Highly robust and sensitive off-the-shelf and custom cancer solutions for high-throughput single-cell targeted DNA sequencing

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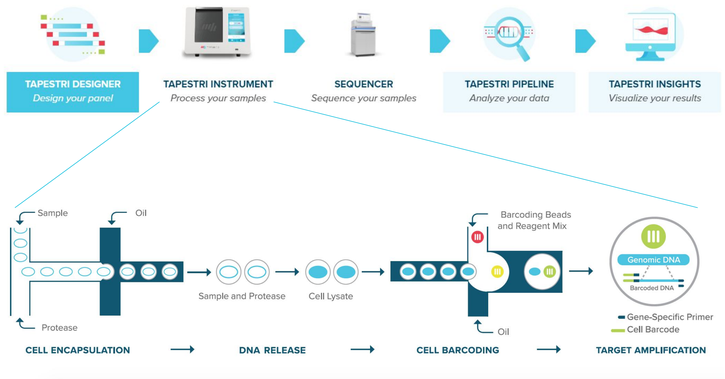
Introduction

Single-cell technologies have advanced our approach to precision medicine, improving our understanding of the genetic variation and drivers of cancer with sensitivity that bulk sequencing cannot provide. To leverage these capabilities, we previously developed a high-throughput single-cell DNA analysis platform (Tapestri®) that employs droplet microfluidics and a multiplex PCR-based targeted DNA sequencing approach, along with several pre-validated catalog cancer panels. Here, we present an update on our catalog panels, including acute myeloid leukemia (AML) (19 genes, 125 amplicons), chronic lymphocytic leukemia (CLL) (32 genes, 274 amplicons) and Myeloid (45 genes, 312 amplicons) panels. Updated features include new biochemistry, panel design, and individual amplicon optimization, resulting in improved performance and sensitivity. Additionally, we show our custom panel design capabilities, including the ability to run high-plex panels (>500 amplicons) with robust panel performance. The workflow from cell prep to sequence-ready libraries can be completed in 2 days, and with new modifications we have validated the performance of the updated AML, CLL and Myeloid panels, demonstrating >95% panel uniformity and >90% of reads mapped to targets. Additionally, we built a custom panel featuring 608 amplicons across 51 genes that demonstrates 85% panel uniformity and 87% reads mapped to targets using standard workflow procedures without any panel optimization. Using mixed cell line samples run with catalog panels, the Tapestri platform demonstrates the sensitivity to detect rare SNV and CNV subclones present at 0.1% and 5% respectively. The Tapestri platform now provides the capability and flexibility of both targeted, robust off-the-shelf and customized cancer panels that can analyze thousands of DNA targets simultaneously, providing unprecedented insight into tumor heterogeneity, clonal evolution and personalized cancer therapies.

Tapestri workflow overview



Mission Bio's Tapestri platform is a complete solution for targeted single-cell DNA sequencing. To begin, customers can select from pre-validated catalog panels, un-validated pre-designed panels with curated content, or design custom panels for any targets in the hg19 exome using our primer design pipeline Tapestri Designer. Custom panels can be built to include whole genes, transcripts, SNVs and indels up to 500 amplicons in size. For unique panel needs, such as non-human models, CRISPR editing targets, and high-plexy panels >500 amplicons, white-glove bioinformatic support is available.

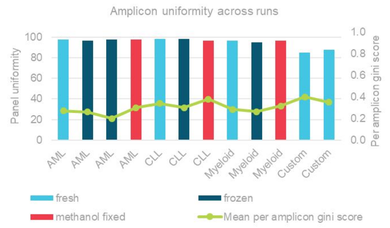
Once a panel is selected, Tapestri experimental set-up, including cell preparation, cell encapsulation and lysis, cell barcoding, target amplification, library preparation and sequencing can be completed in 2 days with minimal hands-on time. Sequencing data is processed and analyzed with fully integrated software, including variant filtering and data visualization with Tapestri Insights.

Panel Performance

a

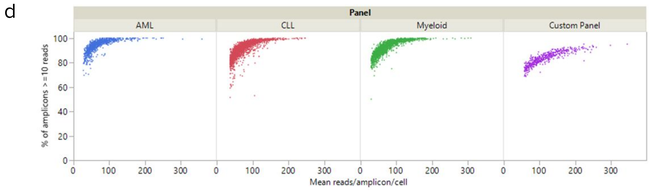
Panel	No. of genes	No. of amplicons	Target region coverage (kb)	Optimized?
AML	19	125	24	Yes
CLL	32	274	53	Yes
Myeloid	45	312	65	Yes
Custom	51	608	144	No

b



c

d



Panel performance with updated chemistry was tested on three catalog panels, AML, CLL and Myeloid, and 1 high plexy custom panel (a). Data for catalog panels represents optimized primer pools, while custom panel data represents first-pass runs without optimization. Multiple runs across AML (4), CLL (3), Myeloid (3) and high plexy custom (2) panels using different cell line-derived cell types (fresh, frozen and fixed) demonstrate consistently high panel uniformity (% >0.2x mean), mapped reads and low mean per amplicon gini scores (perfect uniformity=0), indicating minimal amplicon noise across cells (b and c). Data completeness was analyzed by comparing amplicons with a minimum of 10 reads with the mean number of reads per amplicon per cell (d). Catalog panels achieved >90% data completeness and the custom panel achieved 83% data completeness at the recommended 80x sequencing depth.

