BioSkryb GENOMICS

ResolveDNA® Whole Genome Single-Cell Core Kit Protocol to Prepare WGS-Ready Libraries

96-Well Format

User Guide

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ResolveDNA® Whole Genome Single-Cell Core Kit, 96-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 Whole Genome Amplification (WGA) solution with single cell resolution, enabled by the proprietary Primary Template-directed Amplification (PTA) chemistry.

The optimized reaction parameters employed in this PTA-based kit enable the reproducible recovery of over 97% of the genomes of single cells or nuclei, and robust amplification of limited DNA input samples with industry leading uniformity and accuracy. This complete end-to-end solution kit enables users to go from cells to sequencing-ready libraries in under 8 hours. This kit is configured for working with 96 reactions and contains one adapter set with 96 unique dual indexes (UDIs) to generate 96 libraries for sequencing.

Key features and benefits include the following:

- A simple, user-friendly workflow that requires less than 2.5–hours hands-on time and under 8 hours total run time.
- Specific amplification of single cell genomes recovering >97% of the human genome.
- Excellent allelic balance enables highly sensitive and specific assessment of single nucleotide variation (SNV).
- Compatible with the following:
  - Single cells
  - Multiple cells
  - 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.

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

The ResolveDNA Workflow

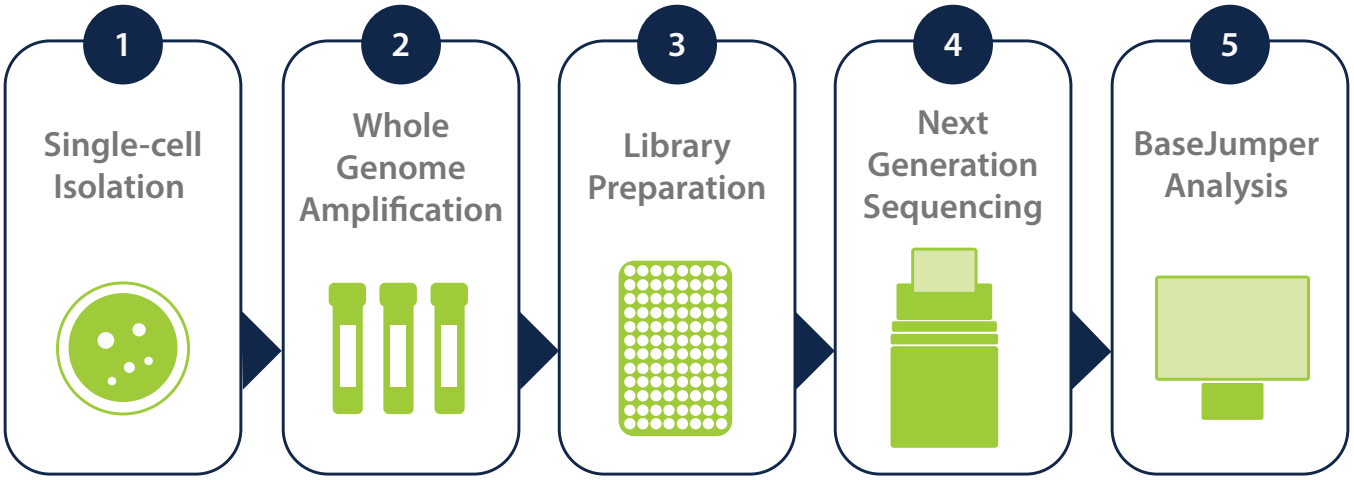


Figure 1. The ResolveDNA Single-Cell Sequencing Workflow

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, 96 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, 96 Reactions and protocol. Library preparation with the kit allows up to 96 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. BaseJumper provides a framework to label cell features and visualize molecular data across multiomic technologies.

