ResolveDNA® Whole Genome Single-Cell Core Kit Protocol to Prepare Library Pool for Exome Hybrid Capture

96-Well Format

User Guide

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ResolveDNA® Whole Genome Single-Cell Core Kit, 96-Well Format, Protocol to Prepare Library Pool for Exome Hybrid Capture

Product Description

The ResolveDNA Whole Genome Single-Cell Core Kit offers the best-in-class Whole Genome Amplification (WGA) solution with single cell resolution, enabled by the proprietary Primary Template-directed Amplification (PTA) chemistry.

The optimized reaction parameters employed in this PTA-based kit enable the reproducible recovery of over 97% of the genomes of single cells or nuclei, and robust amplification of limited DNA input samples with industry leading uniformity and accuracy. This complete end-to-end solution kit enables users to go from cells to a library pool ready for hybrid capture workflows. This kit is configured for working with 96-well format and contains one adapter set with 96 unique dual indexes (UDIs) to generate 96 libraries for sequencing.

Key features and benefits include the following:

- A simple, user-friendly workflow that requires less than 2.5-hours hands-on time and under 8 hours total run time.
- Specific amplification of single cell genomes recovering >97% of the human genome.
- Excellent allelic balance enables highly sensitive and specific assessment of single nucleotide variation (SNV).
- Compatible with the following:
    - Single cells
    - Multiple cells
    - Ultra-low amounts of DNA (4 pg – 10 ng)
- Compatible with any cell collection method that can deliver single, viable cells/nuclei to a reaction well.

For more information, please visit the ResolveDNA product page (BioSkryb.com/ResolveDNA).

The ResolveDNA Workflow for Whole Exome or Panel Sequencing

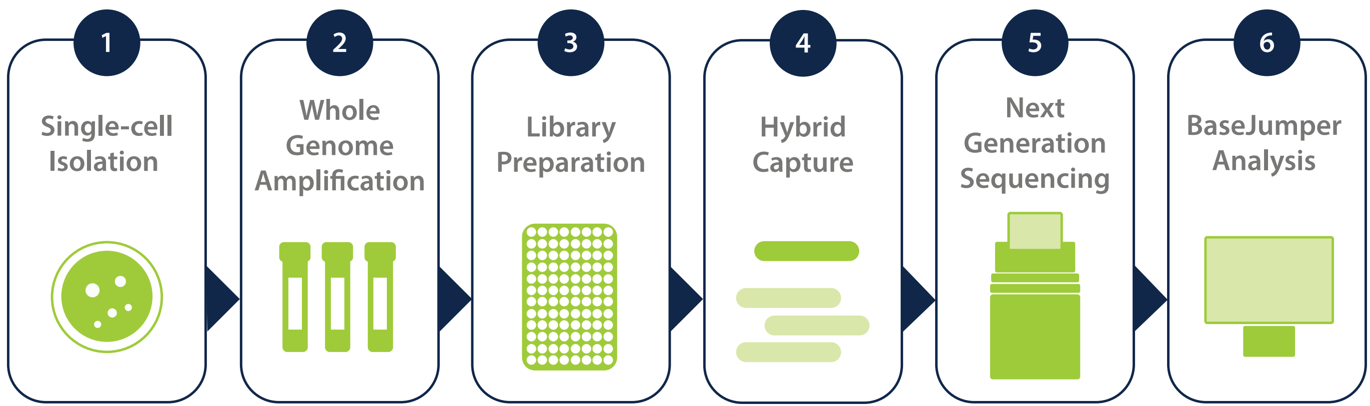


Figure 1. The ResolveDNA single-cell sequencing workflow supporting WES or targeted panel sequencing

I. Single-Cell Isolation

Viable cells of interest are isolated in a microwell plate prior to WGA, either by fluorescence-activated cell sorting (FACS), fluorescence-activated nuclei sorting (FANS), or other means of direct deposition. Cells/nuclei should be delivered into ResolveDNA Cell Buffer or into a dry plate/tube.

II. Whole Genome Amplification

Using the ResolveDNA Whole Genome Single-Cell Core Kit, 96-Well Format and protocol, cells are lysed to release genomic DNA, which undergoes PTA-based WGA to reproducibly achieve uniform and accurate quasi-linear amplification.

III. Library Preparation

The amplified DNA is then used as input into sequencing library preparation using the ResolveDNA Whole Genome Single-Cell Core Kit, 96-Well Format and protocol. Library preparation with the kit and the protocol included in this document allows up to 96 unique, barcoded libraries that are subsequently pooled for multiplex exome or DNA-panel hybrid capture and sequencing on all Illumina® sequencing platforms.

