



ResolveDNA® Whole Genome Single-Cell Core Kit

Protocol to Prepare Library Pool for Exome Hybrid Capture

User Guide

384-Well Format

For Use with the Uno Single Cell Dispenser™, Provided by Tecan

Also compatible with the Duo Digital Dispenser™, Provided by Tecan

ResolveDNA® Whole Genome Single-Cell Core Kit, 384-Well Format Protocol to Prepare Library Pools for Exome Hybrid Capture

Product Description

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

The controlled 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 sequencing-ready libraries in under 8 hours. This kit is configured for working with 384 reactions and contains one adapter set with 384 unique dual indexes (UDIs) to generate 384 libraries for sequencing.

Key features and benefits include the following:

- o A simple, user-friendly workflow that requires less than 2.5-hours hands-on time and under 8 hours total run time.
- o Specific amplification of single cell genomes recovering >97% of the human genome.
- o Excellent allelic balance enables highly sensitive and specific assessment of single nucleotide variation (SNV).
- o Compatible with the following:
 - o Single cells
 - o Multiple cells
 - o Ultra-low amounts of DNA (4 pg – 10 ng)
- o 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](https://www.BioSkryb.com/ResolveDNA) (BioSkryb.com/ResolveDNA).

The ResolveDNA Workflow

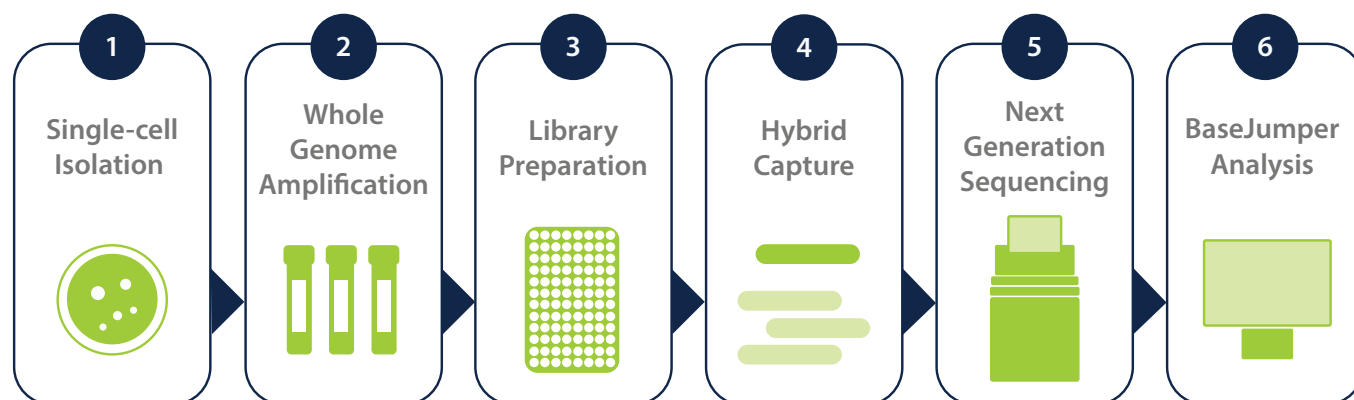


Figure 1. The ResolveDNA single-cell sequencing workflow supporting WES or targeted panel sequencing

I. Single-Cell Isolation

Viable cells of interest are isolated in a microwell plate prior to WGA, either by fluorescence-activated cell sorting (FACS), utilizing the Uno or Duo instruments, or other means of direct deposition. Cells 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, 384 Reactions 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, 384 Reactions and protocol. Library preparation with the kit and the protocol included in this document allows up to 384 unique, barcoded libraries that are subsequently pooled for 96-plex 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](https://bioskryb.com/basejumper/) (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

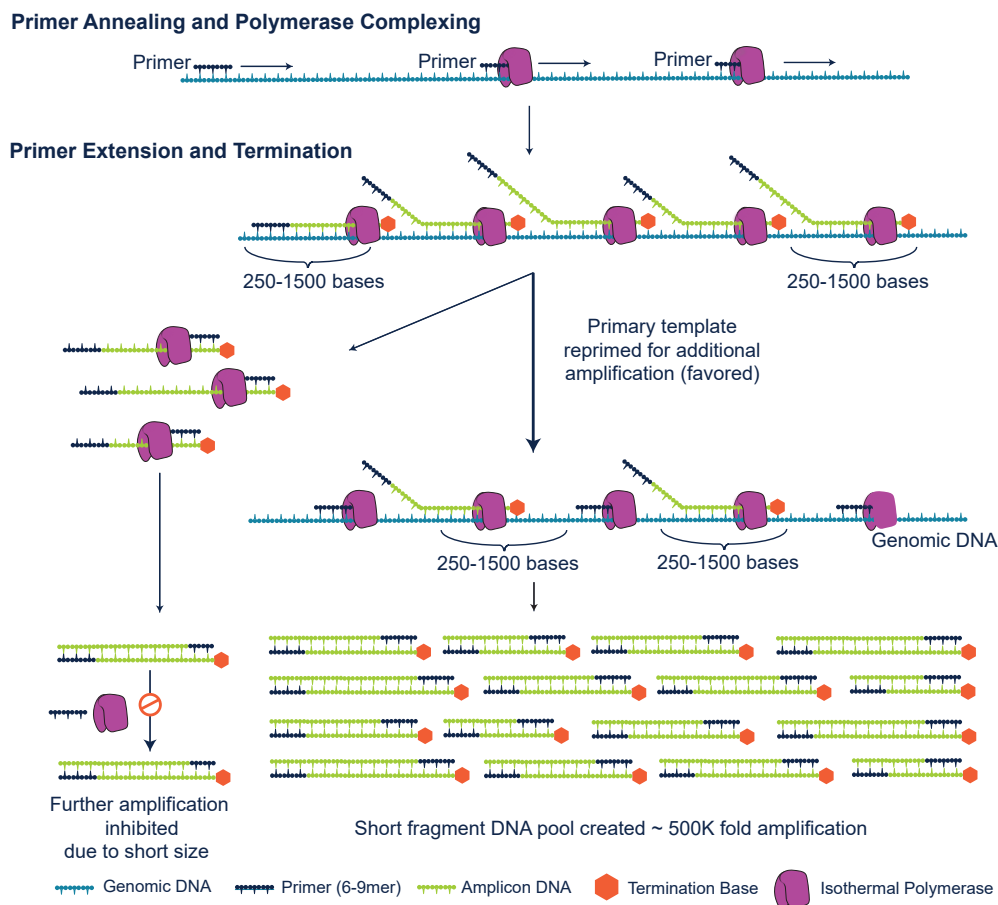


Figure 2. Primary Template-Directed Amplification (PTA). PTA may be performed directly from single cells, multiple cells, or nuclei (collected by FACS, FANS, microfluidic cell separation or other methods), or ultra-low inputs of DNA (4 pg – 10 ng). After cell lysis and genomic DNA denaturation, random 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) (https://youtu.be/GNSLMrZPqRM) for a video on the process.

