BioSkryb GENOMICS

ImTec DIAGNOSTICS NV

ResolveDNA™ Whole Genome Amplification Kit

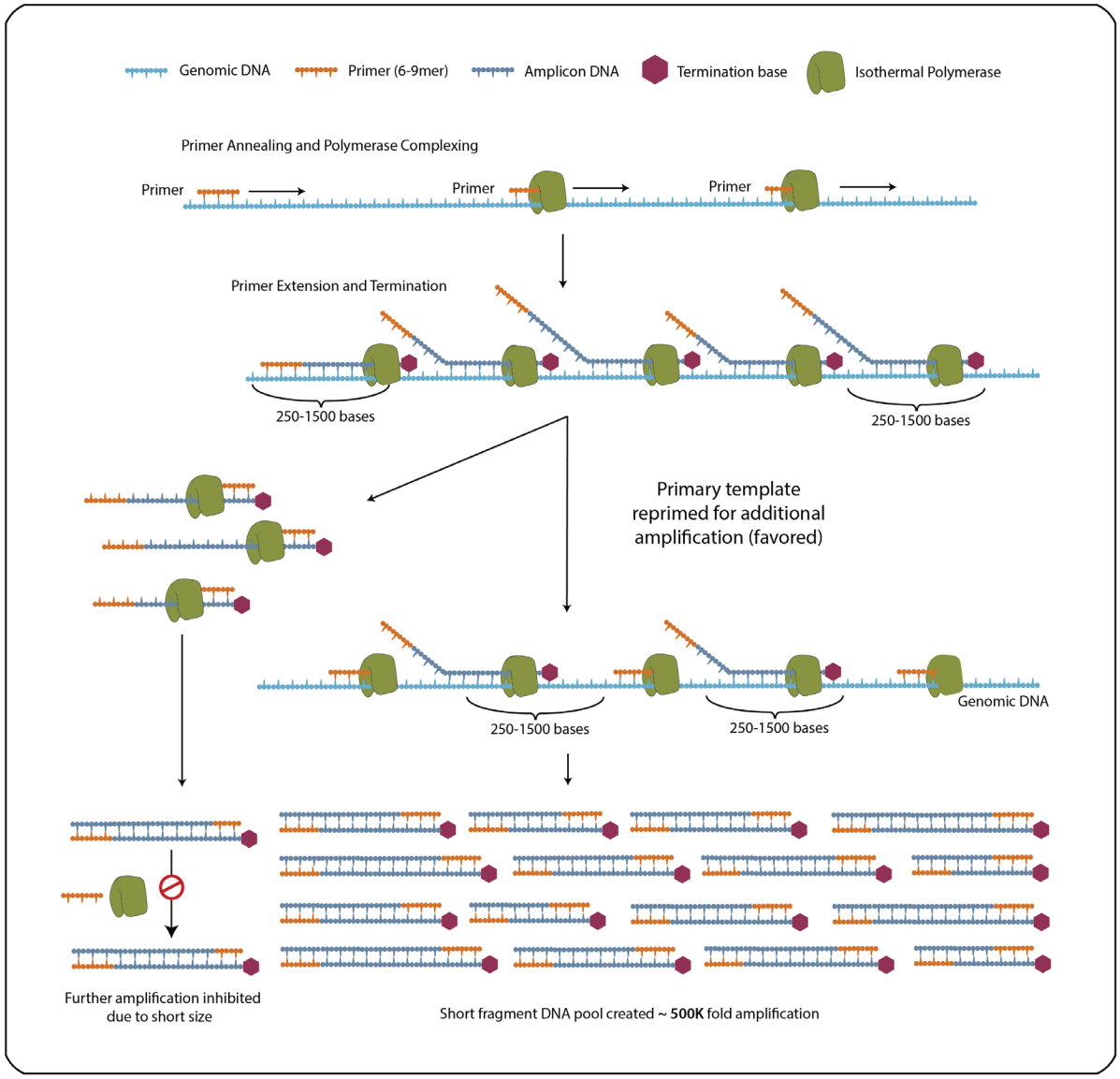
For high-quality single-cell and low-input DNA amplification

