BioSkryb GENOMICS

ResolveDNA® Whole Genome Amplification Kit v2.0

Protocol for Whole Genome Amplification

User Guide

Manual 96 or Automated 384–Well Format

TAS-097 | 01/2026

ResolveDNA® v2.0 Whole Genome Amplification

Product Description

The ResolveDNA Whole Genome Amplification Kit v2.0 offers the best-in-class Whole Genome Amplification (WGA) solution with single cell resolution, enabled by the proprietary Primary Template-directed Amplification (PTA) chemistry.

The 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 kit is configured for working with 96 or 384 reactions.

Key features and benefits include the following:

- Specific amplification of single cell genomes recovering >97% of the human genome.
- Excellent allelic balance enables highly sensitive and specific assessment of single nucleotide variation (SNV).
- A simple, user-friendly workflow that requires less than 30 minutes of hands-on time and a total run time of 3 hours
- Compatible with the following:
  - Single cells
  - Multiple cells
  - Nuclei
  - Ultra-low amounts of DNA (4 pg – 10 ng)
- Compatible with any cell collection method that can deliver single, viable cells/nuclei to a reaction well.

Whole Genome Amplification (WGA) products can be used in:

- Next-generation sequencing, following conversion to sequencing libraries for multiplexed Illumina® sequencing using the ResolveDNA Universal Library Preparation Kit
- Quantitative PCR (qPCR)
- STR/microsatellite analysis
- Genotyping using microarrays
- Comparative genome hybridization studies (CGH)
- Single nucleotide polymorphism (SNP) genotyping
- Haplotyping

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

The ResolveDNA Workflow

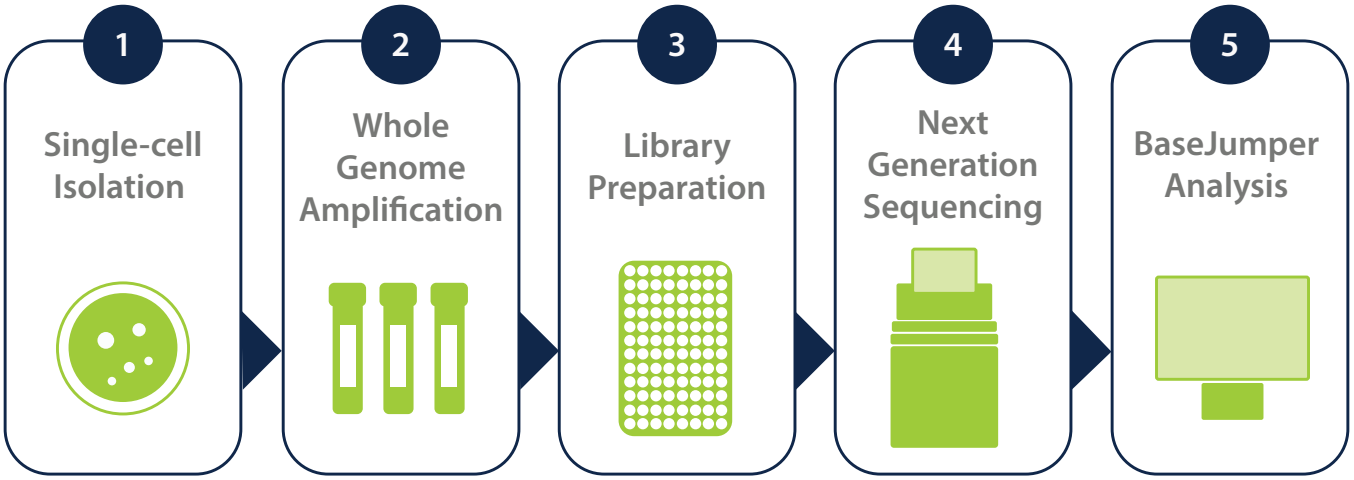

Figure 1. The ResolveDNA Single-Cell Sequencing Workflow

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), 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 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 and protocol. Library preparation with the 384-reaction 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 (bioskryb.com/basejumper/) from Illumina BaseSpace® or BioSkryb Genomics AWS S3, powered by Globus. Analytical pipelines can then be automatically queued to provide analysis of genomic variation.

