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Precise Measurement of Viral Copy Number at Single-Cell Resolution for Cell and Gene Therapy Development

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Introduction

Cell and gene therapies (CGT) 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 lack the resolution and representation to truly reflect sample composition and either report a population average (bulk) or involve laborious and time-consuming clonal outgrowth which can take weeks. 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, as well as measures the single-cell level vector copy number (VCN) for populations of thousands of cells with single nucleotide resolution while reducing sample processing time from weeks to days.

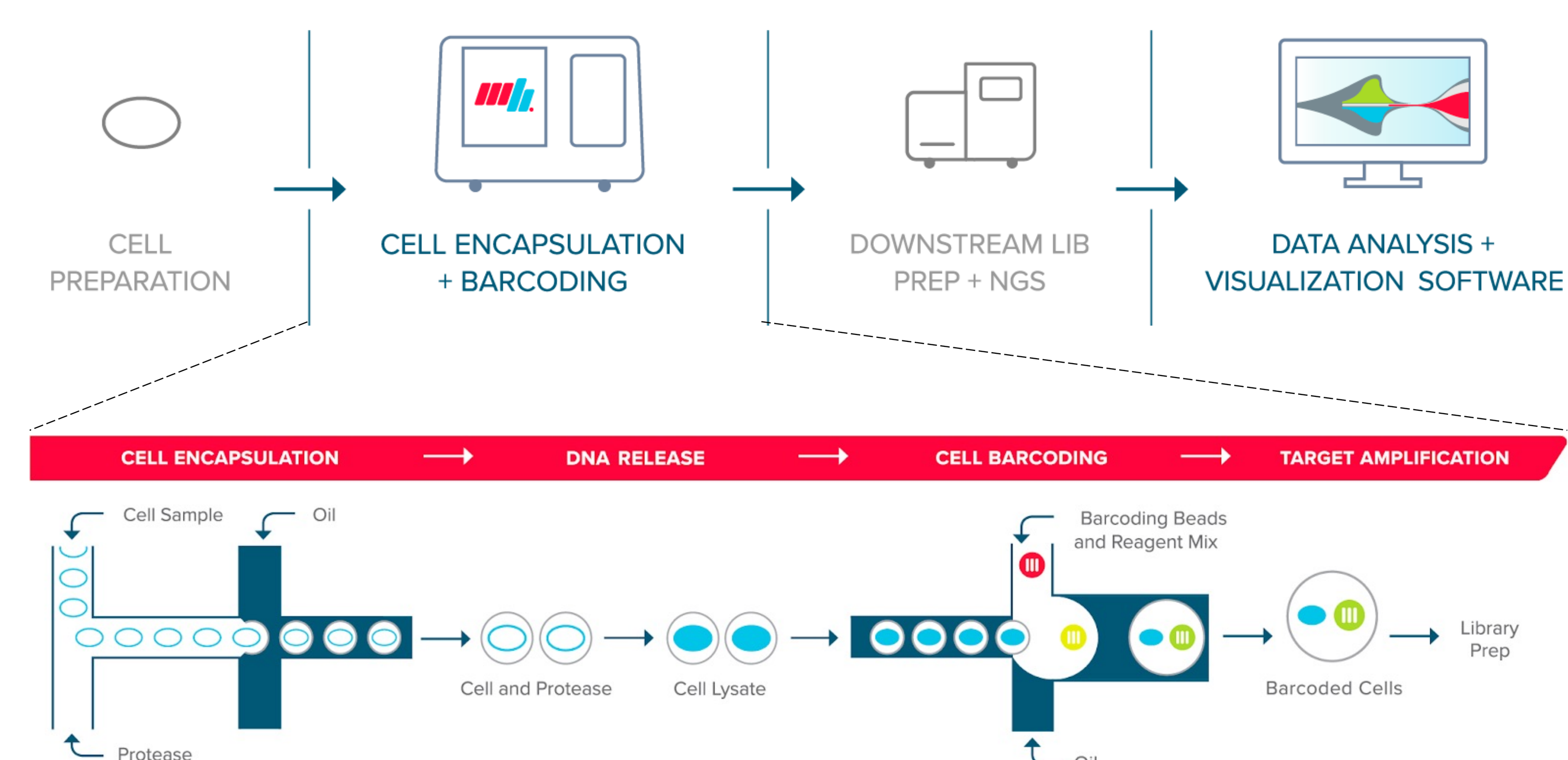
Objective

To demonstrate the quantitative characterization capability of single-cell level vector copy number and transduction efficiency using the Tapestri single-cell DNA sequencing platform in terms of assay specificity, sensitivity, accuracy, and precision.

