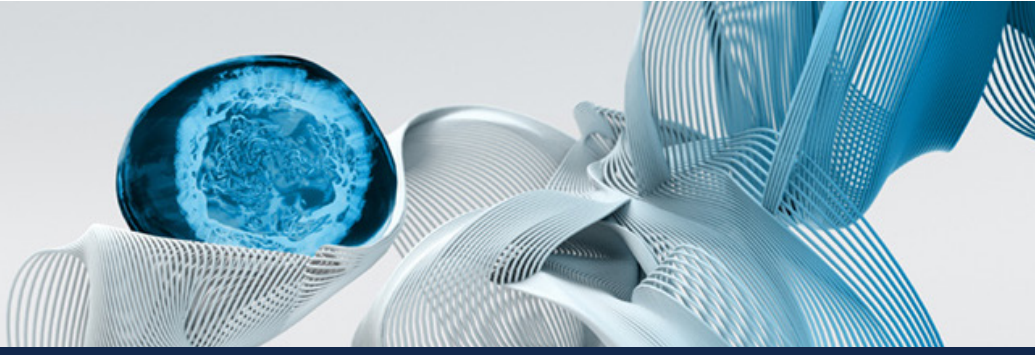
BioSkryb GENOMICS



Technical Note

ResolveDNA® Whole Genome Amplification v2.0

Cells explored. Answers revealed.

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Scalable, Cost-Effective Single-Cell Whole Genome Analysis with ResolveDNA® WGA v2.0

ResolveDNA® whole genome amplification (WGA) technology enables the accurate interrogation of the complete genome at single-cell resolution. The next generation of ResolveDNA® simplifies the workflow, dramatically reducing hands-on time, reaction time, and cost per sample, while increasing sample throughput and improving data quality.

Introduction

The original ResolveDNA® WGA kit was launched in fall 2020 and changed what was possible in single-cell genomics. Based on Primary Template-directed Amplification technology[1], it enabled scientists to answer biological questions not previously addressable. From somatic mutation rates in neurons[2,3], to individual mutation lineage tracing in the tumor microenvironment[4], ResolveDNA has led the way with unparalleled sensitivity and accuracy in single-cell genomics applications. The ResolveDNA WGA Kit v2.0 continues to push the envelope in the pursuit of data quality and completeness, while simplifying the workflow, increasing throughput, and reducing per sample cost.

The benefits of the ResolveDNA v2.0 workflow include:

- Simple, straightforward workflow
- Increased sample throughput
- Reduced cost per sample
- Reduced reagent and plastics use
- Improved reproducibility and robustness through use of automation
- Real-time monitoring triages reactions and saves time, money and reagents
- Execute full workflow – single cell to sequencer – in one day

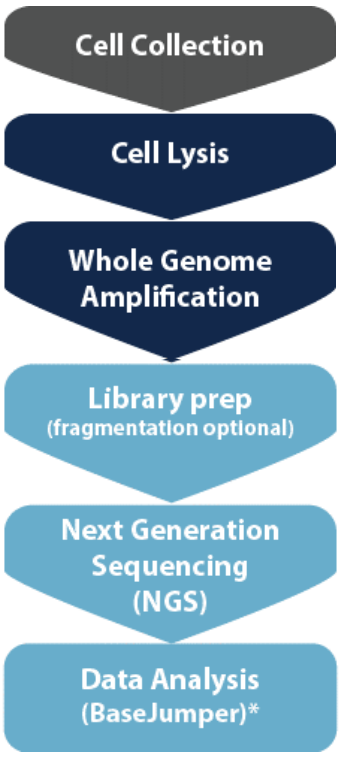


Figure 1. The ResolveDNA v2.0 workflow. The full workflow (cell collection to sequencing) can be completed in one day. *BaseJumper™ sold separately.

Simpler, Quicker Workflow

The ResolveDNA WGA v2.0 workflow can be executed in approximately three hours with less than 30 minutes of hands-on time. The workflow has been streamlined to two simple steps. First, a three component lysis mix is added to isolated single cells. Second, after a 20 minute incubation to lyse cells, a two component reaction mix is added, followed by a 2½ hours incubation at 30°C (Figure 2).