(Formerly SkrybAmp™ EA WGA Kit) PN 100068

P00001 - 02042021

ResolveDNA™ for Single-Cell and Ultra-Low DNAInputs

The BioSkryb ResolveDNA™ Whole Genome Amplification Kit is designed to produce uniform DNA libraries from low inputs of mammalian DNA, including from individual cells. ResolveDNA for single-cell and ultra-low DNA inputs is the result of an innovative system to prevent non-reproducible amplification of the target DNA molecule within the sample. Using the ResolveDNA kit, the user is not required to fragment the DNA prior to library construction for DNA sequencing applications. This is made possible by a proprietary chemical process, Primary Template-directed Amplification (PTA), that prevents long DNA amplicons from being produced during the amplification process. The net effect of this process is the absence of "pile-ups" in the amplified DNA. Typical amplification reactions will generate at least 1 microgram (µg) of DNA from an individual cell (or 5-10 picograms (pg) of input DNA). Yield may vary from the input and quality of DNA. We recommend fragments of DNA > 5kb as the amplification process becomes less efficient with small fragments of DNA.



ResolveDNA - A simple solution with powerful results. ResolveDNA avoids long amplification products that can be reprimed. Repriming of long amplification products generates less accurate representation of the original genome material. By contrast, ResolveDNA, using a high fidelity polymerase, a proprietary nucleotide pool, and a simple primer, amplifies even a single genome uniformly and accurately, providing a true representation of the primary template.

I. Kit Contents:

ResolveDNA Kit Components	Color	Volume (24 Reactions)	Volume (96 Reactions)
Cell Buffer	Clear	500 µL	500 µL
SM2 Reagent	White	500 µL	500 µL
12X SS2 Reagent	Yellow	500 µL	500 µL
SN1 Reagent	Orange	500 µL	500µL
SDX Reagent	Purple	80 µL	360 µL
SB4 Reagent	Blue	138 µL	550 µL
SEZ1 Reagent	Red	27 µL	96 µL
SEZ2 Reagent	Teal	36 µL	144 µL
DNA/Nuclease-Free Water	Natural Opaque	500 µL	500 µL
Control Genomic DNA (50ng/µL)	Gold	10 µL	10 µL

II. Shipping and Storage

This kit is shipped frozen. The kit, including all reagents and buffers, should be stored immediately upon receipt at -20°C in a constant temperature freezer.

III. Reagent Aliquoting

- Avoid contamination of reagents by working in a DNA-freepre-PCR hood and by using separate laboratory equipment (eg. tubes, pipettes, filtered tips).
- Ensure that reagents are kept cool throughout the aliquoting process by keeping tubes on ice or on a cooling block at all times.
- Store aliquots in a -20°C freezer cooling box, or in a -20°C freezer with minimal temperature fluctuations.

IV. Equipment, Materials, and Reagents

The following list of products has been tested with our workflow to provide optimal results. The use of any products not included on this list could result in non-optimal results.

Product Name	Company	Catalog Number
Low Bind 96-Well PCR Plates or Strip Tubes	BioSkryb or General Lab Supplier	100149
PCR Plate Thermal Mixer	BioSkryb	100150
PCR Cooler	Eppendorf	022510541
PCR Plate Sealing Film	ThermoFisher Scientific	AB-0558
PCR Plate Spinner	BioSkryb	100153
ResolveDNA Magnetic Plate or Dual Volume Strip Tube Magnet	BioSkryb	100135 or 100226
ResolveDNA Bead Purification Kit (Elution Buffer included)	BioSkryb	100121/100182
High Sensitivity dsDNA Assay kit	ThermoFisher Scientific	Q32854
Agilent Tapestation	Agilent	—
HS D5000 Screentape and Reagent	Agilent	5067-5592 &5067-5593
ResolveDNA Elution Buffer pH 8.5	BioSkryb	100127
Fluorometer (Qubit 2-4)	ThermoFisher Scientific	—
Thermal Cycler	General Lab Supplier	—
Absolute (200 proof) Ethanol	General Lab Supplier	—
RT-PCR Grade Water	General Lab Supplier	

V. Single Cell Capture by FACS Sorting:

1. Place a Low Bind 96-well PCR plate on PCR cooler.
2. Add 3 µL of Cell Buffer to all the wells where cells will be sorted. Seal the plate with a sealing film and keep it on ice until ready to use.
3. After single cell sorting, seal the plate.
4. Mix the plate for 10 seconds at 1400 RPM on the BioSkryb PCR Plate Thermal Mixer at room temperature, spin plate briefly, and place on ice. Alternatively, plates containing sorted cells must be stored on dry ice with a seal or at -80°C until ready for whole genome amplification.

