mission bio

tapestri
TECHNICAL NOTE

Measuring the efficiency of CRISPR genome-editing systems using the Tapestri® Platform and Tapestri® Single-Cell DNA Custom Panels

Takeaways

- Single-cell data resolves the complexities of mutation zygosity and co-occurrence in a genome-editing experiment targeting multiple loci
- Both on-target and off-target effects can be measured with Tapestri Single-Cell Custom DNA Panels
- High sensitivity enables the detection of low frequency events – down to 0.1% of cells
- Single-cell resolution enables genome-editing system optimization

Abstract

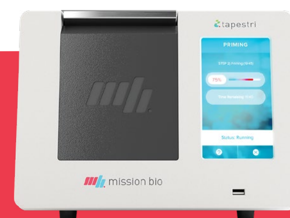
As genome-editing systems move rapidly from the bench to advancing cell therapies, cellular-level understanding of complex systems is the key to informed success. En route to clinical applications, edited cellular systems need to be thoroughly characterized to fully understand the exact nature of induced mutations. Both on-target and off-target effects are equally important to detect and accurately quantify.

As a member of the National Institute of Standards and Technology (NIST) Genome Editing Consortium,[1] Mission Bio is helping to address "the measurements and standards needed to increase confidence and lower the risk of utilizing genome-editing technologies in research and commercial products." Therefore, from development through production, careful analysis of edited cellular systems is a priority for those looking to bring exciting ex vivo and in vivo therapeutics to the clinic.

Introducing the Tapestri Platform

The Tapestri Platform is a complete solution for high-throughput single-cell DNA analysis, delivering high-quality variant calling of single nucleotide variants (SNVs) and indels. Leveraging droplet microfluidics and a novel two-step workflow, high-multiplex PCR, cell barcoding, and targeted amplification is performed on tens of thousands of individual cells simultaneously. Single-cell libraries are prepared using optimized Tapestri kits including cartridges and reagents along with the benchtop Tapestri Instrument, and then sequenced on an Illumina® platform.

Catalog panels are offered in hematology[2] and solid tumor profiling, and panels can also be customized based on specific regions of interest. Altogether, the Tapestri Platform enables the detection of mutation co-occurrence in subclonal populations, preservation of zygosity of mutations, and detection of rare clones down to 0.1%. Additionally, the workflow is amenable



Tapestri Single-Cell Custom DNA Panels

- Customize hundreds of amplicons to specific regions of interest
- Simple custom panel design using an optimized pipeline
- Panel performance typically achieves >85% design coverage and >85% panel uniformity

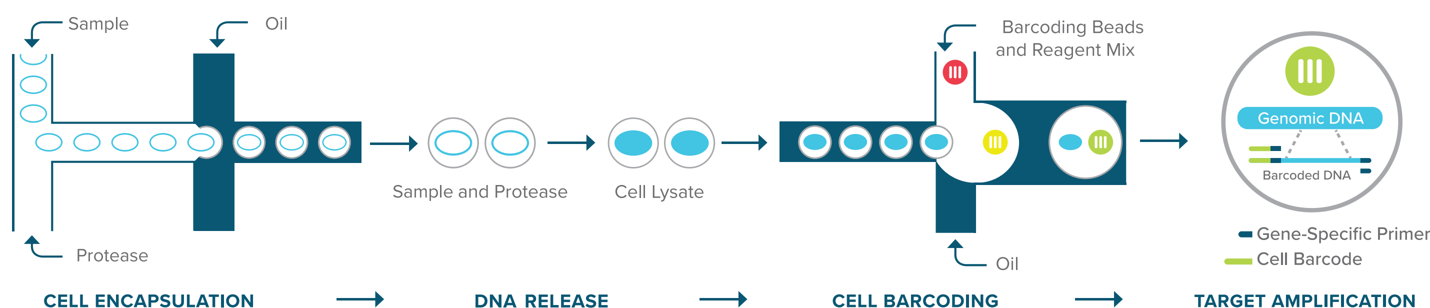


Figure 1. The Tapestri Microfluidic Workflow

to a variety of sample types including cell lines, bone marrow aspirates, PBMCs isolated from whole blood, and nuclei isolated from fresh frozen solid tissue.

To demonstrate the value of the Tapestri Platform for high resolution quality control for genome-editing systems, a Tapestri Single-Cell DNA Custom Panel was designed to measure the efficiency of a multiplex genome-editing system targeting three different loci in primary human T cells. Amplicons for known off-target sites and negative control sites were also included in the custom panel design.