ResolveDNA Amplification Technology

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

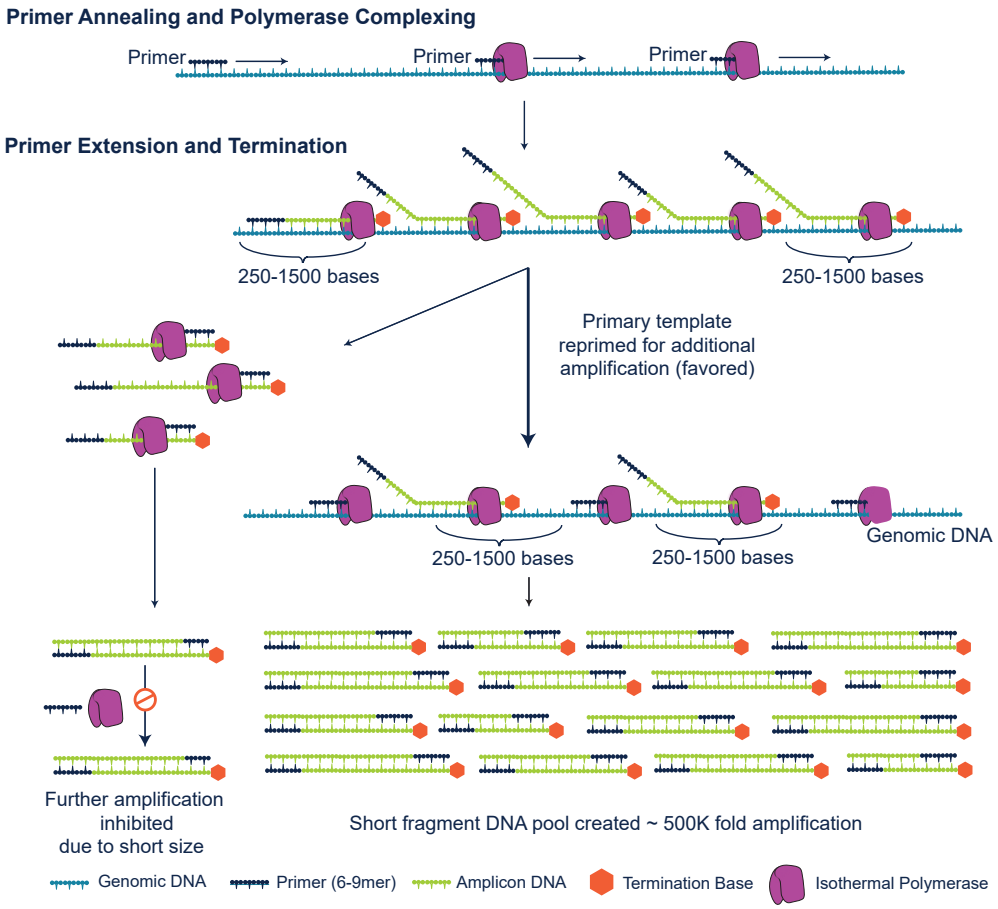


Figure 2. Primary Template-Directed Amplification (PTA). PTA may be performed directly from single cells, multiple cells, or nuclei (collected by FACS, FANS, microfluidic cell separation or other methods), or ultra-low inputs of DNA (4 pg – 10 ng). After cell lysis and genomic DNA denaturation, random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

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

Safety Precautions and Use of Personal Protective Equipment

I. Biosafety Hazards

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

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

II. Chemical Hazard.

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

III. Safety Data Sheets

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

IV. Emergency Response Information

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

North America: +1-800-535-5053

International: +1-352-323-3500

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

Intended Use

The ResolveDNA Whole Genome Single-Cell Core Kit is intended for ***research use only*** and is not intended for prevention, diagnosis, or treatment of disease.

Kit Contents and Storage

I. Kit Contents

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

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 for up to the expiration date, 18 months from the date of manufacture (DOM). Do not exceed 5 freeze/thaw cycles for any individual reagent.

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

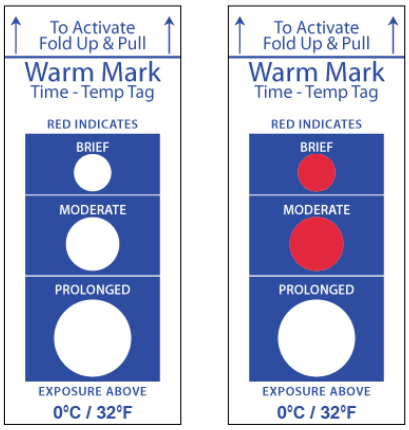


Figure 3. Temperature Indicator Tag Each dry ice shipment includes a temperature tag designed to indicate exposure above 0°C. If the shipment stays below the target temperature, the windows will remain white.

