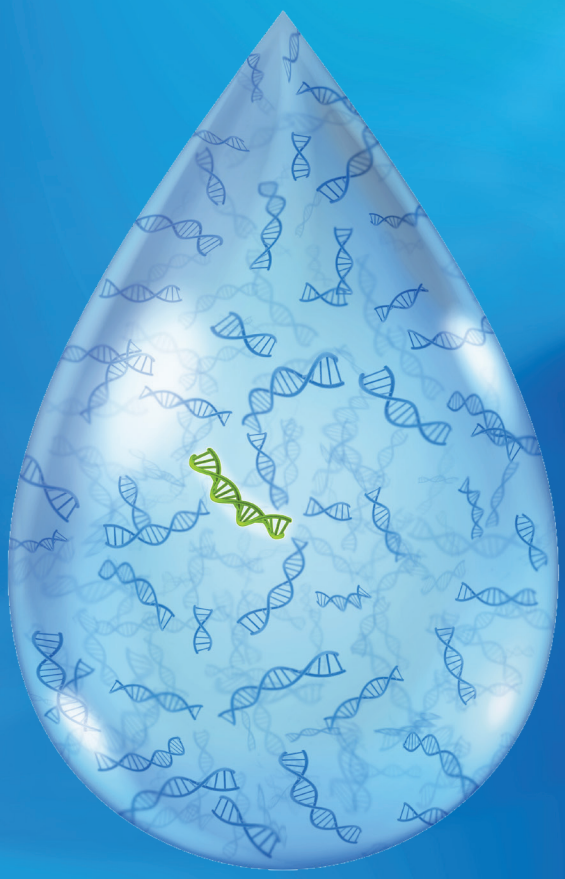


NeXT Personal™

The Next Generation in Molecular Residual Disease Testing and Variant Monitoring for Solid Tumors

