



ResolveDNA® Whole Genome Single-Cell Core Kit

Protocol to Prepare WGS-Ready Libraries

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 Whole Genome Sequencing (WGS)-Ready Libraries

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 Nuclei
 - 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

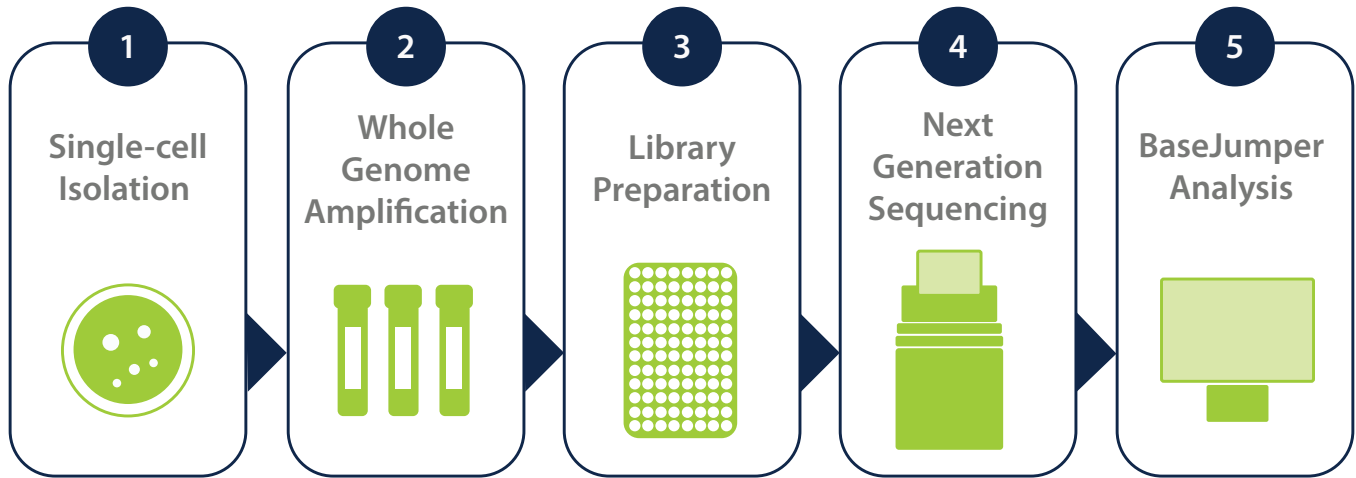


Figure 1. The ResolveDNA Single-Cell Sequencing Workflow

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), utilizing the Uno or Duo instruments, 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, 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 allows up to 384 unique, barcoded libraries suitable for multiplex sequencing on all Illumina® sequencing platforms.

IV. Next-Generation Sequencing

Barcoded libraries are then normalized and pooled prior to next-generation sequencing on an Illumina® sequencing platform.