¹PNAS 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](mailto:TechSupport@BioSkryb.com) (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. The Uno Single Cell Dispenser, Duo Digital Dispenser, and D300e Digital Dispenser are for **research use only**. Not for use in diagnostic procedures.

This user guide focuses on the use of the Uno Single Cell Dispenser, Duo Digital Dispenser, or D300e Digital Dispenser to aid in the execution of the ResolveDNA Whole Genome Single-Cell Core Kit with 384 Reactions.

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	LPOB Reagent*	100795	○ Natural
	LPOE Reagent*	100791	⊗ Clear
	LP1B Reagent	101010	● Teal
	LP1E Reagent	101013	● Purple
	LP2B Reagent	100802	● Blue
	LP2E Reagent	100799	● Gold
	LP3A Reagent	100778	N/A, bottle
	LP3P Reagent	100781	● Red
Library Adapters	Single Use Library Adapter Set	100948, 100949, 100950, or 100951	N/A, plate
ResolveDNA Bead Purification	Resolve Beads	100735	N/A, bottle
	Elution Buffer	100736	N/A, bottle

*LPOB and LPOE 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 temperature of the contents of the box, and the expiration date. When stored as directed, the kit will perform to specifications 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 [BioSkrbyb Genomics Application Support Team](#) if you have any questions about the interpretation of the Temperature Tags.

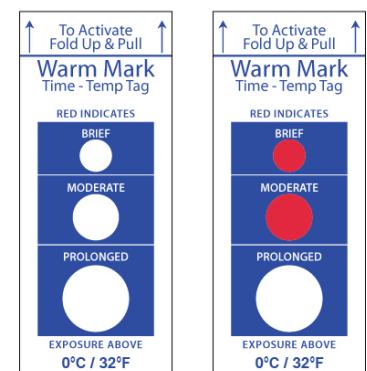


Figure 3. Temperature Indicator Tag

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](mailto:sales@bioskryb.com) (sales@bioskryb.com) for assistance in purchasing these products. Please consult the [BioSkryb Genomics Application Support Team](mailto:TechSupport@BioSkryb.com) (TechSupport@BioSkryb.com) if you have questions about the suitability of any alternative materials or equipment to be used in conjunction with the protocol.

- ① **Important:** This protocol was written for use with the Uno Single Cell Dispenser, the Duo Digital Dispenser, or the D300e Digital Dispenser instruments provided by Tecan, which are automated liquid handlers capable of high accuracy delivery of sub-microliter volumes into a 384-well plate.
- ① **Important:** Users should ensure thermal cycler compatibility with the skirted plates required for the digital dispensers before beginning the protocol.

The [Uno, Duo, and D300e instruments](https://shop.tecan.com/us/en/c/DigitalDispensers) (https://shop.tecan.com/us/en/c/DigitalDispensers) and [consumables](https://shop.tecan.com/us/en/c/SmartConsumables) (https://shop.tecan.com/us/en/c/SmartConsumables) can be purchased from Tecan.

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 Sold by Tecan		
Uno Single Cell Dispenser	HP (sold by Tecan)	30230840
Duo Digital Dispenser	HP (sold by Tecan)	30253250
D300e Digital Dispenser	HP (sold by Tecan)	30100152
C1a Dispensehead Cassettes (Uno)	HP (sold by Tecan)	30230841
D1 Dispensehead Cassettes (Uno)	HP (sold by Tecan)	30230843
T1 Dispensehead Cassettes (Uno)	HP (sold by Tecan)	30230842
C1a+ Dispensehead Cassettes (Duo)	HP (sold by Tecan)	30253251
D4+ Dispensehead Cassettes (D300e/Duo)	HP (sold by Tecan)	30253253
T8+ Dispensehead Cassettes (D300e/Duo)	HP (sold by Tecan)	30253252
Products from Third-Party Suppliers		
Product Name	Company	Catalog Number
384-Well Post Magnet Plate	Permagen	P384
1.5 mL Tube Magnet	General Lab Supplier (GLS)	
VP 74116G Heat Transfer Plate	V & P Scientific, Inc.	—
PCR Plate Sealing Film	ThermoFisher	AB-0558
twin.tec 384-well PCR Plate	Eppendorf	0030128508
8-strip 0.2 mL PCR Tubes	GLS	—

Products from Third-Party Suppliers, Continued

Product Name	Company	Catalog Number
1.5 mL Microcentrifuge Tubes	GLS	—
Single-channel pipet set (P10, P20, P200, P1000) and aerosol barrier tips	Rainin or GLS	—
8-channel pipets (P20, P200) and aerosol barrier tips	Rainin or GLS	—
Agilent TapeStation	Agilent	4200
HS D5000 Screentape	Agilent	5067-5592
HS D5000 Reagents	Agilent	5067-5593
Fluorometer (Qubit 2-4)	ThermoFisher Scientific	—
High Sensitivity dsDNA Assay kit	ThermoFisher Scientific	Q32854
PCR Plate Thermal Mixer	Eppendorf	—
PCR Plate Spinner	GLS	—
Thermal Cycler	GLS	—
Absolute (200 proof) Ethanol	GLS	—
RT-PCR Grade Water	GLS	—

Best Practices

I. Use of Controls

The protocol scripts for dispensing fluids include eight wells of controls useful for evaluating amplification quality:

Well	Purpose	Formulation
A1	No Template Control (NTC)	Cell Buffer Alone
B1	High Input Positive Control (~100 cell equivalents)	600 pg DNA
C1	Mid Input Positive Control (~10 cell equivalents)	60 pg DNA
D1	Low Input Positive Control (~2 cell equivalents)	12 pg DNA
E1	Low Input Positive Control (~2 cell equivalents)	12 pg DNA
F1	Low Input Positive Control (~1 cell equivalents)	6 pg DNA
G1	Low Input Positive Control (~0.2 cell equivalents)	1 pg DNA
H1	No Template Control (NTC)	Cell Buffer Alone

BioSkryb control material is comprised of bulk-isolated human nucleic acids (DNA) from NIST benchmark HG002 (<https://www.nist.gov/programs-projects/genome-bottle>). Use of this material as indicated herein enables customers to both confirm proper execution of the workflow as well as analytically confirm the genomic performance of the assay. In addition to benchmarked genomic values provided by NIST, BioSkryb has extensively tested the material. It is strongly recommended to include these controls, and a negative no template control (NTC) well, with each experimental run for troubleshooting.

The 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

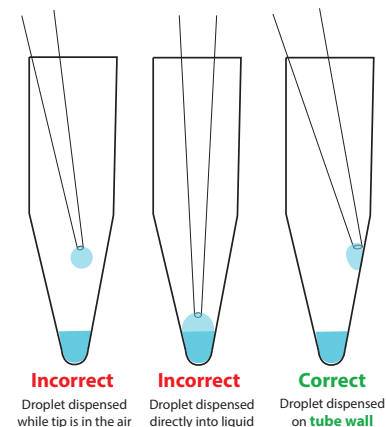
controls help assess the correct execution of the protocol and quantitative accuracy.

II. Protocol Notes

While the individual steps in this protocol are straightforward, specific practices applicable to single-cell work facilitate high-quality outcomes with the ResolveDNA Whole Genome Single-Cell Core Kit.

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

- 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 pipet 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 pipet tip is allowed to come into contact with the reaction mix.




- 2. Gentle and Thorough Mixing:** Once the reagent has been added to the well, 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 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 well.

Figure 4. Pipetting Technique

Once the added droplet has been combined with the reaction components in the bottom of the plate/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 wells.

- 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.
- 5. Digital Dispenser Best Practices**

The D300e Digital Dispenser can be easily substituted with the Uno Single Cell Dispenser or Duo Digital Dispenser.

 **Note:** When using the DispenseControl software, navigate to the file menu and ensure the appropriate device (e.g. Uno, Duo, or D300e) is selected after opening each script.

Before beginning the workflow, align the plate on the Uno. For further information, refer to the user manual found within the DispenseControl (formerly UnoControl) software. Software can be downloaded for [free](http://www.tecan.com/DispenseControl) (www.tecan.com/DispenseControl).

Scripts provided can be modified to customize the number of wells that contain cells by one of the following options

To remove wells:

- Highlight the wells that do not contain cells (non-active wells), right click and select “Remove Fluid”.
- Highlight non-active wells, hit “delete” on the keyboard, select the fluid you would like removed from the well using the drop-down menu, and select “OK”.

To add wells:

- Highlight active wells in the software, right click and select “Copy”. Highlight wells that need to be activated, right click and select “Paste.”
- Highlight wells that need to be activated, select “Set Value”, ensure the correct fluid is selected, add the correct dispense value for the corresponding fluid, select “Apply”, then select “Done.”

Ensure the number of cassettes is sufficient for each run by running the scripts in simulation mode. Consider having extra cassettes on-hand. A list of the minimum required Uno D1 and T1 cassettes for a full 384 plate with cell dispensing is found below:

Protocol Step	D1 cassettes	T1 cassettes
Control gDNA distribution	-	1
Cell Buffer	2	-
Lysis Mix	2	-
Reaction Mix	5	-
FERAT	2	-
Ligation	2	-
Total	13	1

If the Uno is used to deposit cells into a 384-well plate:

- The C1a cell cassette should be warmed up to room temperature before use. Otherwise, the cassette should be stored at 4°C.
- Use a cell concentration of 100–200 cells/μL. Refer to the “Culture Preparation for Dispensing Mammalian Cells” on the Uno Single Cell Dispenser™ [product page](https://lifesciences.tecan.com/products/liquid_handling_and_automation/uno-single-cell-dispenser) (https://lifesciences.tecan.com/products/liquid_handling_and_automation/uno-single-cell-dispenser) for best practices.

Correct pipetting of the cassette is important for proper dispensing. Refer to Tecan’s website, [question 3](https://www.tecan.com/knowledge-portal/d300e-does-not-dispense-correctly-what-to-do) (https://www.tecan.com/knowledge-portal/d300e-does-not-dispense-correctly-what-to-do), for best practices.

It is important to use an aluminum cold block that stays cold for the duration of the reagent dispense. This can be done by storing the aluminum cold block at 4°C or subsequently the aluminum cold block can be stored on ice when not in use. If stored on ice, ensure all ice and liquid is removed from the bottom of the aluminum cold block prior to placing on the Uno dispenser. Moisture on the bottom of the aluminum cold block may cause the plate to shift during dispensing.

Once the aluminum cold block is wiped off, add the plate to the aluminum cold block and remove the plate seal before loading onto the Uno instrument. Removal of the plate seal while the plate is

on the Uno dispenser may cause misalignment of the instrument if too much pressure is used to remove the seal.

The protocol recommends preparing at least 20% overage for each reaction mix. This volume is different than the volume requested by the Uno dispense program; however, the overage suggested is sufficient to run each of the protocols.

If more than one cassette is needed per step and to ensure enough reaction mix volume for all active wells, collect the leftover volume from the used cassette, add back to the original reaction mix tube and distribute into the next cassette.

When loading the Uno dispenser, add the reagent mix to the cassette, wait 30 seconds, then click run. This is critical for proper dispense into all wells for each reaction mix and should be done for EACH cassette.

Seal and spin plates after Uno dispense to ensure all liquid is in the bottom of the wells.

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 μ L 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](mailto:TechSupport@BioSkryb.com) (TechSupport@BioSkryb.com) should you have any questions on sample compatibility.