IV. Hybrid Capture

Hybrid capture for whole exomes or targeted panels is performed using a third party kit and protocol.

V. Next-Generation Sequencing

Barcoded and enriched libraries are then sequenced on an Illumina® sequencing platform.

VI. BaseJumper® Analysis

Sequencing data is imported into the BaseJumper Bioinformatics Platform (bioskryb.com/basejumper/) from Illumina BaseSpace® or BioSkryb Genomics AWS S3, powered by Globus. Analytical pipelines can then be automatically queued to provide analysis of genomic variation.

ResolveDNA Amplification Technology

ResolveDNA makes use of a high fidelity DNA polymerase in combination with random primers to amplify DNA present in a sample. During ResolveDNA amplification the polymerase incorporates proprietary nucleic acid bases which result in the termination of the extension of the amplicon. This process truncates the amplification products. These shorter amplicons are not efficiently amplified by the polymerase, limiting daughter amplicon reamplification. As a result, the original (or primary) template is amplified preferentially, increasing genomic coverage and reducing the propagation of base incorporation errors from daughter amplicons. PTA enables the amplification of genomes of single cells with high coverage and uniformity, superior to other WGA methods.[1]

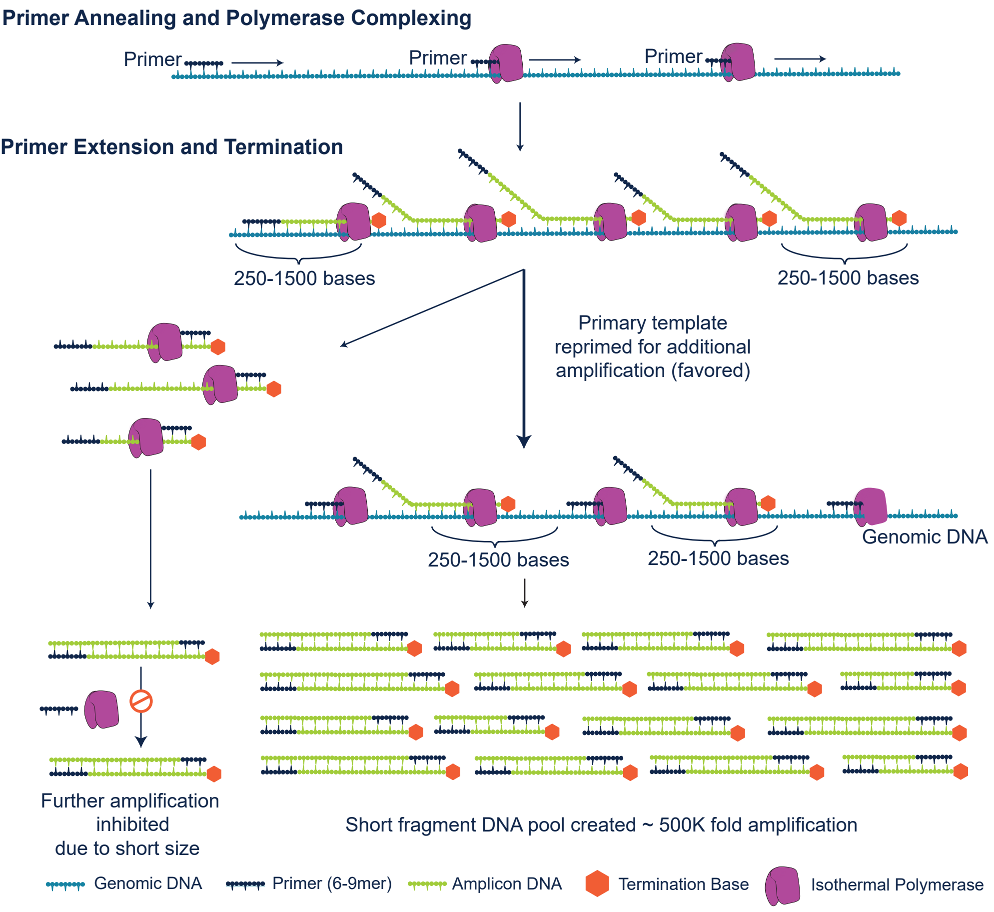


Figure 2. Primary Template-Directed Amplification (PTA). PTA may be performed directly from single cells, multiple cells, or nuclei (collected by FACS, FANS, microfluidic cell separation or other methods), or ultra-low inputs of DNA (4 pg – 10 ng). After cell lysis and genomic DNA denaturation, random primers are annealed. Extension with the included DNA Polymerase and a proprietary nucleotide pool results in amplicons of ~250 bp to 3.5 kb in length. The relatively small size of these amplicons makes them poor targets for subsequent amplification, driving additional priming events to the primary template, thereby limiting the exponential propagation of biases and errors in daughter molecules. In addition, ResolveDNA WGA suppresses the formation of experimental artifacts such as chimeric molecules and non-specific priming. PTA reaction products are double-stranded and may be converted to sequencing libraries for multiplexed sequencing on Illumina® or other platforms using the ResolveDNA Whole Genome Single-Cell Core Kit or other NGS library preparation methods. Click here (https://youtu.be/GNSLMrZPqRM) for a video on the process.

[1] PNAS 2021, Vol. 118, No. 24 e2024176118

Safety Precautions and Use of Personal Protective Equipment

I. Biosafety Hazards

Many samples require handling as biohazards under the Universal Precautions doctrine or other context-specific biosafety protocols.

