Precise Measurement of Transduction Efficiency at Single-Cell Resolution for Cell and Gene Therapy Development

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Introduction

Cell and gene therapies are transformative solutions for a host of inherited and acquired diseases for which existing interventions are ineffective. Many such therapies rely on the introduction of transgenes into host cells using viral or non-viral vectors. The accurate measurement of gene transfer is critical to the development of therapeutic agents and is a key attribute for assessing their safety and efficacy. Yet, conventional methods for measuring gene transfer either report a population average (bulk) or involve laborious and time-consuming clonal outgrowth which can take 4 to 14 days. Mission Bio has developed an end-to-end solution from panel design to data analysis for single-cell targeted DNA sequencing. Here, using the Tapestri platform we demonstrate that single-cell DNA sequencing identifies transduced versus non-transduced cells with exceptional accuracy and precision for populations of up to 10,000 cells while reducing sample processing time from weeks to days.

Objective

Quantify transduced cells at a single cell resolution using the Tapestri single-cell DNA sequencing platform, and demonstrate assay specificity, reproducibility, precision and linearity.

Methods

The Tapestri precision genomic platform is enabled by its novel two-step microfluidic workflow where thousands of cells are each encapsulated and lysed in the first droplet followed by cell-lysate barcoding and target DNA amplification using highly multiplexed PCR in the second droplet. The droplets are then broken, and the amplified products are pooled for DNA library generation using Mission Bio consumables and sequenced on the Illumina NGS system. The final data is analyzed and visualized using the Tapestri Pipeline and Tapestri Insights software.

Figure 1: Tapestri workflow

In this study, a custom panel of 44 amplicons was designed for detection of two viral transduction products, Product 1 and Product 2 (Table 1). 9 amplicons covered common targets across both products, 6 amplicons covered Product 1 specific targets, and 7 amplicons covered Product 2 specific targets. 22 human amplicons covering diploid reference genes TERT and RPPH1 were included for read normalization and cell calling.

Common Viral Targets	Viral Product 1	Viral Product 2	Human TERT	Human RPPH1
Amp 1-6, 13-15	Amp 7-12	Amp 16-22	Amp 23-41	Amp 42-44

Table 1: Custom Panel Design for Viral Products

Samples containing viral transduced cells with unique sequences, Product 1 or Product 2, were thawed from frozen vials and resuspended. Additionally, one sample was diluted with non-transduced cells to achieve roughly 0, 25, 50, 75, and 100% of positively transduced cells. Five replicates from each prepared sample were then quantified using Mission Bio's Tapestri Platform.

Results

Assay amplicon performance demonstrates low variability and high specificity

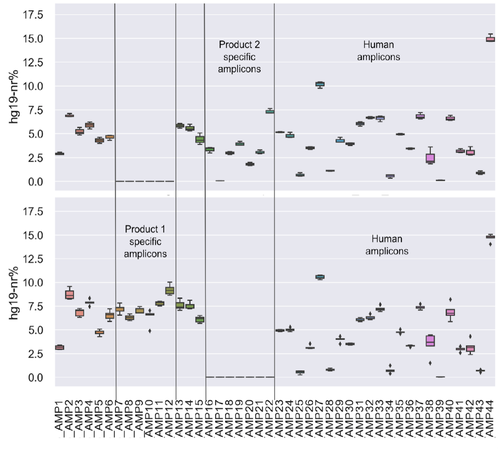

Figure 2: Mean normalized reads (hg19-nr%) across all amplicons in 5 replicates of Product 2 (top) and Product 1 (bottom)

To demonstrate amplicon performance for the assay:
Number of reads for each amplicon was normalized with the number of reads for the human amplicons.
Majority of the amplicons show low dispersion between replicates, confirming amplicon performance was consistent between human amplicons and viral amplicons (Fig 2).

To show the specificity of the viral amplicons:
Virus-specific amplicons for Product 1 (Amp 7-12) and Product 2 (Amp 16-22) were compared.
Amplicons for Product 1 were not amplified in the samples containing Product 2 and vice versa and zero false positive transduced cells were observed, demonstrating high specificity of the assay (Fig 2).