ResolveDNA Amplification Technology

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

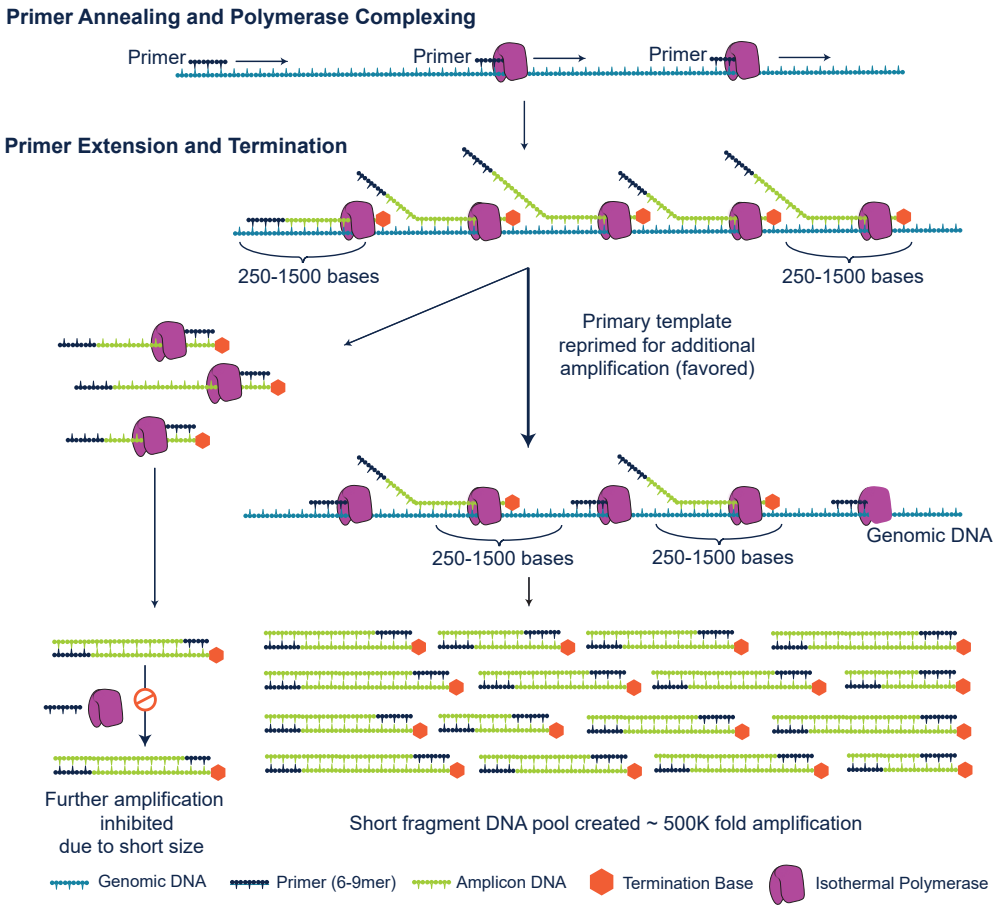

Figure 2. Primary Template-Directed Amplification (PTA). PTA may be performed directly from single cells, multiple cells, or nuclei (collected by FACS, FANS, microfluidic cell separation or other methods), or ultra-low inputs of DNA (4 pg – 10 ng). After cell lysis and genomic DNA denaturation, random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

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

Safety Precautions and Use of Personal Protective Equipment

I. Biosafety Hazards

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

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

II. Chemical Hazard

This kit contains corrosive materials and should be handled only by personnel trained in the safe handling of this type of chemical hazard. Always wear appropriate PPE. Users should consult the relevant Safety Data Sheets for more information.

III. Safety Data Sheets

For access to the safety data sheets for this product, please contact the BioSkryb Genomics Application Support Team (TechSupport@BioSkryb.com).

IV. Emergency Response Information

For 24-hour emergency information pertaining to accidents or spills involving ResolveDNA products, please contact one of the numbers listed below for information on how to clean up and discard the hazardous waste.

North America: +1-800-535-5053

International: +1-352-323-3500

In the event of a life-threatening emergency, please contact local emergency services.

Intended Use

This user guide provides instructions on the manual 96-well and automated 384-well formats, including the use of the Uno Single Cell Dispenser, D300e Digital Dispenser, or Duo Digital Dispenser, to aid in the execution of the ResolveDNA Whole Genome Amplification Kit v2.0.

The ResolveDNA Whole Genome Amplification Kit v2.0 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.

Kit Contents and Storage

I. Kit Contents

Kit Component	Part Number	Cap Color
L1 Reagent	100628	Purple
L2 Reagent	100581	Yellow
L3 Reagent	100523	White
R1 Reagent	100521	Blue
R2 Reagent	100527	Red
Control Genomic DNA (gDNA, 50ng/µL)	101155	Gold
Cell Buffer	100574	Clear

II. Shipping and Storage

Kit components are shipped on dry ice and all reagents and enzymes will be frozen upon arrival.

The labels on the boxes provide essential information including the kit part number, the kit lot number, the recommended storage temperature of the contents of the box, and the kit expiration date. When stored as directed, the kit will perform to specifications for up to the expiration date, 18 months from the date of manufacture (DOM).

Do not exceed 5 freeze/thaw cycles for any individual reagent.

Temperature Tags are shipped with the kit to ensure the shipment has been kept at the intended temperature during transit (Figure 3). Please contact the BioSkryb Genomics Application Support Team if you have any questions about the interpretation of the Temperature Tags.

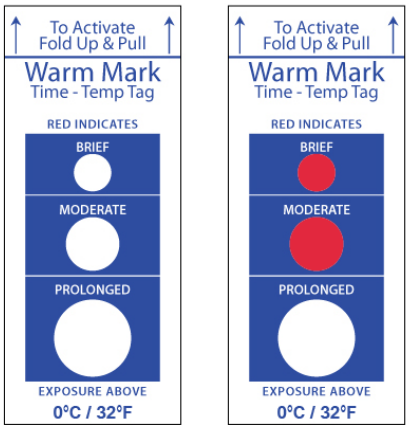

Figure 3. Temperature Indicator Tag
Each dry ice shipment includes a temperature tag designed to indicate exposure above 0°C. If the shipment stays below the target temperature, the windows will remain white.