V. 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

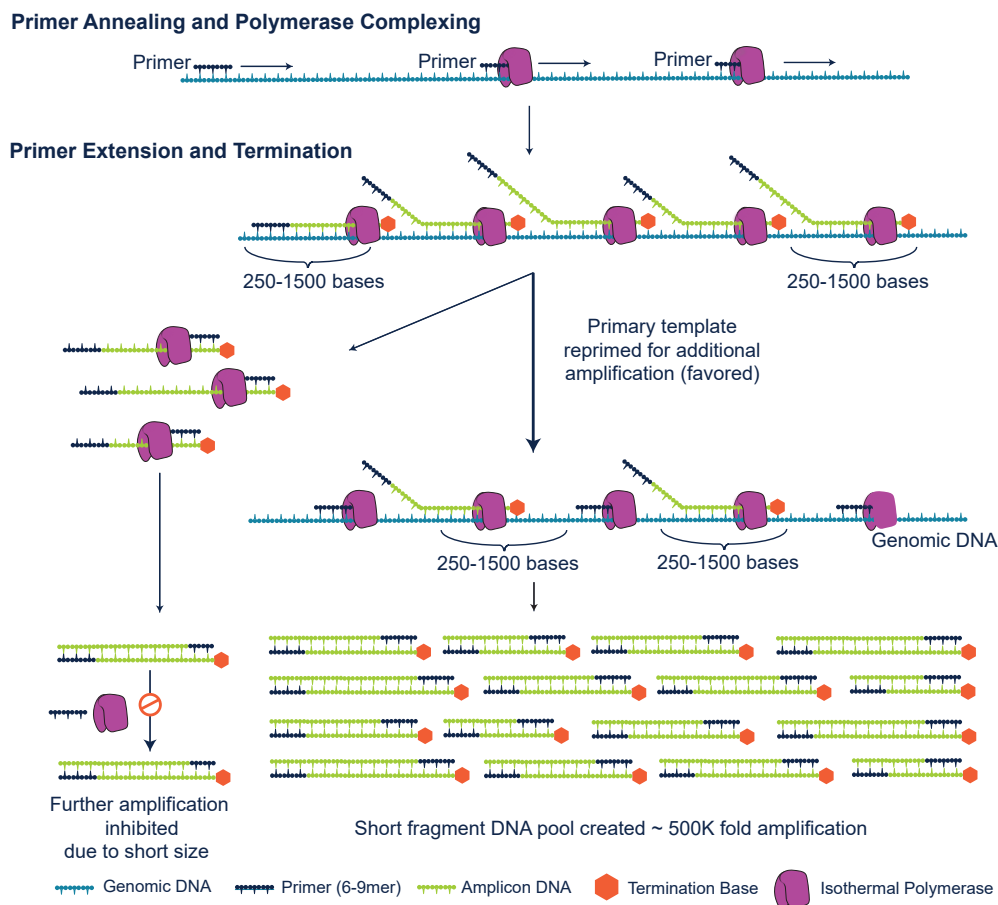


Figure 2. Primary Template-Directed Amplification (PTA). PTA may be performed directly from single cells, multiple cells, or nuclei (collected by FACS, FANS, microfluidic cell separation or other methods), or ultra-low inputs of DNA (4 pg – 10 ng). After cell lysis and genomic DNA denaturation, random 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	LP0B Reagent	100795	○ Natural
	LP0E 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

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 [BioSkryb Genomics Application Support Team](#) if you have any questions about the interpretation of the Temperature Tags.

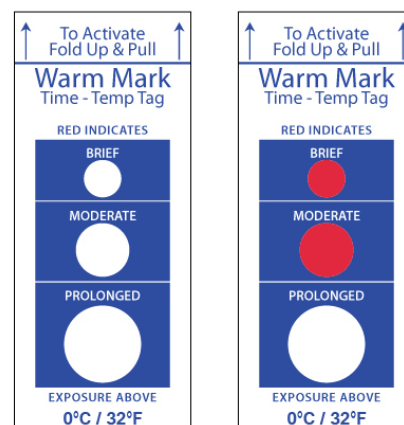


Figure 3. Temperature Indicator Tag

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](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
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
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	General Lab Supplier (GLS)	—
1.5 mL Microcentrifuge Tubes	GLS	—

Products from Third-Party Suppliers, Continued

Product Name	Company	Catalog Number
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

BioSkrbyb 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, BioSkrbyb 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.


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

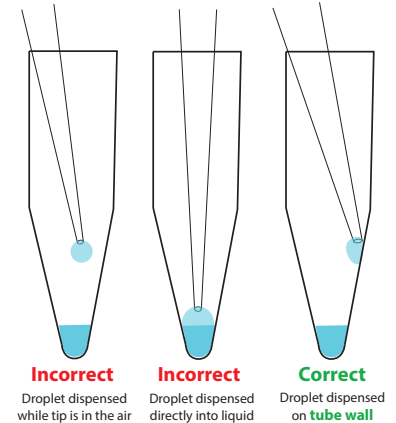


Figure 4. Pipetting Technique

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	-
DNA Prep	2	1
FERAT	2	-
Ligation	2	-
Total	15	2

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/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. 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.
 - ① **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 “BioSkrbyb ResolveDNA Step 1.”