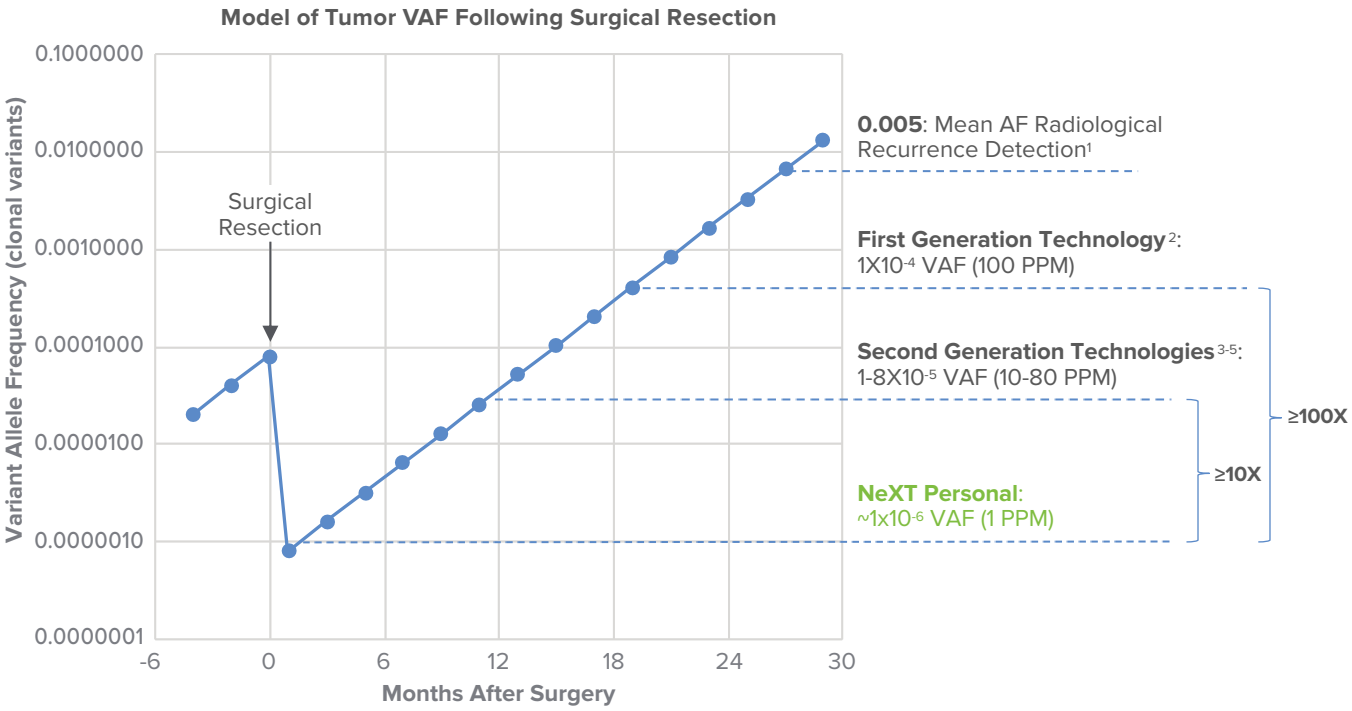
Personalis®

Personalis®

Delivering Industry-Leading Sensitivity to Detect Residual Disease at the Earliest Timepoints

NeXT Personal, an advanced, personalized, and tumor-informed liquid biopsy assay, is designed to detect molecular residual disease (MRD) and cancer recurrence at the earliest timepoints — prior to, during or after treatment — in patients previously diagnosed with cancer. While circulating tumor-derived DNA (ctDNA) is an emerging biomarker for many cancers, the limited sensitivity of current detection methods reduces its utility for diagnosing MRD across a variety of clinical applications. Standard-of-care (SOC) radiological-based technologies, including CT, PET and MRI scans, also remain limited in their ability to detect residual disease during or after surgical or systemic therapy due to the minimum tumor volume required.[1] Therefore reliable, sensitive detection and quantification of MRD remains a key challenge, particularly in early-stage cancers, where timely detection of small micrometastatic lesions may enable treatment that prevents progression to advanced metastatic, incurable disease. To address these challenges, NeXT Personal was developed to deliver industry-leading MRD sensitivity in the range of 1-3 parts per million (PPM) representing a 10-100X increase over other available methods (Figure 1), while requiring only a single tube of blood (4mL plasma/15ng cfDNA), and 1mm^3 of FFPE tumor tissue.

Figure 1: MRD Sensitivity: Targeting Detection at Earliest Timepoints



Model Assumptions: Median breast tumor size detected by mammography: 1.3cm, Median shedding per NSCLC TRACERx study[6]; Residual tumor from surgery: 1%; Volume doubling time (actual VAF time): 2 months

