BioSkryb GENOMICS

Cells Explored. Answers Revealed.

ResolveDNA™ Whole Genome Amplification Kits for Single-Cell and Ultra-Low DNA Inputs

ResolveDNA™ Whole Genome Amplification Kits for single-cell and ultra-low DNA inputs introduce a novel approach to whole-genome amplification (WGA). This addresses the inherent challenges of single-cell genomics that lead to increased sequencing costs and complex data analysis. Our innovative amplification methodology (Primary Template-directed Amplification or PTA[1]) employs controlled reaction parameters to reproducibly recover >95% of the genomes of single cells and low-input samples with unprecedented fidelity and uniformity. This industry-leading performance is highly scalable, supporting robust, reliable and routine single-cell genomic applications.[2]

Key features and benefits:

- Isothermal, quasi-linear PTA method overcomes biases, allelic dropout, low and variable genome coverage, poor reproducibility and artifacts associated with existing WGA approaches
- Versatile and scalable core technology; compatible with all ultra-low input samples, high- and low-throughput sample preparation methods, and downstream applications
- Simple workflow requires less than 45 minutes hands-on time
- Enables high-quality analysis of genomic heterogeneity at single-cell resolution

Key Features

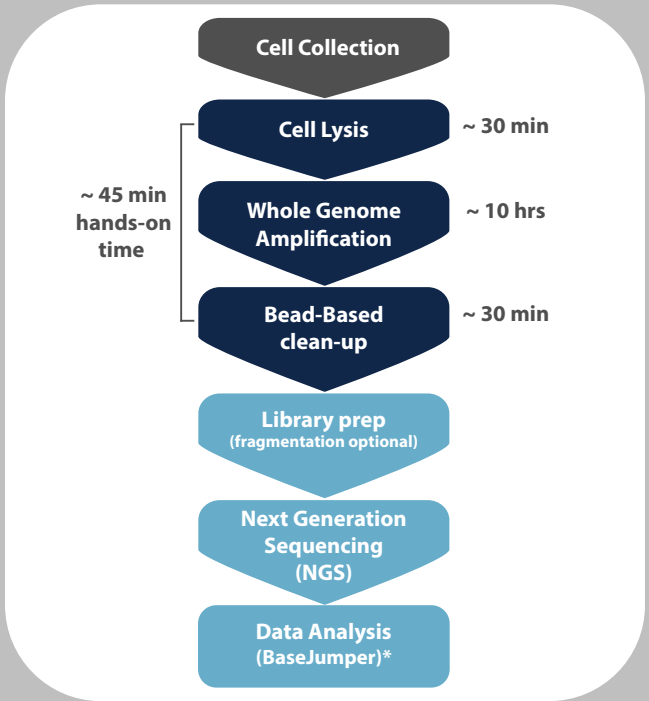
Core Technology for Routine Single-Cell Genomics

BioSkryb Genomics has developed a complete sample-to-analysis workflow solutions to support low- and high-throughput single-cell genomics and ultra-low input applications. Our PTA-based ResolveDNA™ WGA Kits form the core of these workflows, and enable a wide variety of emerging single-cell genomics applications, including:

- Accurate variant analysis (SNPs, indels, SNVs and CNVs) of single cells and sub-nanogram samples
- Detection and characterization of minimal residual disease (MRD)
- Quantitative, genome-wide assessment of CRISPR/Cas9-mediated genome editing at single-cell resolution
- Genome assembly and characterization of rare and unculturable micro-organisms

Simple, Versatile Method

- Human, animal or microbial cells are collected and isolated using FACS, laser-capture micro-dissection, or any other method of choice. ResolveDNA WGA Kits are compatible with single and multiple cells, and ultra-low amounts of DNA (>4 fg – 10 ng). Input material may be prepared in either plate or tube format.
- The ResolveDNA methods consist of three easy steps: cell lysis, whole-genome amplification, and bead-based cleanup of amplified DNA.
- For Next Generation Sequencing (NGS) workflows, WGA products are typically converted to libraries for multiplexed sequencing on the Illumina® platform. Because amplification products are relatively short (~250 to >1,500 bp), no fragmentation is required. Bead-based size selection may be performed after library amplification to select the optimal library fragment distribution for downstream sequencing.
- Data analysis may be performed with tools and pipelines of your choice, or with the cloud-based BioSkryb BaseJumper™ Bioinformatics Platform.



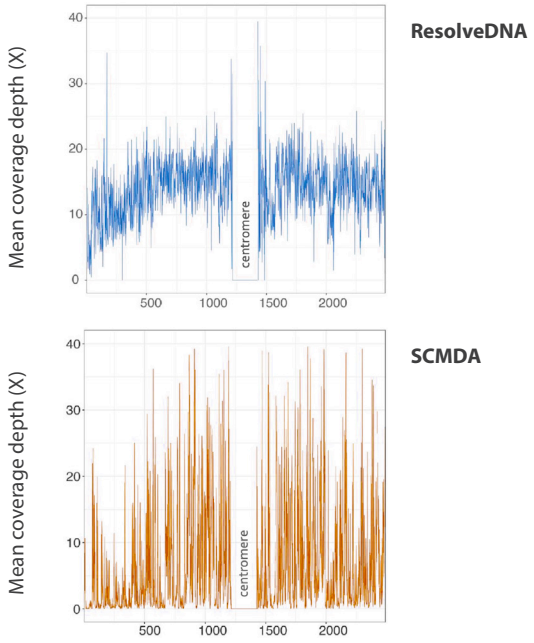
A typical single-cell whole-genome sequencing workflow. Steps highlighted in dark blue are performed with the ResolveDNA WGA Kit. In addition to WGA, amplification products may be used in targeted sequencing or multi-omics workflows. ResolveDNA WGA Kits are also compatible with other genome analysis methods, e.g. microarrays and qPCR. *In development