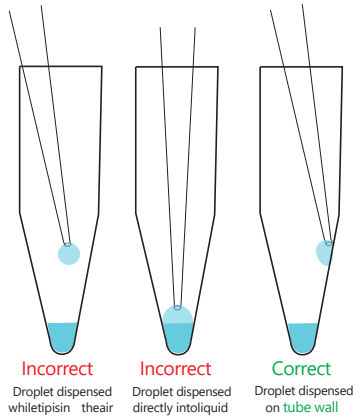
**Note: If cells will be collected by a method other than sorting, ensure the cells are deposited in the bottom of the well. This protocol is optimized for cells collected in 3 µL of cell buffer. See Appendix A for information about positive and negative control setup.*

VI. Single Cell Whole Genome Amplification

IMPORTANT:

1. After adding reagents to plates containing cells, an RPM controlled mixer must be used. **UNDER NO CIRCUMSTANCES SHOULD PLATES CONTAINING SORTED SINGLE CELLS BE MIXED WITH A VORTEXER OR BY PIPETTING**
2. Protocol is compatible with low-bind PCR strip or single tubes
3. Freeze PCR cooler at -20°C for 2 hrs and thaw for 10 min or conduct reactions on ice
4. Assemble reactions in a DNA-free pre-PCR hood.
5. Thaw and keep all reagents on ice until ready to use.
6. Do not remove enzyme from freezer until ready to prepare reaction-enzyme mix (SEZ1 and SEZ2).
7. Before use, vortex each reagent for 10 sec and spin briefly.
8. Dispense ResolveDNA reagents to the wall of the tube without touching cell suspension. (See Figure 1)

Figure 1 - Critical Pipetting Technique



PROCEDURE:

1. Place 96-well PCR plate containing cells on the PCR cooler. If cells were stored at -80°C, thaw the cells on ice for 5 minutes, spin for 10 seconds, then place the plate on the PCR cooler (or ice).
2. Prepare 1X SS2 Reagent Mix by combining reagents, mixing on the vortexer, and briefly spinning tube.

Component	Volume for 24 Reactions	Volume for 96 Reactions
Water	165 µL	330 µL
12X SS2 Reagent	15 µL	30 µL
Total Volume	180 µL	360 µL
* If precipitant is observed in 12X SS2 reagent, warm at 37°C for 10 min and ensure no observable precipitant is present prior to making 1X SS2 buffer mix.		

3. Prepare MS Mix by combining the following reagents, mixing on the vortexer, and briefly spinning tube.

Component	Volume per reaction	Volume for 24 Reactions	Volume for 96 Reactions
SM2 Reagent	1.5 µL	40 µL	160 µL
1X SS2 Reagent	1.5 µL	40 µL	160 µL
Total Volume	3.0 µL	80 µL	320 µL
* Calculations include 10% overages			

4. Add 3 µL of MS Mix to each well.
5. Seal the plate with the sealing film.
6. Spin for 10 sec, mix at room temperature for 1 min at 1400 rpm (plate mixer), spin for 10 sec and place plate back on PCR cooler (or ice).
7. Incubate on PCR cooler or ice for 10 minutes.

8. Add 3 µL of SN1 Reagent.
9. Seal the plate with the plate film.
10. Spin for 10 sec, mix at room temperature for 1 min at 1400 rpm (plate mixer), spin for 10 sec and place plate back on PCR cooler.

