NanOnco Plus Panel v3.0

Background

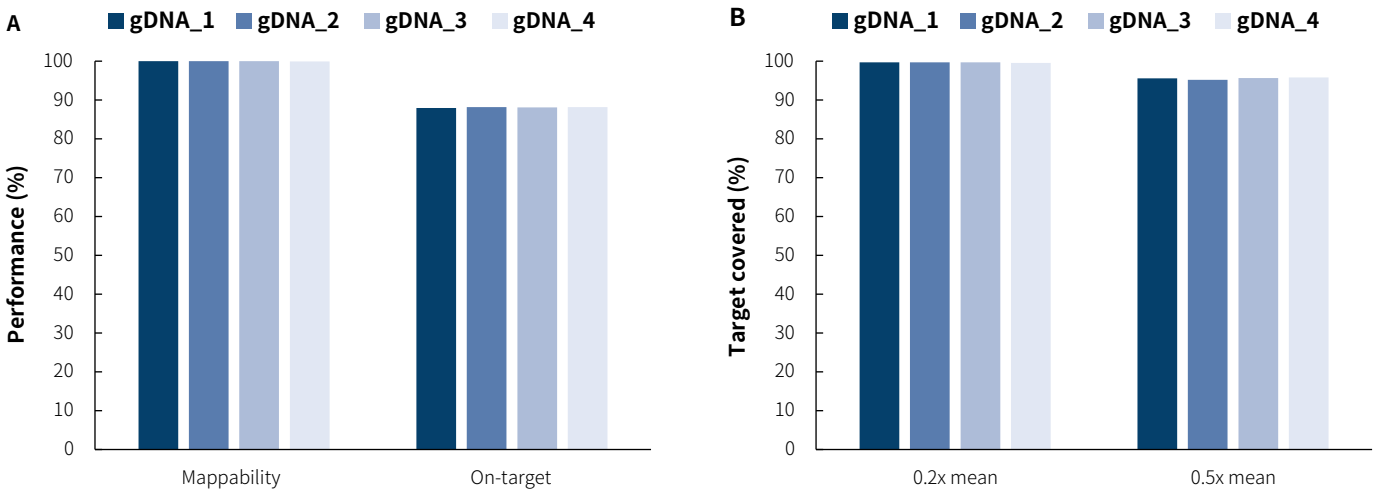
Large pan-cancer panels usually contain hundreds of important tumor-associated genes covering about 1–3 Mb of target regions. If the target region is small enough, it is easy to achieve high sequencing depth, which is beneficial for somatic variant analysis; if the target region is large enough, it can support tumor mutation burden and more stable microsatellite instability assessment; in addition, through optimized combination of different types of target regions, multiple variants information such as SNV, Indel, fusion and CNV can be efficiently obtained simultaneously. After years of development, large pan-cancer panels with target enrichment for NGS has become an important part of targeted tumor therapy and immunotherapy. NanOnco Plus Panel, an excellent large pan-cancer panel, has also become one of the star products of Nanodigmbio. In 2021, Nanodigmbio has upgraded its probe design solution. Based on this design concept, the expanded NanOnco Plus Panel v3.0 has significantly improved sequencing data utilization without increasing the probe coverage area.

Introduction

NanOnco Plus Panel v3.0 targets the full coding regions from 620 genes (HLA genes included) of interest in solid tumor studies, a series of intron regions related to common fusions, classical microsatellite sequences, and chemotherapy-associated polymorphic loci. This panel involves a total number of 637 genes, covering an approximate 2.4 Mb target region of the genome. It supports the enrichment of multiple variants including base substitution, insertion/deletion, gene rearrangement, gene amplification, microsatellite instability and so on.

Performance

Capture performance



Continued

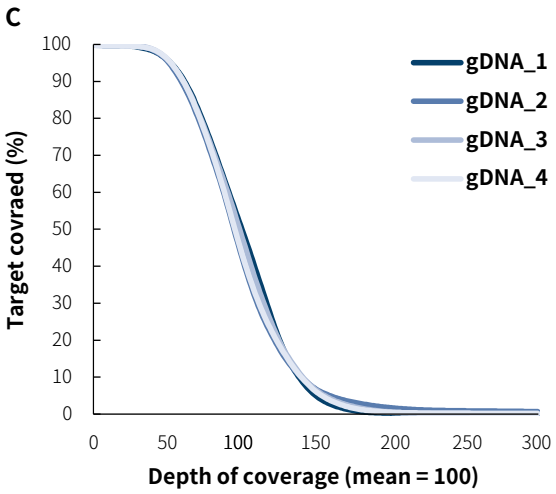


Fig 1. Capture performance of NanOnco Plus Panel v3.0 in different gDNA libraries. Libraries were prepared using NadPrep EZ DNA Library Preparation Kit coupled with NadPrep UDI Adapter (for Illumina®), and sequenced on Illumina Novaseq 6000 with PE150. The BWA was used for alignment to the reference genome hg38 and on-target rate was calculated by the number of reads. **A.** Mappability and on-target rate; **B.** Target covered; **C.** Coverage uniformity and consistency.