Additional Equipment, Materials, and Reagents

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

Products from BioSkryb Genomics		
Product Name	**Company**	**Catalog Number**
ResolveDNA® PTA-Grade Cell Buffer Pack (12X 500 µL)	BioSkryb	100177
ResolveDNA® Cell Buffer Bottle Kit	BioSkryb	100183
Products from Third-Party Suppliers		
Product Name	**Company**	**Catalog Number**
PCR Plate Sealing Film	ThermoFisher	AB-0558
twin.tec 96-well PCR Plate	Eppendorf	0030128648
Magnet PCR Separation Plate	Permagen	MSP750
8-strip 0.2 mL PCR Tubes	General Lab Supplier (GLS)	—
1.5 mL Microcentrifuge Tubes	GLS	—
Single-channel 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	—

Best Practices

I. Use of Controls

The following standard control set is recommended for each experiment:

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

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.

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

II. Protocol Notes

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

1. **Manual Pipetting Technique:** All reagent additions should be dispensed onto the wall of the tube or well as shown in Figure 4. To avoid material loss in the reaction, it is important to avoid direct contact between 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.

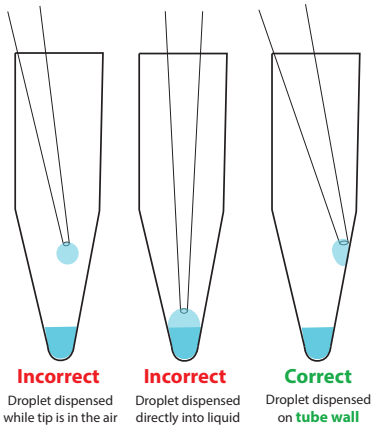


Figure 4. Pipetting Technique

2. **Gentle and Thorough Mixing:** Once the reagent has been added to the tube, it is vital to ensure gentle and thorough mixing of the reaction components. Any non-homogeneity within the reaction will lead to inefficiency and diminish the performance of the kit. To ensure each reagent addition is mixed into the reaction thoroughly, first seal the plate/tubes and briefly spin in a centrifuge/plate spinner (10 seconds at ~750 X g is sufficient). Use just enough force to combine the added droplet with the material in the bottom of the tube.

   Once the added droplet has been combined with the reaction components in the bottom of the tube, place the reaction plate/tubes in a programmable thermal mixer and gently mix according to the instructions in this protocol. After mixing, briefly spin the reactions again to ensure any droplets generated during the mixing process are recombined in the bottom of the plate/tubes.

In summary, for best results ALWAYS pipet reagent additions on the side of the tube, avoiding any contact with the material in the bottom of the tube or well, then SEAL-SPIN-MIX-SPIN. After these steps, proceed with any incubation or move on to the next reagent addition per the protocol.

3. **Quantification:** Use a fluorometric method of quantification (such as Qubit) with the amplification products and sequencing libraries produced with the ResolveDNA Whole Genome Single-Cell Core Kit. The use of spectrophotometric quantification methods (such as Nanodrop) is not recommended.
4. **Plasticware:** Use sterile, DNA-free and nuclease-free polypropylene working stock tubes and containers. Polystyrene tubes and containers are NOT recommended.

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

Sample Selection and Preparation

I. Sample Types Supported

This protocol is generally designed to work with single live mammalian cells, nuclei, or low amounts of DNA input (4 pg – 10 ng). Input can be either single or multiple cells, obtained by common cell collection methods. No upper limit has been established for multiple cell input. Ensure that cells are viable and placed into 3 µL of Cell Buffer, then proceed promptly to the ResolveDNA protocol or freeze the cells at -80°C for short-term storage. Cells may also be sorted "dry" into empty wells if desired. In cases where cells are dry sorted, it will be necessary to add the appropriate volume of Cell Buffer to each well prior to beginning the ResolveDNA protocol.