Wear appropriate Personal Protective Equipment (PPE) such as lab coats, disposable gloves, and safety goggles when working with biohazardous materials.

II. Chemical Hazard.

This kit contains corrosive materials and should be handled only by personnel trained in the safe handling of this type of chemical hazard. 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 ResolveDNA 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 ResolveDNA Whole Genome Single-Cell Core 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

Component Category	Kit Component	Part Number	Cap Color
ResolveDNA Whole Genome Amplification	L1 Reagent	100628	Purple
	L2 Reagent	100581	Yellow
	L3 Reagent	100523	White
	R1 Reagent	100521	Blue
	R2 Reagent	100527	Red
	Control Genomic DNA (50ng/µL)	101155	Gold
	Cell Buffer	100574	Clear
ResolveDNA Universal Library Preparation	LP0B Reagent*	100833	Natural
	LP0E Reagent*	100791	Clear
	LP1B Reagent	100677	Teal
	LP1E Reagent	100680	Purple
	LP2L Reagent	100683	Gold
	LP3A Reagent	100686	N/A, bottle
	LP3P Reagent	100689	Red
Library Adapters	Single Use Library Adapter Set	100940 (or 100941 - 100947)	N/A, plate
ResolveDNA Bead Purification	Resolve Beads	100735	N/A, bottle
	Elution Buffer	100736	N/A, bottle

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

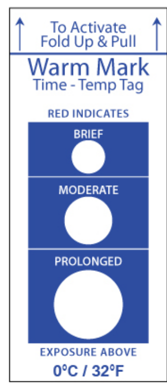
II. Shipping and Storage

Kit components are shipped on dry ice and all reagents and enzymes will be frozen upon arrival, except for the following:

- Resolve Beads and Elution Buffer are shipped in a separate box at ambient temperature and should be stored at 4°C upon receipt.

The labels on the boxes provide essential information including part number, lot number, recommended storage temperatures, and expiration date. 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 the shipment has been kept at the intended temperature during transit (Figure 3). Please contact the BioSkryb Genomics Application Support Team if you have any questions about the interpretation of the Temperature Tags.



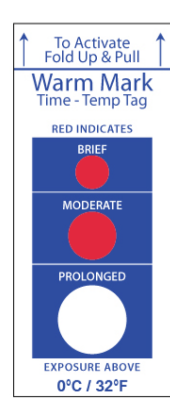


Figure 3. Temperature Indicator Tag. Each dry ice shipment includes a temperature tag designed to indicate exposure above 0°C. If the shipment stays below the target temperature, the windows will remain white.

Additional Equipment, Materials, and Reagents

The following products have been tested with our workflow to provide optimal results. The use of any products not included in this list could result in sub-optimal results. While the listed BioSkryb products are not provided with the kit, interested parties can contact the BioSkryb Genomics Sales Department (sales@bioskryb.com) for assistance in purchasing these products. Please consult the BioSkryb Genomics 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 the protocol.