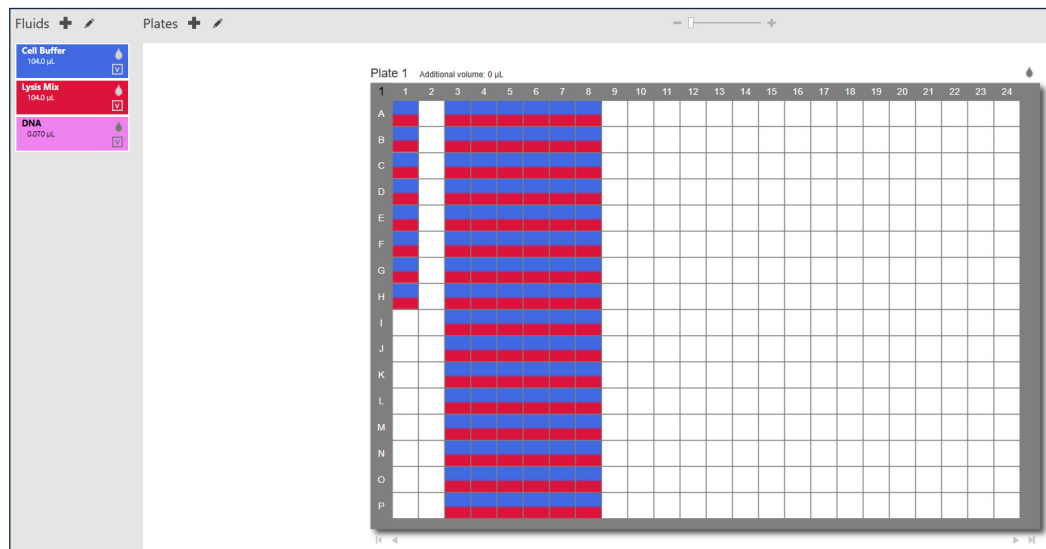


Figure 5. BioSkrbyb ResolveDNA Step 1 template. The fluid classes include Cell Buffer, Lysis Mix, and gDNA stock. The first column has been pre-loaded to create a set of control DNA reactions from the 10 ng/µL control DNA stock (from A–H: NTC, 600 pg, 60 pg, 12 pg, 12 pg, 6 pg, 1 pg, NTC). Single cell 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**.

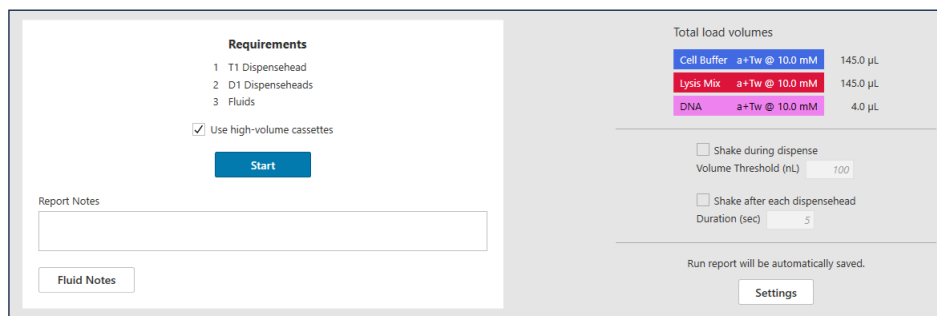


Figure 6. The Pre-Run Summary Screen. Provides a summary of the number and types of dispensehead cassettes necessary as well as the fluid loading volumes required for complete dispensing.

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.

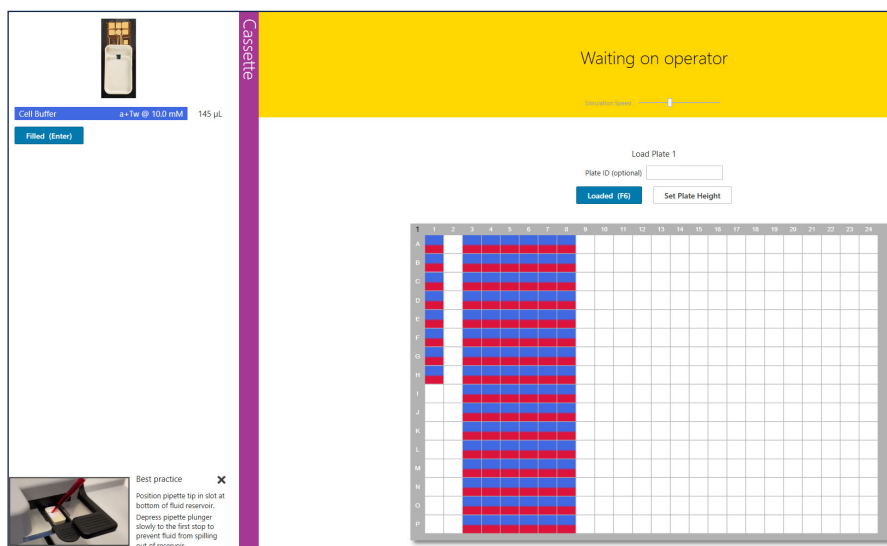


Figure 7. The Run Window Screen. Indicates steps in the operational workflow. Cassettes and fluids are requested on the left panel, plate loading is requested in the center. Dispensing is then reported in real time as the script proceeds. As additional fluids or cassettes are required, the software will 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.**”