Note: The gDNA_1-4 samples were: Human Male Genomic DNA (Promega-male, G1471); Onco Structural Multplex 5% gDNA (Genewell, GW-OGTM001); Onco SNV Multiplex 1–25% gDNA (Genewell, GW-OGTM004); Onco SNV Wildtype gDNA (Genewell, GW-OGTM005), respectively.

Multiple variant analysis

Gene	Variant	Expected Alllele Frequency (%)	Reference observed (%)
NRAS	Q61K	0.46	0.44
NRAS	G12D	2.28	2.39
KRAS	Q61H	5.20	4.95
KRAS	G12D	8.16	8.33
ERBB2	A775-G776insYVMA	0.83	0.74
PIK3CA	E545K	2.56	2.37
PIK3CA	H1047R	7.68	7.47
EGFR	ΔE746-A750	0.99	0.55
EGFR	T790M	13.86	14.04
EGFR	L858R	13.69	12.78

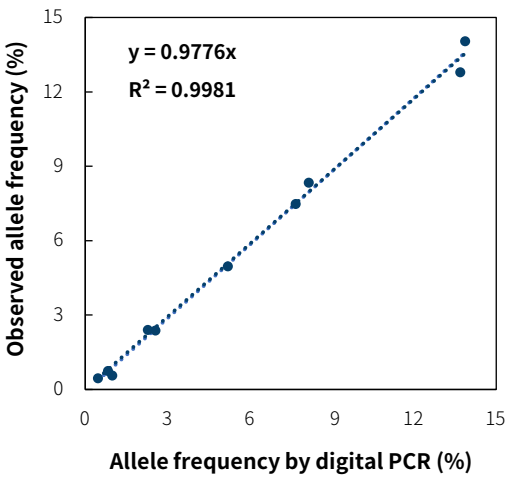


Fig 2. Consistency of the allele frequencies in NanOnco Plus Panel v3.0 capture data with the nominal frequencies of the standards. Libraries were prepared using NadPrep EZ DNA Library Preparation Kit coupled with NadPrep Universal Stubby Adapter (UDI), and sequenced on Illumina Novaseq 6000 with PE150. Variant analysis were performed using Vardict.

Note: Samples were Onco Multiplex gDNA (LDTBIO, custom standards) with an average sequencing depth of 3,518x.

Table 1. Detection of other variant types using NanOnco Plus Panel v3.0.

Type	Variant	Expected allele frequency (%) / copy number	Reference observed (%)	Wild type observed (%)
SNV	AKT1 E17K	5	5.25	0.0
SNV	PIK3CA E545K	5	5.77	0.0
Insertion	EGFR V769_D770insASV	5	4.10	0.0
Deletion	EGFR ΔE746_A750	5	4.42	0.0
Fusion	CD74-ROS1 Fusion	5	5.91	0.0
Fusion	EML4-ALK Fusion	5	3.29	0.0
CNV	MET Amplification	3.5 copies	3.4 copies	2 copies
CNV	ERBB2 Amplification	7.0 copies	8.3 copies	2 copies

Libraries were prepared using NadPrep EZ DNA Library Prepation Kit coupled with NadPrep UDI Adapter (for Illumina®), and sequenced on Illumina Novaseq 6000 with PE150. Variant analysis were performed using Vardict, Delly and CNVkit. The average sequencing depth of GW-OGTM001 and GW-OGTM005 is 966.71x and 1,126.04x, respectively.

Note: The sample was Onco Structural Multplex 5% gDNA (GeneWell, GW-OGTM001); the reference was Onco SNV Wildtype gDNA (GeneWell, GW-OGTM005).