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

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

II. FACS/FANS

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

III. Spatial Cell Picking Technology

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

IV. Other Methods of Single Cell Dispensing

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

ResolveDNA Whole Genome Amplification (WGA) Protocol, 96 Reactions

The ResolveDNA Whole Genome Single-Cell Core Kit with 96 reactions allows the processing of single or multiple cells (or nuclei) and low-input DNA samples. This Kit supports manual operations using 96-well PCR plates or 0.2 mL strip tubes. Reagents should be dispensed utilizing a multichannel 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. Ensure appropriate control samples are added to the reaction plate in 3 µL of **Cell Buffer** ⊗.

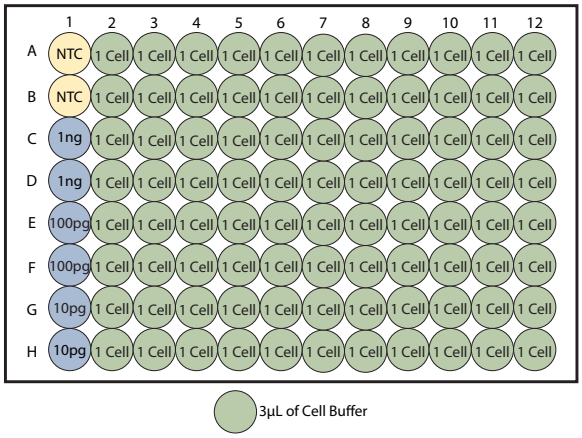


Figure 5. Example 96-well Plate Experimental Layout.
The plate map illustrates a typical reaction setup, including multiple NTC, 1 ng gDNA, 100 pg gDNA and 10 pg gDNA controls added into a 96-well plate containing sorted single cells. 3 µL of Cell Buffer is dispensed into the wells in columns 2 through 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 **Elution Buffer** to a total volume of 40 µL.
2. Add 2 µL of diluted reaction mix to 198 µL Qubit reagent and measure the concentration per manufacturer's instructions.
3. Prepare a 2 ng/µL dilution in a fresh PCR plate by pipetting amplified DNA samples into **Elution Buffer**, seal the plate, vortex briefly, and spin down.
4. Determine fragment size distribution by running 2 µL of each 2 ng/µL diluted sample using a Tapestation HS D5000 Screentape or other fragment analysis instrument per manufacturer's instructions.
5. Refer to Appendix A for example quality control data.
6. Proceed to the ResolveDNA Universal Library Preparation workflow.

ResolveDNA Universal Library Preparation (for downstream WGS)

The ResolveDNA Universal Library Preparation protocol supports multichannel pipet mediated processing of up to 96 samples.

> ⓘ 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.

I. Before You Begin

1. Read through the entire protocol and ensure all required equipment, reagents, and consumables are on hand.
2. Thaw all library preparation kit reagents on ice and maintain the reagents on ice. 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.

   > ⓘ **Important: DO NOT VORTEX** reagents **LP0E, LP1E, LP2L, and LP3A**. These reagents should be mixed by inversion and briefly spun down after thawing. All other reagents should be vortexed for 10 seconds and briefly spun down after thawing.
3. 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.
4. Program thermal cycler to run the DNAPREP Program (Table 4).

   Table 4. DNAPREP Program (lid temperature 105°C)

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

5. Program thermal cycler to run the FERAT Program (Table 5).

   Table 5. FERAT Program (lid temperature 105°C)

Step	Temperature	Time
Hold 1	4°C	30 seconds
Hold 2	30°C	5 minutes
Hold 3	65°C	30 minutes
Hold 4	4°C	∞
Total Time	-	**~40 minutes**

II. DNA Preparation

1. Add 20 ng of each ResolveDNA whole genome amplified (WGA) product to a fresh plate on ice. Add **Elution Buffer** to bring the total volume to 3 µL.

   ⓘ The workflow has been designed for 20 ng DNA input but has been demonstrated to be robust for library prep input volumes of 10–40 ng. Calculating and inputing precise DNA input is optional.

2. Vortex, briefly spin, and return plate to ice.
3. Prepare the **DNA Prep Master Mix** in a 1.5 mL Eppendorf tube on ice by adding the components in Table 6.