Products from BioSkryb Genomics		
Product Name	**Company**	**Catalog Number**
ResolveDNA® PTA-Grade Cell Buffer Pack (12X 500 µL)	BioSkryb	100177
ResolveDNA® Cell Buffer Bottle Kit	BioSkryb	100183
Products from Third-Party Suppliers		
Product Name	**Company**	**Catalog Number**
PCR Plate Sealing Film	ThermoFisher	AB-0558
twin.tec 96-well PCR Plate	Eppendorf	0030128648
Magnet PCR Separation Plate	Permagen	MSP750
8-strip 0.2 mL PCR Tubes	General Lab Supplier (GLS)	—
1.5 mL Microcentrifuge Tubes	GLS	—
Single-channel pipette set (P10, P20, P200, P1000) and aerosol barrier tips	Rainin or GLS	—
8-channel pipettes (P20, P200) and appropriate aerosol barrier tips	Rainin or GLS	—
Agilent Tapestation	Agilent	4200
HS D5000 Screentape	Agilent	5067-5592
HS D5000 Reagents	Agilent	5067-5593
HS D1000 Screentape	Agilent	5067-5584
HS D1000 Reagents	Agilent	5067-5585
Fluorometer (Qubit 2-4)	ThermoFisher Scientific	—
High Sensitivity dsDNA Assay kit	ThermoFisher Scientific	Q32854
PCR Plate Thermal Mixer	Eppendorf	—
PCR Plate Spinner	GLS	—
PCR Cooler	Eppendorf	022510541
Thermal Cycler	GLS	—
Absolute (200 proof) Ethanol	GLS	—
RT-PCR Grade Water	GLS	—
1.5 mL Tube Magnet	GLS	—

Best Practices

I. Use of Controls

The following standard control set is recommended for each experiment:

Purpose	Formulation
No Template Control (NTC)	Cell Buffer Alone
High Input Positive Control	1 ng gDNA
Mid Input Positive Control	100 pg gDNA
Low Input Positive Control (Roughly Equivalent to 1-1.5 Cells)	10 pg gDNA

Each control should be run in duplicate to baseline each ResolveDNA experiment. The no template control (NTC) helps detect contamination such as carryover from adjacent wells or the lab environment. This is critical due to the high sensitivity of ResolveDNA to ultra-low levels of nucleic acid in a sample. Bulk gDNA controls help assess the correct execution of the protocol and quantitative accuracy.

II. Protocol Notes

While the individual steps in this protocol are straightforward, specific practices applicable to single-cell work facilitate high-quality outcomes with ResolveDNA whole genome amplification.

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

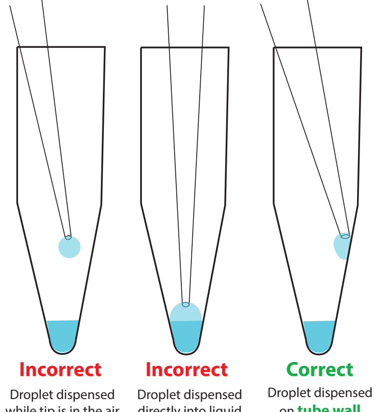


Figure 4. Pipetting Technique

2. **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 in a centrifuge/plate spinner (10 seconds at ~750 X g is sufficient). Use just enough force to combine the added droplet with the material in the bottom of the tube.

   Once the added droplet has been combined with the reaction components in the bottom of the tube, place the reaction plate/tubes in a programmable thermal mixer and gently mix according to the instructions in this protocol. After mixing, briefly spin 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 pipette reagent additions on the side of the tube, avoiding any contact with the material in the bottom of the tube or well, then SEAL-SPIN-MIX-SPIN. After these steps, proceed with any incubation or move on to the next reagent addition per the protocol.

3. **Quantification:** Use a fluorometric method of quantification (such as Qubit) with the amplification

products and sequencing libraries produced with the ResolveDNA Whole Genome Single-Cell Core Kit. The use of spectrophotometric quantification methods (such as Nanodrop) is not recommended.

4. **Plasticware:** Use sterile, DNA-free and nuclease-free polypropylene working stock tubes and containers. Polystyrene tubes and containers are NOT recommended.

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

Sample Selection and Preparation

I. Sample Types Supported

This protocol is generally designed to work with single live mammalian cells, nuclei, or low amounts of DNA input (4 pg – 10 ng). Input can be either single or multiple cells, obtained by common cell collection 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 ResolveDNA protocol or freeze the cells at -80°C for short-term storage. Cells may also be sorted "dry" into empty wells if desired. In cases where cells are dry sorted, it will be necessary to add the appropriate volume of Cell Buffer to each well prior to beginning the ResolveDNA protocol.

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

Please contact the BioSkryb Genomics Application Support Team (TechSupport@BioSkryb.com) should you have any questions on sample compatibility.

