

# Tapestri Platform Resolves Clonality of Heterogeneous Mouse Organoid Cancer Model through Single-Cell DNA Sequencing of Lentiviral Barcodes

## Takeaways

- Tapestri Platform now supports the mouse genome
- Clonality of a heterogeneous mouse organoid bladder cancer model is resolved with the Tapestri Platform
- Custom panel of amplicons to lentiviral barcodes resulted in high sensitivity of subclone detection, below 1%, with co-occurring gene insertions
- A novel method using single-cell DNA sequencing was developed as a system for investigating the functional impact of higher-order genetic interactions in cancer

## Abstract

The genomic heterogeneity of bladder cancer is expansive, with over 30 known oncogenic genetic alterations and each tumor harboring at least 5.<sup>1</sup> Studying these diverse higher-order genetic interactions that drive bladder cancer is difficult with current models of tumorigenesis and limited by bulk sequencing that fail to directly discern clonality and resolve mutational co-occurrence patterns. Here, we demonstrate an organoid model of bladder cancer using lentivirus to introduce gain or loss of function of multiple genes per cell in random combinations. Organoids are selected for in vivo tumorigenesis and profiled through single-cell DNA sequencing using the Tapestri Platform and a custom single-cell DNA panel targeting lentiviral barcodes. This method revealed combinations of altered genes that led to tumorigenesis within immunocompetent and immunodeficient mice.

## Experiment & Methods

Over 30 genes were chosen for gain or loss of function based on recurrent frequencies of at least 5% in bladder cancer. Open reading frames (ORFs) or shRNAs were introduced to cells via lentivirus that also included a DNA barcode for each gene. Primary C57/BL6J mouse bladder urothelial cells were isolated and infected with lentivirus at a multiplicity of infection corresponding to an average of four gene aberrations per cell. In Matrigel, cells formed organoids after 72hrs. Organoids were then grafted into immunocompetent (C57BL/6J) or immunodeficient (NSG) mice. Organoids that formed tumors were sequenced at the single-cell level using the Tapestri Platform with a custom amplicon panel to lentiviral barcodes. Cell barcodes were extracted using umi-tools using pre-filtered sequencing reads. The reads were aligned to our custom reference by bwa (Burrows-Wheeler Aligner) and matrix of cell x amplicon was constructed using python. After additional filtering and read normalization the cell population was defined based on the reads of different amplicons.



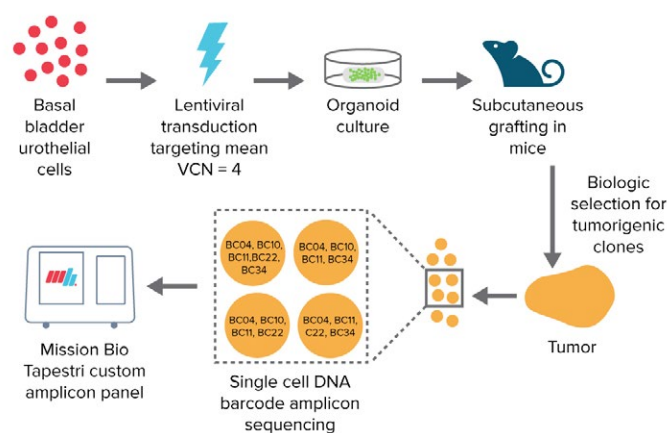


Figure 1 - Schematic of lentiviral transduction, organoid grafting, and single-cell DNA sequencing of a novel mouse model of bladder cancer to uncover higher-order genetic interactions that lead to tumorigenicity.

## Results

### Mouse organoid bladder cancer model enables investigation of higher-order genetic interactions through single-cell DNA sequencing

Traditional technologies to investigate the functional contribution of genetic alterations in cancer are inefficient, low-throughput, and do not address the complexities of higher-order compound genetic interactions. Available genetically defined model systems for cancer severely underrepresent the heterogeneity found in human cancers. To develop a robust, economical, and biologically relevant assay that enables the functional annotation of the compound and context-specific oncogenic drivers of bladder cancer, the Lee lab from the Fred Hutchinson Cancer Research Center combined genetically engineered organoids with mice grafting, and single-cell DNA sequencing (Figure 1).

In this assay, mouse urothelial cells were harvested and infected with a mixture of lentiviruses containing expression cassettes to mimic gain or loss of function of genes relevant to bladder cancer. Lentiviral cassettes also included a genetic barcode for downstream single-cell analysis. Infected cells averaged four

gene aberrations per cell, and were grafted into immunodeficient mice after having formed in vitro organoids. The utilization of organoids in this assay allowed for easy manipulation of cells ex vivo, and tumor formation after grafting into mice.

Organoids that displayed tumorigenicity in mice were then resected and DNA sequenced at the single-cell level using the Tapestri Platform. A custom amplicon panel was designed to sequence lentiviral barcodes in order to distinguish clones containing gain or loss of function cassettes. Using this method, combinations of co-occurring gene aberrations were identified in single cells, establishing clonal populations and giving insight into higher-order genetic interactions that lead to disease.