High sensitivity SNV detection of rare subclones

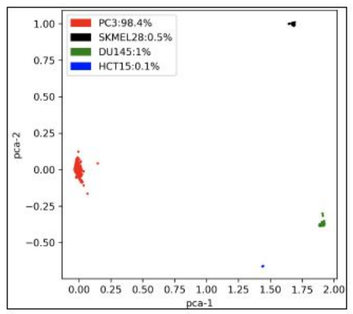
a

THP Truth Variants	PC3	DU145	SKMEL28	HCT15
TP53:chr17:7578515	2	0	0	0
JAK3:chr19:17946054	2	0	0	0
SMO:chr7:128846557	2	2	2	0
BRAF:chr7:140453136	0	0	2	0
ERBB2:chr17:37879588	2	0	0	2
TP53:chr17:7578115	0	2	0	2
TP53:chr17:7578210	0	0	0	2
SMAD4:chr18:48586344	0	0	0	2

b

Cell %	PC3	DU145	SKMEL28	HCT15
Expected	98.5%	1.0%	0.5%	0.1%
Observed	98.7%	0.88%	0.4%	0.04%

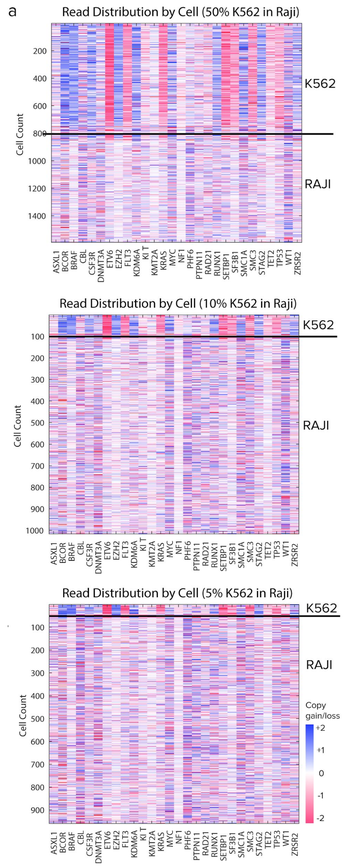
c



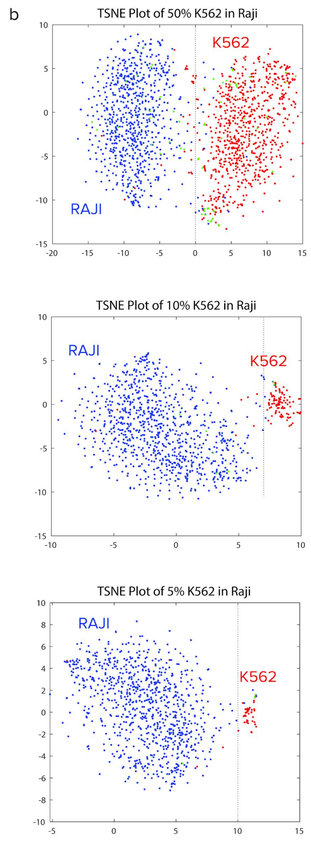
The sensitivity of the Tapestri platform was demonstrated using a 4-cell line mix consisting of 98.4% PC3, 1% DU145, 0.5% SKMEL28, and 0.1% HCT15 run with the Tumor Hotspot panel (THP) (59 genes, 244 amplicons). True positive variants were identified from bulk sequencing of pure cell lines (a) and used in analysis to assign cell types to all single cells identified from Tapestri results. Cell clusters and the resulting percentages assigned by truth variants (b and c) correlate well with expected values, with rare clones at 0.1% detected.

Reliable CNV analysis of rare subclones

a



b



To illustrate the sensitivity of CNV detection on a single-gene basis using the Tapestri platform, K562 cells were mixed with Raji cells at ratios of 50%, 10% and 5%, and run with the Myeloid panel. Tapestri results were analyzed for CNVs using the "tapestri-cnv" package in R, available through Mission Bio's CNV+SNV webpage. Heat maps (a) and t-SNE plots colored by SNV data (b) show clear clustering of the two cell types, demonstrating the ability to detect rare cell populations based on CNVs with existing panels.

Conclusions

- The Tapestri platform offers an end-to-end solution, from amplicon design to variant analysis, for targeted DNA analysis of single cells.
- With improved chemistry, catalog panels demonstrate robust performance, with panel uniformity > 95%, reads mapped > 85% and data completeness >90%.
- Tapestri platform is capable of running high plexy panels >500 amplicons without workflow modifications or panel optimization.
- Tapestri platform co-detects SNVs and CNVs in the same cell with high sensitivity.

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