mission bio

tapestri
APPLICATION NOTE

In-depth Quantification of Cell and Gene Therapy DNA Integrations Enabled with Single-cell Sequencing

Takeaways

- Significantly reduce the time between manufacturing and testing by eliminating clonal outgrowth protocols
- Identify transduced versus non-transduced cells with exceptional precision within populations of up to 10,000 single cells
- Rapidly optimize viral transduction protocols during development and measure the quality of transduced cells during production

Abstract

Cell and gene therapies are altering the treatment landscape of intractable genetic disorders, including cancer and inherited diseases. Single-cell DNA sequencing on the Tapestri Platform provides several advantages over other currently used techniques which require clonal outgrowth followed by qPCR to assess DNA integrations in cell and gene therapy workflows. Relative to qPCR, single-cell sequencing reduces the time to testing results by weeks. In addition, single-cell sequencing quantifies the presence or absence of DNA integrations, including transductions, without the need for lengthy cell culture protocols. The Tapestri Platform provides an unprecedented level of quantification of DNA integrations in significantly less time compared to bulk approaches and does so at single cell resolution. These characteristics allow for the rapid optimization of transduction protocols or HDR protocols during therapy development and streamlined testing and release of transduced or integrated cells during manufacturing of therapeutic products.

Introduction

Cell and gene therapies are transforming the treatment of genetic diseases and are rapidly being seen to cure cancers and inherited diseases that were once thought incurable. These therapies provide hope to patients who live with chronic, life-threatening conditions, such as lymphoma, sickle cell anemia, and Fabry disease. Cell and gene therapies alter the genetic material of cells ex vivo. The modified cells are then placed in the patient and either provide an immune response to diseased cells or proliferate and replace cells responsible for the genetic disease (Figure 1).

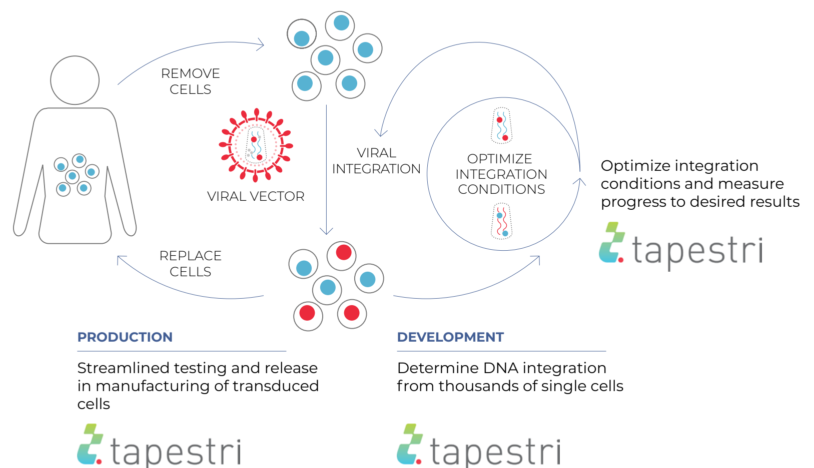

Figure 1 - A schematic of cell and gene therapy during development and production.

Genetic modification occurs through the use of gene-editing tools, such as CRISPR/Cas9, which introduce small insertions and deletions of DNA that can disable genes, or through integration systems, such as viral vectors and homology-directed repair (HDR), which add larger sections of DNA and can repair malfunctioning genes. Several cell therapies leverage viral integration, including CAR-T cell therapies[1], and have already been approved by the FDA[2], while others that treat inherited genetic disorders are rapidly advancing in clinical trials.

Before placing genetically altered cells in a patient, the most rigorous quality control is required to ensure that the correct edit(s) have been achieved. Current quantification of viral integration involves laborious and time-consuming techniques. For instance, clonal outgrowth, which can take 4 to 14 days to perform, may be necessary to assess percent transduction via qPCR analysis. And during this cell culture and outgrowth phase, the nature of the cells in the sample could change via selection. In addition, bulk assessments of transduced cells cannot identify the presence or absence of transduced genes within individual cells, and therefore must infer the percentage of cells in a sample that have been transduced.

In contrast, single-cell sequencing eliminates the need for lengthy clonal outgrowth protocols, reducing sample processing time from a month or more down to a single week. Moreover, single-cell sequencing provides detailed quantification of viral transduction in populations of single cells (Figure 2).

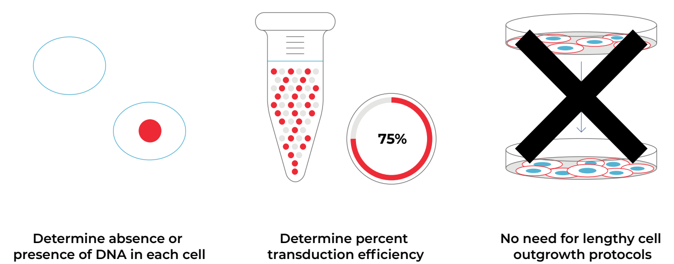

Figure 2 - Single-cell DNA sequencing detects transduced and non-transduced cells.

Experimental Methods and Results

Edits were made via lentiviral particles containing a unique genetic sequence and transduced cells were diluted with non-transduced cells to roughly achieve the following percentages of transduced cells: 0, 25, 50, 75, and 100%. Five replicates from each concentration of transduced cells were quantified using the Tapestri Platform and analyzed with Tapestri Pipeline and Tapestri Insights software (Figure 3).