Gene List

Genes with full coding regions covered

ABL1	*ABL2*	*ACVR1*	*ACVR1B*	*AGO2*	*AJUBA*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*ALOX12B*	*AMER1*	*ANKRD11*	*APC*	*AR*	*ARAF*
ARFRP1	*ARHGAP35*	*ARID1A*	*ARID1B*	*ARID2*	*ARID5B*	*ASXL1*	*ASXL2*	*ATM*	*ATR*	*ATRX*	*AURKA*	*AURKB*	*AXIN1*	*AXIN2*	*AXL*
B2M	*BABAM1*	*BACH1*	*BAP1*	*BARD1*	*BBC3*	*BCL10*	*BCL2*	*BCL2L1*	*BCL2L11*	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BIRC3*	*BLM*
BMPR1A	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*	*BTK*	*CALR*	*CARD11*	*CARM1*	*CASP8*	*CBFB*	*CBL*	*CCND1*
CCND2	*CCND3*	*CCNE1*	*CD22*	*CD274*	*CD276*	*CD70*	*CD74*	*CD79A*	*CD79B*	*CDC42*	*CDC73*	*CDH1*	*CDK12*	*CDK4*	*CDK6*
CDK8	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*	*CEBPA*	*CENPA*	*CFTR*	*CHD2*	*CHD4*	*CHEK1*	*CHEK2*	*CIC*	*COL4A6*	*CREBBP*
CRKL	*CRLF2*	*CSDE1*	*CSF1*	*CSF1R*	*CSF3R*	*CTCF*	*CTLA4*	*CTNNA1*	*CTNNB1*	*CTSO*	*CUL3*	*CUL4A*	*CXCR4*	*CYLD*	*CYP17A1*
CYP2D6	*CYSLTR2*	*DAXX*	*DCUN1D1*	*DDR1*	*DDR2*	*DDX3X*	*DICER1*	*DIS3*	*DNAJB1*	*DNMT1*	*DNMT3A*	*DNMT3B*	*DOT1L*	*DROSHA*	*DUSP4*
E2F3	*EED*	*EGFL7*	*EGFR*	*EIF1AX*	*EIF4A2*	*EIF4E*	*ELF3*	*EME1*	*EMSY*	*EP300*	*EPAS1*	*EPCAM*	*EPHA3*	*EPHA5*	*EPHA7*
EPHB1	*EPHB4*	*ERBB2*	*ERBB3*	*ERBB4*	*ERCC1*	*ERCC2*	*ERCC3*	*ERCC4*	*ERCC5*	*ERF*	*ERG*	*ERRFI1*	*ESR1*	*ETV1*	*ETV6*
EWSR1	*EXO1*	*EZH1*	*EZH2*	*EZR*	*FAAP20*	*FAM175A*	*FAM46C*	*FAM58A*	*FANCA*	*FANCC*	*FANCD2*	*FANCE*	*FANCF*	*FANCG*	*FANCI*
FANCL	*FANCM*	*FAS*	*FAT1*	*FAT2*	*FAT4*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGFR1*
FGFR2	*FGFR3*	*FGFR4*	*FH*	*FLCN*	*FLT1*	*FLT3*	*FLT4*	*FOXA1*	*FOXL2*	*FOXO1*	*FOXP1*	*FRS2*	*FUBP1*	*FYN*	*GABRA6*
GALNT12	*GATA1*	*GATA2*	*GATA3*	*GATA4*	*GATA6*	*GEN1*	*GID4*	*GLI1*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GPR124*	*GPS2*	*GREM1*
GRIN2A	*GRM3*	*GSK3B*	*GTF2I*	*H3F3A*	*H3F3B*	*H3F3C*	*HDAC1*	*HDAC2*	*HFM1*	*HGF*	*HIST1H1C*	*HIST1H2BD*	*HIST1H3A*	*HIST1H3B*	*HIST1H3C*
HIST1H3D	*HIST1H3E*	*HIST1H3F*	*HIST1H3G*	*HIST1H3H*	*HIST1H3I*	*HIST1H3J*	*HIST2H3C*	*HIST2H3D*	*HIST3H3*	*HLA-A*	*HLA-B*	*HLA-C*	*HLA-DMA*	*HLA-DMB*	*HLA-DOA*
HLA-DOB	*HLA-DPA1*	*HLA-DPB1*	*HLA-DQA1*	*HLA-DQA2*	*HLA-DQB1*	*HLA-DQB2*	*HLA-DRA*	*HLA-DRB1*	*HLA-DRB3*	*HLA-DRB4*	*HLA-DRB5*	*HMGA2*	*HNF1A*	*HOXB13*	*HRAS*
HSD3B1	*HSP90AA1*	*ICOSLG*	*ID3*	*IDH1*	*IDH2*	*IFNGR1*	*IGF1*	*IGF1R*	*IGF2*	*IKBKE*	*IKZF1*	*IL10*	*IL7R*	*INHA*	*INHBA*
INPP4A	*INPP4B*	*INPPL1*	*INSR*	*IRF2*	*IRF4*	*IRS1*	*IRS2*	*JAK1*	*JAK2*	*JAK3*	*JUN*	*KAT6A*	*KBTBD4*	*KDM5A*	*KDM5C*
KDM6A	*KDR*	*KEAP1*	*KEL*	*KIT*	*KLF4*	*KLHL6*	*KMT2A*	*KMT2B*	*KMT2C*	*KMT2D*	*KNSTRN*	*KRAS*	*LATS1*	*LATS2*	*LMO1*
LRP1B	*LTK*	*LYN*	*LZTR1*	*MAF*	*MAGI2*	*MALT1*	*MAP2K1*	*MAP2K2*	*MAP2K4*	*MAP3K1*	*MAP3K13*	*MAP3K14*	*MAPK1*	*MAPK3*	*MAPKAP1*
MAX	*MC1R*	*MCL1*	*MDC1*	*MDM2*	*MDM4*	*MECOM*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	*MET*	*MGA*	*MITF*	*MKNK1*	*MLH1*
MLH3	*MPL*	*MRE11*	*MSH2*	*MSH3*	*MSH6*	*MSI1*	*MSI2*	*MST1*	*MST1R*	*MTAP*	*MTOR*	*MUTYH*	*MYC*	*MYCL*	*MYCN*
MYD88	*MYH9*	*MYOD1*	*NBN*	*NCOA3*	*NCOR1*	*NEGR1*	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NKX3-1*	*NOTCH1*	*NOTCH2*	*NOTCH3*
NOTCH4	*NPM1*	*NRAS*	*NRG1*	*NRIP1*	*NSD1*	*NT5C2*	*NTHL1*	*NTRK1*	*NTRK2*	*NTRK3*	*NUF2*	*NUP93*	*P2RY8*	*PAK1*	*PAK3*
PAK7	*PALB2*	*PARK2*	*PARP1*	*PARP2*	*PARP3*	*PARP4*	*PAX5*	*PBRM1*	*PDCD1*	*PDCD1LG2*	*PDGFB*	*PDGFRA*	*PDGFRB*	*PDK1*	*PDPK1*
PEG3	*PGR*	*PHOX2B*	*PIK3C2B*	*PIK3C2G*	*PIK3C3*	*PIK3CA*	*PIK3CB*	*PIK3CD*	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PIK3R3*	*PIM1*	*PLCG2*	*PLK2*