Methods

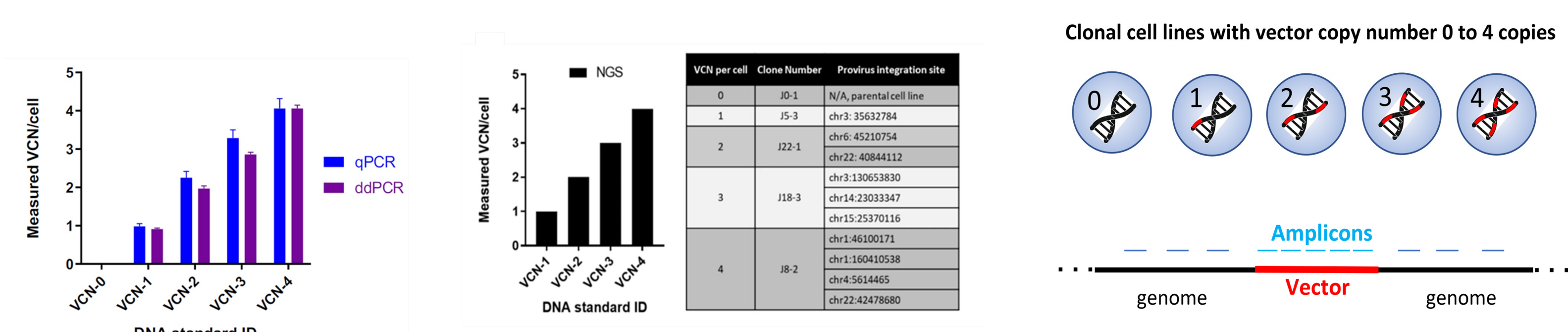
The Tapestri precision genomic platform is enabled by a novel two-step microfluidic workflow where thousands of cells are each encapsulated and lysed in the first droplet followed by cell-lysate barcoding and target DNA amplification using highly multiplexed PCR, in the second droplet. The droplets are 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).

Figure 1: Tapestri workflow



Characterized and experimental lentiviral transduced Jurkat clonal cell lines with vector copy number of 0, 1, 2, 3 and 4 copies were used in this study [1]. A custom panel of amplicons was designed to enable the vector copy number analysis. Pure clonal cell lines of VCN 0-4 and three (3) mixtures with non-transduced Raji cell spike-in were processed in triplicate for single-cell sequencing using Mission Bio's Tapestri platform. Tapestri data from a control cell line with known VCN, as well as unknown samples, were combined and analyzed with a copy number caller to produce single-cell level VCN calls, as well as a population average VCN and a percent transduction for each sample. A median of 5,116 cells were analyzed for each Tapestri sample.

Figure 2: Lentiviral transduced VCN cell lines and vector assay design[1]



Results

Tapestri vector copy number assessment tool is precise and accurate

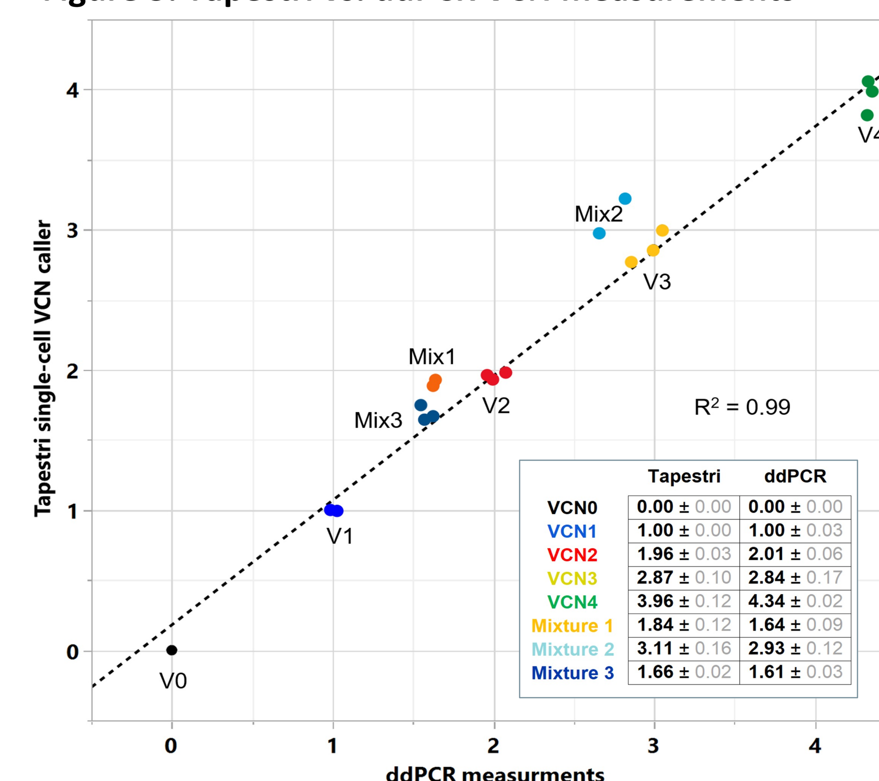
Table 1: Combinations of controls and test samples' average VCN

