Incubate the tube at room temperature for 2 minutes to elute DNA off the beads.
23. Briefly spin tube and place on magnet for 2 minutes or until the liquid is clear.
24. Carefully transfer 400 µL of the DNA in water to a new tube using a P200 pipette. Be careful not to disturb the beads.

25. Perform a second cleanup of the 400 µL of water-eluted DNA from step 24. Add 1X sample volume of Resolve Beads (400 µL). Repeat steps 5–20.
26. Resuspend the beads in 210 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 close the lid and vortex the tube.
27. Incubate the tube at room temperature for 2 minutes to elute DNA off the beads.
28. Briefly spin the tube and place on the magnet for 2 minutes or until the liquid is clear.
29. Carefully transfer 200 µL of the DNA in water to a new tube using a P200 pipette. Be careful not to disturb the beads.
30. Place the tube on ice if proceeding to library pool quantification, fragment size analysis, and hybrid capture. Otherwise, store library pool at -20°C.

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 17.

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. Prepare the RNA Library Prep plate by adding 20 ng of RNA (cDNA) sample diluted with **Elution Buffer** ☒ from the 4°C box to a total volume of 6 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 volumes of 10–40 ng. Calculating and inputing precise cDNA input is optional.
3. Initiate OMEv2–ERAT program on thermal cycler (Table 15). Allow the thermal cycler to cool to 4°C and pause the program.

Table 15. OMEv2–ERAT (lid temperature 105°C, reaction volume 10 µL)

Step	Temperature	Time
Hold 1	4°C	30 seconds
Hold 2	30°C	5 minutes
Hold 3	65°C	20 minutes
Hold 4	4°C	∞
Total time		**~ 30 minutes**

4. Calculate the volume of reagents for the **Fragmentation Mix** (Table 16) based on the number of samples with 20% overage using the formula:

Number of samples X volume per reaction X 1.2

5. Assemble the **Fragmentation Mix** on ice by combining the components in the order listed in Table 16, vortex for 10 seconds, spin briefly, and place on ice.

Table 16. Volume of Components in Fragmentation Mix

Product Name	Volume (µL) per number of reactions (rxn)		
	1 rxn	96 rxn*	__ rxn
LP1B Reagent 🟢	0.8	92.2	
LP1E Reagent 🟣	1.2	138.2	
Elution Buffer ☒	2	230.4	
Total Volume	**4**	**460.8**	
			*20% overage included

6. Vortex for 10 seconds, spin briefly, and place on ice.
7. Add 4 µL of **Fragmentation Mix** into the appropriate wells of the RNA Library Preparation plate.
8. Seal and spin down for 10 seconds.
9. Vortex for 10 seconds.
10. Spin down for 10 seconds and place on ice.
11. 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.
12. While incubating, retrieve the following reagents from Box 2 in -20°C storage and place them on ice for 30 minutes to thaw (if not previously thawed for DNA Fraction Library Preparation):
    - **LP2L Reagent** 🟡
    - **Single Use Adapter Plate(s)**
    - **LP3A Reagent** ⊠
    - **LP3P Reagent** 🔴
13. 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.
2. Briefly spin thawed single use adapter plate, vortex for 10 seconds, and spin down for 10 seconds.
3. Add 5 µL of library adapter from a Single Use Adapter Plate, carefully noting which sample receives which adapter.
4. Add 5 µL **LP2L Reagent** 🟡 to each sample.

    Note: Do not vortex the **LP2L Reagent** 🟡.
5. Seal, vortex for 10 seconds, and spin down for 10 seconds.
6. Incubate at 20° C for 15 minutes in a thermal cycler with an unheated lid.
7. Remove the plate from the thermal cycler, spin down, and place on ice.
8. Proceed immediately to Library Amplification.

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

1. Initiate OMEv2–LIB-AMP program on thermal cycler (Table 17). Allow the thermal cycler to warm up to temperature and pause the program.

Table 17. OMEv2–LIB-AMP (Lid 105°C, Reaction volume 40 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	45 seconds	
Final extension	72°C	1 minutes	1
Hold	4°C	∞	1

2. Calculate the volume of reagents needed for the **Library Amplification Reaction Mix** (Table 18) based on the number of samples with 10% overage using the formula:

   Number of samples X volume per reaction X 1.1

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** with reagents listed in Table 18.