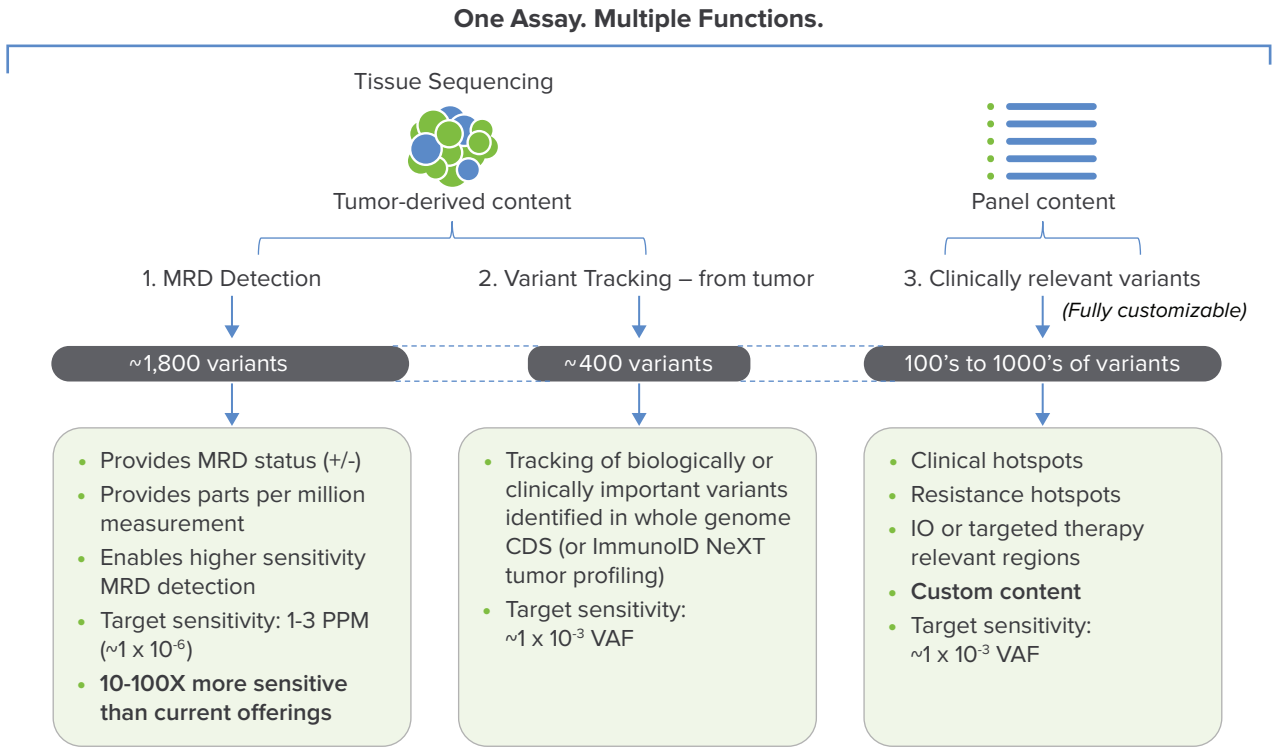
In the biopharma setting, MRD is rapidly emerging as a key biomarker in therapy development, whereby more sensitive detection and quantification of MRD may provide substantial benefits versus less sensitive methods through the reduction of false negative detection of cancer. These benefits include:

- More efficient cohort enrichment of study participants that are MRD positive.
- Faster time to surrogate endpoint analysis through earlier detection of MRD.
- Enhanced accuracy of recurrence and survival risk.
- Greater resolution of therapy response.

Unique Assay Design Provides Unprecedented Insights Into Longitudinal Disease Monitoring

While the MRD component of NeXT Personal provides a highly sensitive and aggregated measurement of tumor burden in plasma, the ability to simultaneously track individual variants longitudinally can also be utilized to further our understanding of tumor biology and its dynamic response to therapy. Therefore, in addition to MRD, NeXT Personal provides the ability to track and annotate individual variants over the whole evolution of a cancer patient's trajectory in a single panel design. Variants tracked by NeXT Personal in the blood are derived from those detected in the tissue, as well as those specified for panel inclusion by the user (Figure 2).

Figure 2: NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD & Variant Tracking



For every panel, NeXT Personal boasts the capacity to include up to 400 single nucleotide variants (SNVs) detected in the tumor to track in plasma. NeXT Personal automatically filters for variants that lie in coding regions of clinically relevant genes, prioritizing those in genes with known relevance to cancer. This ensures that the most biologically and clinically important variants are tracked longitudinally to deliver insights on evolving tumor biology (Figure 2.2).