This dramatic reduction in sample processing time means that, paired with an efficient library prep workflow, a sample can be processed from single cell to sequencing in a single day.

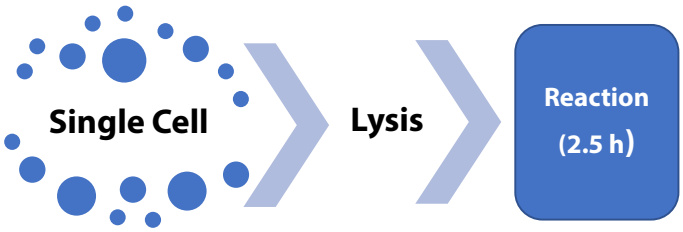


Figure 2. The ResolveDNA v2.0 Workflow. A streamlined workflow requires just two simple steps: lysis and amplification. The process takes less than 30 minutes of hands-on time and can be completed in approximately three hours.

Enabling Throughput

The simplicity of the ResolveDNA WGA v2.0 workflow has increased the feasibility of scaling up experiments to include greater numbers of cells. While incredible insights are evident in the comprehensive analysis of the genomes of small numbers of cells[4,5], the scale enabled by ResolveDNA WGA v2.0 will facilitate new modalities of single cell genomic analyses. The recommended workflow, a high throughput (HT) workflow, has been optimized for scale and processing of hundreds of cells simultaneously. It is executed in 384-well plates in a reduced reaction volume, and using this workflow each ResolveDNA WGA v2.0 kit is capable of running a full plate.

Built for Automation

To facilitate the successful and efficient execution of workflows at a 384-well scale, we recommend the use of automated liquid dispensing to enable the speed and accuracy necessary. The protocol has been tested and optimized using HP digital dispensing technology, available on the HP® D100 and D300e (Figure 3). Other capable instruments include, but are not limited to, the Formulatrix® Mantis® and the SPT Labtech dragonfly®. In some instances, the viscosity of the Reaction Mix may require volumetric offsets for accurate dispensing. The use of automated liquid dispensing platforms dramatically reduces operator time, facilitates throughput, eliminates human error, reduces consumable usage, and improves reproducibility, accuracy and data quality.

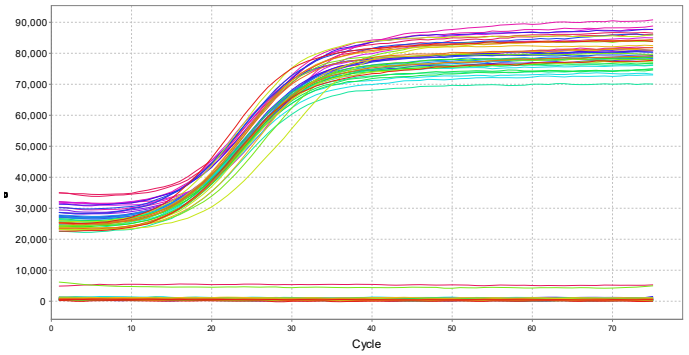
Figure 3. HP D300e Digital Dispenser. The platform allows the dispensing of low volumes (from 11 pl to 10 µl). Accurate volumetric dispensing is critical to enabling the full capabilities of ResolveDNA v2.0.

Workflow Flexibility

While the opportunity to increase sample throughput has been a focus of ResolveDNA v2.0, in many cases there is a need for higher total DNA yield or flexibility of the workflow to accomodate larger initial sample input volumes. To facilitate these needs, the components of the kit also operate in a high yield (HY) workflow in a 96-well plate or strip tube format. This workflow increases the reaction volume while maintaining the simplified workflow of ResolveDNA WGA v2.0. Notably, these increased volumes mean the kit will perform fewer overall reactions relative to the high throughput workflow.