Table 18. Volume of Components of Library Amplification Mix

Product Name	Volume (µL) per number of reactions (rxn)		
	1 rxn	96 rxn	__rxn
LP3A Reagent ☒	18	1901	
LP3P Reagent 🔴	2	211	
Total Volume	**20**	**2112**	
*10% overage included			

6. Add 20 µL of **Library Amplification Mix** to each reaction.
7. Seal, vortex for 10 seconds, spin down for 10 seconds, and place on ice.
8. Place on the thermal cycler and run the OMEv2-LIB-AMP program (Table 17).
9. During this incubation, allow **Resolve Beads** ☒ and **Elution Buffer** ☒ to equilibrate to room temperature (if not previously equilibrated for DNA Fraction Library Preparation).
10. Proceed immediately to Post Library Amplification Cleanup.

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

ⓘ For most efficient operation, samples, or an aliquot of each sample, may be pooled immediately after Library Amplification and cleaned up as one sample to prepare for sequencing. This may result in increased variability in sequencing depth of samples.

ⓘ For greatest downstream flexibility, including most accurate sequencing balancing, samples may be cleaned up individually, then pooled for 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** ☒ (30 µL per individual well, e.g. 75 µL beads per 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 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.

7. Wash by adding 200 µL of 80% ethanol to each tube or well, incubate for 30 seconds at room temperature, then remove and discard the wash solution (1st wash).
8. Repeat the wash step in step 7 a second time.
9. Remove any remaining ethanol using a P20 pipet.
10. Let stand for 2–3 minutes to dry the beads (do not over-dry the beads).
11. Remove from the magnet and add 42 µL of **Elution Buffer** ☒ to each well (or a volume approximately equal to the input sample volume if samples have already been pooled).
12. Seal, vortex for 10 seconds, and spin down for 3 seconds.
13. Incubate for 2 minutes.
14. Place on the magnet for 3 minutes, or until the supernatant clears.
15. Transfer the eluted cDNA (RNA Fraction) to a new plate/tube.

    ✎ **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.

16. 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.

ⓘ **IMPORTANT:** The final sequencing pool of RNA (cDNA) libraries should be subjected to a second Resolve Bead Cleanup step with 0.75X beads (i.e. for 100 µL of pooled sample volume, add 75 µL of beads), follow workflow steps as described in Step XVIII. RNA (cDNA) Fraction Library Prep: Post Library Amplification Cleanup.

Appendix A: Post-Amplification QC Analysis

Amplified DNA from human cells typically results in yields from 500 ng to over 1000 ng and amplified RNA from 500 ng to over 1000 ng (Figure 6). Lower yields may be sufficient for successful library preparation and higher yields do not necessarily correlate with better sequencing outcomes.

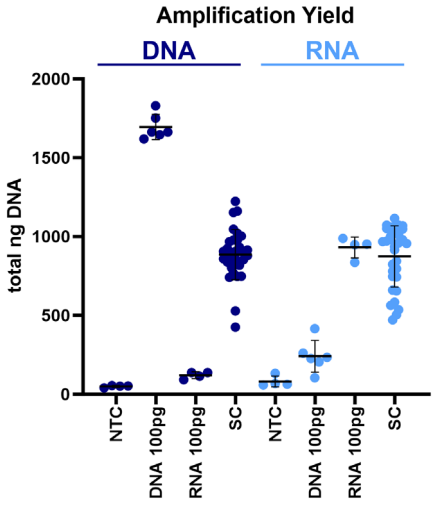


Figure 6. Example of Post Amplification DNA and RNA Fraction Yield.

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).