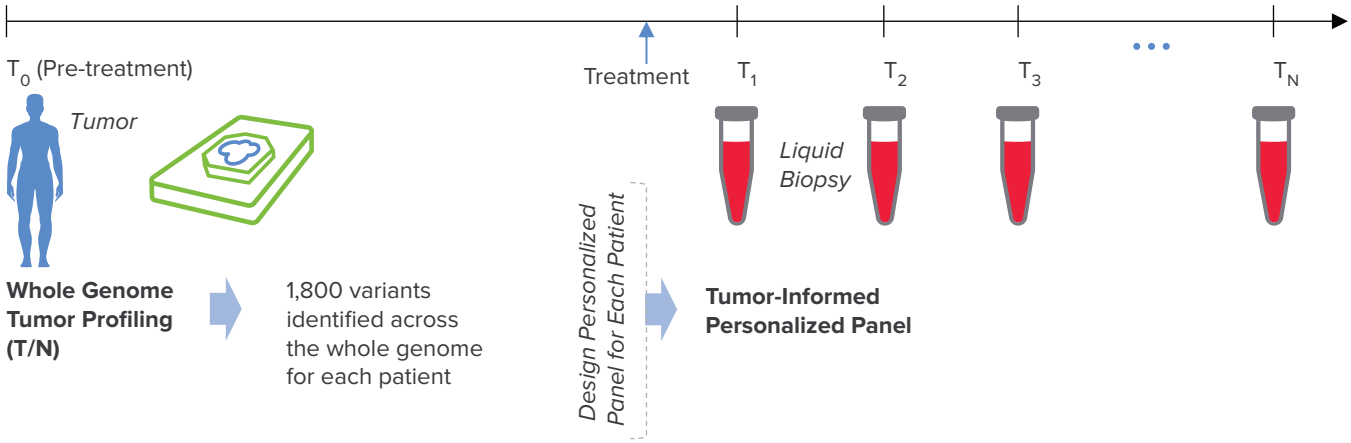
Similarly, variants not detected in the tumor may also be added during the panel design process. As custom content, users may define their own set of variants that may be representative of known clinical hotspots (ex. EGFR T790M), resistance hotspots, driver mutations (which may allow detection of a second primary tumor), or other therapy-specific and biologically important variants studied or monitored in clinical research. Capacity for user-supplied content can include up to thousands of variants. Alternatively, users may also leverage clinically relevant content developed and curated by Personalis in addition to, or instead of, user-supplied content (Figure 2.3).

Target sensitivity for individual variant detection is 0.1% VAF.

Leveraging Tissue-Based Whole Genome Sequencing (WGS) Yields Best-In-Class MRD Performance in Plasma: 1-3 PPM Sensitivity

Central to the MRD performance of NeXT Personal is the number of tumor-derived mutations interrogated in patient plasma samples. Analytical and clinical MRD sensitivity can be increased by expanding coverage of loci containing tumor-specific mutations.[7] To this end, NeXT Personal leverages the broad array of SNVs detected with >30x WGS coverage of a patient's tumor (and accompanying normal) to inform the selection of 1,800 SNVs for inclusion in a personalized MRD panel (Figure 3). The majority of these patient-specific SNVs are located in non-coding regions of the genome. Finally, Personalis' extensive investment into the Illumina® NovaSeq® series enables ultra-deep sequencing (~100,000 raw depth), while keeping costs in line with other MRD tests that interrogate many fewer loci.

Figure 3: NeXT Personal: Advanced, Higher Sensitivity, Personalized, Tumor-Informed Liquid Biopsy for MRD & Variant Tracking



Upon completion of sequencing with the tumor-informed personalized panel, data generated across these 1,800 targeted loci are analyzed and an aggregated measurement of tumor burden is reported in a PPM readout with accompanying MRD status (MRD+/-). Rather than using a predetermined minimum number of mutations to define MRD status, NeXT Personal uses advanced, proprietary statistical analyses to distinguish signal from noise for each individual patient panel and sample. Guided by a P-value threshold of <0.001 (derived from a specificity target of >99.9%), NeXT Personal is optimized for both sensitivity and specificity.

Assessing the prospective utility of individual MRD targets is an important aspect of tumor-informed panel design. Tissue WGS often yields tens of thousands of mutations, but selecting those that are most likely to be (1) shed into the blood and (2) have high signal-to-noise profiles is of critical importance. While the human genome harbors a plethora of inherently noisy regions, Personalis, leveraging its extensive experience with WGS, is the first company to develop methods to filter for targets that may be difficult to resolve if detected in plasma. Further, the abundance of variants identified via WGS allows the selection of predominately truncal mutations with high allele frequencies; those that are more likely to be shed into the blood.