II. Uno Single Cell Dispenser or Duo Digital Dispenser

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

III. FACS

Fluorescence-activated cell sorting (FACS) 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. Refer to the BioSkryb Genomics Cell Sorting Protocol for more details.

IV. Spatial Cell Picking Technology

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

V. Other Methods of Single Cell Dispensing

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

ResolveDNA Whole Genome Amplification (WGA) Protocol, 384 Reactions

The ResolveDNA Whole Genome Single-Cell Core Kit with 384 reactions allows the processing of single or multiple cells (or nuclei) and low-input DNA samples. This Kit supports an automated liquid handler mediated high-throughput protocol allowing the parallel processing of up to 384 samples. The genome amplification takes place in a 2.5 hour isothermal incubation which is carried out in a thermal cycler.

The 384-reaction protocol must be executed using an automated liquid handler capable of high accuracy delivery of sub-microliter volumes into a 384-well plate. This workflow has been optimized on the Uno and D300e instruments. Please refer to the respective instrument and software documentation for general operation of each instrument. To obtain this documentation please contact Tecan. The Post WGA Quality Control Checkpoint is performed using manual multichannel pipetting. Please refer to Table 1 for a summary of reagent dispensing methods for WGA and Quality Control steps.




BioSkryb provides two protocol script files, “BioSkryb ResolveDNA Step 1” and “BioSkryb ResolveDNA Step 2”, for execution of the WGA workflow. Refer to the “Before You Begin” section below for information on obtaining the scripts.

Cells should be placed into a 384-well plate containing 1 μ L 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, the protocol script is set up to allow the addition of 1 μ L **Cell Buffer** to each well during the run.

Table 1. Summary of Dispensing Methods for WGA and Quality Control Steps

Workflow Step(s)	Reagent Dispensing Method
BioSkryb ResolveDNA Step 1 (Cell Buffer, Lysis Mix, gDNA stock)	Uno
BioSkryb ResolveDNA Step 2 (Reaction Mix)	Uno
Post WGA Quality Control Checkpoint	Manual multichannel pipetting

I. Before You Begin

1. Read through the entire protocol and ensure all required equipment, reagents, and consumables are on hand.
2. Contact the [BioSkryb Genomics Application Support Team](#) to obtain the required protocol script files.
 -  **Note:** When using the DispenseControl software, navigate to the file menu and ensure the appropriate device (e.g. Uno, Duo, or D300e) is selected after opening each script.
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.
 -  **Note:** 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.
 -  **Note:** 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** ●.
 -  **Note:** 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.
 -  **Note:** Lab cooling blocks designed to keep reactions chilled during handling are recommended (such as a [V&P Scientific Heat Transfer Plate](#) that has been pre-chilled).
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 384-well block installed to run the DNA Amplification program (Table 2).

Table 2. 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 Kit from -20°C storage.
2. Place **L2** ● at room temperature to thaw for 30 minutes to 1 hour.
3. Place **L1** ●, **L3** ○, **R1** ●, **Control gDNA** ●, and **Cell Buffer** ⊗ 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 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 gDNA stock for 5 seconds, briefly spin down, and place on ice.
 -  **(Optional)** Verify that the 10 ng/μL 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. 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.
9. Open the DispenseControl (or UnoControl) Software.
 10. Open the script file “**BioSkrzyb ResolveDNA Step 1.**”

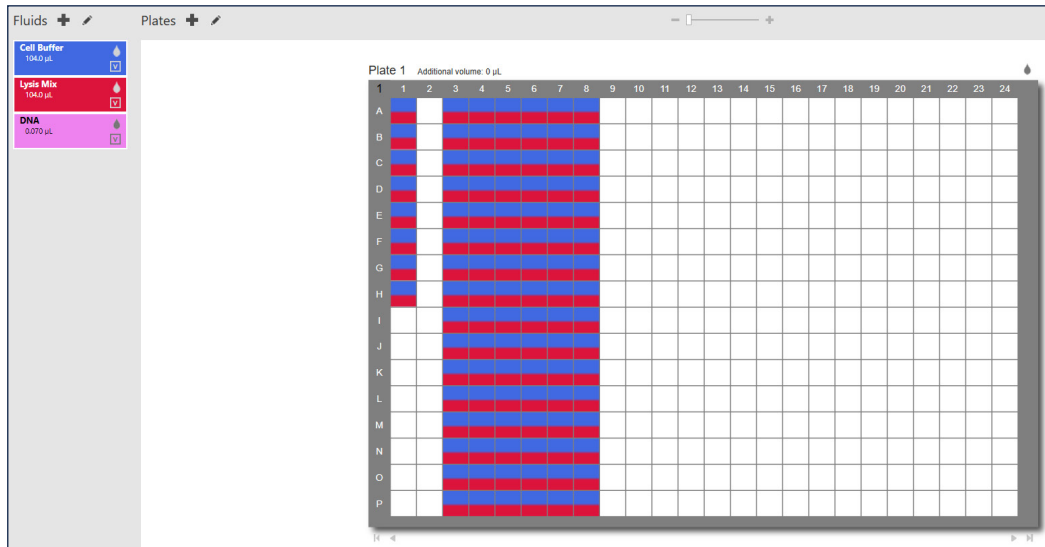


Figure 5. BioSkrzyb ResolveDNA Step 1 template. The fluid classes include Cell Buffer, Lysis Mix, and gDNA stock. The first column has been pre-loaded to create a set of control DNA reactions from the 10 ng/µL control DNA stock (from A–H: NTC, 600 pg, 60 pg, 12 pg, 12 pg, 6 pg, 1 pg, NTC). Single cell wells are listed in columns 3–8. Copy and paste single cell wells to cover all reaction wells up to the full plate. For wells that already contain 1 µL Cell Buffer, use the “Remove Fluid” setting to define that no additional Cell Buffer will be dispensed.

11. Do not change the settings of column 1 to enable control reactions. Ensure all wells in which reactions will be run indicate the dispensing of 1000 nL of **Cell Buffer** and 1000 nL **Lysis Mix** by cutting and pasting to make the colored areas on the plate map match the sample layout.
 - ① **Important:** If cells are already suspended in 1 µL **Cell Buffer**, select all non-control wells, right-click and choose “Remove Fluid”, and choose from the drop-down menu “**Cell Buffer**”. This will omit the **Cell Buffer** addition step in the script. This step is only necessary for “dry” sorted plates.
12. Save the file with a unique file name to preserve the integrity of the original script file.
13. Click “Run” in the top left to reach the protocol “Pre-Run Summary” page with cassette and fluid volume requirements. Use this information when creating the **Lysis Mix**.