11. Add 3 µL of SDX Reagent.
12. Seal the plate with the plate film.
13. Spin for 10 sec, mix at room temperature for 1 min at 1400 rpm (plate mixer), spin for 10 sec.
14. Incubate at room temperature for 10 min.
15. During the incubation step, prepare the Reaction Mix by combining the components in the order listed below. Mix gently and thoroughly by pipetting up and down 10 times, and spin briefly.

Component	Volume per reaction (µL)	Volume for 24 Reactions**	Volume for 96 Reactions**
SB4 Reagent*	5.0 µL	132.0 µL	528.0 µL
1X SS2 Reagent	1.0 µL	26.4 µL	105.6 µL
SEZ1 Reagent*	0.8 µL	21.1 µL	84.5 µL
SEZ2 Reagent*	1.2 µL	31.7 µL	126.7 µL
Total Volume	8.0 µL	211.2 µL	844.8 µL
*Be sure to spin reagents down before use **Calculations include 10% overage			

16. When the incubation is completed, place plate on PCR cooler (or ice) and proceed immediately to the next step.

17. Add 8 µL of Reaction Mix to each sample while the plate is still on the PCR cooler (or ice), seal the plate and spin briefly.
18. Mix at room temperature for 1 min at 1000 rpm in plate mixer, then spin briefly.
19. Place the plate on thermal cycler (lid set to 70°C) and run the following program:
    - 30°C for 10 hrs
    - 65°C for 3 min
    - 4°C Hold
20. Discard any unused thawed aliquots.

VII. Amplified DNA Cleanup

CONSUMABLES:

Component	Source
BioSkryb ResolveDNA Bead Purification Kit	BioSkryb
BioSkryb ResolveDNA Magnetic Plate or Dual Volume Strip Tube Magnet	BioSkryb
80% Ethanol (freshly prepared)	General Lab Supplier

**Note: This protocol is optimized for 20 µL reactions. Adjust reagent volumes accordingly if your final reaction volume is different.*

PROCEDURE:

1. Allow BioSkryb ResolveDNA Beads to equilibrate to room temperature for 30 min.
2. Mix BioSkryb ResolveDNA Beads thoroughly, and then add 40 µL of beads to each

reaction well (vortex and spin).
*Aspirate (pipet up and down) beads prior to each dispensing step.

3. Incubate at room temperature for 10 minutes and briefly centrifuge sample plate.
4. Place plate on magnet for 3 minutes or until the supernatant clears.
5. While on magnet, remove and discard supernatant, being careful not to disturb the beads. Do not discard beads containing amplified DNA.
6. While on magnet, add 200 µL of freshly prepared 80% ethanol to the beads and incubate for 30 seconds at room temperature.
7. While on magnet, remove and discard the first ethanol wash, taking care not to disturb the beads.
8. While on the magnet, add another 200 µL of freshly prepared 80% ethanol to the beads, and incubate for 30 seconds at room temperature.
9. While on the magnet, remove and discard the second ethanol wash, taking care not to disturb the beads.
10. Remove any remaining ethanol from the wells. Incubate at RT for 5 minutes to air-dry beads (DO NOT OVER-DRY).
11. Remove plate from the magnet
12. Re-suspend beads in 40 µL of ResolveDNA Elution Buffer.
13. Wait for 2 minutes at room temperature.
14. Place plate on the magnet for 3 minutes, or until the supernatant clears.
15. Transfer 38 µL of the eluted DNA to a new plate, and proceed to DNA quantification.
16. DNA is ready to use in downstream applications such as PCR or Real Time PCR. For library preparation refer to the BioSkryb ResolveDNA™ Library Preparation Kit Protocol.

VIII. DNA Quantification

- Quantitate DNA using the High Sensitivity dsDNA Assay kit (Qubit) as per manufacturer.
- Size fragment analysis should be completed to ensure proper amplification product size.
- Determine fragment size distribution by running 1 µL of 2 ng/µL on a Tapestation HS D5000 Screen Tape (Agilent PN 5067-5592).
- See Appendix B for example yield and fragment size.

