NeXT Dx™

Personalis NeXT Dx™ Test

The Personalis NeXT Dx Test provides clinicians with a comprehensive and accurate next- generation sequencing (NGS) based testing solution for solid tumors, to help guide and optimize therapeutic options for patients including enrolling into cancer clinical trials.

The NeXT Dx test is a tumor-normal comprehensive genomic tumor profiling test based on whole exome and transcriptome sequencing. It is intended for profiling a solid tumor's genomic and molecular characteristics using NGS technology to report small nucleotide variants (SNVs), small insertions and deletions (indels), copy number alterations (CNAs), fusions, microsatellite instability (MSI) and exome-wide tumor mutational burden (TMB). Pathogenic and likely pathogenic germline mutations detected in certain cancer-related genes are also reported as incidental findings from sequencing of the matched normal sample. In addition, mutations in genes involved in homologous recombination repair (HRR) are highlighted when identified.

Clinical Report

The Personalis NeXT Dx Test is analytically validated for the detection of SNVs, indels, CNAs, and fusions in ~20,000 genes and MSI and TMB reporting.

- A clinical report includes SNVs, indels and CNAs detected in 401 cancer-related genes, fusions in 284 genes, MSI from 117 loci, exome-wide TMB results based on tumor-normal analysis, and germline small variants (SNVs and indels) identified from 59 cancer-related genes using matched normal tissue (**Figure 1**).
- Based on the tumor's molecular profile, the report delivers relevant therapy recommendations and a custom-generated list of clinical trial matches, including sponsor contact details for trial enrollment.
- We also report clinically important genes, specific to the patient's tumor type, in which no variants were detected.
- We use our Future-Driven™ Personalis NeXT Platform® to provide high accuracy, clinical-grade next generation sequencing and analysis. Each case is reviewed by a team of board-certified molecular geneticists and genetic counselors.
- Test results are provided to clinicians by fax or secure email, and can be accessed via the Personalis Clinical Portal.

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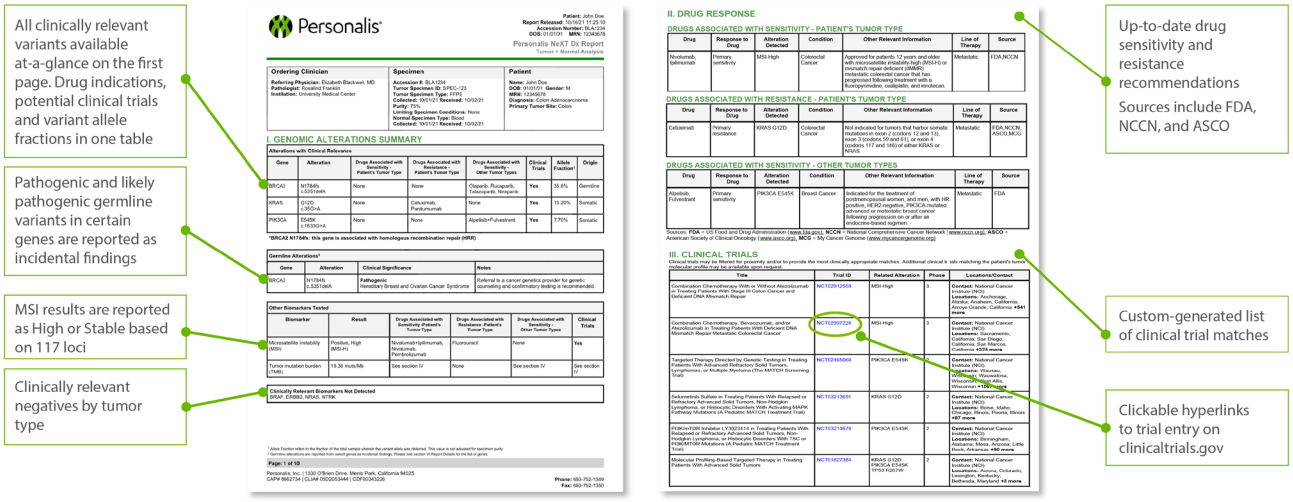


Figure 1: Clinical report example

What Makes Our Test Unique

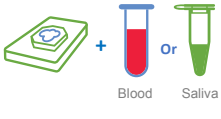
The Personalis NeXT Dx Test goes beyond typical cancer genomics tests in a few key areas:

1. Sensitive, RNA-Based Fusion Detection

Novel and known fusion detection using RNA sequencing[1] with deeper coverage compared to other transcriptome tests.