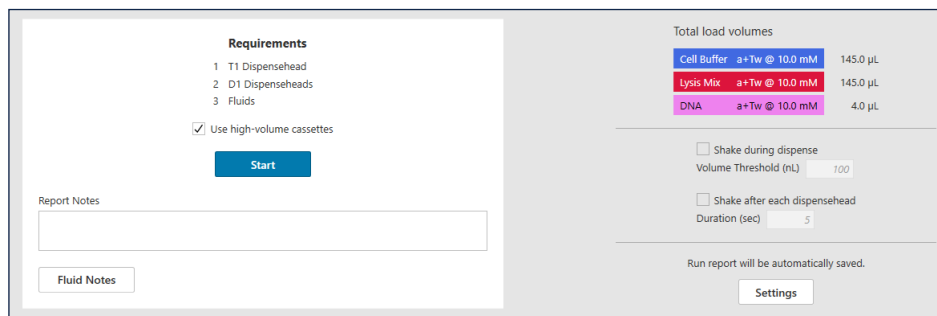


Figure 6. The Pre-Run Summary Screen. Provides a summary of the number and types of dispensehead cassettes necessary as well as the fluid loading volumes required for complete dispensing.

14. Prepare Lysis Mix by combining the following reagents in a microcentrifuge tube (Table 3).






-  **Note:** If running fewer than 384 reactions, use the Volume per Reaction to calculate the required reagent volumes and add 30% overage as noted. If the volume calculated is less than the load volume requested on the pre-run summary, calculate an increased reaction number to create the requested lysis mix.

Table 3. Lysis Mix

Component	Volume per Reaction (µL)	Volume per 384 Reactions (µL)*
L1 Reagent 	0.56	280
L2 Reagent 	0.04	20
L3 Reagent 	0.4	200
Total Volume	1.0	500
*30% overage included		

15. Vortex the **Lysis Mix** for 10 seconds to mix, briefly spin down, and place on ice.
16. Remove the plate seal from the plate and load the plate into the plate tray on the instrument.
17. Proceed from the Pre-Run Summary page by clicking “Start”. Follow the prompts from the software to insert dispensehead cassettes and fluids, confirming your actions by clicking the blue button (Filled, Loaded, etc.). **Cell Buffer**  will be added first (if not already in wells), then **Lysis Mix**, then 10 ng/µL gDNA stock.

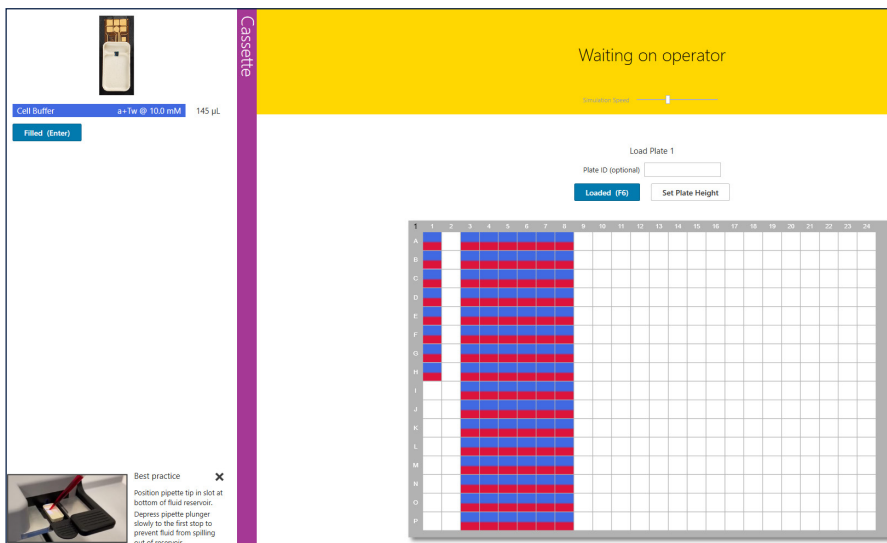


Figure 7. The Run Window Screen. Indicates steps in the operational workflow. Cassettes and fluids are requested on the left panel, plate loading is requested in the center. Dispensing is then reported in real time as the script proceeds. As additional fluids or cassettes are required, the software will prompt for user action. Make sure to follow these prompts carefully.

18. After the script has completed, seal the plate and spin down for 10 seconds to combine components.
19. Incubate in the thermal mixer, mixing at room temperature for 20 minutes at 1,400 rpm.

During incubation complete the following steps:

20. Start the DNA Amplification protocol (see Table 2) on the thermal cycler and allow the block to reach the amplification reaction set point of 30°C. Pause the thermal cycler.
21. Prepare the **Reaction Mix** on ice by combining the components in the following order (Table 4).

Table 4. Reaction Mix

Component	Volume per Reaction (µL)	Volume per 384 Reactions (µL)*
R1 Reagent ●	1.8	900
R2 Reagent ●	0.2	100
Total Volume	2.0	1000
*30% overage included		

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

Note: Avoid creating air bubbles while mixing with a pipet.