   Note: Sufficient volume is provided for up to five 24–48 reactions of DNA Prep.

   Table 6. DNA Prep Master Mix

Reagent Name	Number of Samples	
	24–48 Reactions	49–96 Reactions*
LP0B Reagent ○	192 µL	384 µL
LP0E Reagent ⊗	0.5 µL	1.0 µL
Total Volume	**192.5 µL**	**385 µL**
		*33% overage included

4. Vortex 10 seconds to mix, briefly spin down and place on ice.
5. Add 3 uL of **DNA Prep Master Mix** to each well.
6. Briefly spin, vortex, spin again, and place in thermocycler.
7. Initiate the DNAPREP protocol.

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

1. Vortex **LP1B Reagent** ● for 5 seconds and briefly centrifuge to collect all liquid in the bottom of the tube.
2. Invert **LP1E Reagent** ● 10 times to homogenize and ensure complete mixing. Briefly centrifuge to collect all liquid in the bottom of the tube.
3. Prepare the **FERAT Master Mix** in a 1.5 mL Eppendorf tube on ice by adding the components in Table 7.

   Table 7. FERAT Master Mix

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

4. Vortex the **FERAT Master Mix** on medium speed for 5 seconds to ensure equal mixing and briefly spin to collect all liquid in the bottom of the tube.

   ✎ **Note:** this mixture is stable on ice for up to 4 hours.

5. Once the DNAPREP program is complete, remove the plate from the thermal cycler, briefly spin to collect all liquids at the bottom of the wells, and place plate on ice.
6. Add 4 µL of the **FERAT Master Mix** to each well on ice.
7. Seal the plate and briefly spin to get the liquid to the bottoms of the wells.
8. Vortex the plate at medium speed to homogenize the reaction.
9. Spin for 10 seconds to collect the samples at the bottoms of the wells then place the plate back on ice.

   ✎ **Note:** Complete mixing is critical to achieve desired fragment lengths.

10. Place the plate into the preheated thermal cycler and initiate the FERAT Program (Table 5).
11. While the thermal cylcer is running, remove **ResolveDNA Bead Purification** components from storage and allow to equilibrate to room temperature for at least 30 minutes.
12. While the thermal cylcer is running, thaw the **Single Use Library Adapter Set** plate on ice.

IV. Ligation

1. Once the FERAT program is complete, remove the plate from the thermal cycler, briefly spin to collect all liquids at the bottom of the wells, and place plate on ice.
2. Invert the **LP2L Reagent** ● ten times to homogenize **(DO NOT VORTEX)** and place on ice.
3. Vortex thawed **Single Use Library Adapter Set** plate briefly and centrifuge.
4. Add 5 µL of **Single Use Library Adapters** to each sample in the plate.

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

5. Add 5 µL of **LP2L Reagent** ● to each sample in the plate.

   ✎ **Note: LP2L Reagent** ● is viscous. Pipette carefully.

6. Seal the plate, mix by vortexing at medium speed, and briefly spin to collect all liquid at the bottom of the wells.
7. Incubate the plate at 20°C for 15 min in a thermal cycler with the lid temperature disabled or set to 50°C.
8. Proceed immediately to library amplification.

V. Library Amplification

1. Initiate LIB-AMP program on a thermal cycler (Table 8). Allow the thermal cycler to warm up to temperature and pause the program.

Table 8. LIB-AMP program (lid temperature 105°C)

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

2. Invert **LP3A Reagent** several times to mix **(DO NOT VORTEX)**.
3. Vortex **LP3P Reagent** 🔴 and briefly spin down.
4. Prepare **Amplification Master Mix** by assembling the components in Table 9 in a new tube.

Table 9. Amplification Master Mix

Reagent Name	Volume per Number of Reactions	
	One (1) Reaction	96 Reactions*
LP3A Reagent	18 µL	1900 µL
LP3P Reagent 🔴	2 µL	211 µL
Total Volume	**20 µL**	**2111 µL**
		*10% overage included

5. Add 20 µL of the **Amplification Master Mix** to each well of the plate containing adapter-ligated DNA.
6. Seal the plate with a film and mix thoroughly by vortexing at medium speed followed by a brief spin.
7. Load plate into preheated thermal cycler and initiate the LIB-AMP program in Table 6.
8. Place the sample plate on ice and proceed directly to post-amplification cleanup.