Strong Pairwise Pearson correlation for intra- and inter-group human amplicon performance showing assay reproducibility

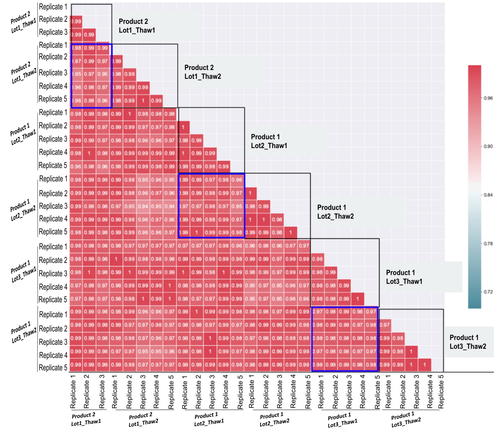

Figure 3: Pearson Correlation coefficient >0.94

To assess the amplicon performance and the assay reproducibility, Pearson correlation analysis was performed across 28 Tapestri runs between the groups of replicates across different products, independently manufactured lots, and different thaws.

It was found that the pairwise Pearson correlation for the human amplicons' performance was 94%, suggesting a strong association between replicates and high assay reproducibility (Fig 3).

Transduction efficiency can be detected with high precision

To identify transduced cells, positive viral amplification assessment was performed using an hr19-nr% threshold of 0.25%. Transduction % results in 28 runs performed across products, independently manufactured lots, and thaws demonstrated low variance and high precision (Table 2). The results suggest that the assay is applicable to detect differences across manufactured lots of viral transduction products (Fig 4).

Sample	Product 2 Lot1_thaw1	Product 2 Lot1_Thaw2	Product 1 Lot2_Thaw1	Product 1 Lot2_Thaw2	Product 1 Lot3_Thaw1	Product 1 Lot3_Thaw2
Average % transduction	60.34%	60.76%	81.32%	80.52%	86.39%	88.38%
CV% of %transduction	2.87%	2.41%	0.78%	1.41%	0.66%	0.47%

Table 2: CV% of %transduction efficiency across product lots

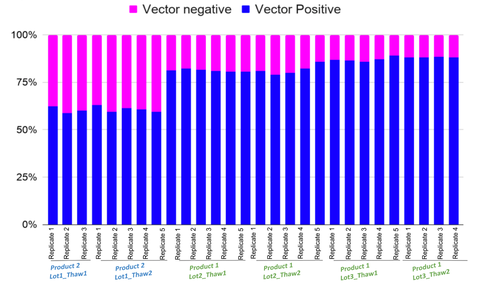

Figure 4: Fractional abundance (positive amp>=0.25% fraction read)

Assessment of linearity with a mixture of transduced cells and non-transduced cells

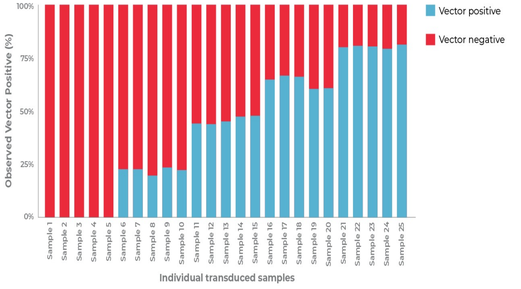

Figure 5a : Transduction% in transduced to non-transduced mixtures at 0:100, 25:75, 50:50, 75:25, and 100:0 ratios

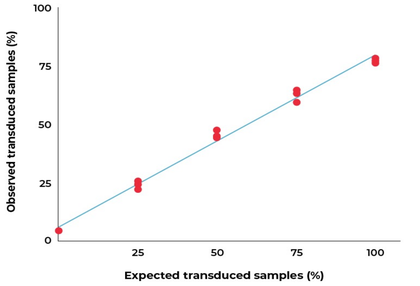

Figure 5b: Linear fit (r^2=0.992) for 0%,25%, 50%, 75% and 100%

To assess the linearity of transduction calling, Product 1 was serially diluted for transduced cell concentrations of 0%, 25%, 50%, 75% and 100% with 5 replicates each. Single-cell sequencing across these 25 samples showcased the ability to detect and quantify transduced versus non-transduced cells at expected ratios (Figure 5a). The false positive rate was below 0.03% for the 5 non-transduced samples. The 5 samples that made up the starting point of the titration and the dilution series thereafter showed excellent linearity and precision among replicates between the expected and observed transduction percentages (Figure 5b).

Conclusion

Using the Mission Bio Tapestri single-cell DNA sequencing platform, this study shows a consistent and reliable assay for in-depth quantification of cell and gene therapy transduction efficiency. Single-cell analysis of viral-vector transduced cells showed strong correlation between the sample groups, high analytical linearity between expected and observed percentages of transduced cells and exceptional precision in specificity and reproducibility among replicates. Together, these characteristics and data demonstrate the accuracy of the assay and streamline both the therapy development and early release testing of manufactured clinical cell and gene therapy products into the market.