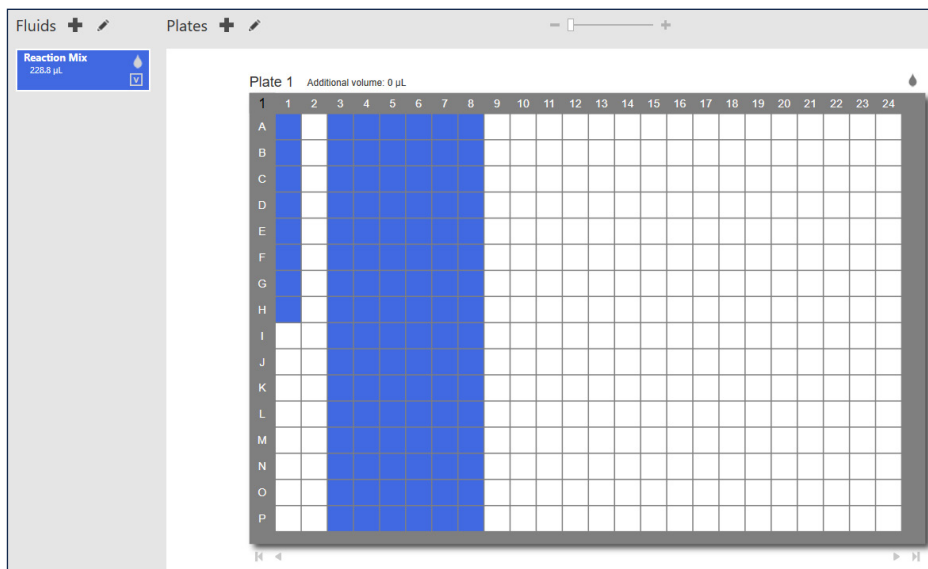



Figure 8. BioSkryb ResolveDNA Step 2 template. A single fluid class, Reaction Mix, should be indicated to dispense in each reaction well. Copy and paste as necessary to cover all reaction wells up to the full plate.

- 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.

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

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.

 **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 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 WGS)

- ① The following library preparation protocol creates libraries compatible with whole genome sequencing. **A separate library preparation protocol is required to create libraries compatible with hybridization enrichment for downstream whole exome or targeted panel sequencing.** Contact our [Application Support Team](#) (TechSupport@BioSkryb.com) to obtain the library preparation protocol compatible with hybridization enrichment.

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 three protocol script files, “BioSkryb Universal Library Prep DNAPrep,” “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
DNA Prep	Uno
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 (such as a [V&P Scientific Heat Transfer Plate](#) that has been pre-chilled).

- ① **Important: DO NOT VORTEX** reagents **LPOE, LPIE, 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.

- 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. DNA Preparation

- Prepare 5–30 ng of each ResolveDNA WGA DNA product in 3µL of **Elution Buffer** in a 384-well plate, seal plate, briefly spin, and place on ice.

 **Note:** This workflow has been designed for using an input of 20 ng ResolveDNA WGA DNA product, but has been demonstrated to work robustly for DNA input amounts of 5–30 ng. The input tolerance range (5–30 ng) enables the use of a constant volume of PTA product (i.e. 1µL) across samples for input into library preparation in place of individual sample quantification and normalization.

- Program a thermal cycler to run the DNAPREP Program (Table 6). Initiate the run to preheat the block and then pause the thermal cycler until samples are ready.

Table 6. DNAPREP Program (lid temperature 105°C)

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


- Open the DispenseControl (or UnoControl) Software.
- Open the script file “**BioSkryb Universal Library Prep DNAPrep.**”
- Ensure all wells in which reactions will be run indicate the dispensing of 1041 nL of “DNA prep mix”
 -  **Note:** For wells that do not have reactions, right-click and choose “Remove Fluid”, and choose “DNA prep mix” from the drop-down menu.
- Save the file with a unique file name to preserve the integrity of the original script file.
- Click “Run” in the top left to reach the protocol “Pre-Run Summary” page with cassette and fluid volume requirements.
- Prepare the **DNA Prep Master Mix** by combining the reagents in Table 7 in a microcentrifuge tube on ice.