VI. Post Amplification Cleanup

1. Make sure **ResolveDNA Bead Purification** components are equilibrated to room temperature before use.
2. Vortex **Resolve Beads** thoroughly immediately before use to ensure even distribution of beads.
3. Add 30 µL of **Resolve Beads** to each reaction with a multichannel pipet.

> **Note:** Maintain a 0.75:1 volume ratio of beads to sample. If your sample volume varies from the 40 µL (+/-10%) reaction volume used in this protocol, adjust the bead volume accordingly to maintain this ratio.

4. Seal the plate with film and vortex on high for 10 seconds.
5. Incubate the plate at room temperature for 5 minutes.
6. Briefly spin the plate for 10 seconds.

7. Place plate on a magnet for 3 minutes or until the liquid is clear.
8. While on the magnet, remove and discard the supernatant using a multichannel pipet.
   - **Note:** Take care not to disturb the beads here and in the upcoming wash steps.
9. Keep the plate on the magnet and add 200 µL of freshly prepared 80% ethanol to each well, being careful not to disturb the beads.
10. Incubate the plate on the magnet at room temperature for 30 seconds.
11. With the plate on the magnet, carefully remove and discard the ethanol.
12. Perform a second ethanol bead wash. With the plate on the magnet, add another 200 µL of freshly prepared 80% ethanol to each well, being careful not to disturb the beads.
13. Incubate the plate on the magnet at room temperature for 30 seconds.
14. With the plate on the magnet, carefully remove and discard the ethanol.
15. Seal the plate, spin briefly, return to the magnet, and carefully remove the seal.
16. Incubate the plate on the magnet at room temperature for 1 minute or until the supernatant clears.
17. Remove any remaining ethanol using a P20 pipet.
18. Allow the beads to dry for 3 minutes.
    - **Note: DO NOT** over dry the beads, this will result in reduced yields.
19. Remove the plate from the magnet.
20. Resuspend the beads in 42 µL of **Elution Buffer**. Pipette multiple times to mix well or seal the plate and vortex.
    - **Note:** pipetting carefully will minimize bubbling and allow for greater library recovery.
21. Incubate the plate at room temperature for 2 minutes to elute DNA off the beads.
22. Briefly spin plate and place on magnet for 2 minutes or until the liquid is clear.
23. Carefully transfer 40 µL of the DNA in elution buffer to a new plate. Be careful not to disturb the beads.
24. Place the plate on ice if proceeding to library quantification and fragment size analysis. Otherwise, store samples at -20°C.

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 additonal 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