Additional Equipment, Materials, and Reagents

I. Manual 96-Well Format

The following products have been tested with our workflow to provide optimal results. The use of any products not included in this list could result in sub-optimal results. While the listed BioSkryb products are not provided with the kit, interested parties can contact the BioSkryb Genomics Sales Department (sales@bioskryb.com) for assistance in purchasing these products. Please consult the BioSkryb Genomics Application Support Team (TechSupport@BioSkryb.com) if you have questions about the suitability of any alternative materials or equipment to be used in conjunction with the protocol.

Products from BioSkryb Genomics		
Product Name	**Company**	**Catalog Number**
ResolveDNA® PTA-Grade Cell Buffer Pack (12X 500 µL)	BioSkryb	100177
ResolveDNA® Cell Buffer Bottle Kit	BioSkryb	100183
Products from Third-Party Suppliers		
Product Name	**Company**	**Catalog Number**
PCR Plate Sealing Film	ThermoFisher	AB-0558
twin.tec 96-well PCR Plate	Eppendorf	0030128648
Magnet PCR Separation Plate	Permagen	MSP750
8-strip 0.2 mL PCR Tubes	General Lab Supplier (GLS)	—
1.5 mL Microcentrifuge Tubes	GLS	—
Single-channel pipet set (P10, P20, P200, P1000) and aerosol barrier tips	Rainin or GLS	—
8-channel pipets (P20, P200) and appropriate aerosol barrier tips	Rainin or GLS	—
Agilent Tapestation	Agilent	4200
HS D5000 Screentape	Agilent	5067-5592
HS D5000 Reagents	Agilent	5067-5593
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	—
10 mM Tris pH 8.5, PCR Grade	GLS	—

II. General Automated 384-Well Format

The following products have been tested with our workflow to provide optimal results. The use of any products not included in this list could result in sub-optimal results. While the listed BioSkryb products are not provided with the kit, interested parties can contact the BioSkryb Genomics Sales Department (sales@bioskryb.com) for assistance in purchasing these products. Please consult the BioSkryb Genomics Application Support Team (TechSupport@BioSkryb.com) if you have questions about the suitability of any alternative materials or equipment to be used in conjunction with the protocol.

ⓘ **Important:** This protocol requires an automated liquid handler capable of high accuracy delivery of sub-microliter volumes into a 384-well plate.

ⓘ **Important:** Users should ensure thermal cycler compatibility with the plates required for the automated liquid handler that will be utilized.

Products from BioSkryb Genomics		
Product Name	**Company**	**Catalog Number**
ResolveDNA® PTA-Grade Cell Buffer Pack (12X 500 µL)	BioSkryb	100177
ResolveDNA® Cell Buffer Bottle Kit	BioSkryb	100183
Products from Third-Party Suppliers		
Product Name	**Company**	**Catalog Number**
384-Well Post Magnet Plate	Permagen	P384
VP 741I6G 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	—
Single-channel pipet set (P-10, P-20, P200, P1000) and appropriate aerosol barrier tips	Rainin or GLS	—
8-channel pipets (P-20, P-200) and appropriate aerosol barrier tips	Rainin or GLS	—
Agilent Tapestation	Agilent	4200
HS D5000 Screentape	Agilent	5067-5592
HS D5000 Reagents	Agilent	5067-5593
Fluorometer (Qubit 2-4)	ThermoFisher Scientific	—
High Sensitivity dsDNA Assay kit	ThermoFisher Scientific	Q32854
PCR Plate Thermal Mixer	Eppendorf	—
PCR Plate Spinner	GLS	—
Thermal Cycler	GLS	—
Absolute (200 proof) Ethanol	GLS	—
RT-PCR Grade Water	GLS	—
10 mM Tris pH 8.5, PCR Grade	GLS	—

III. Automated 384-Well Format with Uno Single Cell Dispenser, D300e, or Duo Digital Dispenser

The following products have been tested with our workflow to provide optimal results. The use of any products not included in this list could result in sub-optimal results. While the listed BioSkryb products are not provided with the kit, interested parties can contact the BioSkryb Genomics Sales Department (sales@bioskryb.com) for assistance in purchasing these products. Please consult the BioSkryb Genomics Application Support Team (TechSupport@BioSkryb.com) if you have questions about the suitability of any alternative materials or equipment to be used in conjunction with the protocol.

ⓘ **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.

ⓘ **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) and consumables (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 741I6G 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	—
10 mM Tris pH 8.5, PCR Grade	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 Amplification v2.0 Kit.