Single-cell sequencing using the Tapestri Platform was performed on 5 replicates from each concentration of transduced cells. Single-cell sequencing across 25 samples in a serial dilution experiment showcased the ability to detect and quantify transduced versus non-transduced cells at expected ratios (Figure 4a). For the 5 samples made up of non-transduced cells, the false positive rate was below 0.03%. For the 5 samples that

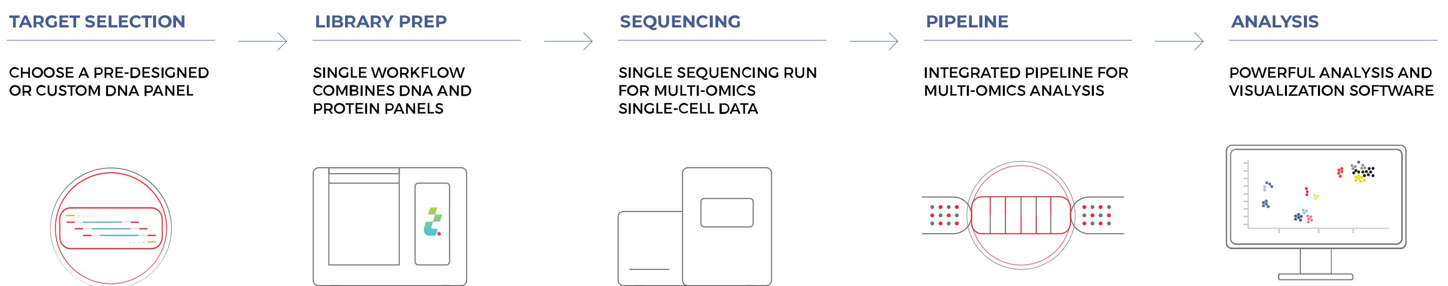

Figure 3 - Tapestri workflow.

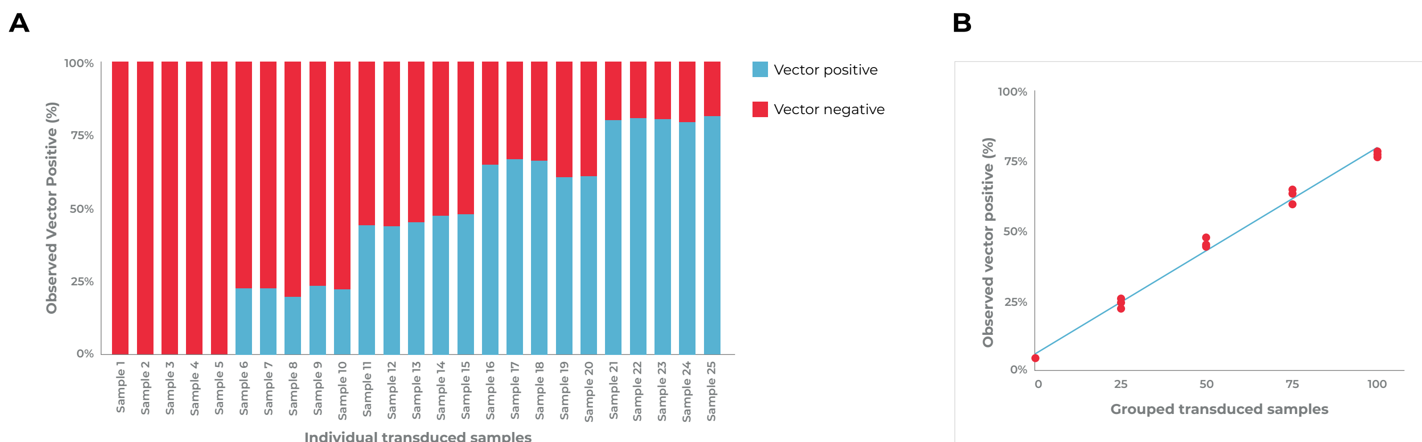

Figure 4 - Assessment of linearity with admixture of transduced cells and non-transduced cells.

made up the starting point, while not at 100% transduced, the dilution series thereafter showed excellent linearity and precision among replicates between the expected and observed transduction percentages (Figure 4b). Overall, these data showcase the ability to use the Mission Bio Tapestri Platform to interrogate 1000s of single cells per sample, and garner the percentage of transduced to non-transduced cells in the sample, without the need for any lengthy clonal outgrowth cell culture assays upfront of analysis.

Conclusion

Single-cell sequencing technology offers exciting new capabilities for the development of in vivo and ex vivo cell and gene therapies. By precisely measuring the presence or absence of DNA integrations from thousands of single cells, researchers can better optimize their protocols and reduce the time to go to market. When cells were transduced with a viral vector, single-cell analysis showed high correlation between the expected and observed percentages of transduced cells and exceptional precision among sample replicates. In addition, single-cell sequencing shortened the time from manufacturing to testing results from weeks, which is required for clonal outgrowth, down to days. These characteristics of single-cell sequencing streamline both therapy development and release testing of manufactured clinical cell and gene therapy products.

References

1. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *N Engl J Med*. 2018;378(5):439-448. doi:10.1056/NEJMoa1709866
2. Braendstrup P, Levine BL, Ruella M. The long road to the first FDA-approved gene therapy: chimeric antigen receptor T cells targeting CD19. *Cytotherapy*. 2020;22(2):57-69. doi:10.1016/j.jcyt.2019.12.004

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