Average copy number for various combinations of controls and test samples		Test Sample											
		VCN1 rep1	VCN1 rep2	VCN1 rep3	VCN2 rep1	VCN2 rep2	VCN2 rep3	VCN3 rep1	VCN3 rep2	VCN3 rep3	VCN4 rep1	VCN4 rep2	VCN4 rep3
Control Sample	VCN1 rep1	1.00	1.00	1.00	2.10	2.00	2.00	3.00	3.00	3.26	4.34	4.46	4.33
	VCN1 rep2	1.00	1.00	1.00	2.00	2.00	2.00	3.00	3.00	3.00	4.12	4.23	4.01
	VCN1 rep3	1.00	1.00	1.00	2.01	2.00	2.00	3.00	3.00	3.05	4.09	4.32	4.10
	VCN2 rep1	1.01	1.03	1.03	2.00	2.00	2.00	3.00	3.00	3.00	4.08	4.27	4.19
	VCN2 rep2	1.02	1.03	1.03	2.01	2.00	2.00	3.00	3.00	3.05	4.18	4.35	4.18
	VCN2 rep3	1.01	1.01	1.02	2.00	2.00	2.00	3.00	3.00	3.00	4.09	4.38	4.34
	VCN3 rep1	1.09	1.08	1.10	2.10	2.08	2.07	3.00	3.11	3.13	4.37	4.64	4.46
	VCN3 rep2	1.03	1.03	1.04	2.00	2.00	2.00	3.00	3.00	3.00	4.18	4.43	4.27
	VCN3 rep3	1.06	1.06	1.06	2.02	2.01	2.00	3.00	3.00	3.00	4.22	4.42	4.29
	VCN4 rep1	1.00	1.01	1.01	2.00	2.00	2.00	2.99	3.00	3.00	4.01	4.34	4.11
	VCN4 rep2	1.00	1.00	1.00	1.94	1.98	1.96	2.77	2.96	2.83	3.85	4.04	3.98
	VCN4 rep3	1.00	1.00	1.01	2.00	2.00	2.00	2.92	3.00	2.96	4.00	4.31	4.03

Clonal populations of engineered VCN cells (VCN1-4) were single-cell sequenced and individually processed through the Tapestri VCN caller (n=3). We demonstrate that the single-cell VCN caller can use any cell population with a known number of VCNs to characterize a test sample's VCN with high accuracy and precision (Table 1). Using the VCN caller model established from VCN 1 to VCN 4 each as reference points for determining average VCN produces coefficient of variation of 2.76%, 1.62%, 2.48% and 4.04%; and percent error of 2.17%, 0.43%, 0.05% and 5.56% respectively across all combinations and runs.