II. FACS/FANS

Fluorescence-activated cell sorting (FACS) or fluorescence-activated nuclei sorting (FANS) are currently the most common methods 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 ResolveDNA protocol, cells should be sorted into the ResolveDNA Cell Buffer in tube or plate format. Cells may also be sorted "dry" into empty wells if desired. In cases where cells are dry sorted, it will be necessary to add the appropriate volume of Cell Buffer to each well prior to beginning the protocol. Refer to the BioSkryb Genomics Cell Sorting Protocol for more details.

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 Dispensing

Most methods of live cell isolation are compatible with the ResolveDNA protocol.

ResolveDNA Whole Genome Amplification (WGA) Protocol, 96-Well Format

The ResolveDNA Whole Genome Single-Cell Core Kit with 96-well format allows the processing of single or multiple cells (or nuclei) and low-input DNA samples. This Kit supports manual operations using 96-well PCR plates or 0.2 mL strip tubes. Reagents should be dispensed utilizing a multichannel pipette. The genome amplification takes place in a 2.5 hour isothermal incubation which is carried out in a thermal cycler.

Cells should be placed into the appropriate plate containing BioSkryb Cell Buffer and may be used immediately or frozen at -80°C until needed. Cells may also be sorted "dry" into empty wells if desired. In cases where cells are dry sorted, it will be necessary to add the appropriate volume of Cell Buffer to each well prior to beginning the protocol.

I. Before You Begin

1. Read through the entire protocol and ensure all required equipment, reagents, and consumables are on hand.
2. Input samples must be suspended in 3 µL of **Cell Buffer** ⊗ in a 96-well plate.
3. The ResolveDNA WGA process should be carried out in a DNA-free, pre-amplification workspace or PCR hood enclosure to avoid the possible introduction of exogenous DNA from the operator or the lab environment.
   - Including a no-template control allows for detection of DNA carryover in reactions.
4. Run positive control reactions at a range of input concentrations. See "Use of Controls" in the Best Practices section for an in-depth discussion of this critical topic.
   - Failure to run positive and negative controls can make it difficult to interpret results.
5. Use a vortex mixer to thoroughly mix all reagents after thawing except **R2** ●.
   - DO NOT use traditional vortex mixers on multiwell plates containing cells, lysates, etc. during the protocol. Always mix multiwell plates in a thermal plate mixer. (See "Protocol Notes: Gentle and Thorough Mixing" in the "Best Practices" section for an in-depth discussion of this topic).
6. Always keep reactions and reagents on ice unless otherwise instructed.
   - Lab cooling blocks (such as the Eppendorf PCR Cooler) designed to keep reactions chilled during handling are recommended.
7. 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.
8. Program thermal cyclers with a 96-well block installed to run the DNA Amplification program (Table 1).

Table 1. DNA Amplification (lid temperature 70°C)

Step	Temperature	Time
Hold 1	30°C	2.5 hours
Hold 2	65°C	3 minutes
Hold 3	4°C	∞
Total Time	-	**~2.6 hours**

II. ResolveDNA WGA Procedure

1. Retrieve the ResolveDNA Whole Genome Amplification components from -20°C storage.
2. Place **L2** ●, **Control gDNA** ●, and **Cell Buffer** ⊗ at room temperature to thaw for 30 minutes to 1 hour.
3. Place **L1** ●, **L3** ○, and **R1** ● on ice to thaw for 30 minutes to 1 hour.
4. **R2** ● should be left in -20°C storage until needed.
5. Once the reagents from steps 2 and 3 have thawed, vortex for 5 seconds, briefly spin down, and place on ice.

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

6. Prepare a 10 ng/µL gDNA stock by adding 2 µL of **Control gDNA** ● to 8 µL of **Cell Buffer** ⊗ in a labeled microcentrifuge tube.
7. Vortex the 10 ng/µL **Control gDNA** ● for 5 seconds, briefly spin down, and place on ice.

   (Optional) Verify that the 10 ng/µL **Control gDNA** ● stock is at the intended concentration using a Qubit fluorometer.

   Note: If the concentration deviates from the expected concentration 10 ng/µL by more than 10%, modify the dilution factor in subsequent dilutions to achieve the desired concentration.

8. Dilute the 10 ng/µL gDNA stock in **Cell Buffer** ⊗ to create 1 ng/µL, 100 pg/µL and 10 pg/µL stocks. This can be done via serial dilution and manual addition to the reaction plate, or by in situ dilution using an automated liquid handler capable of accurate nanoliter-scale pipetting.
9. Place the plate containing samples on ice.

   If cells were stored at -80°C, thaw the cells on ice for 5 minutes, spin for 10 seconds, and place on ice.

   If cells are fresh, maintain on ice and proceed with amplification promptly.