The powerful combination of WGS to enable target breadth, coupled with robust error and noise reduction methods, results in MRD sensitivity in the 1-3 PPM range with ≥99.9% specificity (Figures 4 and 5).

Figure 4: Cell line dilution series, HCC38, demonstrates the linearity of tumor signal across the dilution series down to the 1-3PPM range (C50 and C95, respectively). Orthogonal confirmation using droplet digital PCR (ddPCR) demonstrates near-perfect concordance down to ddPCR LOD (750 PPM).

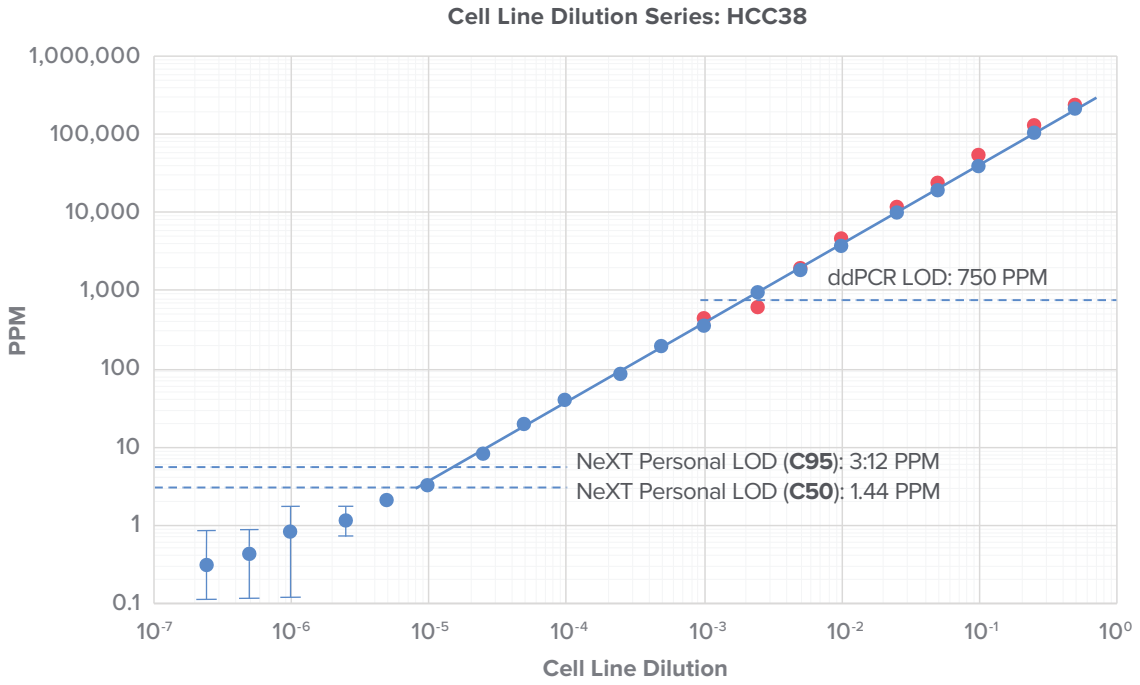
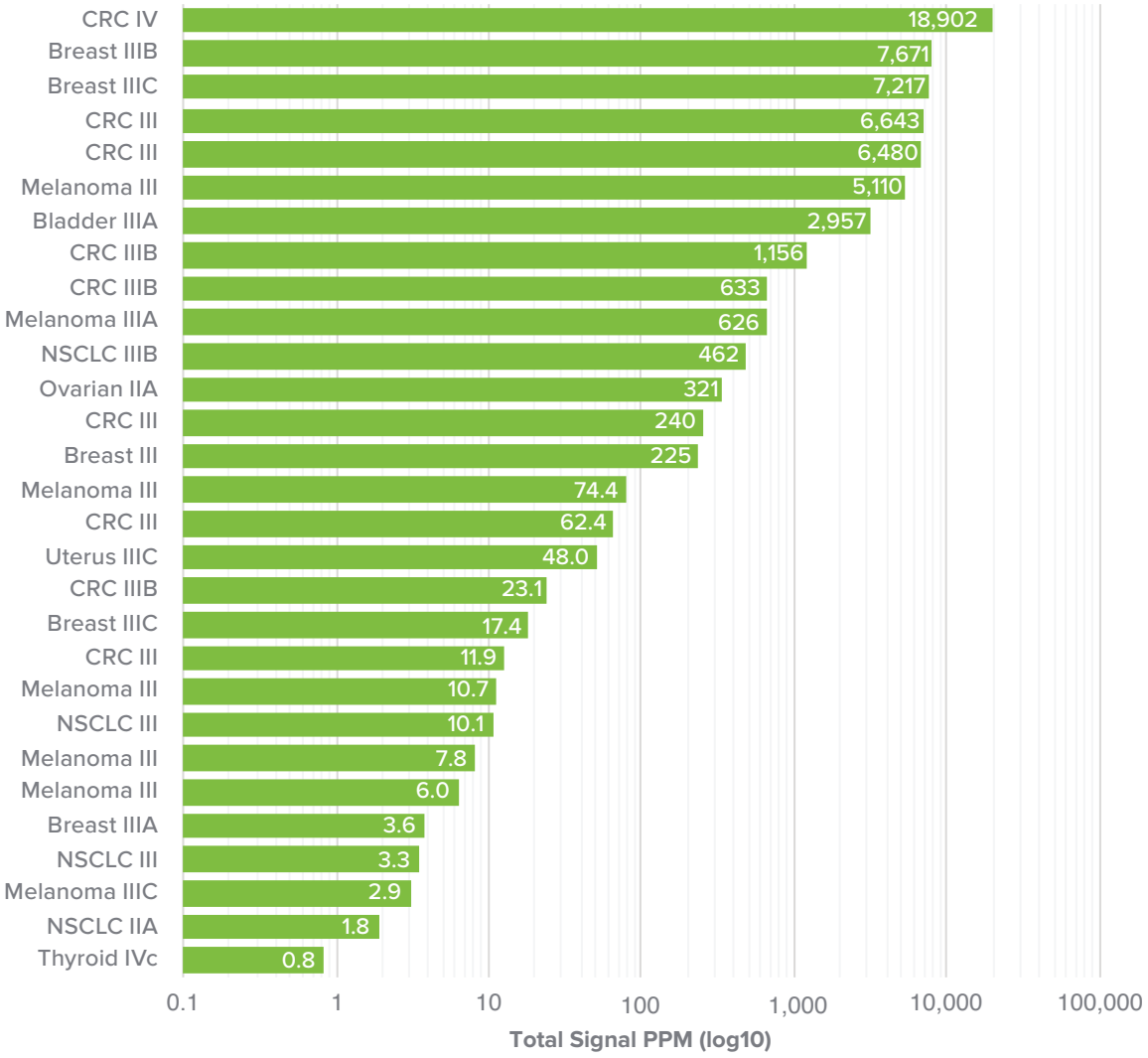