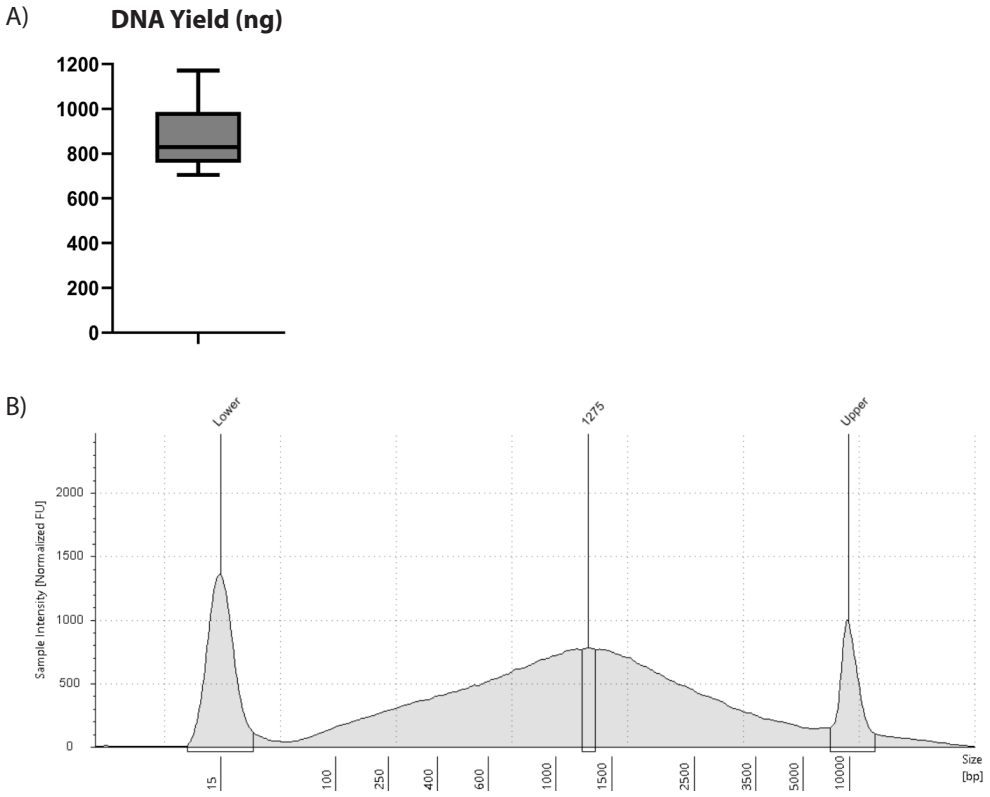


Figure 6. Examples of Total Amplification Yields and Fragment Size Distribution.

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

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

Appendix B: Post Library Amplification Quality Control Example Data

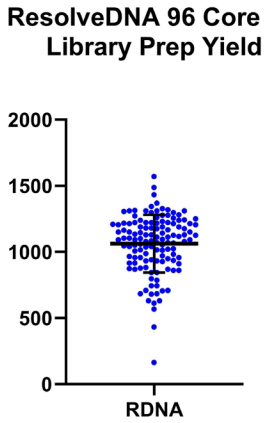
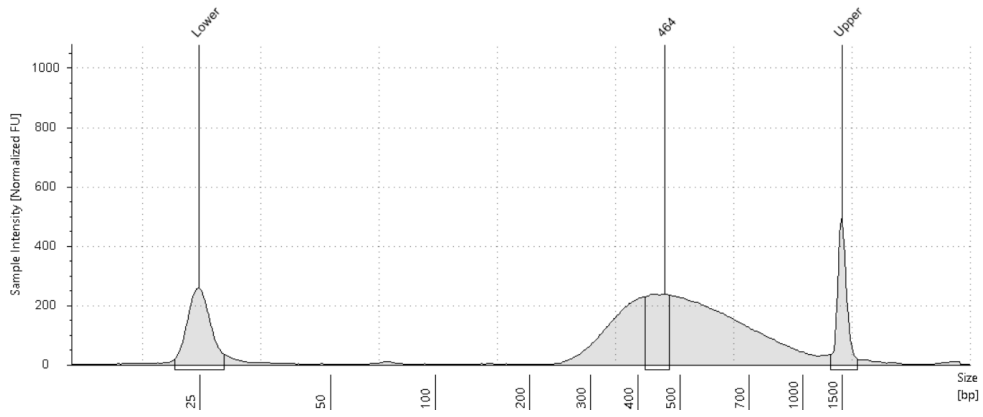


Figure 7. Examples of DNA Library Yields.

DNA library yield for the ResolveDNA Whole Genome Single-Cell Core Kit from single human cells. Measurement unit is nanograms (ng). Lower yields are sufficient for successful sequencing.



Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
50	1000	522	1490	5140	92.36		

Figure 8. Example DNA Library Fragment Size Distribution.

The electropherogram represents a sample after library preparation using the ResolveDNA Whole Genome Single-Cell Core Kit, 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 (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) 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).

Appendix D: Library Prep Adapter Sequences

For a complete list of BioSkryb Library Prep Adapter Sequences, please contact our technical support team.

For more information please contact:

BioSkryb, Inc
2810 Meridian Pkwy, Suite 110
Durham, NC 27713

www.bioskryb.com
techsupport@bioskryb.com
orders@bioskryb.com
(P) +919-370-0841

24 Hr Emergency Response
North America: +1-800-535-5053
International: +1-352-323-3500