23. Open the script file “**BioSkryb ResolveDNA Step 2.**”

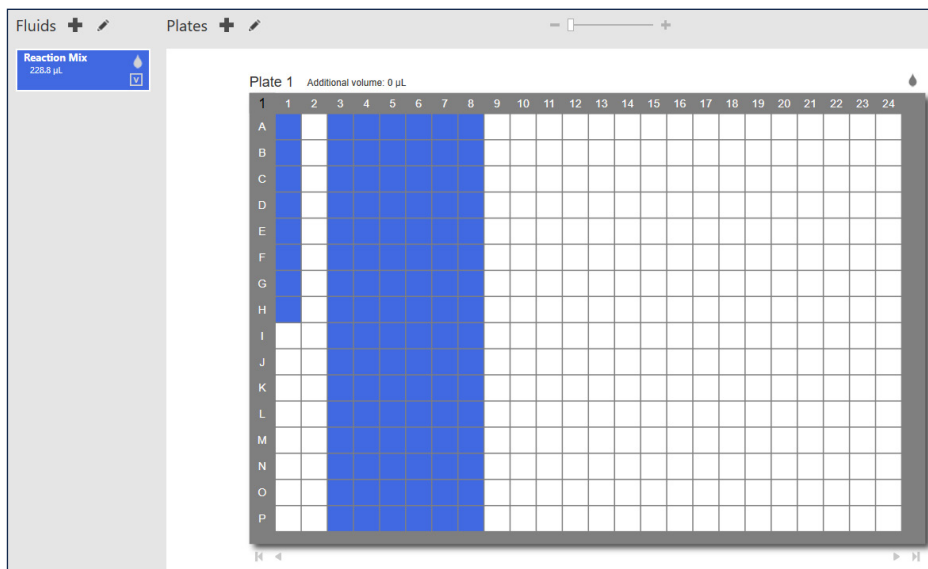


Figure 8. BioSkryb ResolveDNA Step 2 template. A single fluid class, Reaction Mix, should be indicated to dispense in each reaction well. Copy and paste as necessary to cover all reaction wells up to the full plate.

24. Ensure that all reaction wells (including controls) are set to receive 2200 nL of **Reaction Mix** by cutting and pasting to make the blue areas on the plate map match the sample layout.

Note: The dispense volume (2200 nL) is greater than the target volume to be dispensed (2000 nL or 2 µL). This offset is required due to the high viscosity of the **Reaction Mix** and has been optimized by BioSkryb Genomics.

25. Save the file with a unique file name to preserve the integrity of the original script file.
26. Click “Run” in the top left to reach the protocol “Pre-Run Summary” page with cassette and fluid

volume requirements. Ensure that sufficient **Reaction Mix** has been prepared; the volume offset means that the instrument will use ~10% less fluid than requested.

Once incubation is complete, continue with the following steps:

27. Remove the plate from the thermal mixer, spin down for 10 seconds, remove the plate seal and load onto the digital dispensing instrument.
28. Proceed from the Pre-Run Summary page by clicking “Start”. Follow the prompts from the software to insert dispensing cassettes and fluids, confirming your actions by clicking the blue buttons (Filled, Loaded, etc).
29. Seal the plate and spin down for 10 seconds.
30. In the thermal mixer, mix at room temperature for 1 minute at **1000 rpm**.
31. Spin down for 10 seconds and place on ice.
32. Load the plate in the preheated thermal cycler and unpause the DNA Amplification program (Table 2).
33. After the program is complete, remove the plate, spin down for 10 seconds, and place on ice.
34. Continue with the Quality Control procedure or store samples overnight at -20°C .

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

 **Safe Stop:** Samples may be stored overnight at -20°C before proceeding to the Quality Control Checkpoint.

Post WGA Quality Control Checkpoint

1. To assess DNA yield, dilute each reaction by adding **Elution Buffer** to a total volume of $20\ \mu\text{L}$.
2. Add $2\ \mu\text{L}$ of diluted reaction mix to $198\ \mu\text{L}$ Qubit reagent and measure the concentration per manufacturer’s instructions.
3. Prepare a $2\ \text{ng}/\mu\text{L}$ 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 $2\ \mu\text{L}$ of each $2\ \text{ng}/\mu\text{L}$ 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)

- ① The following library preparation protocol **creates libraries with fragment sizes (~325–400 bp) 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.

- ① 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 ResolveDNA Universal Library Prep Kit, 384 Reactions is optimally executed using an automated liquid handler capable of high accuracy delivery of sub-microliter volumes into a 384-well plate. This workflow has been optimized on the Uno and D300e instruments. Please refer to the respective instrument and software documentation for information on the general operation of each instrument. To obtain this documentation, please contact Tecan. Some steps in this protocol are performed using manual multichannel pipetting. Please refer to Table 5 for a summary of reagent dispensing methods for BioSkryb Universal Library Preparation and Quality Control steps.

BioSkryb Genomics provides two protocol script files, “BioSkryb Universal Library Prep FERAT Mix” and “BioSkryb Universal Library Prep Ligation Mix,” for execution of the workflow. Refer to the “Before You Begin” section below for instructions on how to obtain these scripts.

Table 5. Summary of Dispensing Methods for Library Preparation and Quality Control Steps

Workflow Step(s)	Reagent Dispensing Method
Library input sample preparation	Manual multichannel pipetting
FERAT	Uno
Addition of adapters	Manual multichannel pipetting
Ligation	Uno
Amplification Mix	Manual multichannel pipetting
Bead cleanup(s)	Manual multichannel pipetting
Post Amplification Quality Control Checkpoint	Manual multichannel pipetting

I. Before You Begin

1. Read through the entire protocol and ensure all required equipment, reagents, and consumables are on hand.
2. Contact the [BioSkryb Genomics Application Support Team](#) to obtain the required protocol script files.
 -  **Note:** When using the DispenseControl software, navigate to the file menu and ensure the appropriate device (e.g. Uno, Duo, or D300e) is selected after opening each script.
3. Thaw all library preparation kit reagents on ice and maintain the reagents on ice. Always keep reactions and reagents on ice unless otherwise instructed.
 -  **Note:** Lab cooling blocks designed to keep reactions chilled during handling are recommended.
 - ① **Important: DO NOT VORTEX** reagents **LP0E, LP1E, LP2E, 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. Open the script file “**BioSkryb Universal Library Prep FERAT Mix**” in the DispenseControl (or UnoControl) Software.
2. Ensure that all wells in which reactions will be run indicate the dispensing of 1041 nL of **FERAT Mix**.
 -  **Note:** For wells that do not have reactions, right-click and choose “Remove Fluid”, and choose “FERAT Mix” from the drop-down menu.
 -  **Note:** For Uno, the correct settings are fluid class “Aqueous + Tween20 + Glycerol” within the aqueous fluid group.
3. Save the file with a unique file name to preserve the integrity of the original script file.
4. Click “Run” in the top left to reach the protocol “Pre-Run Summary” page with cassette and fluid volume requirements.
5. Add 20 ng of each ResolveDNA whole genome amplified (WGA) product to a fresh 384-well plate on a PCR cooler or ice. Add Elution Buffer to bring the total volume to 4 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.
6. Vortex **LPIB Reagent**  for 5 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
7. Invert **LPIE Reagent**  10 times to homogenize or vortex on low speed for 10 seconds to ensure complete mixing. Briefly centrifuge to collect all liquid in the bottom of the tube.
8. Program a thermal cycler to run the FERAT-HC Program (Table 6). Initiate the run to cool the block to 4°C and pause the program once cooled.