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

1. **Automated Pipetting with Liquid Handlers:** The 384 format protocol requires the use of a high precision automated liquid handler (ALH) to ensure timely and accurate dispensing of low volumes of reagents into the wells of a 384–well plate. Suitable ALH platforms must be able to accurately dispense sub-microliter volumes (<5% error dispensing a 1 µL volume) and should be capable of dispensing an entire 384–well plate in less than 10 minutes. Examples of instruments meeting these specifications are the HP® D100 Digital Dispenser, the HP® D300e Digital Dispenser, Uno Single Cell Dispenser sold by Tecan, Duo Digital Dispenser sold by Tecan, and the Formulatrix® Mantis® Microfluidic Liquid Handler, and other similar devices.

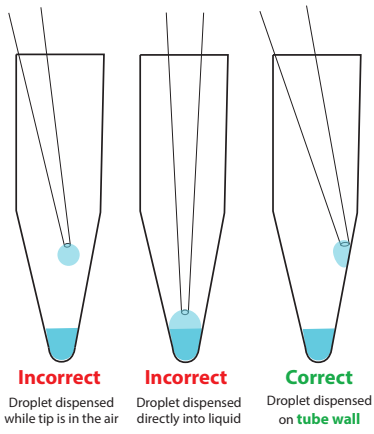

Figure 4. Pipetting Technique

2. **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 (Figure 4).

3. **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.

4. **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 Kit. The use of spectrophotometric quantification methods (such as Nanodrop) is not recommended.

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

6. 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 (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	-
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) for best practices.

Correct pipetting of the casssette 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), 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 1 µL of Cell Buffer, then proceed promptly to the ResolveDNA protocol or freeze the cells at -80°C for short-term storage. Cells may also be sorted "dry" into empty wells if desired. In cases where cells are dry sorted, it will be necessary to add the appropriate volume of Cell Buffer to each well prior to beginning the ResolveDNA protocol.

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

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

II. FACS/FANS

Fluorescence-activated cell sorting (FACS) or fluorescence-activated nuclei sorting (FANS) are currently the most common methods used to enrich cell populations of interest. Cells can be sorted based on surface markers, fluorescent staining, and light scattering properties. In preparation for the ResolveDNA protocol, cells should be sorted into the ResolveDNA Cell Buffer in tube or plate format. Refer to the BioSkryb Genomics Cell Sorting Protocol for more details.

III. 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) for best practices.

IV. Spatial Cell Picking Technology

A number of systems enable fully-automated cell picking. Refer to the BioSkryb "Integrated Workflow for Spatial Single Cell Genome Analysis" for one example (bioskryb.com/eap-cellcelector/).

V. Other Methods of Single Cell Dispensing

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

ResolveDNA WGA v2.0 Protocol, Manual 96 Reaction Format

The ResolveDNA WGA v2.0 Kit used for 96 reactions allows the processing of single or multiple cells (or nuclei) and low-input DNA samples. This protocol supports manual operations using 96-well PCR plates or 0.2 mL strip tubes. Reagents should be dispensed utilizing a multichannel pipet. The genome amplification takes place in a 2.5 hour isothermal incubation which is carried out in a thermal cycler.

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

I. Before You Begin

1. Read through the entire protocol and ensure all required equipment, reagents, and consumables are on hand.
2. Input samples must be suspended in 3 µL of **Cell Buffer** ⊗ in a 96-well plate.
3. The ResolveDNA WGA process should be carried out in a DNA-free, pre-amplification workspace or PCR hood enclosure to avoid the possible introduction of exogenous DNA from the operator or the lab environment.
   - ✎ Including a no-template control allows for detection of DNA carryover in reactions.
4. Run positive control reactions at a range of input concentrations. See "Use of Controls" in the Best Practices section for an in-depth discussion of this critical topic.
   - ✎ Failure to run positive and negative controls can make it difficult to interpret results.
5. Use a vortex mixer to thoroughly mix all reagents after thawing except **R2** 🔴.
   - ✎ DO NOT use traditional vortex mixers on multiwell plates containing cells, lysates, etc. during the protocol. Always mix multiwell plates in a thermal plate mixer. (See "Protocol Notes: Gentle and Thorough Mixing" in the "Best Practices" section for an in-depth discussion of this topic).
6. Always keep reactions and reagents on ice unless otherwise instructed.
   - ✎ Lab cooling blocks (such as the Eppendorf PCR Cooler) designed to keep reactions chilled during handling are recommended.
7. When instructed to "briefly spin down," the intent is to ensure any droplets dispersed within a tube are collected. A quick pulse (10 seconds) on a benchtop microcentrifuge is usually sufficient.
8. Program thermal cyclers with a 96-well block installed to run the DNA Amplification program (Table 1).

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

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

II. ResolveDNA WGA Procedure

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

   ⓘ **Important:** Once **L2** 🟡 has reached room temperature, vortex thoroughly ***until any precipitate is fully dissolved***, briefly spin down, and place on ice.
6. Prepare a 10 ng/µL gDNA stock by adding 2 µL of **Control gDNA** 🟡 to 8 µL of **Cell Buffer** ⊗ in a labeled microcentrifuge tube.
7. Vortex the 10 ng/µL **Control gDNA** 🟡 for 5 seconds, briefly spin down, and place on ice.

