mission bio

tapestri
APPLICATION NOTE

Deep Understanding of Cell and Gene Therapy Genome Editing Protocols Enabled With Single-cell Sequencing

Takeaways

- Replace multiple traditional bulk assays with one single-cell sequencing assay
- Simultaneously identify edits, zygosity, co-occurrence and translocations in up to 10,000 single cells per sample
- Optimize genome editing protocols during development and get superior control during production manufacturing and release testing

Abstract

Cell and gene therapies are yielding new treatments, which were inconceivable only a few decades ago, for genetic disorders including cancers and inherited diseases. Single-cell DNA sequencing on the Tapestri Platform provides the most sensitive and nuanced quantification of genetic edits made to single cells with a simple one-day high-throughput workflow. Quantification of on- and predicted off- target edits for multiple targets, zygosity, and translocations are all measured simultaneously from a single assay for up to 10,000 single cells per sample. After making genomic edits, single-cell sequencing garners richer information than bulk assays, resulting in accurate quantification of all genome editing cell outcomes without the need for multiple cumbersome assays and cell culture. The Tapestri Platform integrates with cell and gene therapy workflows during development as well as during final product testing and release in manufacturing. Having faster turnaround and more complete data at these steps will aid in reducing the time and cost for cell and gene therapies to go to market.

Introduction

Cell and gene therapy is revolutionizing the treatment of many intractable genetic disorders, including hemophilia, sickle-cell anemia, Huntington's disease, and cancer. With these therapies, patients get a reprieve from life-threatening diseases or finally find relief from life-long, debilitating symptoms. Cell and gene therapy repairs genetic errors in a population of cells. After the cells are edited, they are placed in the patient where they either begin replacing the dysfunctional cells or provide an immune response against cancerous cells (Figure 1).

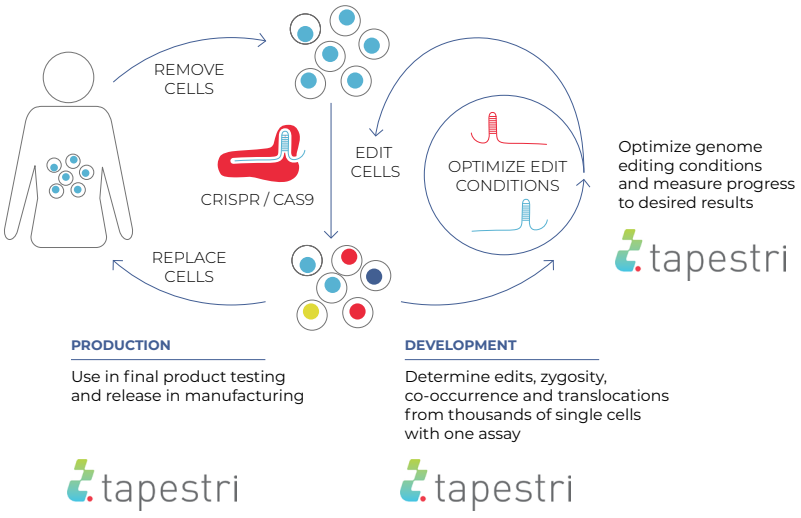

Figure 1 - A schematic of cell and gene therapy during development and production.

Two types of systems are commonly used to edit genomes for cell and gene therapies. The first method uses genome editing tools, such as CRISPR/Cas9 or other similar technologies, to target the gene or DNA sequence of interest and disable the malfunctioning gene. The second uses integration systems, such as viral vectors or homology directed repair (HDR) to incorporate large pieces of DNA into the genome. Some of these edits result in the restoration of normal gene function.

Before genetically modified cells can be placed in a patient, they must undergo rigorous testing to identify both desirable and undesirable edits. For the genome editing approach, careful selection and optimization of genome editing protocols, including the selection and design of guide RNAs (gRNAs), is an important initial step when creating cell and gene therapies. After execution of these genome editing protocols, the Tapestri Platform is used to thoroughly assess the results and quality of the protocol and can be used to help understand the ideal gRNA with the highest editing efficiency. Specifically, single-cell DNA sequencing with the Tapestri Platform identifies expected on-target edits, discerns whether the locus or loci is homozygous or heterozygous, and finds the proportion of cells that have co-occurrence of edits in multiplexing experiments (>1 target per cell). All of these characteristics are important to measure as they affect the efficacy of the therapy. In addition, undesirable events, including off-target edits and translocations, are quantified to determine the efficiency of the gene editing protocol and the possibility of dangerous changes to gene function. It is the selection of the right gRNAs and the characterization of the overall genome editing protocol that is critical for getting cell and gene therapies to market faster.