Table 7. DNA Prep Master Mix


Reagent Name	Volume per Number of Reactions	
	96 Reactions*	384 Reactions*
LPOB Reagent ○	115.2 µL	460.8 µL
LPOE Reagent ⊗	0.6 µL	2.4 µL
Total Volume	115.8 µL	463.2 µL
*20% overage included		

9. Vortex 10 seconds to mix, briefly spin down and place on ice.
10. Remove the plate seal from the plate and load the plate into a chilled plate block on the instrument.
11. Proceed from the “Pre-Run Summary” page by clicking “Start.” Follow the prompts from the software to insert dispensehead cassettes and fluids and confirm your actions by clicking the blue button (Filled, Loaded, etc.).

 **Note:** One (1) µL of DNA Prep Master Mix will be dispensed per well. After this step each well will have 4 µL of total volume.

12. After the script has completed, seal the plate and spin down for 10 seconds to combine components.
13. Vortex the plate for 10 seconds to mix and briefly spin down.
14. Place the plate into the preheated thermal cycler and initiate the DNAPREP Program (Table 6).

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

1. While the DNAPREP Program is running, 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.
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. Prepare the **FERAT mix** by combining the components in Table 8 in listed order in a centrifuge tube on ice. Vortex 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 FERAT Mix.

Table 8. FERAT Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	384 Reactions*
LP1B Reagent 	0.4 µL	200 µL
LP1E Reagent 	0.6 µL	300 µL
Total Volume	1 µL	500 µL
*30% overage included		

- Vortex for 10 seconds to mix, briefly spin down and place the **FERAT mix** on ice.
- Once the thermal cycler has completed the DNAPREP Program, remove the plate from thermal cycler, spin down for 10 seconds, and place on ice. Proceed immediately to the next steps.
- Program a thermal cycler to run the FERAT Program (Table 9). Initiate the run to cool the block to 4°C and then pause the thermal cycler until samples are ready.

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

Step	Temperature	Time
Hold 1	4°C	30 seconds
Hold 2	37°C	5 minutes
Hold 3	65°C	30 minutes
Hold 4	4°C	∞
Total Time	-	~35 minutes

- 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 button (Filled, Loaded, etc).

 **Note:** One (1) µL of FERAT Mix will be dispensed per well. After this step each well will have 5 µL of total volume.

- After the script has completed, seal and centrifuge the PCR plate for 10 seconds.
- Vortex briefly to mix and briefly centrifuge the plate to collect all liquid at the bottom of the wells.
- Place the plate into the prechilled thermal cycler and initiate the FERAT Program (Table 9).
- While the thermal cycler is running, thaw the **Single Use Library Adapter Set** plate on ice.

IV. Ligation

- 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.
- 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.

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. Prepare the **Ligation Mix** by combining the components in Table 10 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 10 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 10. Ligation Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	384 Reactions*
LP2B Reagent 	0.9 μ L	449.3 μ L
LP2E Reagent 	0.1 μ L	49.9 μ L
Total Volume	1 μL	499.2 μL

*30% overage included

6. Once the thermal cycler has completed the FERAT Program, remove the plate from thermal cycler, spin down for 10 seconds, and place on ice.
7. Program a thermal cycler to run the Ligation Program (Table 11) 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 11. Ligation Program (lid temperature 50°C)

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

8. Vortex thawed **Single Use Library Adapter Set** plate briefly and centrifuge.
9. Add 1 μ L of **Single Use Library Adapters** to each sample in the plate.
 - ① **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](#).
10. Remove the plate seal and load the plate into a chilled plate block onto the digital dispensing instrument.

11. 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:** One (1) μL of Ligation Mix will be dispensed per well. After this step each well will have 7 μL of total volume.

12. 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 11).

V. Library Amplification

1. Prepare the **Amplification Mix** by combining the components in Table 12 in listed order in a centrifuge 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 12 to calculate the required reagent volumes for the number of reactions being run and add 20% overage.

Table 12. Amplification 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		

2. Once the thermal cycler has completed the Ligation Program, remove the plate from thermal cycler, spin down for 10 seconds, and place on ice/in a cold block.
3. Start the Amplification Program on the thermal cycler and allow the block to reach the start temperature of 98°C. Pause the thermal cycler until samples are ready.

Table 13. Amplification Program (lid temperature 105°C, total volume per well 15 μL)

Step	Temperature	Time	Cycles
Hot Start	98°C	45 seconds	1
Denature	98°C	15 seconds	8
Anneal	60°C	30 seconds	
Extend	72°C	45 seconds	
Final Extension	72°C	1 minute	1
Hold	4°C	∞	1
Total Time	-	~15 minutes	

4. Manually dispense 7 μL of **Amplification Mix** into each well of the plate. Each well will have a total

volume of 14 μ L after manually dispensing the **Amplification Mix**.