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

   ✎ **Note:** If the concentration deviates from the expected concentration 10 ng/µL by more than 10%, modify the dilution factor in subsequent dilutions to achieve the desired concentration.
8. Dilute the 10 ng/µL gDNA stock in **Cell Buffer** ⊗ to create 1 ng/µL, 100 pg/µL and 10 pg/µL stocks. This can be done via serial dilution and manual addition to the reaction plate, or by in situ dilution using an automated liquid handler capable of accurate nanoliter-scale pipetting.
9. Place the plate containing samples on ice.

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

   ✎ **If cells are fresh**, maintain on ice and proceed with amplification promptly.
10. If cells are suspended in less than 3 µL, add **Cell Buffer** ⊗ to bring them up to 3 µL total volume.
11. Dispense DNA controls (Figure 5). Ensure appropriate control samples are added to the reaction plate in 3 µL of **Cell Buffer** ⊗.

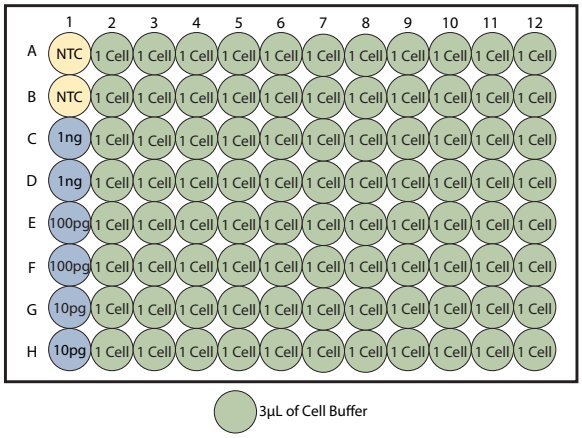

Figure 5. Example 96-Well Plate Experimental Layout.
The plate map illustrates a typical reaction setup, including multiple NTC, 1 ng gDNA, 100 pg gDNA and 10 pg gDNA controls added into a 96-well plate containing sorted single cells. 3 µL of Cell Buffer is dispensed into the wells in columns 2 through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

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

Table 2. Volume of Components in Lysis Mix.

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

13. Vortex 10 seconds to mix, briefly spin down, and place on ice.
14. Using a P-20 pipet, add 3 µL of **Lysis Mix** to each well.

> **If processing more than 16 reactions**, divide the **Lysis Mix** equally across the wells of an 8-strip PCR tube to act as a reservoir and use an 8-channel P-20 pipet to minimize time required for reagent additions.

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

During incubation complete the following steps:

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

Table 3. Volume of Components in Reaction Mix

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

18. 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 pipet mixing.

Once incubation is complete, continue with the following steps:

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

    If processing more than 16 reactions, divide the **Reaction Mix** equally across the wells of an 8-strip PCR tube to act as a reservoir and use an 8-channel P-20 pipet to minimize time required for reagent additions.

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

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

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

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

Post WGA Quality Control Checkpoint

1. To assess DNA yield, dilute each reaction by adding 10mM Tris pH 8.5 to a total volume of 40 µL.
2. Add 2 µL of diluted reaction mix to 198 µL Qubit reagent and measure the concentration per manufacturer's instructions.
3. Prepare a 2 ng/µL dilution in a fresh PCR plate by pipetting amplified DNA samples into 10mM Tris pH 8.5, 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.

ResolveDNA WGA v2.0 General Automation Protocol, 384 Reactions

The ResolveDNA WGA v2.0 Kit used for 384 reactions allows the processing of single or multiple cells (or nuclei) and low-input DNA samples. This protocol supports an automated liquid handler mediated high-throughput protocol allowing the parallel processing of up to 384 samples. 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. The genome amplification takes place in a 2.5 hour isothermal incubation which is carried out in a thermal cycler.

Cells should be placed into a 384-well plate containing 1 µL **Cell Buffer** and may be used immediately or frozen at -80°C until needed. Cells may also be sorted "dry" into empty wells if desired. In cases where cells are dry sorted, it will be necessary to add 1 µL **Cell Buffer** to each well before starting the protocol.

I. Before You Begin

1. Read through the entire protocol and ensure all required equipment, reagents, and consumables are on hand.
2. 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.
3. 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.
4. 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).
5. 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).
6. 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.
7. Program thermal cyclers with a 384-well block installed to run the DNA Amplification program (Table 4).

Table 4. DNA Amplification (lid temperature 70°C)

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

II. ResolveDNA WGA Procedure

1. Retrieve the ResolveDNA Whole Genome Amplification components from -20°C storage.
2. Place **L2** 🟡, **Control Genomic DNA** (gDNA) 🟡, and **Cell Buffer** ⊗ at room temperature to thaw for 30 minutes to 1 hour.
3. Place **L1** 🟣, **L3** ⚪, and **R1** 🔵 on ice to thaw for 30 minutes to 1 hour.
4. **R2** 🔴 should be left in -20°C storage until needed.
5. Once the reagents 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. Dilute the 10 ng/µL stock in **Cell Buffer** ⊗ to create 600 pg/µL, 60 pg/µL, 12 pg/µL, 6 pg/µL, and 1 pg/µL stocks. This can be done via serial dilution and manual addition to the reaction plate, or by in situ dilution using an automated liquid handler capable of accurate nanoliter-scale pipetting.
9. Place the plate containing samples on ice.
   - **If cells were stored at -80°C**, thaw the cells on ice for 5 minutes, spin for 10 seconds, and place on ice.
   - **If cells are fresh**, maintain on ice and proceed with amplification promptly.
10. If cells are suspended in less than 1 µL, add **Cell Buffer** ⊗ to bring them up to 1 µL.
11. Dispense 1 µL of each DNA control (Figure 6).
12. Prepare Lysis Mix by combining the following reagents in a microcentrifuge tube (Table 5).