Figure 5: Patient T/N/P trios across 8 tumor types and 3 stages were sequenced demonstrating range of detection; 0.8 – ~19,000 PPM



Target Breadth Mitigates Challenging Sample Conditions

Target breadth is important to achieve higher sensitivity, but it is also important to overcome challenging sample conditions.The success of tumor-informed liquid biopsy approaches is dependent on the number of targeted mutant fragments present in the sample. Key variables that determine successful MRD detection are: (1) number of genomic equivalents (GEs) in the plasma sample, (2) tumor fraction in the plasma sample, and (3) tumor mutational burden (TMB) in the tissue sample. Under ideal sample conditions — characterized by a high number of GEs in plasma, a high tumor fraction in plasma, and a high TMB in tissue — narrower tumor-informed panel footprints (ex. those constrained by the tissue exome-seq) and fixed content panels may be sufficient to detect MRD.

However, ideal sample conditions are often not attainable:

- Tumor fraction in plasma in early-stage cancers is often low due to limited shedding of DNA molecules from small, localized lesions.[8]
- Plasma volume can be difficult to obtain due to patient variability, study design, and other blood analyte testing requirements.
- Finally, tissue TMB is highly variable and less dependent on tumor type and tumor stage[9], with low TMB tumors reducing the number of high-quality variant candidates for MRD panel inclusion, materially impacting assay performance.

NeXT Personal mitigates these challenges by ensuring high coverage (1,800 loci) of the tumor-derived DNA molecules, increasing the detection probability of mutant fragments even when there are few. Therefore, even in challenging sample conditions, NeXT Personal is able to deliver superior assay performance.

Combining ImmunoID NeXT™ and NeXT Personal Yields Multidimensional View Into Tumor Biology

In addition to NeXT Personal, Personalis' flagship tissue-based immunogenomics platform, ImmunoID NeXT, may also be run from the same tissue sample with no additional tumor material required. For the first time, investigators can comprehensively characterize both the tumor- and immune-related components of the tumor microenvironment while still retaining the ability to longitudinally monitor MRD and variants in the blood with industry-leading sensitivity, even in sample-limited scenarios.

Table 1

Key NeXT Personal Specifications		
General	Cancer Stage	All
	Cancer Type	Solid tumors
	cfDNA Input	15 ng
	Blood / Plasma Volume	10 mL (1 tube) / 4 mL
	Assay Type	Tumor-informed
	Front-End Tissue Sequencing	WGS
	Tissue Sequencing Coverage	>30X
	Plasma Sequencing	Targeted panel
	Plasma Sequencing Coverage	~100,000X (raw)
	Assay Multifunctionality	1. MRD 2. Tumor variant tracking 3. Customer-specified variant tracking
MRD	# MRD targets	1,800
	Analytical Sensitivity	1-3 PPM
	Analytical Specificity	≥99.9%
Tumor Variant Tracking	Variant capacity	400
	Source of variants	Coding regions only
	Analytical Sensitivity	0.1% VAF
Panel Variant Tracking	Variant capacity	1,000's
	Source of variants	Custom / Customer specified
	Analytical Sensitivity	0.1% VAF

Personalis, Inc.

Unique Features of NeXT Personal

Deep Sequencing

~100,000X raw coverage for plasma is performed across 1,800 loci, enabling sequencing dollars to be directed to variants of highest value. >30X WGS coverage for tumor and normal tissue is performed to detect SNVs for targeted panel inclusion.

Industry Leading MRD Performance

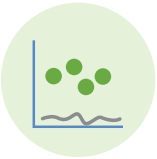
1-3 PPM MRD LOD is 10-100X more analytically sensitive than other MRD solutions on the market, aiding the detection of MRD and recurrence at the earliest timepoints. ≥99.9% specificity minimizes the rate of false positive results.

Assay Multifunctionality

NeXT Personal is not just an MRD solution: In addition to MRD, NeXT Personal tracks and annotates individual variants — those that are detected in the tumor, and those specified for panel inclusion by the user — to advance knowledge of tumor biology and observe emergence of known clinical and resistance hotspots.

Low Input Sample Requirements

NeXT Personal requires only 15ng of cfDNA, or 1 tube of blood (4mL of plasma), to deliver exceptional performance. Additionally, Personalis' protocols enable flexible sample formats — blood, plasma, and cfDNA — to be processed in the lab. Additionally, as little as 1mm^3 of FFPE tumor tissue is required for WGS.



Advanced Error Suppression

Robust, proprietary error suppression techniques reduce the error rate from sequencing, PCR and other sample processing steps.

Panel Identification

Included in every NeXT Personal design, Personalis deploys a SNP-based quality control measure to ensure a patient's panel is never mixed with another patient's sample.

Personalis-Provided Panel Content

Personalis provides the option to include a carefully curated list of clinically actionable and resistance hotspots in addition to or in the absence of panel content specified by the user.

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