5. Seal and centrifuge the PCR plate. Vortex briefly and then briefly centrifuge the plate to collect all liquid at the bottom of the wells.
6. Place the plate into the preheated thermal cycler and initiate the Amplification Program (Table 13).


VI. Post Amplification Cleanup

 **Note:** The following steps are written for performing the cleanup on individual wells. Pooling wells is also acceptable. If samples are pooled, maintain a 0.75x **Resolve Beads** to total Amplification Reaction product volume ratio.


1. Allow the **ResolveDNA Bead Purification** components to equilibrate to room temperature for 30 minutes.
2. Once the thermal cycler has completed the Amplification Program, remove the plate from thermal cycler, spin down for 10 seconds, and proceed directly to library cleanup.
3. Vortex **Resolve Beads** thoroughly immediately before use to ensure even distribution of beads.
4. Add **Resolve Beads** at 0.75x the total volume of Amplification Reaction product. For example, add 10.5 μ L **Resolve Beads** to 14 μ L Library Amplification reaction volume.
5. Mix thoroughly by vortexing and/or pipetting up and down multiple times.
6. Incubate the plate/tube(s) at room temperature for 5 min to bind DNA to the beads.
7. Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear, approximately 3 minutes.
8. Carefully remove and discard the supernatant.

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

9. Keep the plate/tube(s) on the magnet and add 20 μ L of freshly prepared 80% ethanol to each well. Incubate the plate/tube(s) on the magnet at room temperature for \geq 30 seconds.
10. With the plate on the magnet, carefully remove and discard the ethanol.
11. Perform a second bead wash with ethanol. With the plate/tube(s) on the magnet, add 20 μ L of freshly prepared 80% ethanol to each well.
12. Incubate the plate/tube(s) on the magnet at room temperature for \geq 30 sec.
13. With the plate on the magnet, carefully remove and discard the ethanol.
14. Seal the plate, spin briefly, return to the magnet, and carefully remove the plate seal.
15. Incubate the plate on the magnet at room temperature for 1 minute or until the supernatant clears.
16. Using a clean pipet tip, remove any residual ethanol (~5–10 μ L) without disturbing the beads.
17. Dry the beads at room temperature for ~1 minute.

 **Note:** **Resolve Beads** dry quickly and are often dried within 1–2 minutes of removing the ethanol. If working with >6 columns, add **Elution Buffer** (step 19) immediately to wells after removal of ethanol from 6 columns then proceed with the remainder of the columns to prevent over drying.

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

18. Remove the plate/tube(s) from the magnet.
19. Thoroughly resuspend the beads in 22 μL of either **Elution Buffer** or PCR-grade water. Always use PCR-grade water if proceeding to target enrichment and capture.
 -  **Note:** If samples were pooled for the Post Amplification Cleanup, use a volume of **Elution Buffer** or PCR-grade water equivalent to approximately twice the **ResolveDNA Bead** volume used.
20. Incubate the plate/tube(s) at room temperature for 3 minutes to elute DNA off the beads.
21. Place the plate/tube(s) on a magnet to capture the **Resolve Beads**. Incubate at least 2 minutes or until the supernatant is clear.
22. Transfer 20 μL of the eluted DNA to a new tube or plate, place on ice, and proceed to library quantification.

Post Library Amplification Quality Control Checkpoint

1. To assess library yield, add 2 μL of amplified library to 198 μL Qubit reagent and measure the concentration using the High Sensitivity dsDNA Assay kit, as per the manufacturer's instructions.
2. Prepare a 2 ng/ μL dilution of samples in a fresh PCR plate by diluting libraries with **Elution Buffer**, seal the plate, vortex briefly, and spin down.
3. Determine fragment size distribution by running 2 μL of each 2ng/ μL diluted library using a TapeStation HS D1000 ScreenTape or other fragment analysis instrument using manufacturer's instructions.
4. Refer to Appendix B for example quality control data.
5. Refer to Appendix C for more information on multiplexing, dilution, and denaturation for sequencing.
6. **The final sequencing pool should be subjected to an additional ResolveDNA Bead Purification step with 0.75X beads prior to sequencing (i.e. for 100 μL of pooled sample volume, add 75 μL of beads). Follow workflow steps as described in Step VI. Post Amplification Cleanup.**