Table 6. 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

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

Table 7. FERAT Master Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	384 Reactions*
LPIB Reagent 	0.4 µL	199.7 µL
LPIE Reagent 	0.6 µL	299.5 µL
Total Volume	1.0 µL	499.2 µL
*30% overage included		

10. Vortex the **FERAT Master Mix** on low speed for 10 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.

11. Load the plate into a chilled plate block onto the digital dispensing instrument.

12. Proceed from the “Pre-Run Summary” page by clicking “Start.” Follow the prompts from the software to insert dispensing cassettes and fluids and confirm your actions by clicking the blue button (Filled, Loaded, etc).

 **Note:** Let the reagent sit in the cassette for 30 seconds before starting the program.

13. Seal the plate and briefly spin to get the liquid to the bottoms of the wells.

14. Vortex the plate at medium speed to homogenize the reaction.

15. 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.

16. Place the plate into the preheated thermal cycler and initiate the FERAT-HC Program (Table 6).

17. While the thermal cycler is running, remove **Resolve Beads** from storage and allow to equilibrate to room temperature for at least 30 minutes.

18. While the thermal cycler is running, thaw the **Single Use Library Adapter Set** plate on ice.

III. Ligation

1. While the FERAT program is running on the thermal cycler, open the script file “**BioSkryb Universal Library Prep Ligation Mix**” in the DispenseControl (or UnoControl) Software.

2. Ensure that all wells in which reactions will be run indicate the dispensing of 1041 nL of **Ligation Mix**.

 **Note:** For wells that do not have reactions, right-click and choose “Remove Fluid”, and choose “Ligation Mix” from the drop-down menu.

 **Note:** For Uno, the correct settings are fluid class “Aqueous + Tween20 + Glycerol” within the aqueous fluid group.

3. Save the file with a unique file name to preserve the integrity of the original script file.

4. Click “Run” in the top left to reach the protocol “Pre-Run Summary” page with cassette and fluid volume requirements.

- Prepare the **Ligation Mix** by combining the components in Table 8 in listed order in a microcentrifuge tube on ice. Vortex the mix for 10 seconds and briefly centrifuge to collect all liquids in the bottom of the tube.




 **Note:** If running fewer than 384 reactions, use the volumes for one reaction in Table 8 to calculate the required reagent volumes for the number of reactions being run and add 30% overage. If the total volume calculated is less than the load volume requested on the pre-run summary, increase the reaction number to create the requested volume of Ligation Mix.

Table 8. Ligation Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	384 Reactions*
LP2B Reagent 	0.9 µL	449.3 µL
LP2E Reagent 	0.1 µL	49.9 µL
Total Volume	1 µL	499.2 µL
*30% overage included		

- Once the thermal cycler has completed the FERAT-HC Program, remove the plate from thermal cycler, spin down for 10 seconds, and place on a PCR Cooler or ice.
- Program a thermal cycler to run the Ligation Program (Table 9) and allow the block to reach the ligation temperature of 20°C and lid temperature of 50°C. Pause the thermal cycler until samples are ready.

Table 9. Ligation Program (lid temperature 50°C)

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

- Vortex thawed **Single Use Library Adapter Set** plate briefly and centrifuge.
- Using a multichannel pipette, add 1 µL of **Single Use Library Adapters** to each sample in the plate.
 - Important:** Ensure each sample well receives a unique adapter. If fewer than 384 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](#).
- Remove the plate seal and load the plate into a chilled plate block onto the digital dispensing instrument.
- Proceed from the “Pre-Run Summary” page by clicking “Start.” Follow the prompts from the software to insert dispensing cassettes and fluids and confirm your actions by clicking the blue buttons (Filled, Loaded, etc).
 - Note:** Let the reagent sit in the cassette for 30 seconds before starting the program.
- After the script has completed, seal and centrifuge the PCR plate for 10 seconds.

13. Vortex briefly and briefly centrifuge the plate to collect all liquid at the bottom of the wells.
14. Place the plate into the thermal cycler pre-set to 20°C and initiate the Ligation Program (Table 9).
15. Upon completion of the Ligation Program, proceed immediately to Library Amplification.

IV. Library Amplification

1. Initiate the LIB-AMP program on a thermal cycler (Table 10). Allow the thermal cycler to warm up to temperature and pause the program.

Table 10. 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 11 in a new tube.


Table 11. Amplification Master Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	384 Reactions*
LP3A Reagent	6.5 µL	2995.2 µL
LP3P Reagent	0.5 µL	230.4 µL
Total Volume	7 µL	3225.6 µL
*20% overage included		


5. Vortex the **Library Amplification Master Mix** for 10 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
6. Split the **Library Amplification Master Mix** evenly across each tube of one 0.2 mL 8-strip tube.
7. Manually dispense 7 µL of **Library Amplification Mix** to each well of the plate using a multi-channel pipette.
8. Seal the plate, vortex for 10 seconds, spin down for 30 seconds, and place on ice.
9. Load the plate into the preheated thermal cycler and initiate the LIB-AMP program in Table 10.
10. After completion of the LIB-AMP program, place the plate on a PCR Cooler or ice.
11. Proceed to post-amplification cleanup.

V. Post Amplification Cleanup

 **Note:** The following steps pool equal volumes of 96 individual libraries for a total of four 96-plex enrichment pools per set of 384 individually prepped libraries. Pooling is followed by two sequential cleanups using 1X the sample volume of Resolve Beads. Alternatively, each library can be cleaned up individually (without pooling) also using two sequential cleanups with 1X the sample volume of Resolve Beads (e.g. 40 μ L of library volume plus 40 μ L 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 96 DNA samples together by adding 7.5 μ L of each DNA sample well into a 1.5 mL Eppendorf tube for a total volume of 720 μ L. Spin tube down for 10 seconds.