10. If cells are suspended in less than 3 µL, add **Cell Buffer** ⊗ to bring them up to 3 µL total volume.
11. Dispense DNA controls. Ensure appropriate control samples are added to the reaction plate in 3 µL of **Cell Buffer** ⊗.

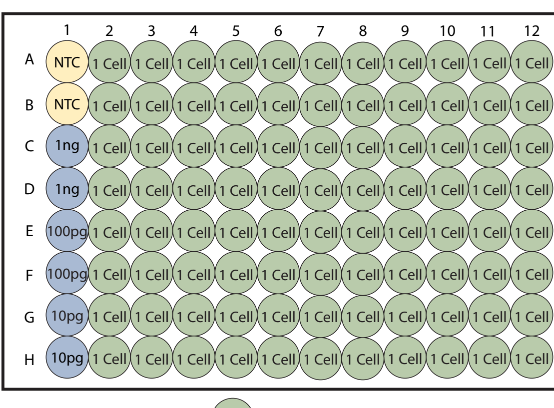


Figure 5. Example 96-well Plate Experimental Layout. The plate map illustrates a typical reaction setup, including multiple NTC, 1 ng gDNA, 100 pg gDNA and 10 pg gDNA controls added into a 96-well plate containing sorted single cells. 3 µL of Cell Buffer is dispensed into the wells in columns 2 through 12. Cells are then sorted into these wells (FACS/FANS etc.) Prior to processing, 1 µL of the control samples are added to column 1, and 2 µL Cell Buffer added to bring the total volume to 3 µL.

12. Prepare **Lysis Mix** by combining the following reagents in a microcentrifuge tube (Table 2).

Table 2. Volume of Components in Lysis Mix.

Component	Volume per Reaction (µL)	Volume per 96 Reactions (µL)*	Volume per _ Reactions (µL)
L1 Reagent	1.68	210	
L2 Reagent	0.12	15	
L3 Reagent	1.2	150	
Total Volume	**3.0**	**375**	
			*30% overage included

13. Vortex 10 seconds to mix, briefly spin down, and place on ice.
14. Using a P20 pipette, add 3 µL of **Lysis Mix** to each well.

    If processing more than 16 reactions, divide the **Lysis Mix** equally across the wells of an 8-strip PCR tube to act as a reservoir and use an 8-channel P20 pipette to minimize time required for reagent additions.

15. Seal and spin down for 10 seconds to combine components.
16. Incubate in the thermal mixer, mixing at room temperature for 20 minutes at 1,400 rpm.

During incubation complete the following steps:

17. Start the DNA Amplification protocol (see Table 1) on the thermal cycler and allow the block to reach the amplification reaction set point of 30°C. Pause the thermal cycler. Prepare the **Reaction Mix** on ice by combining the components in the following order (Table 3).

Table 3. Volume of Components in Reaction Mix

Component	Volume per Reaction (µL)	Volume per 96 Reactions (µL)*	Volume per _ Reactions (µL)
R1 Reagent	5.4	675	
R2 Reagent	0.6	75	
Total Volume	**6.0**	**750**	
			*30% overage included

18. Pipette the **Reaction Mix** up and down 10 times with the pipette set to 50% of the total volume to mix, briefly spin down, and place on ice.

    Note: Avoid creating air bubbles while pipette mixing.

    Once incubation is complete, continue with the following steps:

19. Remove plate from thermal mixer, spin down for 10 seconds, and place on ice.
20. Using a P20 pipette, add 6 µL of the **Reaction Mix** to each well.

    If processing more than 16 reactions, divide the **Reaction Mix** equally across the wells of an 8-strip PCR tube to act as a reservoir and use an 8-channel P20 pipette to minimize time required for reagent additions.

21. Seal and spin down for 10 seconds.
22. In the thermal mixer, mix at room temperature for 1 minute at 1000 rpm.
23. Spin down for 10 seconds and place on ice.

    Note: Keep the plate on ice until the thermal cycler has reached 30°C.

24. Load the plate and unpause the thermal cycler program.
25. After the program is complete, remove the plate, spin down for 10 seconds, and place on ice.
26. Continue with the Quality Control procedure or store samples overnight at -20°C.

    Safe Stop: Samples may be stored overnight at -20°C before continuing.

