High-throughput Single-cell Targeted DNA Sequencing Using an Updated Tapestri™ Platform Reveals Rare Clones and Clonal Evolution for Multiple Blood Cancers

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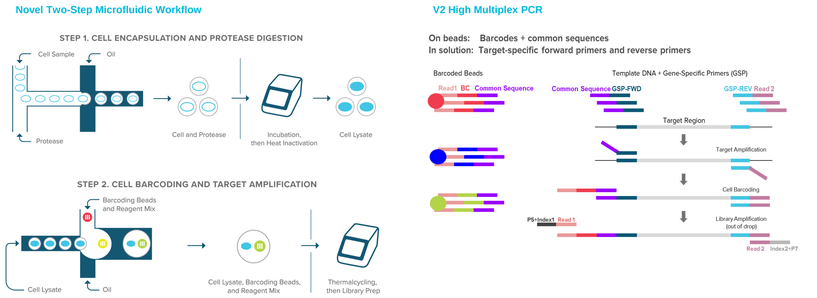
Abstract

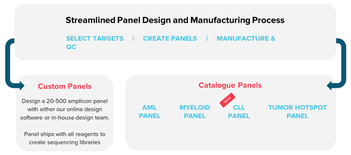
The challenge in precision medicine has been improving the understanding of cancer heterogeneity and clonal evolution, which has major implications in targeted therapy selection and disease monitoring. However, current bulk sequencing methods are unable to unambiguously identify rare pathogenic or drug-resistant cell populations and determine whether mutations co-occur within the same cell. Single-cell sequencing has the potential to provide unique insights on the cellular and genetic composition, drivers, and signatures of cancer at unparalleled sensitivity. **Methods:** Previously we have developed a high-throughput single-cell DNA analysis platform (Tapestri™) that leverages droplet microfluidics and a multiplex-PCR based targeted DNA sequencing approach, and demonstrated the generation of high-resolution maps of clonal architecture from acute myeloid leukemia (AML) tumors. Here we present an update to the Tapestri Platform which employs new biochemistry and features improved firmware, software, workflow, and data analysis solution resulting in higher throughput, better sensitivity, specificity and unprecedented flexibility. **Results:** From cell prep to sequencing-ready libraries, the workflow can be completed within 2 days. We have validated the performance of an AML (19 genes, 50 amplicons) and a CLL (chronic lymphocytic leukemia) (34 genes, 286 amplicons) panel. We also developed a robust web-based design portal for custom targets. The updated biochemistry enables easy addition of new gene and loci targets into existing panels for improved coverage and updated studies. Using longitudinal AML and CLL samples, we were able to detect rare subclones of <0.1% prevalence, identify mutation co-occurrence, and characterize clonal evolution due to disease progression and drug treatment. **Conclusion:** We demonstrate that single-cell DNA sequencing can reveal the heterogeneity of blood cancers and map the clonal architecture and clonal evolution with higher sensitivity than bulk NGS methods. This is critical in patient stratification and drug selection over the entire course of treatment. Besides the catalog AML and CLL panels, the flexibility of system allows for analyzing SNV and indel mutations of any custom cancer DNA targets. Additionally, the system provides capabilities for quality control of gene edited cells, further advancing research into cancer therapies.

The Foundations of Tapestri Technology



The Tapestri precision genomic platform is enabled by a novel two-step microfluidic workflow and a high multiplex PCR biochemistry scheme. The two-step microfluidics allows for efficient access to DNA for downstream genomic reactions and provides flexibility to adapt for additional applications and multi-omics. The multiplex PCR chemistry is developed and co-optimized with an AI-powered panel design pipeline and enables direct and efficient amplification of targeted genomic regions within barcoded individual cells. Taken together the platform produces high genomic coverage, low allele dropout rate, highly uniform amplification in thousands of cells from single run, is compatible with diverse and difficult samples, and is easily deployable for custom content.

Build Desired Targeted Panels



TAPESTRI SINGLE-CELL DNA PANELS

	# genes	# amplicons	Target regions coverage (Kbp)	Panel Uniformity
AML Panel	19	50	~8.5	>90%
CLL Panel	34	286	~54.9	>90%
Myeloid Panel	47	330	~66.0	>80%
Tumor Hotspot Panel	59	244	~40.0	>80%

19-GENE AML PANEL

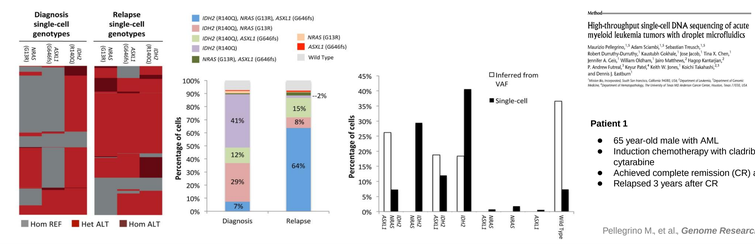
ASXL1	GATA2	KIT	PTPN11	TP53
DNMT3A	IDH1	KRAS	RUNX1	U2AF1
EZH2	IDH2	NPM1	SF3B1	WT1
FLT3	JAK2	NRAS	SRSF2	