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


3. Vortex **Resolve Beads** thoroughly immediately before use to ensure even distribution of beads.
4. Add 720 μ L of **Resolve Beads** to each amplified library pool (Table 12).




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

Table 12. Resolve Bead Cleanup Mix

Component	Volume per Reaction
96 pooled libraries	720 μ L
Resolve Beads	720 μ L
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 μ L 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 μ L 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 μ L.
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 210 μ L 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 200 μ L 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 200 μ L of water-eluted DNA from step 24. Add 1X sample volume of Resolve Beads (200 μ L). Repeat steps 5–20.
26. Resuspend the beads in 110 μ L 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.
 -  **Note:** pipetting carefully will minimize bubbling and allow for greater library recovery.
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 100 μ L 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 yield, add 2 μ L of amplified library to 198 μ L 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/ μ L 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 μ L of each 2ng/ μ L 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

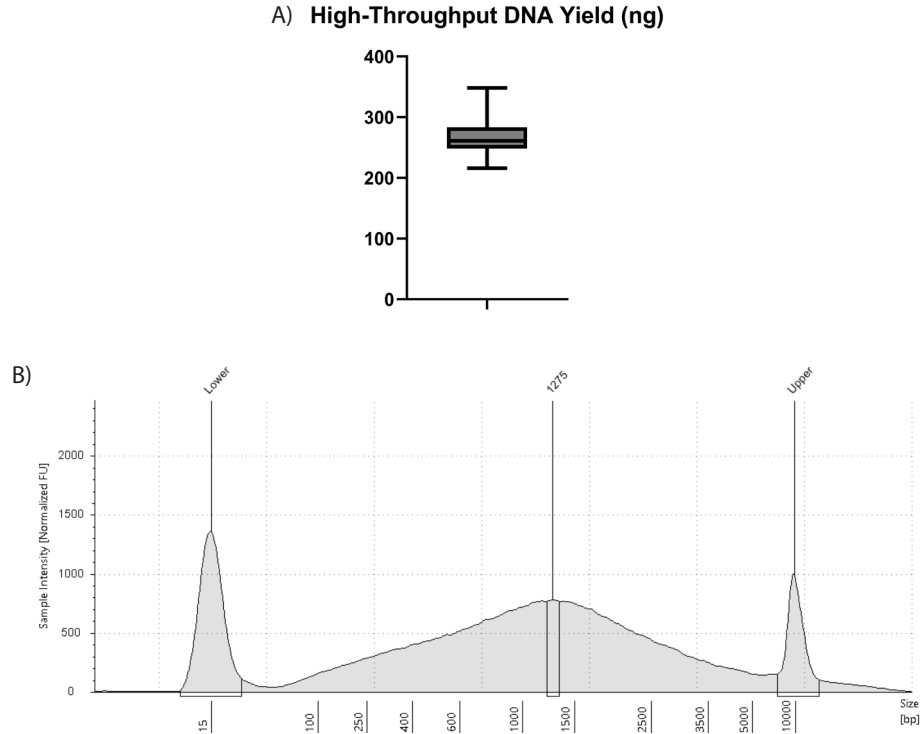


Figure 9. Examples of Total Amplification Yields and Fragment Size Distribution.

A) DNA amplification yield for the high-throughput, 384-reaction protocol. Average yield is 260 ng from single human cells.
B) The electropherogram represents a sample amplified using ResolveDNA WGA, which has been normalized to 2 ng/ μ L 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

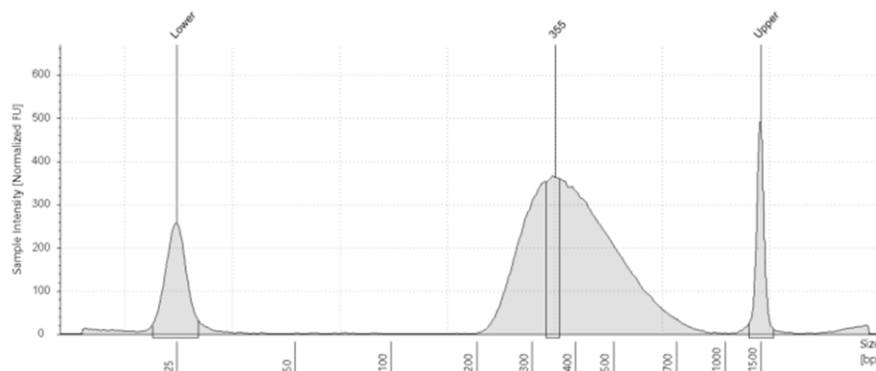


Figure 10. Example DNA Library Fragment Size Distribution.

The electropherogram represents a sample after pooled library preparation as described in this document, which has been normalized to 2 ng/ μ L and run on a TapeStation using the HS D1000 ScreenTape. Average expected library size is around 325-400 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
IDT™	xGen™ Exome Hyb Panel v2*	xGen Hybridization Capture of DNA Libraries Protocol "Tube Protocol" (version:8)	4	6	96	12 µg (2x vendor recommended)	20M paired-end reads (10M clusters) per library X 96 libraries = 1920M paired-end reads (960M clusters) per pool
Twist Bioscience	Alliance CNTG Hereditary Oncology Panel (0.2 MB)	Twist Target Enrichment Fast Hybridization Protocol (version: DOC-001066 REV 4.0)	4	13	96	8 µg (2x vendor recommended)	1M paired-end reads (500K clusters) per library X 96 libraries = 96M paired-end reads (48M clusters) per pool
IDT™	xGen™ Pan-Cancer Hybridization Panel (0.8 MB)	xGen Hybridization Capture of DNA Libraries Protocol "Tube Protocol" (version:8)	16	10	96	12 µg (2x vendor recommended)	1M paired-end reads (500K clusters) per library X 96 libraries = 96M paired-end reads (48M clusters) per pool

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

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

Appendix D: Sequencing and Analysis using BaseJumper®

Sequencing Library Preparation

The 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/) (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/), 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) (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](mailto:techsupport@bioskryb.com) (techsupport@bioskryb.com).



BioSkryb

GENOMICS

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