Experiment and Methods

Tapestri Workflow with a Tapestri Custom DNA Panel and with enriched, edited primary T cells

In this experiment, stimulation of human PBMCs, followed by lentiviral transduction and mRNA electroporation, was performed using standard protocols. After culturing cells for at least 5 days post transduction and electroporation, cells were enriched for primary T cells (>95%), cryopreserved, and then analyzed using the Tapestri Platform.

The Tapestri Platform is an end-to-end solution for single-cell DNA analysis. Using a novel two-step workflow, thousands of individual cells were first encapsulated with a protease in droplets running the Tapestri cartridge on the Tapestri Instrument (Figure 1). Proteolytic action lyses the cells, exposing the DNA. Following lysate preparation, the proteases were inactivated via heat denaturation on a thermal cycler. In the second step on the Tapestri Instrument, individual cell lysates were returned to the same cartridge and then partitioned with barcoded beads and multiplex-PCR primers and reagents to target specific regions of the genome. Here, a Tapestri Single-Cell DNA Custom Panel with 20 amplicons was designed to target three on-target sites and three known off-target sites. Four negative control sites were also included to determine false positive rates and are defined as neither on-target nor known off-target sites. Additionally, the panel included 10 amplicons to assess allele dropout rate. These amplicons target SNVs known to be highly polymorphic across a variety of populations.

After barcode incorporation and target amplification were complete, library amplification, purification, and quantification were performed prior to sequencing on Illumina platforms. Fastq files were processed through Tapestri Pipeline Software to group reads by the cell barcode, thereby reconstructing the mutational profile for each cell. Thereafter, custom analysis was performed to precisely determine the distribution and frequency of on-target and off-target mutations in the pool of cells.

Results

High quality panel performance and sequencing metrics

Cryopreserved T cells generated high quality panel and sequencing data (Table 1). Two technical replicates were separately processed through the Tapestri workflow and

both were multiplexed on a single MiSeq® sequencing run. An average of 6,600 cells were sequenced per replicate. Panel uniformity was 90% or greater, and the average depth of coverage of 39.5 reads per amplicon per cell ensured high quality variant calling for each cell. Additionally, allele dropout rates (ADO) were below 10% for both replicates, where ADO represents the percentage of cells within a run, averaged across several loci known to be heterozygous in many populations, where the known heterozygous SNV was incorrectly genotyped as either homozygous wild type or homozygous mutant.

Replicate	Seq. read pairs	Cells	Panel uniformity	Reads per amplicon per cell	ADO
1	12.9M	8,142	95	43	8.4%
2	10.0M	5,058	90	36	7.4%
Average	**11.5M**	**6,600**	**92.5**	**39.5**	**7.9%**

Table 1. Panel and sequencing metrics for two technical replicates. Panel uniformity is the percentage of panel where coverage depth is at least 20% of the mean coverage depth.

Characterizing the zygosity and distribution of every induced mutation across cells

While many genome-editing systems often target homozygous edits, there is a large number of potential combinations as a consequence of different editing efficiencies at each target site, especially when inducing multiple edits. In a diploid organism, there are 3 different outcomes for an intended edit at each target locus: no edit, a heterozygous edit (successful on 1 allele), and a homozygous edit (successful on both alleles). With 3 intended target sites (e.g. locus 1, locus 2, locus 3), each with 3 possible edit results, there are 27 total possible scenarios (26 edit combinations, 1 without any edits). In a single experiment across thousands of cells, the Tapestri Platform revealed the distribution of the zygosity and co-occurrence of the edits induced by the genome-editing system and resolved every possible on-target edit combination (Figure 2). Significantly, the Tapestri Platform revealed that when an unoptimized genome-editing system was employed, only ~3% of cells were edited as expected (both alleles at all three sites, see 6 edits in Figure 2B). None of the three on-target sites were edited in 22.45% of cells. The largest effect was a homozygous mutation on a single target locus (target site 1, 2 edits in 21.47% of cells). Compared to a pseudo bulk sample analysis (Figure 2A), performed by informatically removing cell barcoding assignments from Tapestri single-cell data, the data available from single-cell analysis using the Tapestri Platform is much more comprehensive and accurate in its resolution of every mutation combination.