Tapestri VCN measurements correlate with orthogonal ddPCR technology

Figure 3: Tapestri vs. ddPCR VCN measurements

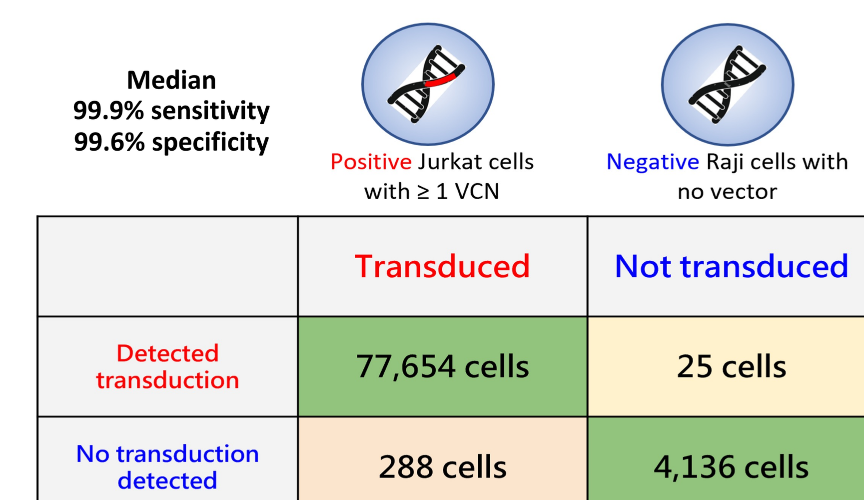


X-Y scatter plot of the average VCN for each sample called using Tapestri VCN caller vs. ddPCR measurement. Pure cell lines (VCN 0-4) or mixtures of cell lines (Mixture 1-3) were analyzed in triplicate by both Tapestri and ddPCR. Each sample's average VCN was then calculate based on single-cell level VCN (y-axis) and plotted against orthogonal ddPCR measurements (Bio Rad QX200 [1], x-axis, n=3). The average copy number of the population estimated using Tapestri correlates well with ddPCR measurements (R^2= 0.99). The average and standard deviation of replicate measurements (n=3) are shown in the bottom right table.

Transduction efficiency is detected with high sensitivity and specificity

Tapestri also provides vector amplicon specific measurements that can be used to determine transduction efficiency. A mixture of non-transduced Raji and vector-containing Jurkat cells was used for the study. The sample's transduction efficiency was calculated based on detection of vector amplicon reads and validated using the assignment from SNPs specific to Raji (negative) and Jurkat (positive) cell lines. The Tapestri VCN can detect transduction efficiency with >99.6% and >99.9% median specificity and sensitivity, respectively.

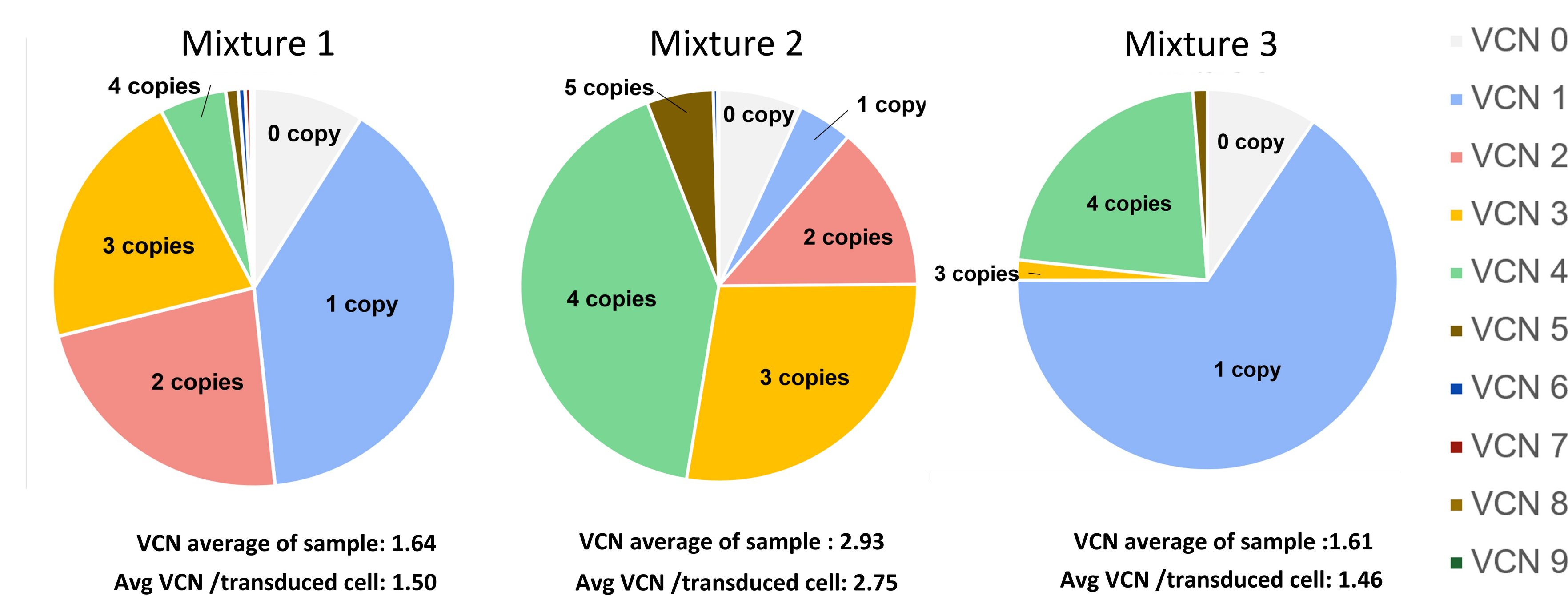
Figure 4: Transduction efficiency sensitivity and specificity