    ✎ **Note:** If running fewer than 384 reactions, use the Volume per Reaction to calculate the required reagent volumes and add 30% overage to enable automated pipetting.

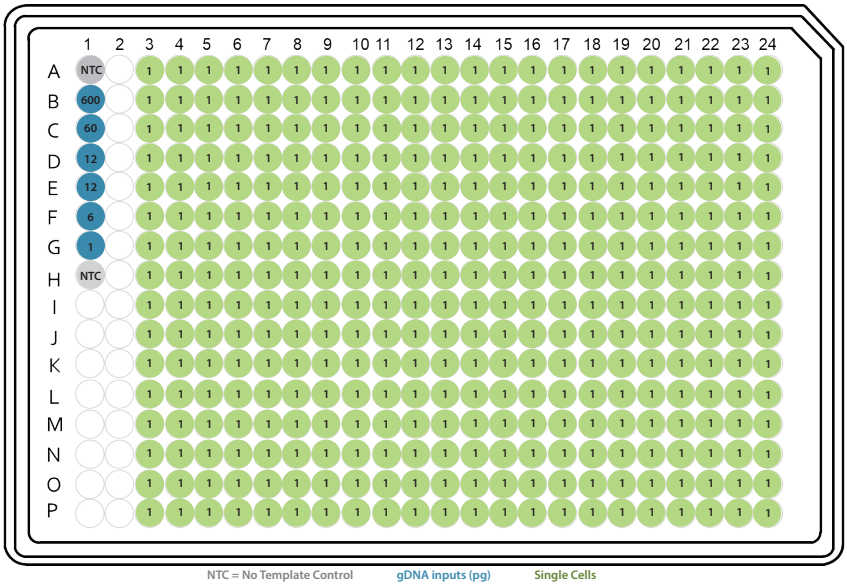

Figure 6. Example 384-Well Plate Experimental Layout. The plate map illustrates a typical reaction setup, including multiple NTC and multiple inputs of gDNA added into a 384-well plate containing sorted single cells. 1 µL of **Cell Buffer** is dispensed into the wells in columns 3 through 24. Cells are then sorted into these wells (FACS/FANS etc.) Prior to processing, 1 µL of the control samples are added to column 1 in the listed order.

Table 5. Volume of Components in 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

13. Vortex the **Lysis Mix** for 10 seconds to mix, briefly spin down, and place on ice.
14. Using an automated liquid handler, add 1 µL of Lysis Mix to each well.
15. Seal and spin down for 10 seconds to combine components.
16. Incubate in the thermal mixer, mixing at room temperature for 20 minutes at 1,400 rpm.

During incubation complete the following steps:

17. Start the DNA Amplification protocol (see Table 1) on the thermal cycler and allow the block to reach the amplification reaction set point of 30°C. Pause the thermal cycler.
18. Prepare the **Reaction Mix** on ice by combining the components in the following order (Table 6).

Table 6. 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

19. 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.

Once incubation is complete, continue with the following steps:

20. Remove the plate from the thermal mixer, spin down for 10 seconds, and place on ice.
21. Using an automated liquid handler, add 2 µL of the **Reaction Mix** to each well.

    ⓘ **Important:** Due to the viscosity of the **Reaction Mix**, it may be necessary to include an offset in the volume settings of the ALH software. For example, the HP D100/D300e instruments require a +10% offset in the dispense volume setting (i.e. 2,200 nL to achieve a 2.0 µL volume delivery).

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

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

25. Load the plate and unpause the thermal cycler program.
26. After the program is complete, remove the plate, spin down for 10 seconds, and place on ice.
27. 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 10mM Tris pH 8.5 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 10mM Tris pH 8.5, 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.

ResolveDNA WGA v2.0 Tecan Automation Protocol, 384 Reactions

The ResolveDNA WGA v2.0 Kit used for 384 reactions allows the processing of single or multiple cells (or nuclei) and low-input DNA samples. This protocol 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 µL 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 µL **Cell Buffer** to each well during the run.

Table 7. 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 8).