### Single-cell tumor analysis reveals four clonal populations with co-occurring genetic aberrations

As a proof-of-concept of the assay, and to develop a genetically defined model for squamous cell carcinoma of the bladder, genetically modified organoids were generated with combinations of lentiviral vectors expressing PPARG, PVRL4, YWHAZ, FGFR3 S243C, and PIK3CA E545K. These genes corresponded with lentiviral barcodes BC04, BC10, BC11, BC22, and BC34, respectively. The organoids were grafted into immunodeficient mice and allowed to grow into tumors. In one mouse that developed a tumor from the grafted organoid, the tumor was resected and then analyzed using the Tapestri Platform for single-cell DNA sequencing. 2,933 cells were analyzed from the tumor using custom amplicons for detection of the lentiviral barcodes.

Single-cell DNA sequencing revealed four distinct populations of cells within the heterogeneous tumor sample (Table 1). Each clonal population had several co-occurring genetic aberrations, with clones detectable under 2%. The most abundant clone had been modified with all five genes at over 90% of the tumor cell population. PPARG and YWHAZ expressing lentiviral vectors were found in all four clones, suggesting a critical role in bladder cancer formation.

LV Barcodes	PPARG	PVRL4	YWHAZ	FGFR3 S243C	PIK3CA E545K	Clonal Frequency
BC04, BC10, BC11, BC22, BC34	+	+	+	+	+	91.65%
BC04, BC10, BC11, BC34	+	+	+	-	+	3.92%
BC04, BC10, BC11, BC22	+	+	+	+	-	2.73%
BC04, BC11, BC22, BC34	+	-	+	+	+	1.70%

Table 1 - Single-cell tumor clonality analysis reveals four clonal populations, each with multiple co-occurring genetic aberrations, within a heterogeneous sample.

### a

LV Barcodes	PPARG	PVRL4	YWHAZ	FGFR3 S243C	PIK3CA E545K	Clonal Frequency
BC04, BC10, BC11, BC22, BC34	+	+	+	+	+	89.70%
BC04, BC10, BC11, BC34	+	+	+	-	+	8.70%
BC04, BC10, BC11, BC22	+	+	+	+	-	0.76%
BC04, BC11, BC22, BC34	+	-	+	+	+	0.75%

### b

LV Barcodes	PPARG	PVRL4	YWHAZ	FGFR3 S243C	PIK3CA E545K	Clonal Frequency
BC04, BC10, BC11, BC22, BC34	+	+	+	+	+	87.89%
BC04, BC10, BC11, BC34	+	+	+	-	+	6.38%
BC04, BC10, BC11, BC22	+	+	+	+	-	0.00%
BC04, BC11, BC22, BC34	+	-	+	+	+	5.73%

Table 2 - Single-cell tumor clonality analysis of tumors propagated in NSG mice, which are highly immunodeficient (a) and C57BL/6J mice, which are immunocompetent (b), showing loss of clone that lacks PIK3CA E545K expression. 10,282 and 3,666 cells were analyzed from NGS or C57BL/6J mice, respectively.

## Changes in heterogeneity of tumors observed in immunocompetent versus immunodeficient mice through single-cell DNA sequencing

The tumor that developed from initial lentiviral transduction of vectors expressing PPARG, PVRL4, YWHAZ, FGFR3 S243C, and PIK3CA E545K was

propagated in immunodeficient and immunocompetent mice in order to observe population changes based on immunity. Propagated tumors were then analyzed using the Tapestri Platform.

In immunodeficient mice, all four clones previously observed were still detectable (Table 2a). In contrast, only three clones were detectable in immunocompetent mice (Table 2b). The only clone that lacked expression of PIK3CA E545K was undetectable, suggesting a cell-intrinsic role of PIK3CA in activating mutations in tumor immune evasion.

Subclonal populations from these samples were detectable down to 0.75%, again illustrating extremely high sensitivity of single-cell DNA sequencing using the Tapestri Platform.

## Conclusion

The introduction of systematic genetic aberrations to model heterogeneous tumors mixed with single-cell DNA sequencing results in a next-generation functional genomics assay to deconvolute complex genotype-to-phenotype relationships in cancer.

Multiplexed lentiviral transduction of primary mouse urothelial cells enabled interrogation of higher-order genetic interactions in cancer initiation and progression. Single-cell DNA sequencing was able to resolve multiple clones with co-occurring genetic aberrations from a heterogeneous mouse tumor sample at extremely high sensitivity. Sequencing based on lentiviral barcodes enabled robust screening for loss or gain of function of targeted genes. Tumor comparison between tumors propagated in immunodeficient or immunocompetent mice using single-cell sequencing revealed a potential role for PIK3CA in immune evasion.

## References

- <https://www.ncbi.nlm.nih.gov/pubmed/28988769>