Following gene editing protocols, cell populations contain a mixed assemblage of different edits. Current methods used to identify genomic edits are both time-consuming and do not provide a detailed understanding of the individual edited cells. qPCR, ddPCR, TIDE (Tracking of Indels by DEcomposition), and ICE (Inference of CRISPR Edits) are the most common techniques used to analyze the genetic edits made to cells. These techniques return bulk data for the entire mixed population of cells or require clonal outgrowth to provide data on individual cells. Alternatively, researchers use karyotyping to identify large chromosomal translocations, but can only obtain information from a handful of cells.

Mission Bio's Tapestri Platform sequences DNA and protein expression in up to 10,000 single cells per sample after cells are edited in cell and gene therapy protocols. Single-cell sequencing provides the most detailed analysis of on- and predicted off-target edits, combination of edits, zygosity and co-occurrence within single cells, and the detection of very low-frequency events like translocations, which can have important effects on the safety of the therapy (Figure 2). This information can then be used to optimize edit conditions during development and then used for final product testing and release in manufacturing.

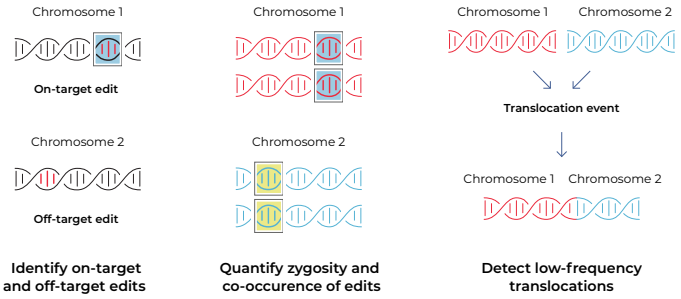

Figure 2 - Single-cell DNA sequencing detects desirable genetic edits, such as on-target events and the zygosity and co-occurrence of edits in a single cell and aberrant events including low-frequency translocations.

Experimental Design and Results

In partnership with Agilent Technologies, three genes, *HBB*, *CLTA* and *RAB11A* were edited with CRISPR/Cas9 in a cancer cell line. Agilent Technologies' CRISPR SureGuide Chemically

Figure 3 - Tapestri Workflow

Synthesized sgRNAs were used to ensure a high on-target to predicted off-target ratio of edits.[1] High-throughput single-cell sequencing was performed using the Tapestri Platform and analyzed with Tapestri Pipeline and Tapestri Insights software (Figure 3).

Single-cell sequencing quantified all on- and predicted off-target events for the three genes in the multiplexing CRISPR experiment (Figure 4a). While *CLTA* had only 0.08% predicted off-target edits, the *HBB* gene had 26%, showing the value of quantifying multiple on- and predicted off-target events for several genes in the same assay. This allows researchers to select gRNAs and optimize conditions that increase the proportion of on- to predicted off-target edits for all of the genes simultaneously.

Measuring the zygosity and co-occurrence of edits at multiple loci in single cells helps determine the efficacy of the cell and gene therapy. If the therapy is most effective when all of the targeted sequences have homozygous edits, then understanding the distribution of edits within the population of single cells is essential. Single-cell sequencing