2. Gold-Standard Tumor Mutational Burden

Optimized exome-wide TMB assessment based on matched tumor-normal approach, the gold standard established by the Friends of Cancer Research[3].



3. Personalized Tumor-Normal Approach

More accurately identifies somatic alterations with fewer false positives than a tumor-only approach, while also identifying incidental, cancer-related germline variants*.

**Requires confirmatory germline testing.*

4. Future-Driven™ Platform

Comprehensive whole exome and whole transcriptome analytically validated to enable faster integration of new clinically actionable biomarkers as the field of oncology evolves.

5. Leading Technology

Proprietary ACE (Accuracy and Content Enhanced) Technology® delivers augmented coverage of ~20,000 genes including difficult-to-sequence regions[2] missed by conventional methods.

Test Performance Specifications

Sensitivity	
Single Nucleotide Variants (at mutant allele frequency ≥5%)	>99%
Small Insertions and Deletions (at mutant allele frequency ≥5%)	>98%
Copy Number Alterations (at ≥30% tumor content)	98%
Gene Fusions	96%
Positive Predictive Value*	
Single Nucleotide Variants (at mutant allele frequency ≥5%)	>99%
Small Insertions and Deletions (at mutant allele frequency ≥5%)	>99%
Copy Number Alterations (at ≥30% tumor content)	>99%
Gene Fusions	>98%
Additional Assay Specifications	
Microsatellite Instability (MSI)**	>99% accuracy
Tumor Mutation Burden (TMB)+	Calculated from whole exome by measuring the number of mutations per megabase (mut/Mb) is reported
Type of Sequencing	DNA and RNA sequencing using whole exome and transcriptome sequencing
Typical Median Depth Whole exome (Tumor) Boosted region (247 cancer-related genes) (Tumor) Whole exome (blood or saliva as matched normal samples) Whole Transcriptome (Tumor)	 ~500x >1500x ~150x 200M total reads
Sample Types++	Tumor: FFPE Normal: blood or saliva
Regions Analyzed	Coding and relevant non-coding regions of 401 genes
Turn Around Time	About 2 weeks from sample receipt
Test Requisition Form Required	Yes

*Positive predictive value is calculated by comparing variants detected by the NeXT Dx Test to those detected by a validated TSO500 test.

**MSI status is determined by measuring nucleotide repeats from 117 loci.

+TMB is reported as the number of mutations per megabase (muts/Mb) from tumor-normal analysis. Please note that there is currently no standard cut-off to define a high TMB for different tumor types.

++Decalcified bone is not an acceptable specimen. Additionally, specimens collected in New York State are not acceptable at this time.

NeXT Dx Reportable Gene List

Single nucleotide variants, small insertions and deletions, copy number alterations, and gene fusions involving the genes below may be reported in the test.