Post WGA Quality Control Checkpoint

1. To assess DNA yield, dilute each reaction by adding **Elution Buffer** to a total volume of 40 µL.
2. Add 2 µL of diluted reaction mix to 198 µL Qubit reagent and measure the concentration per manufacturer's instructions.
3. Prepare a 2 ng/µL dilution in a fresh PCR plate by pipetting amplified DNA samples into Elution Buffer, seal the plate, vortex briefly, and spin down.
4. Determine fragment size distribution by running 2 µL of each 2 ng/µL diluted sample using a Tapestation HS D5000 Screentape or other fragment analysis instrument per manufacturer's instructions.
5. Refer to Appendix A for example quality control data.
6. Proceed to the ResolveDNA Universal Library Preparation workflow.

ResolveDNA Universal Library Preparation (for downstream hybrid capture and whole exome or targeted panel sequencing)

The ResolveDNA Universal Library Preparation protocol supports multichannel pipette mediated processing of up to 96 samples. The volume overages recommend for master mixes in this protocol are to facilitate multichannel pipetting from an 8-strip tube.

> ⓘ The following library preparation protocol **creates 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.

I. Before You Begin

1. Read through the entire protocol and ensure all required equipment, reagents, and consumables are on hand.
2. 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)
3. 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.

4. 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.

II. Enzymatic Fragmentation, End Repair, and A-Tailing (FERAT)

1. Add 20 ng of each ResolveDNA whole genome amplified (WGA) product 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 ResolveDNA WGA product. Alternatively, an average concentration can be calculated by obtaining Qubit measurements for a random sampling of ResolveDNA WGA reactions. Post-capture heterogeneity may increase when using an averaged concentration.

2. Vortex **LP1B Reagent** ● for 5 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
3. 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.
4. Program a thermal cycler to run the FERAT-HC Program (Table 4). Initiate the run to cool the block to 4°C and pause the program once cooled.

Table 4. 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**

5. Prepare the **FERAT Master Mix** in a 1.5 mL Eppendorf tube on ice by adding the components in Table 5.

Table 5. FERAT Master Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	96 Reactions*
LP1B Reagent	0.8 µL	92.2 µL
LP1E Reagent	1.2 µL	138.2 µL
Elution Buffer	2.0 µL	230.4 µL
Total Volume	**4.0 µL**	**460.8 µL**
		*20% overage included

6. 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.

7. Add 4 µL of the **FERAT Master Mix** to each well while the plate is on a PCR Cooler.
8. Seal the plate and briefly spin to get the liquid to the bottoms of the wells.
9. Vortex the plate at medium speed to homogenize the reaction.
10. 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.

11. Place the plate into the preheated thermal cycler and initiate the FERAT-HC Program (Table 4).
12. While the thermal cycler is running, remove **Resolve Beads** from storage and allow to equilibrate to room temperature for at least 30 minutes.
13. While the thermal cycler is running, thaw the **Single Use Library Adapter Set** plate on ice.

III. 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 6).

Table 6. 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 cycler and initiate the LIG Program (Table 6).
9. After completion of the LIG Program, proceed immediately to Library Amplification.

IV. Library Amplification

1. Initiate the LIB-AMP program on a thermal cycler (Table 7). Allow the thermal cycler to warm up to temperature and pause the program.

Table 7. LIB-AMP program (lid temperature 105°C)

Step	Temperature	Time	Cycles
Hold 1, Hot Start	98°C	45 seconds	1
Hold 2, Denaturation	98°C	15 seconds	8
Hold 3, Annealing	60°C	30 seconds	
Hold 4, Extension	72°C	45 seconds	
Hold 5, Final Extension	72°C	60 seconds	1
Hold 6	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 8 in a new tube.

Table 8. Amplification Master Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	96 Reactions*
LP3A Reagent	18 µL	1901 µL
LP3P Reagent 🔴	2 µL	211 µL
Total Volume	**20 µL**	**2112 µL**
		*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 LIB-AMP program in Table 7.
8. After completion of the 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.

V. 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 cleanup with 1X the sample volume of Resolve Beads (e.g. 40 µL of library volume plus 40 µL of bead volume, performed twice)

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 9).

    Note: Use caution to avoid spills.

Table 9. 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 samples at -20°C.

Post Library Amplification Quality Control Checkpoint

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.