Table 8. 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 **"BioSkryb ResolveDNA Step 1."**

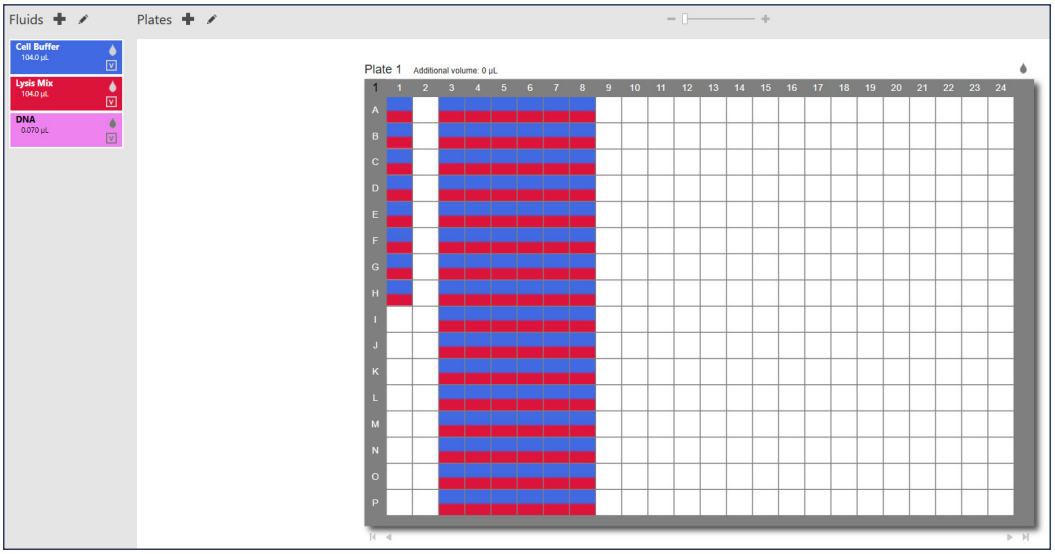

Figure 7. BioSkryb ResolveDNA Step 1 template. The fluid classes include Cell Buffer, Lysis Mix, and gDNA stock. The first column has been pre-loaded to create a set of control DNA reactions from the 10 ng/µL control DNA stock (from A-H: NTC, 600 pg, 60 pg, 12 pg, 12 pg, 6 pg, 1 pg, NTC). Single cell 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.

    ⓘ **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.**

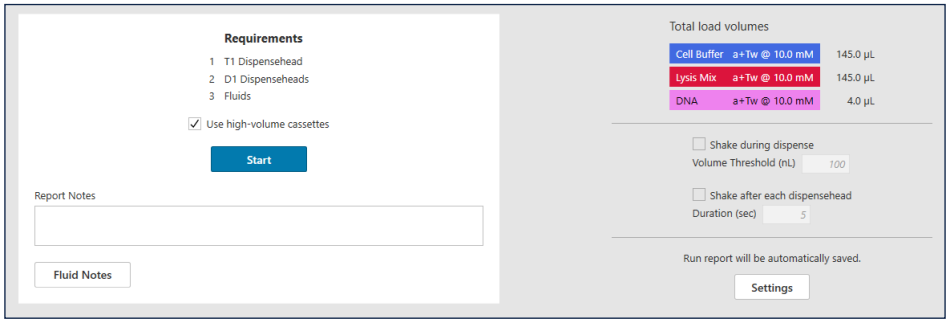

Figure 8. The Pre-Run Summary Screen. Provides a summary of the number and types of dispensehead cassettes necessary as well as the fluid loading volumes required for complete dispensing.

14. Prepare Lysis Mix by combining the following reagents in a microcentrifuge tube (Table 9).

> **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 9. 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.

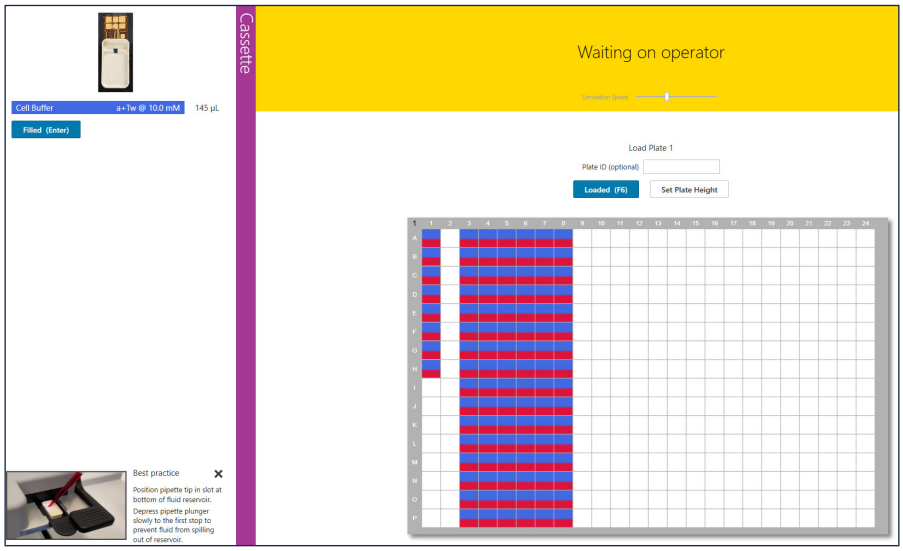

Figure 9. The Run Window Screen. Indicates steps in the operational workflow. Cassettes and fluids are requested on the left panel, plate loading is requested in the center. Dispensing is then reported in real time as the script proceeds. As additional fluids or cassettes are required, the software will 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 10).

