Applying NeXT Liquid Biopsy™, an exome-scale platform, to monitor and discover tumor variants in a broad set of cancer types

#6385

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Introduction

Circulating tumor cell-free DNA (ctDNA) has become a biomarker for prognosis and disease monitoring. However, studies typically utilize assays limited to a small set of genes that may miss biologically important and clinically actionable mutations. To address this limitation, we have developed a whole-exome scale cfDNA platform, NeXT Liquid Biopsy (NeXT LB), that enables sensitive detection and tracking of somatic mutations in plasma samples across ~20,000 genes. The NeXT LB platform monitors tumor variants and discovers novel mutations in the plasma, through analysis of tumor, normal and plasma samples from the same patient. The NeXT LB platform enables the identification of somatic variants in liquid biopsy samples, following interventions such as surgery and treatment therapies.

Methods

To enable sensitive detection across the exome in solid tumor and liquid biopsies, we developed an enhanced whole-exome assay and chemistry that augments challenging genomic regions to enable more uniform coverage across the exome. Additionally, we achieve a mean depth of coverage of ~2,000X across the exome, with boosted depth (~5,000X) for 247 clinically relevant oncogenic or tumor suppressor genes to further enhance sensitivity. We apply NeXT LB to sequence over 100 plasma samples at 250 gigabases (G) and their corresponding matched tumor and normal samples. Finally, we developed computational algorithms to sensitively monitor and discover somatic mutations in liquid biopsies without compromising specificity.

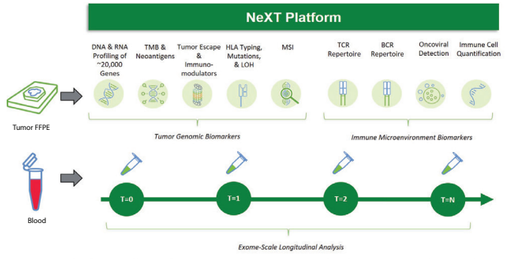

Figure 1: NeXT platform allows for comprehensive sample characterization at the solid tumor and cfDNA

Results – NeXT LB sensitivity

We validated with two reference standardsOur NeXT LB platform successfully and consistently detected all 25/25 (100%) known SNVs across each of the 2% and 1% dilution replicates, and detected 24/25 (96%) events in the majority of the 0.5% dilution Seracare sample replicates. Further, we achieved 100% sensitivity in each of the 5%, 2.5%, and 1% Horizon dilution samples. Additionally, the observed allele frequencies (AFs) were well correlated with the expected AFs in both the Seracare and Horizon samples.

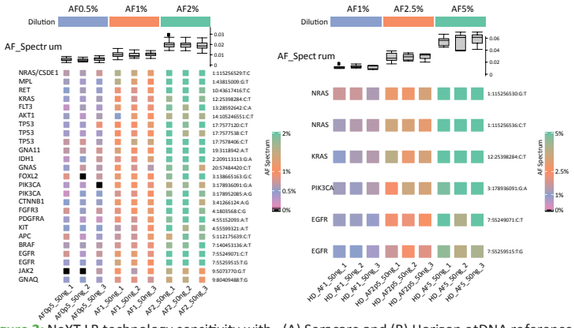

Figure 2: NeXT LB technology sensitivity with (A) Seracare and (B) Horizon ctDNA reference standards.

Using a combination of reference samples and a cell media system, we estimated the NeXT LB performance for boosted and coding regions. NeXT LB detects variants in two modes: variants can be monitored if they were observed in the solid tumor; or *De novo*. We find the monitor sensitivity is above 90% for variants with AF >= 0.5%. Conversely, we estimate a sensitivity above 90% for variants with AF >= 1% in boosted regions and AF >= 5% in CDS for *De novo* - variants not observed in the solid tumor.

Sensitivity				
Mutation Type	Analysis Type	Design Region	AF	Sensitivity - Measured
SNVs	*De novo*	Boosted regions	@0.5%	39%
			@1.0%	91%
			@2.0%	96%
		All CDS	≥0.5%	70%
			≥1.0%	74%
			≥2.0%	85%
			≥3.0%	88%
			≥5.0%	94%
			≥10.0%	100%
	Monitoring	Boosted regions	@0.5%	93%
			@1.0%	100%
			@2.0%	100%
		All CDS	≥0.5%	90%
			≥1.0%	94%
			≥2.0%	98%
			≥3.0%	99%
			≥5.0%	100%
			≥10.0%	100%

Table 1: NeXT LB performance for Single Nucleotide Variants (SNVs) across design regions and AF thresholds

We generate low-pass Whole Genome Sequencing (lpWGS) in order to estimate ctDNA fraction patients with bladder and head and neck tumors in conjunction with NeXT LB. As expected, we noted stronger monitor and *De novo* performance when the low-pass derived tumor fraction was larger than 3%, which is consistent with the stated performance of our platform.