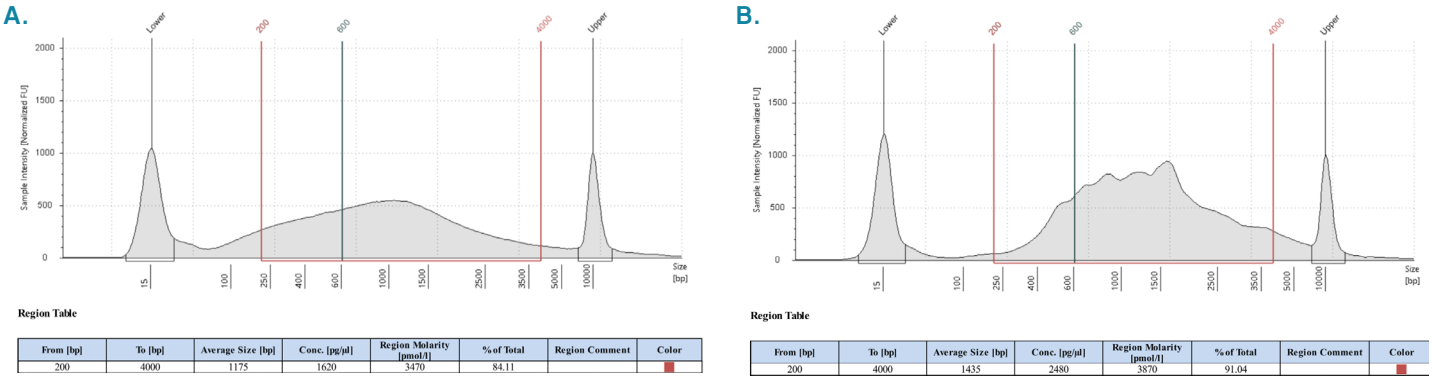


Figure 7. Example of Post Amplification DNA (A) and RNA (B) Fraction Size Distribution. The blue and red lines define the fraction between 0.2 kb – 4 kb. Samples analyzed on the Agilent TapeStation, using a HS D5000 Tape.

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

The pre-hybrid capture DNA library pool 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 range from 1000 ng-2000 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 325-375 bp. RNA libraries should have a peak between 400–550 bp (Figure 9). Typically, only samples with promising yields and size distribution proceed to sequencing.

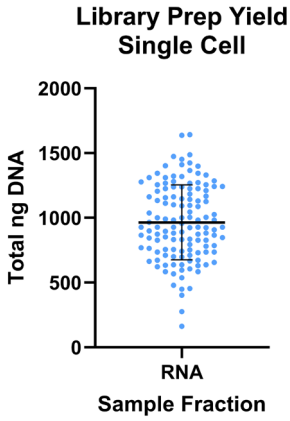
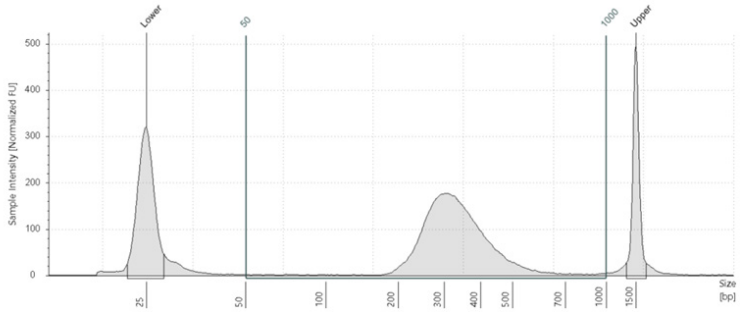


Figure 8. Example of RNA Fraction Library Yields.

A.



B.

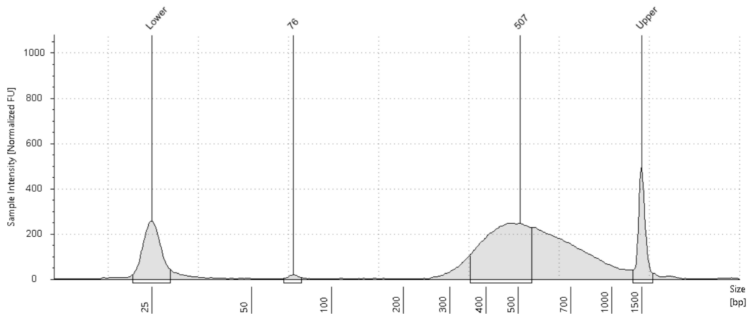


Figure 9. Example of Library DNA (Top) and RNA (Bottom) Fraction Size Distribution. Samples analyzed on the Agilent TapeStation, using a HS D1000 Tape.

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**
					48	8 µg (2x vendor recommended)	20M paired-end reads (10M clusters) per library X 48 libraries = **960M paired-end reads (480M 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**
					48	6 µg (1x vendor recommended)	20M paired-end reads (10M clusters) per library X 48 libraries = **960M paired-end reads (480M 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**
					48	8 µg (2x vendor recommended)	1M paired-end reads (500K clusters) per library X 48 libraries = **48M paired-end reads (24M 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**
					48	12 µg (2x vendor recommended)	1M paired-end reads (500K clusters) per library X 48 libraries = **48M paired-end reads (24M 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:

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