Table 10. 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."**

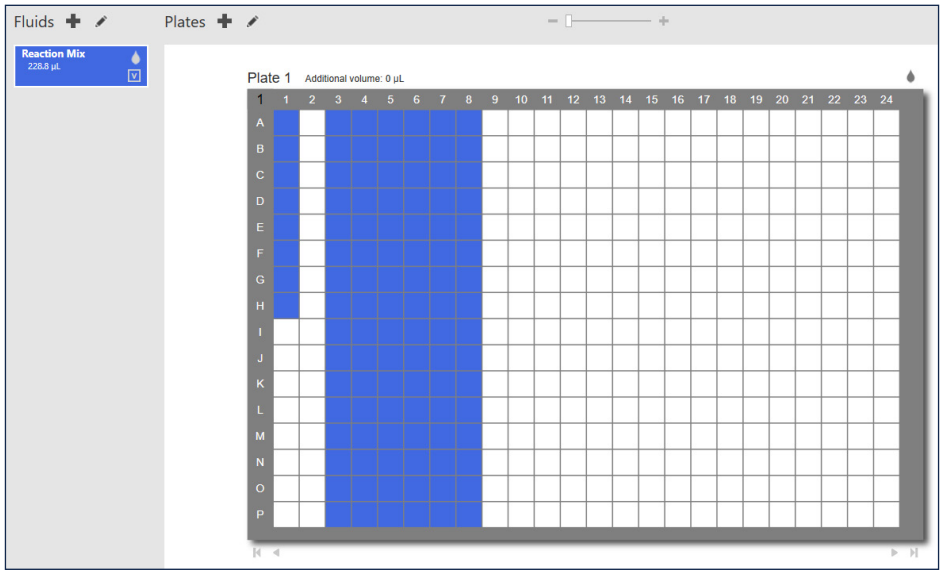

Figure 10. BioSkryb ResolveDNA Step 2 template. A single fluid class, Reaction Mix, should be indicated to dispense in each reaction well. Copy and paste as necessary to cover all reaction wells up to the full plate.

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 cylcer and unpause the DNA Amplification program (Table 8).
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 10mM Tris pH 8.5 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 10mM Tris pH 8.5, 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.

Appendix A: Post WGA Quality Control Example Data

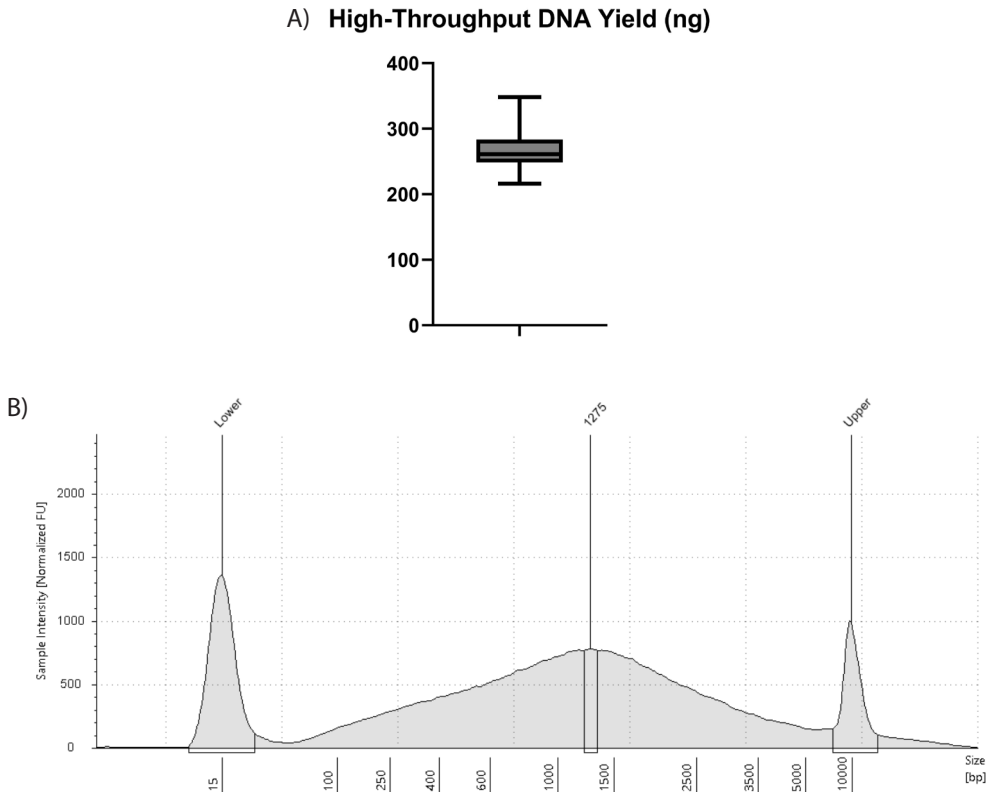

Figure 11. Examples of Total Amplification Yields and Fragment Size Distribution.

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/µL and run on a Tapestation using the D5000 HS Screentape. Average fragment size in this sample is 1275 bp, which is typical.

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