34-GENE CLL PANEL

ATM	CD79B	EZH2	MAP2K1	PAX5	SPEN
BCOR	CHD2	FAT1	MED12	PLCG2	TP53
BIRC3	CREBBP	FBXW7	MYD88	POT1	XPO1
BRAF	CXCR4	KLHL6	NFKBIE	RPS15	ZMYM3
BTK	DDX3X	KRAS	NOTCH1	SETD2	-
CARD11	EGR2	LRP1B	NRAS	SF3B1	-

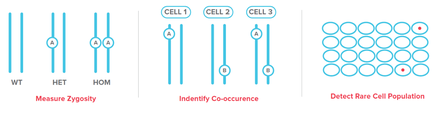
We launched a simple intuitive cloud-based Tapestri Designer this year to enable users to focus time and budget and easily build custom panels for their relevant targets, multiplexed at 20 to 500 amplicons. The targets can be whole genes, transcripts, chromosome regions, or target SNPs and small indels. Catalogue panels are also available for AML, Myeloid, CLL, and Tumor Hotspot, to initiate the studies immediately or use as a base panel. For unique target sequences such as CRISPR edited cells, targets outside the exome, or for non-human animal models, expert bioinformatic support can be provided in custom project format.

Clonal Evolution in Longitudinal AML Samples



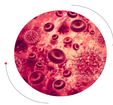
Pellegrino M., et al., ***Genome Research*** (2018)

The Power of Single Cell DNA Analysis



Parameter	Bulk Sequencing	Single-Cell Sequencing
VAF	Inferred	Quantifiable
Zygosity	Heterozygous (assumed)	Quantifiable
Clonal architecture	Inferred	Quantifiable
Mutation co-occurrence	Inferred	Quantifiable
Non-neoplastic cell contamination	Undetectable	Detectable & Removable

Hematology



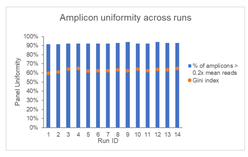
Solid Tumor

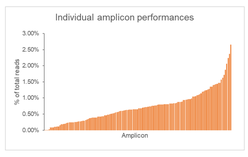
Gene Editing QC

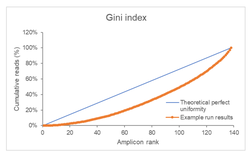
Tumors are genetically and phenotypically complex samples. MOST cancers have many clones with different mutations. Rare cells may be the most informative for clinical intervention and mutational co-occurrence patterns may be implicated in disease progression. Bulk sequencing methods typically fail to resolve complex heterogeneity and mutation co-occurrence patterns. Single cell sequencing enables not only detection of rare clones, co-occurrence of mutations, but also quantifiable VAF, zygosity, clonal architectures, and a paradigm shift in hematology, solid tumor, and gene editing.

Performances of Testing Panels

Panel ID	Species	Targets	# gene	# amplicon	Target region coverage (Kbp)	Mapped to Genome %	Mapped to Target %	Panel Uniformity* (% >0.2x mean)
Testing Panel 1	Human	Gene exons, hotspot, ADOs	20	138	~24.2	97.7	94.4	92.4
Testing Panel 2	Human	Gene exons and hotspot	266	287	~53.0	91.9	90.0	88.9



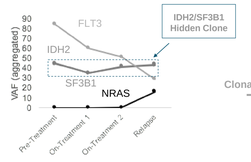




To test the performances of the updated chemistry and designer pipeline, we designed three panels with 31, 138, and 287 (small, medium, large) amplicons respectively. The primer pools were pooled in equal volumes without any optimization, in order to simulate a standard custom panel at first pass (Mission Bio can also provide white-glove services to rescue specific designs and optimize individual amplicon performances after wet QC). Multiple runs were conducted for each panel with different cell types. An updated Tapestri analysis pipeline was used in analyzing the FASTQ data, collapsing cell barcodes, finding cells and calling genotypes. Typical cell outputs range from 5000 to 7,000 cells in these runs (30-40x sequencing depth), with <8% cell mixing (50/50 cell mix experiments) and <10% allele dropout rate. The run to run consistency data were shown above from multiple runs of Testing Panel 1. Bottom graphs show the panel uniformity for the runs and how the panel uniformity metrics were calculated and visualized.