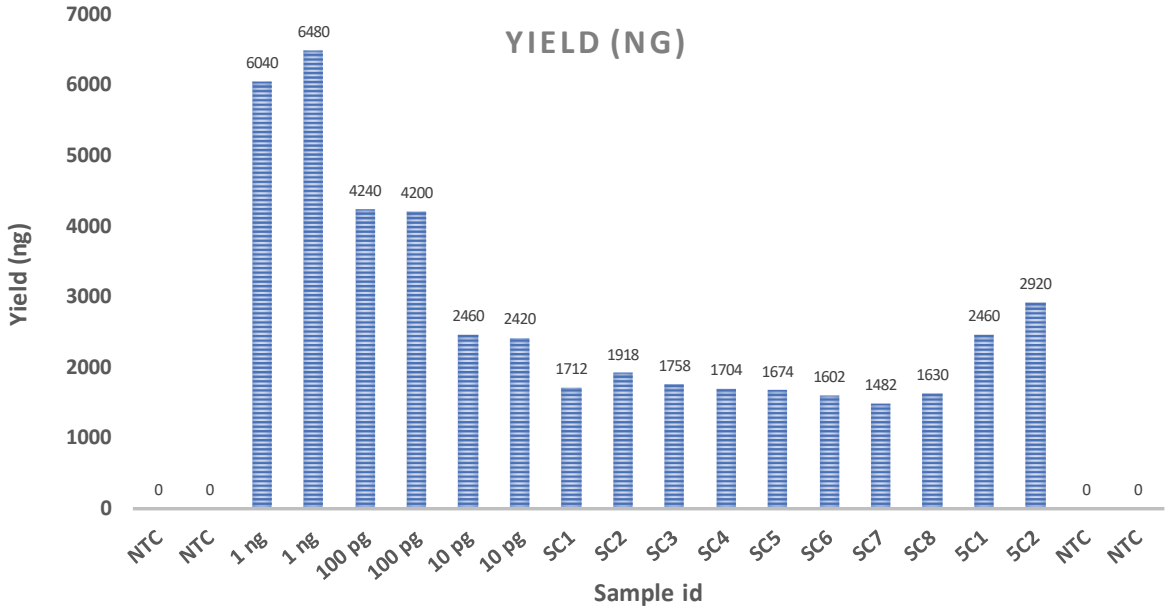
IX. Appendix A: Positive and Negative Controls

A positive and negative control should be included in every experiment. A negative control is included to detect test contamination and avoid false positive results. Positive controls should be included to assess the sensitivity of the assay. Yield and DNA size distribution should be determined by Qubit dsDNA High Sensitivity Assay and/or Agilent's BioAnalyzer DNA High Sensitivity tracings.

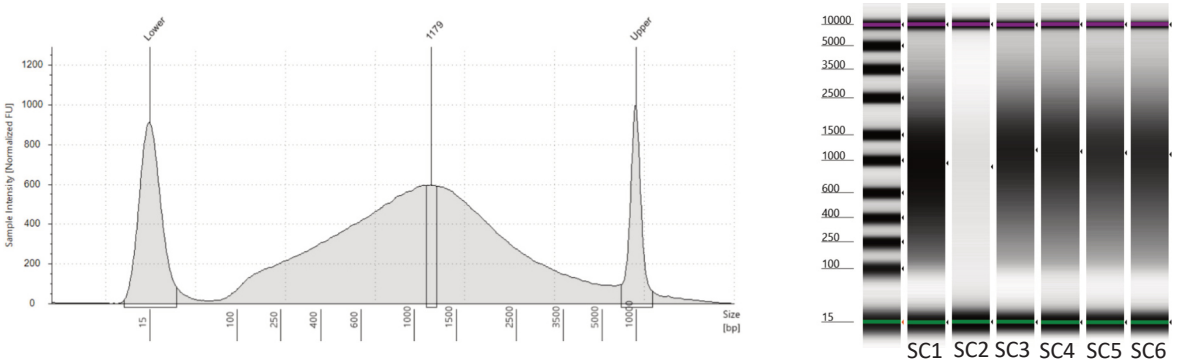
- Negative Control:
  - To each negative control well: add 3 µL of Cell Buffer. This will be the starting material to undergo amplification.
  - Less than 100 ng of DNA should be detected in the negative control.
  - If there is contamination, the experiment should be repeated using new aliquots.