Real Time Monitoring

A novel feature enabled in the ResolveDNA WGA Kit v2.0 is the intrinsic ability to monitor the amplification of samples in real time using an intercalating dsDNA specific reporter dye. When amplified on a qPCR-capable thermal cycler, the rate of amplification can be tracked through emission in the SYBRGreen channel (488 nm). The identification of an inflection point where the level of fluorescence crosses a particular threshold correlates strongly with the yield of DNA, identifies failed reactions without the use of additional reagents and processing steps, and correlates with downstream sequencing



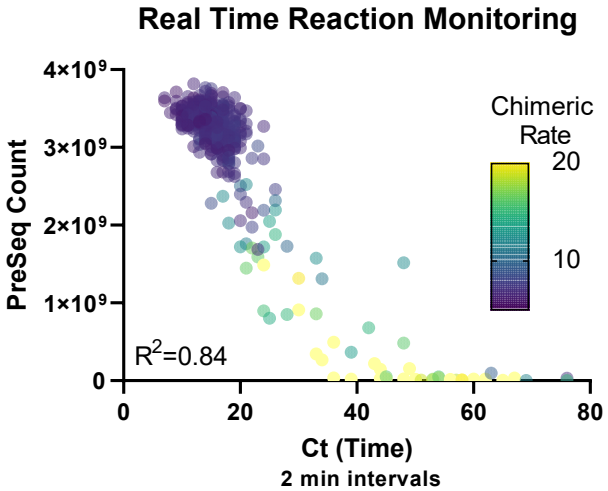


Figure 4. Real time monitoring identifies failing samples. (Above) Reaction curves from wells containing a single cell amplify reproducibly. (Below) Ct value calculated from the cycle during which the reaction fluorescence first exceeds 30% above the baseline predicts which samples will sequence poorly based on PreSeq count and chimer rate analysis.

Metric	ResolveDNA v1.0 (100136)	ResolveDNA v2.0 HY Workflow (100545)	ResolveDNA v2.0 HT Workflow (100545)
Plate Format	96	96	**384**
Input Volume	3 µL	3 µL	**1 µL**
Setup Steps	4	**2**	**2**
Setup Hands on Time	~45 min	**~30 min**	**~30 min**
Reaction Time	10 hours	**2.5 hours**	**2.5 hours**
Reaction Volume	20 µL	**12 µL**	**4 µL**
DNA Yield	1-3 µg ± 2 µg	**1-1.5 µg ± 200 ng**	**300-400 ng ± 50 ng**
Automation / Manual	Manual	Manual	**Automated**
Real Time Monitoring	No	**Yes**	**Yes**
Genomic Coverage	>95%	>95%	>95%
Chimer Rate	~10-15%	**<8%**	**<8%**

Table 1. Features of ResolveDNA v2.0 compared. The two workflows of ResolveDNA v2.0 (HY for high yield and HT for high throughput) are compared to the classic ResolveDNA workflow.

quality (Figure 4). These capabilities allow triaging of samples during the amplification reaction itself, and eliminates the requirement for extraneous reagents, plastics, consumables, and time to quantify the DNA yield of every sample.

Improved Data Quality

ResolveDNA has always delivered the highest quality genomic coverage, sensitivity, precision, and allelic balance available from a single cell (Figure 5). It enables the interrogation and analysis of genomic variation in greater than 95% of the human genome based on sequencing sufficient reads to mathematically cover the genome 20X. To demonstrate the performance of the ResolveDNA v2.0 chemistry, quality control data from individual cells of two recently produced ResolveDNA lots, an original ResolveDNA WGA lot and a pilot production lot of ResolveDNA WGA v2.0, were analyzed and compared using our BaseJumper™ Bioinformatics Platform, a cloud-based single-cell genomics analytical platform. Four cells with amplified genomes using the original ResolveDNA WGA Kit (labeled v1) and six cells with genomes amplified using ResolveDNA WGA v2.0 underwent whole genome sequencing at sequencing depths ranging from 13.5X to 31.2X (3.48×10^8 – 9.41×10^8 total reads). Despite largely having up to 50% less sequencing depth per sample, samples from ResolveDNA v2.0 covered a higher proportion of the genome (average 95.8%) as v1.0 (average 93.5%) (Figure 5A). Comparisons of allelic balance (Figure 5B), sensitivity, and precision (Figure 5C) showed similarly that ResolveDNA v2.0 achieved equivalent levels of performance, albeit using fewer reads.