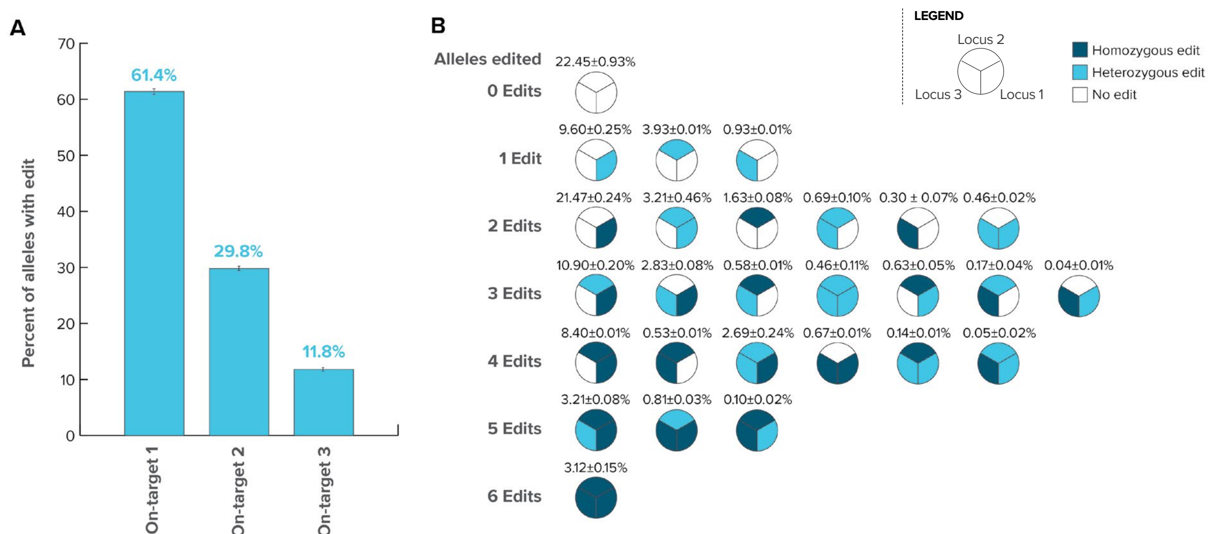


Figure 2. A. Percent of edited alleles inferred from pseudo bulk sample analysis. B. Percent of alleles edited, at single-cell level, using the Tapestri Platform. The Tapestri Platform resolves the distribution of zygosity and co-occurrence of all 27 possible on-target mutation combinations induced by genome-editing system at three on-target loci.

Characterizing off-target edits at low frequency

The ability to confidently measure both high and low frequency off-target edits is also critical when analyzing genome-editing systems. Figure 3 illustrates that the Tapestri Platform was able to detect known off-target mutations in as few as ~0.1% of alleles (allele edit frequency). Furthermore, false positive events were reported in ~0.01% of alleles, based on the mutation of negative control sites (neither on-target nor known off-target sites).

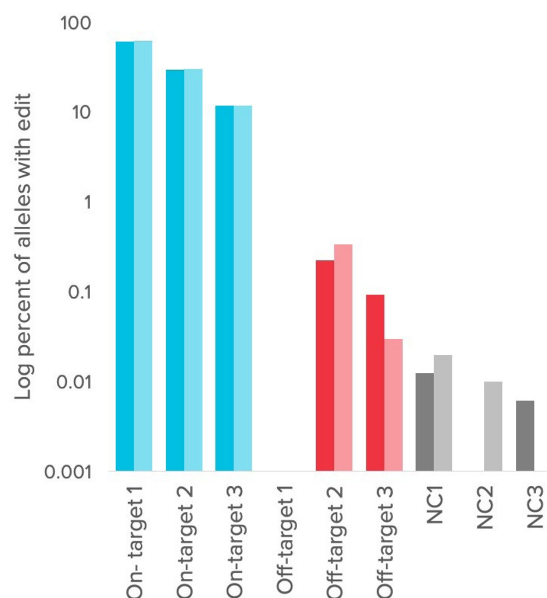


Figure 3. The Tapestri Platform reveals the extent of known off-target mutations at ~0.1% allele edit frequency, and mutations at negative control sites at ~0.01% allele edit frequency, while simultaneously detecting high frequency on-target effects in the same experiment. NC = Negative control. 1 of 4 NCs not shown.

Conclusions

- The Tapestri Platform offers a turnkey solution to the complex challenge of characterizing genome-edited systems.
- The zygosity, co-occurrence, and distribution pattern of every induced mutation in thousands of cells can be characterized in a single experiment.
- Off-target effects at low frequency can be easily identified.
- Genome-editing system optimization becomes significantly easier employing the Tapestri Platform.
- Mission Bio is the only single-cell DNA analysis technology to join the NIST Genome Editing Consortium.[1]

References

1. The National Institute of Standards and Technology Genome Editing Consortium: https://www.nist.gov/programs-projects/nist-genome-editing-consortium Last accessed November 2018.

2. M. Pellegrino *et al.*, High-throughput single-cell DNA sequencing of acute myeloid leukemia tumors with droplet microfluidics. *Genome Res.* **28**, 1345-1352 (2018).

QUESTIONS?

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