	Transduced	Not transduced
Detected transduction	77,654 cells	25 cells
No transduction detected	288 cells	4,136 cells

Single-cell level vector copy number distribution characterization

Figure 5: Single-cell VCN distribution per sample

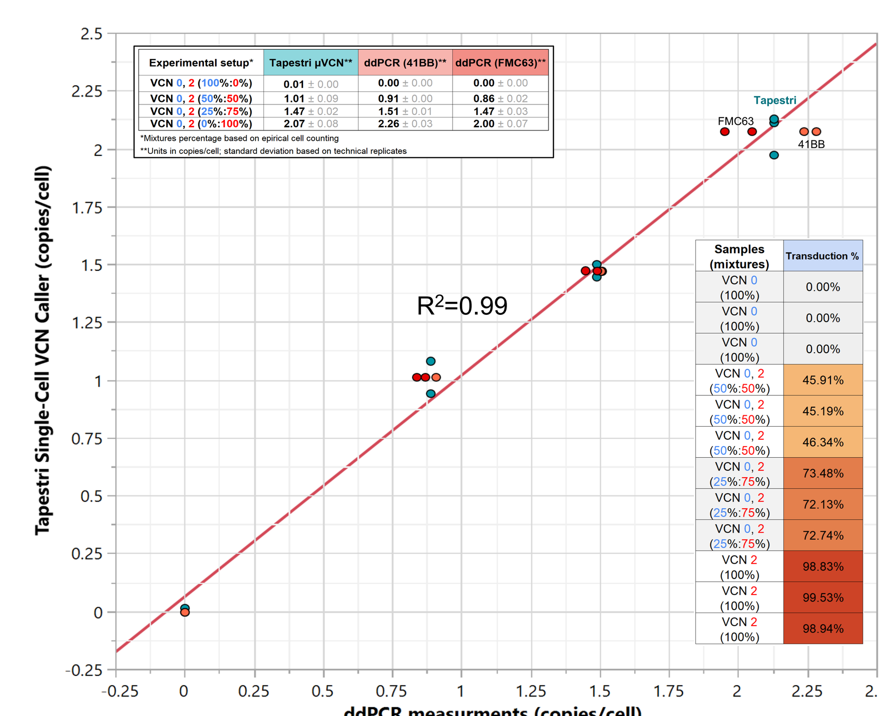


Besides the measurement of average VCN per sample, Tapestri VCN caller calculates VCN distribution of a particular sample at single-cell level. Figure 5 shows the VCN distribution calculated for each of the three mixtures, Mixture 1, Mixture 2, and Mixture 3 each with transduction % of 91.76%, 94.02% and 90.72%. Note that "Mixture 1" (VCN 1-4's respective proportions = 39.4%, 22.8%, 21.3%, 5.4%) and "Mixture 3" (VCN 1-4's respective proportions = 65.6%, 0%, 1.7%, 22.1%) VCN distributions are drastically different despite having similar average populational VCN value of 1.64 and 1.61 (average VCN for transduced cell 1.50 vs. 1.46), respectively. Furthermore, Tapestri also provides the average VCN per transduced cell (below each pie chart).

Dual CD19 X CD20 CAR-T Vector Characterization

Bi-cistronic CD19/CD20 dual CAR Jurkat clonal cell lines of VCN 0 and 2 were used in this study (100% VCN 0, 2 and two mixtures at 50:50 and 25:75). Figure 6 shows the X-Y scatter plot of the average VCN for each sample called using Tapestri VCN caller vs. ddPCR measurement (two separate ddPCR probe assays targeting 41BB and FMC63 region; albumin as reference; BioRad QX200, n=2). The average copy number of the population estimated using Tapestri correlates well with ddPCR measurements (R^2= 0.99). Note that the sample average VCN measured by two separate ddPCR assays may not necessarily agree with each other, based on assay dependent performance and the degree of optimization. The transduction % characterized for each sample is shown in the bottom right table (n=3).

Figure 6: Tapestri vs. ddPCR on avg VCN; % TXN table



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 vector copy number distribution, and populational average. The single-cell level VCN distribution, as well as single-cell vector sequence validation provides unprecedented resolution and insight to assess the potential functional efficacy and safety for CGT products. Together, these characteristics and data demonstrate the potential to accelerate and streamline both the development and release testing of cell and gene therapy products.

Reference

1.https://doi.org/10.1038/s41598-020-79698-w