Core Benefits

Industry-leading Performance

The most commonly used strategies for WGA rely on traditional PCR with degenerate primers (DOP-PCR); multiple-displacement amplification (MDA) with isothermal, strand-displacement DNA polymerases; or a hybrid approach (e.g. MALBAC[3]). The LIANTI (Linear Amplification via Transposon Insertion) method utilizes transposases and *in vitro* transcription[4]. All of these methods have drawbacks, such as amplification bias, poor uniformity, errors and artifacts, low genome coverage, inability to address all variant classes, low accuracy, poor reproducibility, and/or complex protocols that are difficult to automate or scale.

In a recent single-cell whole-genome sequencing study[2] (see below), ResolveDNA™ WGA Kits (based on the novel, isothermal PTA method) were shown to outperform all other WGA methods with respect to data quality and variant calling metrics, making it the first commercially available WGA technology capable of supporting robust, reliable, routine single-cell genomic applications.



Superior coverage and uniformity in single-cell WGS. WGA was performed with the ResolveDNA WGA Kit (top) or single-cell MDA (bottom). Plots show a portion of chromosome 1 (100 kb bins). The central area (poorly covered with both methods) corresponds to the centromere.

Method	ResolveDNA	Mixed Method A	MDA A	MDA B	Mixed Method B	Mixed Method C	DOP-PCR
Genome Mapping	97%	91%	88%	55%	88%	55%	52%
Genome Recovery	97%	73%	65%	59%	50%	33%	20%
CV of Coverage	0.8	1.3	1.8	2.3	2.6	3.2	3.6
SNV Sensitivity	92%	70%	65%	55%	45%	30%	19%
SNV Precision	99%	88%	87%	88%	28%	35%	35%

ResolveDNA WGA Kits outperform other methods with respect to data quality and variant calling metrics. Ten single cells were isolated from a human B-lymphocyte cell culture (CEPH1463/NA12878/GM12878 human genome reference standard). WGA was performed on individual cells, using either the ResolveDNA WGA Kit, or single-cell MDA (SCMDA[5]). WGA products were converted to indexed libraries and subjected to high-coverage whole-genome sequencing (WGS) on the Illumina® platform targeting 25X coverage. For other WGA methods, low-pass WGS data (generated from individual BJ1 fibroblasts), were obtained from a previously published studies[2,4].

To achieve a fair comparison of the various WGA methods, raw data for all samples were aligned and pre-processed for variant calling using the same pipeline. All metrics shown in the table were generated from randomly subsampled BAM files (450 million reads per cell). Note that the metrics for all methods other than ResolveDNA are overestimates, due to the way in which data analysis was performed in the original study.

As shown in the table, the ResolveDNA WGA Kit outperformed all other WGA methods with respect to data quality and variant calling sensitivity and precision. In addition, ResolveDNA amplified single-cells returned results comparable to those for bulk samples in all coverage and variant calling analyses performed, whereas results from the other WGA methods were consistently less accurate and more variable (data not shown). CV represents coefficient of variation and SNV represents single nudoetide variant.

Product Information

WGA reagents and computational tools

ResolveDNA™ Kits contain all of the enzymes and reagents needed for whole-genome amplification from single cells or ultra-low DNA inputs. We continue to expand our portfolio to support complete workflows, from cell sorting and processing, to reaction cleanups and NGS library construction.

Our BaseJumper™ Bioinformatics Platform uses standardized best practices for sequencing analysis, and enables easy visualization of alignments, coverage, SNV calling, and data quality.

Product Ordering Information

Part number	Description
100068/100136	ResolveDNA Whole Genome Amplification Kit – For single-cell and low-input DNA amplification (24 or 96 rxns)
100121/100182	ResolveDNA Bead Purification Kit – For reliable WGA reaction and library cleanup (24 or 96 rxns)
100135	ResolveDNA Magnetic Plate – For optimum bead cleanup performance
100226	ResolveDNA Dual Volume Strip Tube Magnet – For optimum bead cleanup performance
100137	ResolveDNA Complete Starter Pack – Cell sorting and WGA equipment, reagents and consumables
100180	ResolveDNA™ Consumables Only Starter Pack – Cell sorting and WGA consumables
100080	ResolveDNA™ Library Preparation Kit – Optimal library construction for Illumina sequencing
100181	ResolveDNA™ Multi-Use Library Adapters – Compatible with Illumina sequencers
100199	ResolveDNA™ FACS Kit – For optimum FACS-based cell sorting

Contact us for a complete product list.

References:

1 BioSkryb 2020. Enabling routine single-cell genomics (brochure).
2 Gonzalez, V *et al*. PNAS, 2021. 118 (24) doi.org/10.1073/pnas.2024176118
3 Zong, C *et al. Science* 2012; 338: 1622. doi:10.1126/science.1229164.
4 Chen, C *et al. Science* 2017; 356: 189. doi:10.1126/science.aak9787.
5 Dong, X *et al. Nat Methods* 2017; 14: 491. doi:10.1038/nmeth.4227.

Learn more about BioSkryb

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