- Bulk DNA Positive Control:
  - To each positive control well: add 2 µL of Cell Buffer and 1 µL of positive control DNA. This will be the starting material to undergo amplification.
  - 1 ng/µL positive control gDNA is prepared by diluting 50 ng/µL gDNA control (included in the kit) with Elution Buffer. The concentration of diluted 1 ng/µL DNA should then be confirmed with the HS dsDNA Assay Kit before diluting further to 100 pg/µL and 10 pg/µL.
  - All positive controls must show positive amplification.
  - When 1 ng of the positive control DNA is used in the reaction, a yield ~4-6 µg DNA should be expected.
  - Positive controls that better resemble the type of sample being tested should also be included. The amount of input DNA should be optimized by the user so the yield always falls in an acceptable range pre-determined by the user. We recommend using 1000 pg, 100 pg, and 10 pg of input DNA for amplification controls.

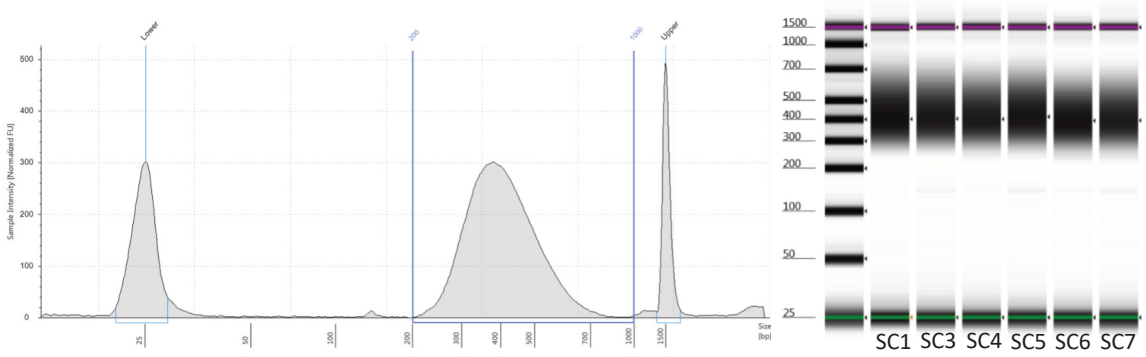
X. Appendix B: Example Yield and Size Fragments - ResolveDNA Kit and Library Creation Process



Typical yield from ResolveDNA amplification of DNA and single cells - Using the ResolveDNA Whole Genome Amplification Kit, a range of DNA inputs and several single cells were amplified. Reactions that contain no template (NTC) do not generate detectable products, as measured by the High Sensitivity dsDNA Qubit Analysis. The amplification of 1 µg of free DNA generates greater than 6 µg of amplified product, whereas lower inputs, 100 pg and 10 pg, generate greater than 4µg and 2µg, respectively. Amplification of single cells (NA12878 cell line sorted by FACS) typically generate greater than 1 µg of DNA/cell. Wells containing 5 cells (controls) demonstrate amplification yield of greater than 2 µg. Yield may vary based on cell state and health. Occasionally, a sample/cell will have very low yield, which typically results from the absence of a cell (or sample), or due to improper pipetting.



ResolveDNA product size distribution - Products generated using the Primary Template-directed Amplification (PTA) typically create fragments from a size range 200-4000 bp (above-left). As shown, samples that fail to amplify also generate no detectable products based on TapeStation analysis, while the remaining samples have good reproducibility.



Typical product size distribution from library creation of PTA amplified genomic DNA - Using the ResolveDNA PTA amplification products, libraries were created to allow downstream NGS analysis. Product size range is typically between 250 and 700 bp (above-right). Library products from mutiple reactions demonstrate appropriate consistency between samples (above-left). Yield of the reactions is typically greater than 10 ng/µl, but is dependent on the elution volume of the bead-based library DNA purification with a starting input of 500 ng of PTA amplified material. We do not recommend creating libraries from individual samples that have a high degree of yield variance from the majority of samples.

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