Appendix A: Post WGA Quality Control Example Data

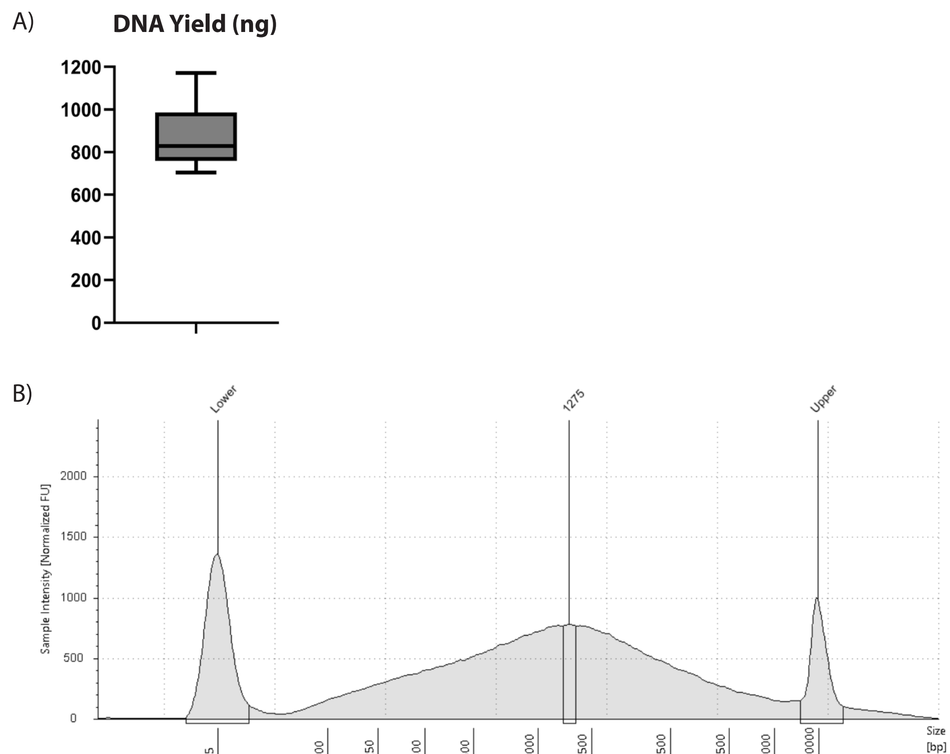


Figure 6. Examples of Total Amplification Yields and Fragment Size Distribution.

A) DNA amplification yield for ResolveDNA WGA. Average yield is over 800 ng from single human cells.

B) The electropherogram represents a sample amplified using ResolveDNA WGA, which has been normalized to 2 ng/μL and run on a Tapestation using the D5000 HS Screentape. Average fragment size in this sample is 1275 bp, which is typical.

Appendix B: Post Library Amplification Quality Control Example Data

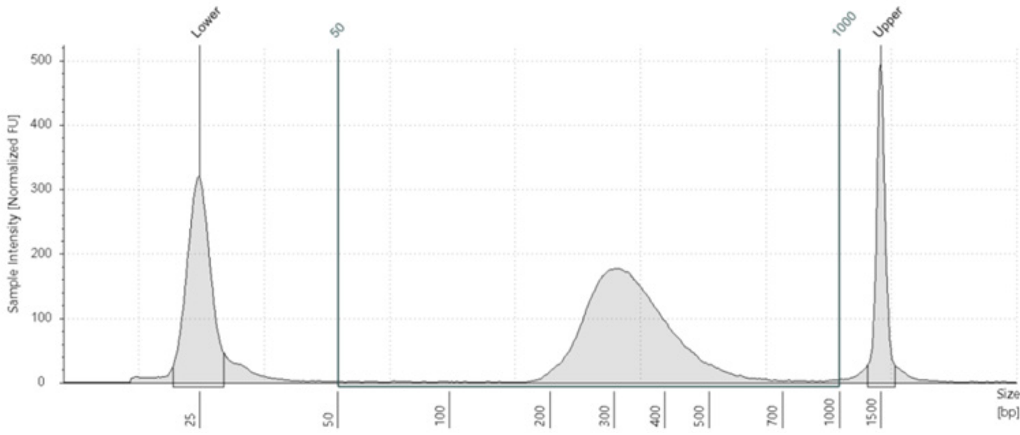


Figure 7. Example DNA Library Fragment Size Distribution.

The electropherogram represents a sample after pooled library preparation as described in this document, which has been normalized to 2 ng/μL and run on a Tapestation using the HS D1000 ScreenTape. Average expected library size is around 325-375 bp when using the library preparation protocol described in this document.

Appendix C: Hybrid Capture Recommendations for ResolveDNA 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 ResolveDNA 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 ResolveDNA Whole Genome 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 ResolveDNA 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 ResolveDNA 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.

Data Analysis using BioSkryb BaseJumper Bioinformatics Platform

ResolveDNA 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 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.

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 Applications Support Team (techsupport@bioskryb.com).

For more information please contact:

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