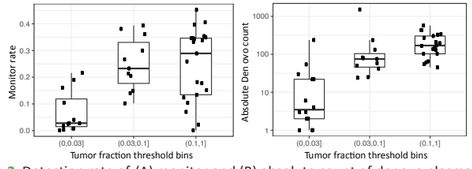

Figure 3: Detection rate of (A) monitor and (B) absolute count of denovo plasma variants across tumors fractions measured by lpWGS.

Results – Pan-cancer cohort application of NeXT LB

We run NeXT LB on nearly 150 plasma time points from over one hundred patients. We prioritize the characterization of circulating tumor DNA (ctDNA) deriving from tumors with high incidence and mortality in the general population.

Tissue	Number of patients	Number of plasma time points
Breast	16	16
Lung	15	15
Gastro intestinal	14	42
Head and Neck	14	28
Colorectal	12	12
Melanoma	9	9
Prostate	6	6
Kidney	5	5
Uterus	5	5
Thyroid	3	3
Ovary	3	3
Bladder	1	1

Table 2: Number of patients and plasma time points in the pan-cancer cohort

Collectively, we detect tumor somatic variants in plasma in over 1,000 distinct genes, thereby demonstrating the breadth and performance of our whole-exome scale liquid biopsy platform. Our platform not only identified ctDNA variants in driver genes characterized by commercial targeted panels, but greatly expanded the list of genes with identifiable mutations to a comprehensive list of driver and clinically relevant genes.

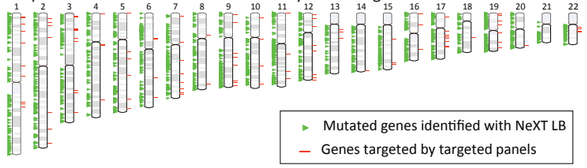

Figure 4: Distribution of plasma variants identified across a pan-cancer cohort in NeXT LB.

We further filter tumor, normal and plasma matched samples with stronger evidence of ctDNA shedding to evaluate the patterns of allele frequency and localization patterns of variants detected in plasma.

As expected, we find evidence that, compared to tumor variants absent in plasma, monitor variants tend to have higher allele frequencies in solid tumors. These results indicate that clonal variants are more likely to be observed in plasma, however, NeXT LB is also able to detect non-clonal variants in plasma.

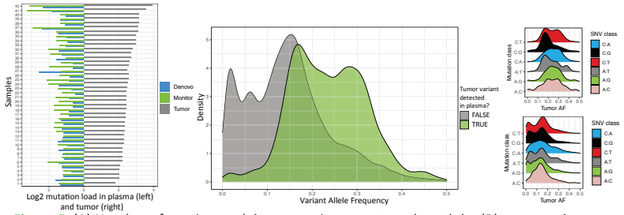

Figure 5: (A) Number of monitor and denovo variants per sample and the (B) comparative distribution of AF for variants detected in plasma. (C) Breakdown of AF by SNV class

Finally, we investigate the incidence of ctDNA variants overlapping driver genes for tumors with solid evidence of shedding. We identify most driver gene mutations present in the solid tumor with AF >= 20%. Variants in driver genes tended to overlap hot-spots; however, we also find variants in less studied regions. These results suggest that NeXT LB can be leveraged to identify common variants and explore and identify novel mutations using

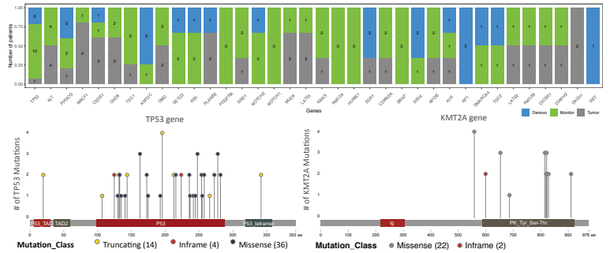

Figure 6: (A) Monitor and Denovo counts of variants in driver genes (B) Tumor variants detected in plasma coincide with mutation hotspots.

Conclusion

We developed an exome-scale NeXT LB technology that enables sensitive monitoring and detection of somatic SNVs and indels from cfDNA. The NeXT LB platform covers a much broader landscape of tumor mutations from the plasma than existing targeted platforms, thereby enabling broader monitoring and discovery of mutations related to therapies, mechanisms of resistance, intra- and inter-tumor heterogeneity, among others.

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