ABCB1†	*CAMTA1*†	*CSF1R*†	*EWSR1*†	*GATA1*†	*LIG4*	*MUTYH*†	*PDGFA*	*PTPN11*†	*SDHA**	*TMPRSS2*†
ABL1†	*CBFB*†	*CSF3R*†	*EXO1*	*GATA2**†	*LRP1B*	*MYC*†	*PDGFB*†	*PVRL4*†	*SDHAF2**	*TNFRSF4*†
AKAP9†	*CBL*†	*CTAG2*†	*EZH2*†	*GEN1*	*MAGEA3*†	*MYCL*	*PDGFRA**†	*RAD21*†	*SDHB**†	*TNFRSF8*†
AKT1†	*CCNA1*	*CTDNEP1*	*EZHIP*†	*GLI2*†	*MAGEA4*†	*MYCN*†	*PDGFRB*†	*RAD50**†	*SDHC**†	*TNFRSF10B*
AKT2†	*CCNA2*	*CTLA4*†	*FAM175A*	*GNA11*†	*MAML1*	*MYD88*†	*PGR*†	*RAD51*†	*SDHD**†	*TP53**†
AKT3†	*CCNB1*	*CTNNA1*	*FAN1*	*GNAQ*†	*MAP2K1*†	*MYH11*†	*PHF1*†	*RAD51B*†	*SETBP1*†	*TSC1**†
*ALK**†	*CCNB2*	*CTNNA2*	*FANCA*†	*GNAS*†	*MAP2K2*†	*MYOD1*†	*PIK3CA**†	*RAD51C**†	*SETD2*	*TSC2**†
*APC**†	*CCNB3*†	*CTNNA3*	*FANCB*†	*GPNMB*†	*MAP2K4*†	*NAB2*†	*PIK3CB*†	*RAD51D**†	*SF3B1*†	*TYRO3*
APOBEC3B	*CCND1*†	*CTNNB1*†	*FANCC*†	*H3F3A*	*MAP3K1*†	*NBN*	*PIK3CD*†	*RAD52*	*SHFM1*	*U2AF1*†
AR†	*CCND2*†	*CUX1*†	*FANCD2*†	*HDAC1*	*MAPK1*†	*NCSTN*	*PIK3CG*†	*RAD54B*	*SHH*†	*USH2A*
ARAF†	*CCND3*†	*DDR2*†	*FANCE*†	*HDAC2*	*MAPK11*	*NF1**†	*PIK3R1*†	*RAD54L*	*SLX4*†	*VEGFA*†
AREG†	*CCNE1*†	*DDX3X*	*FANCF*†	*HEY1*†	*MAPK3*	*NF2**†	*PIK3R2*	*RAF1*†	*SMAD4**†	*VEGFB*†
ARID1A†	*CCNE2*	*DEK*†	*FANCG*†	*HNF1A*†	*MAX**	*NFE2L2*†	*PML*†	*RARA*†	*SMARCA4*†	*VEGFC*
ARID1B	*CD274*†	*DKK1*†	*FANCI*†	*HRAS*†	*MBTD1*†	*NKX2-1*†	*PMS1*	*RB1**†	*SMARCB1*†	*VGLL2*†
ARID2	*CD276*†	*DLL3*†	*FANCL*†	*HSP90AA1*†	*MCL1*†	*NOTCH1*†	*PMS2**†	*RBBP8*	*SMC1A*†	*VHL**†
ASXL1†	*CD40*†	*DLL4*	*FANCM*†	*IDH1*†	*MCPH1*	*NOTCH2*†	*POLD1**	*RBM15*†	*SMC3*†	*WEE1*†
*ATM**†	*CDH1**†	*DNMT3A*†	*FBXW7*†	*IDH2*†	*MDC1*	*NOTCH3*†	*POLD2*	*RECQL4*	*SMO*†	*WRN*
ATR†	*CDH3*†	*DOT1L*	*FCER2*†	*IGF1R*†	*MDM2*†	*NOTCH4*	*POLE**†	*RELA*†	*SRC*†	*WT1**†
ATRX†	*CDK1*	*EED*	*FGF2*†	*IKBKE*	*MDM4*†	*NPAP1*	*POLQ*	*RET**†	*SRSF2*†	*WWTR1*†
AURKA†	*CDK2*	*EGFR**†	*FGF4*	*IKZF1*†	*MECOM*†	*NPM1*†	*PPM1D*	*RFC1*	*SS18*†	*XPO1*†
AXL†	*CDK4**†	*EIF1AX*	*FGF19*†	*IL2RA*†	*MEN1**†	*NR4A3*†	*PPP2R1A*	*RFC2*	*SSBP1*	*XRCC1*†
*BAP1**†	*CDK6*†	*EML4*†	*FGFR1*†	*JAG1*	*MERTK*	*NRAS*†	*PPP2R2A*	*RFC3*	*STAG2*†	*XRCC2*
*BARD1**	*CDK9*†	*EP300*†	*FGFR2*†	*JAK1*†	*MET**†	*NRG1*†	*PRAME*†	*RFC4*	*STAT3*†	*XRCC3*
BCL2†	*CDK12*	*EPCAM*†	*FGFR3*†	*JAK2*†	*MGAM*	*NTRK1*†	*PRKACA*†	*RFC5*	*STAT5B*†	*XRCC4*
BCL6†	*CDKN1A*†	*EPHA2*	*FGFR4*†	*JAK3*†	*MKL1*†	*NTRK2*†	*PRKCA*†	*RHEB*	*STAT6*†	*XRCC5*