A major data quality advantage of ResolveDNA v2.0 is a reduction in the rate of chimeric reads (Figure 5D). Chimeric reads are defined as read pairs where the reads do not map near one another reading 5'->3' toward its pair. These occur for various reasons and can complicate long read sequencing and analysis of insertions and deletions. With the optimized chemistry in ResolveDNA WGA v2.0, the chimeric rate has been reduced by approximately half. This will dramatically improve

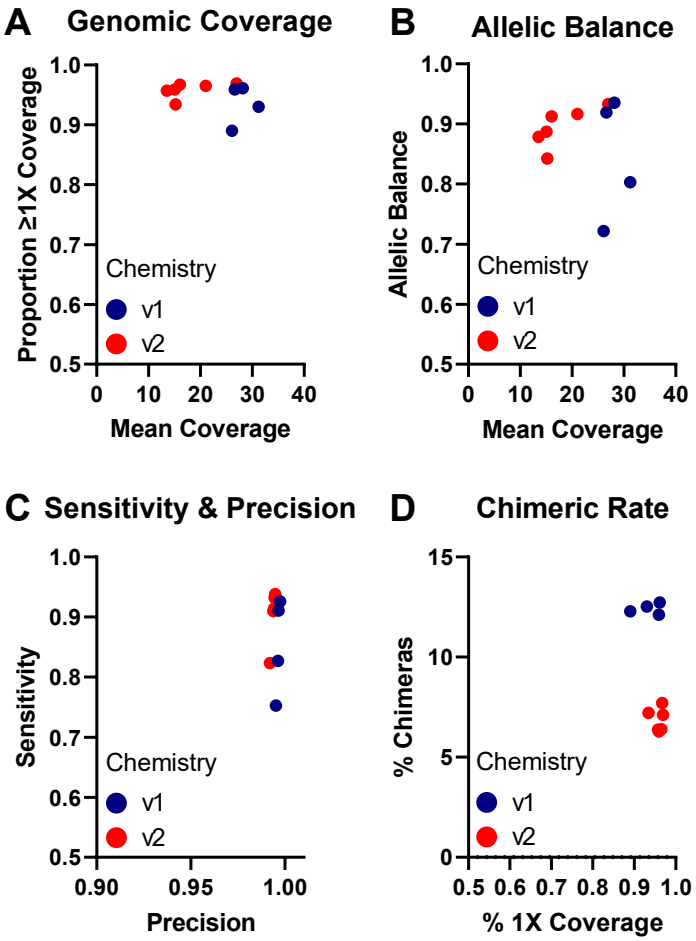


Figure 5. Similar, more reproducible performance of ResolveDNA v2.0 in whole genome sequencing. (A) Genomic coverage is similar, acheiving greater than 95%, even with reduced sequencing depth. Less variability is observed between samples. (B) Both alleles are recovered in greater than 90% of the genome in both ResolveDNA v1.0 and v2.0 as assessed by known heterozygous site accuracy. (C) Sensitivity and precision are highly consistent between chemistries. (D) The rate of chimeric reads is dramatically reduced in ResolveDNA v2.0, enabling high confidence analysis of insertions, deletions, and structural rearrangements.

the ability of ResolveDNA to detect insertions, deletions and structural rearrangements in individual cells.

Summary

The ResolveDNA WGA v2.0 kit includes a number of features and benefits. It improves on the already market-leading technology, while reducing reaction time four-fold, halving the workflow steps, and quadrupling the throughput (Table 1). The best performance is realized when utilizing an automated liquid handler to execute the recommended high throughput workflow. This workflow allows for a full 384 samples to proceed from single cells to sequencing in one day. Most importantly, the workflow results in higher quality, more robust genomic data. ResolveDNA WGA v2.0 improves across the board on ResolveDNA and is the optimal method for single-cell genomics.

Citations

1. Gonzalez-Pena, V., et al., Accurate genomic variant detection in single cells with primary template-directed amplification. Proc Natl Acad Sci USA, 2021. 118(24).

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3. Luquette L.J., et al., Single-cell genome sequencing of human neurons identifies somatic point mutation and indel enrichment in regulatory elements. Nat Genet. 2022 Oct;54(10):1564-1571.

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