Appendix A: Post WGA Quality Control Example Data

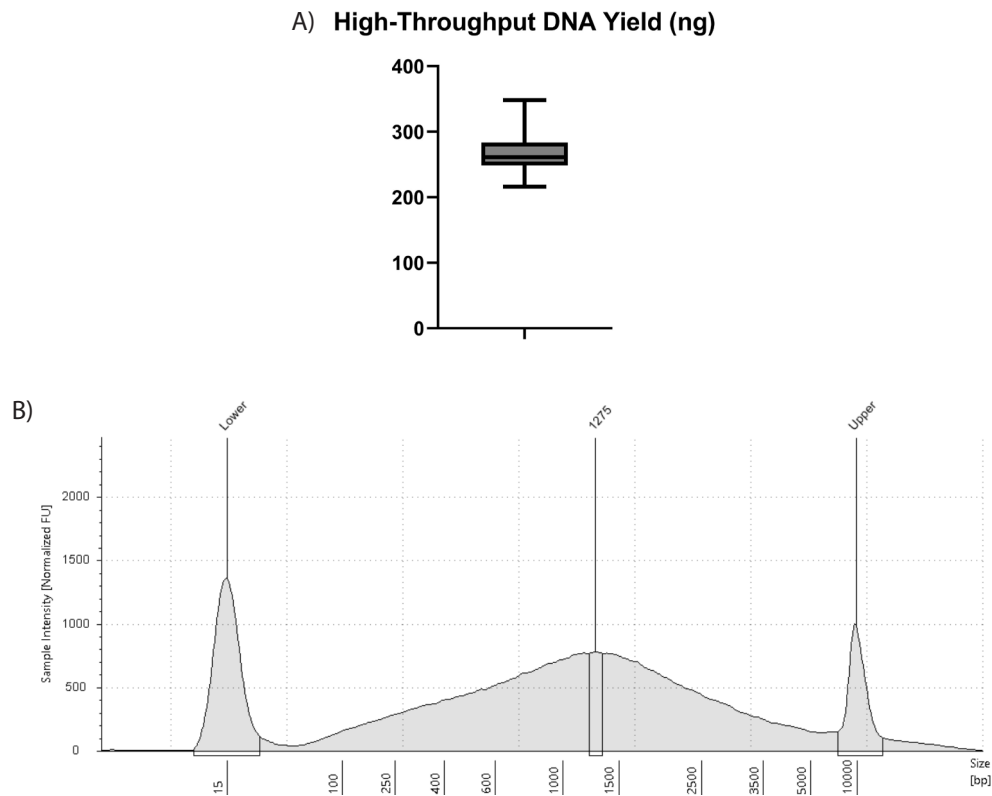


Figure 9. Examples of Total Amplification Yields and Fragment Size Distribution.

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

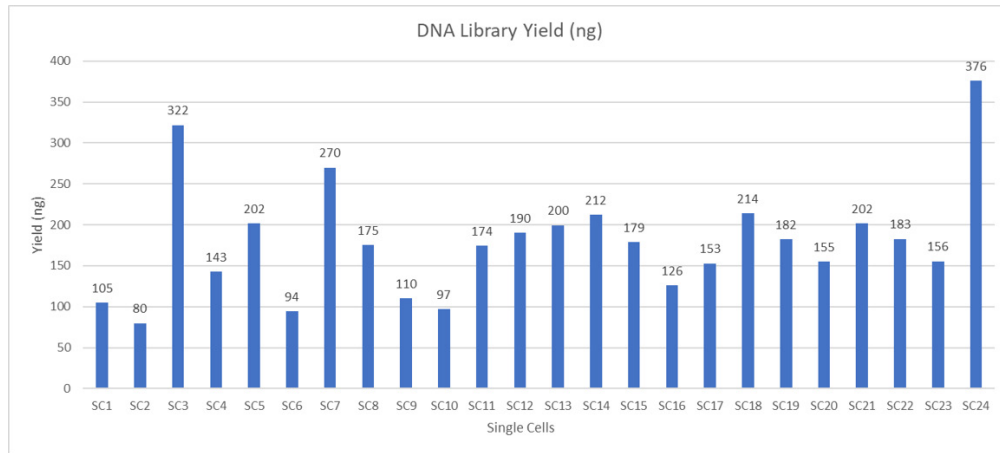
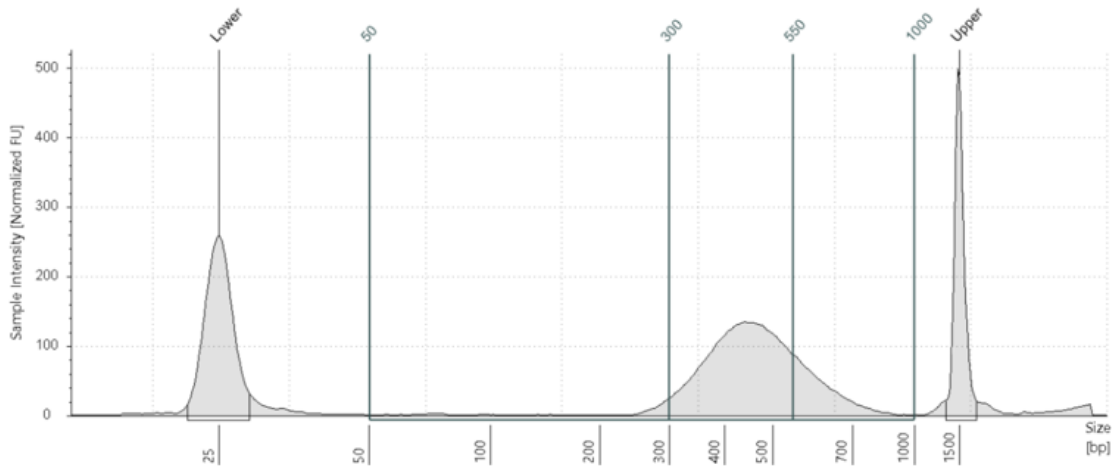