BCOR†	*CDKN1B**†	*ERBB2*†	*FH**†	*KDM5C*	*MLH1**†	*NTRK3*†	*PRKCB*†	*RICTOR*†	*STK11**†	*XRCC6*
BCORL1†	*CDKN2A**†	*ERBB3*†	*FIGF*	*KDM6A*†	*MLH3*	*NUP214*†	*PRKCD*†	*ROS1*†	*SUFU**	*YAP1*†
BCR†	*CDKN2B*†	*ERBB4*†	*FLCN**†	*KDR*†	*MLLT3*†	*NUTM2A*†	*PRKCE*†	*RPA1*	*SULT1A1*†	*YES1*†
BLM	*CDKN2C*	*ERCC1*	*FLT1*†	*KEAP1*	*MPL*†	*OTX2*	*PRKCG*†	*RPA2*	*SUZ12*†	*YWHAE*†
BRAF†	*CEBPA**†	*ERCC2*	*FLT3*†	*KIT**†	*MRE11A**†	*PALB2**†	*PRKCI*†	*RPA3*	*SYK*†	*ZMYM3*
*BRCA1**†	*CHEK1*†	*ERCC3*	*FLT4*†	*KLB*†	*MS4A1*†	*PARP1*†	*PRKCQ*†	*RPA4*	*TEK*	*ZRSR2*†
*BRCA2**†	*CHEK2**†	*ERCC4*	*FOLR1*†	*KMT2A*†	*MSH2**†	*PARP2*	*PRKCZ*†	*RPN1*†	*TERT**†	
BRD4†	*CIC*†	*ERCC5*	*FOXL2*†	*KMT2C*	*MSH3*	*PAX3*†	*PRKDC*	*RPTOR*	*TET2*†	
*BRIP1**†	*CREBBP*†	*ERCC6*	*FOXO1*†	*KMT2D*	*MSH6**†	*PBRM1*	*PSCA*†	*RTEL1**	*TFE3*†	
BTK†	*CRKL*†	*ESR1*†	*FRK*	*KRAS*†	*MSLN*†	*PCNA*	*PTCH1**†	*RUNX1**†	*TGFBR1*†	
C11orf30	*CRLF2*†	*ESR2*†	*FUS*†	*LAG3*†	*MST1R*	*PDCD1*†	*PTEN**†	*RUNX1T1*†	*TGFBR2*†	
CALR†	*CRTC1*†	*ETV6**†	*FYN*†	*LIG3*	*MTOR*†	*PDCD1LG2*†	*PTK2*†	*RYR1*	*TMEM127**	

* Represents genes in which likely pathogenic/pathogenic germline variants will be reported, in addition to somatic variants, as incidental findings

† Represents fusion genes

References

1. Solomon, J.P. et al. NTRK fusion detection across multiple assays and 33,997 cases: diagnostic implications and pitfalls. *Mod Pathol 33*, 38-46 (2020).
2. Patwardhan, A. et al. Achieving high-sensitivity for clinical applications using augmented exome sequencing. *Genome Med 7*, 71 (2015).
3. Vega, D.M. et al. Aligning tumor mutational burden (TMB) quantification across diagnostic platforms: phase II of the Friends of Cancer Research TMB Harmonization Project. *Ann Oncol 32*, 1626-1636 (2021).

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Personalis NeXT Dx™ Test: This laboratory developed test (LDT) will be performed in a CLIA/CAP accredited laboratory. The test was developed and its performance characteristics determined by the Personalis Clinical Laboratory. It has not been cleared or approved by the United States Food and Drug Administration (FDA). The Personalis Clinical Laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing.

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