Tapestri Reveals Therapy Resistant Clones

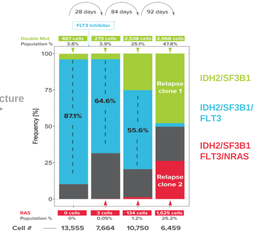
Aggregated Single-Cell VAFs



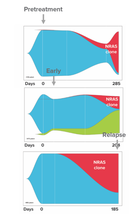
- Expansion of **double-mutant relapse clone** never detected w/bulk NGS
- Acquisition of **RAS clone** detected too late w/bulk NGS

Clonal Architecture Resolved Over Time

Resolve Clonal Architecture



Early identification of Gilteritinib resistant clones

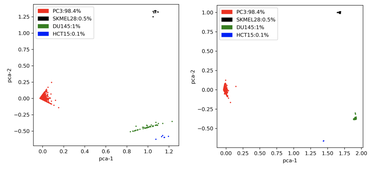


McMahon C.M., et al., ***submitted*** (2019)

Tapestri Precision Genomics Platform Overview

Mission Bio has developed an end-to-end solution from panel design to data analysis for single cell targeted DNA-sequencing. Typical workflow starts from online Tapestri Portal (newly launched this year) either design a custom panel or order a catalogue panel. Actual experiments take two days from sample prep to cell encapsulation, barcoding to sequencing-ready libraries with < 2 hours hands-on time.

High Sensitivity Detection of Rare Cell Populations



Panel A Truth Variants	PC3	DU145	SKMEL28	HCT15
SORCS3:chr10:106721610	0	0	2	0
TP53:chr17:7577610	0	0	0	2
TP53:chr17:7578115	0	2	0	2
TP53:chr17:7578210	0	0	0	2
KIT:chr4:55599436	2	1	0	1

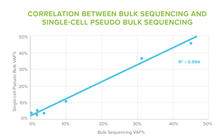
Panel B Truth Variants	PC3	DU145	SKMEL28	HCT15
TP53:chr17:7578515	2	0	0	0
JAK3:chr19:17946054	2	0	0	0
SMO:chr7:128846557	2	2	2	0
BRAF:chr7:140453136	0	0	2	0
ERBB2:chr17:37879588	2	0	0	2
TP53:chr17:7578115	0	2	0	2
TP53:chr17:7578210	0	0	0	2
SMAD4:chr18:48586344	0	0	0	2

Cell Percentages	PC3	DU145	SKMEL28	HCT15
Designed Input Cell Composition	98.5%	1%	0.5%	0.1%
Panel A run results	98.6%	0.95%	0.35%	0.11%
Panel B run results	98.7%	0.88%	0.40%	0.04%

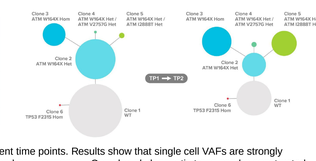
To test the sensitivity and specificity of the system, we mixed four types of cells at different concentrations (98.4%, 1%, 0.5%, and 0.1%), and run the mixed cell population on Tapestri with two targeted panels (Panel A-50 amplicons and Panel B-244 amplicons). The truth variant data from the four cell types were generated from bulk sequencing of pure cell lines, then used in Tapestri data analysis to determine cell types for all single cells from the Tapestri results. The resulted percentages matched closely to the desired cell input composition. Clones of 0.1% cells can be detected using both panels.

Single-Cell DNA Sequencing Resolves the Genetic Complexity Underlying CLL Progression

CORRELATION BETWEEN BULK SEQUENCING AND SINGLE-CELL PSEUDO BULK SEQUENCING

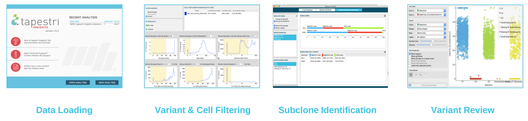


Variant	TP 1 WT	TP 1 Het	TP 1 Hom	TP 2 WT	TP 2 Het	TP 2 Hom
ATM p.W164X	44.4%	37.7%	17.9%	31.6%	45.6%	22.8%
ATM p.V2757G	97.4%	2.6%	0%	95.8%	4.2%	0%
ATM p.I2888T	95.4%	4.6%	0%	78.6%	21.4%	0%
TP53 p.F231S	98.5%	0%	1.5%	98.5%	0%	1.5%



In collaboration with Dr Esteban Braggio at Mayo Clinic, we have tested patient CLL samples at different time points. Results show that single cell VAFs are strongly correlated with bulk sequencing VAFs, but only single cell sequencing can resolve mutation zygosity and co-occurrence. Complex phylogenetic trees can be constructed from the single cell variant information to represent the clonal evolution (circle sizes correlate to subclone cell percentages).

Single Cell DNA-Seq Data Analysis and Visualization



A significant challenge for high-throughput single cell sequencing is analyzing the complex data and visualizing results in biologically-meaningful way. We have updated the Tapestri pipeline and Tapestri Insight software, to more accurately and quickly reconstruct cell-level mutation profiles across thousands of cells, and explore clone distribution and progression with key annotations.

Conclusions

The Tapestri Precision Genomics Platform utilizes novel droplet microfluidics to access the genomic DNA. Together with updated chemistry and designer, it generates high coverage uniformity and low allele dropout for thousands of single cells, and enables detection of rare subclones down to 0.1%. Researchers now have a highly sensitive, targeted, and customizable solution for investigating genomic variation and clonal evolution in complex biological samples, as demonstrated in multiple AML and CLL longitudinal studies.

References:

Pellegrino M., et al., ***Genome Research*** (2018)

McMahon C.M., et al., ***submitted*** (2019)