MiniPerspective

BioSkryb GENOMICS

Gene Edited Off-Target Events Identified Through Single-Cell Whole Genome Sequencing

CRISPR/Cas9 genome editing introduces off-target mutations

Early studies using CRISPR/Cas9 in U2OS.EGFP cells detected off-site mutations at a frequency of 5.6% to 125% (mean of 40%) of on-site mutations, as determined by a T7 Endonuclease I (T7EI) assay[1]. As guide-RNA (gRNA) targeting strategies, Cas9 variants, and predictive off-target algorithms have been developed to improve fidelity (reviewed [2]), off-site mutational frequency has decreased. For example, single-cell whole genome sequencing following CRISPR/Cas9 genome editing of U2OS cells demonstrated the VEGFA gRNA introduced 11 off-target mutations versus one mutation with the EMX1 gRNA[3].

CRISPR/Cas9 off-target mutations are variable between individual cells

In cell culture, CRISPR/Cas9 genome editing results in mixed populations of edited and non-edited cells. Comparisons between expanded monoclonal cultures of genome-edited cells reveals different off-target effects. In single-cell whole genome sequencing following CRISPR/Cas9 genome editing of U2OS cells with the VEGFA gRNA, of the 11 off-target mutations observed, only two were recurrent across single cells[3]. Importantly, establishing and passing monoclonal cell lines can further drive the genomic divergence of genome-edited cells[4].

CRISPR/Cas9 mutations are detected by single-cell whole genome sequencing

Multiple methods for detecting on- and off-target mutations arising during genome editing have been described, including whole genome sequencing (reviewed [5]). Recently, a new method for whole genome amplification, primary template-directed amplification (PTA), was described[3]. Importantly, PTA enables whole genome sequencing at a single-cell level, facilitating characterization of genome editing on a cell-by-cell basis[3].

References

1. Fu et al., "High frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells". *Nat Biotechnol*. (2013)
2. Naeem et al., "Latest Developed Strategies to Minimize the Off-Target Effects in CRISPR-Cas-Mediated Genome Editing". *Cells*. (2020)
3. Gonzalez-Pena et al., "Accurate genomic variant detection in single cells with primary template-directed amplification". *PNAS*. (2021).
4. Panda et al., " Clonally Selected Lines After CRISPR-Cas Editing Are Not Isogenic". *CRISPR J*. (2023)
5. Atkins et al., " Off-Target Analysis in Gene Editing and Applications for Clinical Translation of CRISPR/Cas9 in HIV-1 Therapy". *Front. Genome Ed.* (2021)

*Primary template-directed amplification (PTA) technology is only available in **ResolveDNA®** and **ResolveOME™** kits.*

ResolveDNA® and ResolveOME™ Assay Performance

Table 1: ResolveOME WGS DNA Performance*	
Characteristic	**Observed Values**
Accuracy	99.5%
Sensitivity	97.1%
Specificity	99.2%
Allelic Balance	98.4%
Genomic Coverage	97.1%

Table 1: Assay performance characteristics of DNA isolated using ResolveOME. Analysis of FACS-sorted NA12878 single cells prepared with ResolveOME versus gold-standard reference. WGS: whole genome sequencing.

Table 2: ResolveOME WTS RNA Performance	
Characteristic	**Observed Values**
Genes Detected	4,546
Reportable Range	6,057
Average Concordance	0.91
Reproducibility (CV)	43.3%

Table 2: Assay performance characteristics of RNA isolated using ResolveOME. Analysis of FACS-sorted NA12878 single cells prepared with ResolveOME versus gold-standard reference WTS: whole transcriptome sequencing.

**DNA amplified using ResolveDNA and ResolveOME have comparable DNA performance characteristics. All data on file.*

Custom Services

We offer custom service packages from our end-to-end single-cell multiomic pipeline, from singulating cells to ready-to-publish figures. All services include quality control verification. Services include:

- Cell sorting from fresh or frozen cells and tissues
- Whole genome amplification or whole genome and transcriptome amplification
- Library preparation for downstream applications, including whole genome or exome sequencing
- Sequencing of 550M quality reads capturing >97% of the human genome from each cell
- Analysis using our bioinformatics platform, BaseJumper™

Products

Code	Product	Description
100500	ResolveOME™ Whole Genome and Transcriptome Amplification System	PTA-based kit for accurate and reproducible whole genome and transcriptome amplification.
100545	ResolveDNA® Whole Genome Amplification Kit	PTA-based kit for accurate and reproducible whole genome amplification from single cells and low-input DNA inputs.
100605	BaseJumper™ Bioinformatics Platform	A complete bioinformatics solution for multiomic data analysis and visualization. https://www.bioskryb.com/basejumper/

For a complete list of services, products, and pricing, email a member of our team, info@bioskryb.com

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All data on file.

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