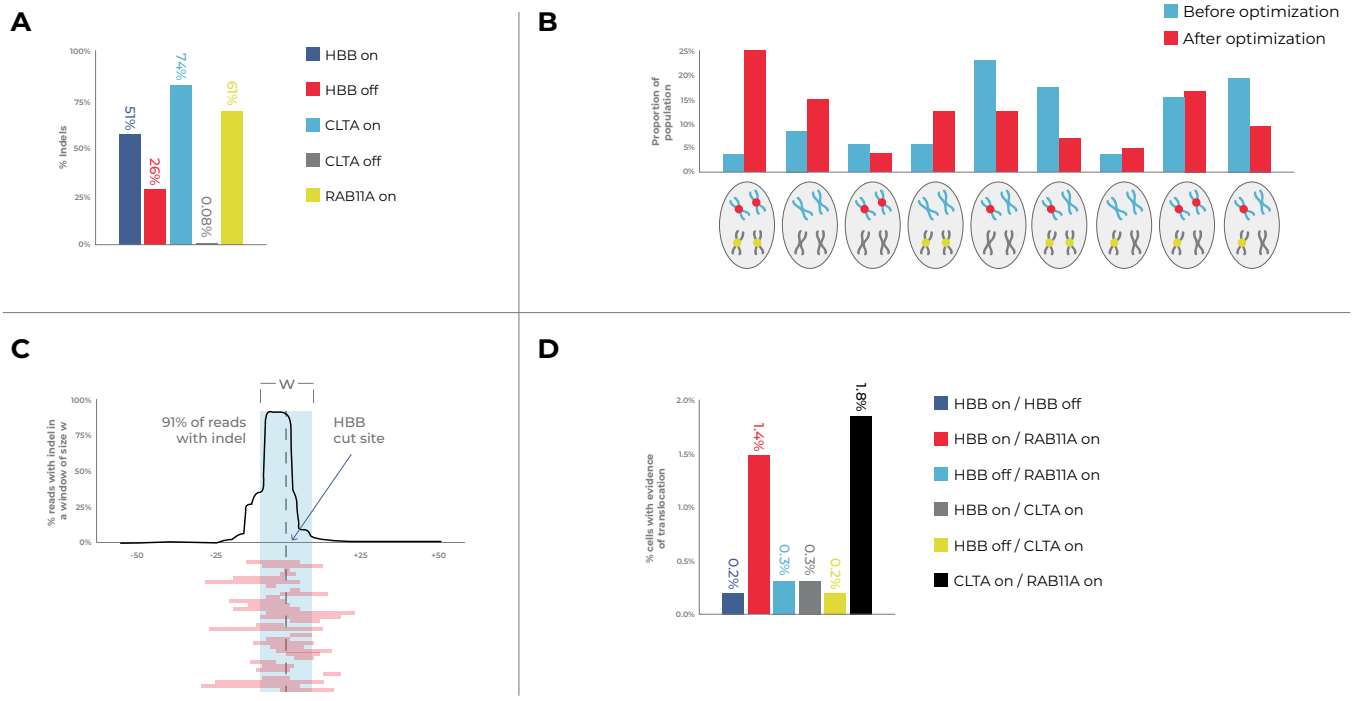

Figure 4 - Simultaenous measurement of on- and predicted off-target edits (a), co-occurrence, zygosity (b), indels (c) and translocations (d) from thousands of single cells.

provides an in-depth quantification of all possible combinations of zygosity and edit co-occurrence, and this information is used to improve CRISPR editing protocols to increase the occurrence of homozygous edits at all target sequences (Figure 4b).

The characterization of CRISPR-induced indels helps define the potential effects of frameshift and in-frame mutations on gene function. The benefits of using a sequencing technology are that the exact indels are measured directly from the DNA, and not extrapolated from reporter molecules. Data showed indel length and location varied considerably around the cut site in the *HBB* gene, providing detailed analysis of exactly what changes and at what quantities were being made to the DNA at the cut site of interest (Figure 4c).

Finally, a translocation in an edited cell can alter gene and cell function in unexpected, deleterious ways, which is a known-side effect of genome-editing but hard to quantify in a high-throughput fashion using traditional karyotyping methods. The ability to measure amplicons that arise from mis-matched primer pairs allows the direct quantification of translocation events, while simultaneously quantifying on- and predicted off-target events, co-occurrence, and zygosity of edits in the same single cells. More complete information is obtained from a single assay concerning the changes to each cell after cell and gene therapy protocols have been employed. Here we show rare translocations between 0.2% and 1.8% that occur in this multi-gene CRISPR experiment.

Conclusion

Single-cell sequencing technology offers exciting new capabilities for the development of cell and gene therapies. By simultaneous assessing desired and undesired editing events at the single-cell level, researchers can better optimize their protocols to build both in vivo and ex vivo cell and gene therapies. When edits were made to cancer cells using CRISPR technology, single-cell DNA sequencing characterized the frequency of on-target and predicted off-target events, the zygosity and co-occurrence of edits, indel length, location, and frequency, and translocations within a populations of single cells. Rare events, such as translocations were identified in as few as 0.2% of the sequenced cells. The high sensitivity and ability to provide comprehensive information from up to 10,000 cells per sample make single-cell sequencing an ideal approach to integrate into cell and gene therapy workflows from development to production.

References

1. Hendel A, Bak RO, Clark JT, et al. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. *Nat Biotechnol.* 2015;33(9):985-989. doi:10.1038/nbt.3290