Figure 10. Examples of DNA Library Yields.

DNA library yield for the ResolveDNA Whole Genome Single-Cell Core Kit, 384 Reactions from single human cells. Measurement unit is nanograms (ng).



Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [pg/μl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
50	1000	469	695	2540	88.58		█
300	550	437	528	1910	67.25		█

Figure 11. Example DNA Library Fragment Size Distribution.

The electropherogram represents a sample after library preparation using the ResolveDNA Whole Genome Single-Cell Core Kit, 384 Reactions which has been normalized to 2 ng/μl and run on a TapeStation using the HS D1000 ScreenTape.

Appendix C: Sequencing and Analysis using BaseJumper®

Library Multiplexing, Dilution and Denaturation

Follow Illumina manufacturers instructions for dilution, multiplexing and denaturation of sequencing libraries.

Load and initiate sequencing run on an appropriate Illumina sequencing platform following manufacturers instructions.

Initial Low-pass Sequencing

It is highly recommended that users perform an initial round of low depth sequencing (50 base paired-end, 2 – 5 million reads per cell) to check library complexity and ensure the single-cell libraries are uniformly amplified as desired and to estimate data quality prior to performing high-depth sequencing.

Typically, samples sequenced to this level are suitable for use in copy number variation (CNV) analysis. We recommend up to 5 million reads/cell for CNV analysis. For single nucleotide variant analysis (SNV) and analysis of other genomic structural variation (such as detection of fusions and indels), deep sequencing (25 – 30X genomic coverage) will be required.

Data Analysis for Sample QC and Triage for Deep Sequencing

[The BioSkryb BaseJumper Bioinformatics platform](https://bioskryb.com/basejumper/) (bioskryb.com/basejumper/) contains an analytical pipeline, BJ-DNA-QC, which utilizes algorithms designed to evaluate low depth sequencing data to predict genome coverage at higher depth sequencing levels and for visualizing other general performance metrics.

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

Samples which meet minimum performance standards may be further analyzed via high depth sequencing (25 – 30X genome coverage).

Data Analysis using BioSkryb BaseJumper Bioinformatics Platform

ResolveDNA users can choose from analytic processes that characterize the genomic variability detected in the samples using BaseJumper platform. BaseJumper currently supports the following pipeline capabilities:

- **BJ-DNA-QC (Quality Control)** – The Quality Control pipeline uses low-pass sequencing data and generates several QC metrics which can help to assess whether single-cell libraries are suitable for high-depth sequencing.
- **BJ-WGS** – The BJ-WGS pipeline processes WGS sequencing data and performs comprehensive evaluation of single-cell libraries and calls SNP/indel variants.

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

Appendix D: Library Prep Adapter Sequences

For a complete list of BioSkryb Library Prep Adapter Sequences, please contact the [BioSkryb Genomics Application Support Team](mailto:TechSupport@BioSkryb.com) (TechSupport@BioSkryb.com)



BioSkryb

GENOMICS

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