Continued

PMAIP1	*PMS1*	*PMS2*	*PNRC1*	*POLD1*	*POLE*	*POT1*	*PPARG*	*PPM1D*	*PPP2R1A*	*PPP2R2A*	*PPP4R2*	*PPP6C*	*PRDM1*	*PRDM14*	*PREX2*
PRKAR1A	*PRKCI*	*PRKD1*	*PRKDC*	*PRSS1*	*PRSS8*	*PTCH1*	*PTEN*	*PTP4A1*	*PTPN11*	*PTPRD*	*PTPRO*	*PTPRS*	*PTPRT*	*QKI*	*RAB35*
RAC1	*RAC2*	*RAD21*	*RAD50*	*RAD51*	*RAD51B*	*RAD51C*	*RAD51D*	*RAD52*	*RAD54B*	*RAD54L*	*RAF1*	*RANBP2*	*RARA*	*RASA1*	*RB1*
RBBPB	*RBM10*	*RECQL*	*RECQL4*	*REL*	*RET*	*RFWD2*	*RHBDF2*	*RHEB*	*RHOA*	*RIC8A*	*RICTOR*	*RINT1*	*RIT1*	*RNF43*	*ROBO2*
ROS1	*RPA1*	*RPA3*	*RPS6KA4*	*RPS6KB2*	*RPTOR*	*RRAGC*	*RRAS*	*RRAS2*	*RSPO2*	*RTEL1*	*RUNX1*	*RUNX1T1*	*RXR4*	*RYBP*	*SDHA*
SDHAF2	*SDHB*	*SDHC*	*SDHD*	*SESN1*	*SESN2*	*SESN3*	*SETD2*	*SETD8*	*SF3B1*	*SGK1*	*SH2B3*	*SH2D1A*	*SHKBP1*	*SHOC2*	*SHQ1*
SIN3A	*SLFN11*	*SLIT2*	*SLX4*	*SMAD2*	*SMAD3*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMARCD1*	*SMO*	*SMYD3*	*SNCAIP*	*SOCS1*	*SOS1*	*SOX10*
SOX17	*SOX2*	*SOX9*	*SPEN*	*SPINK1*	*SPOP*	*SPRED1*	*SPTA1*	*SRC*	*SRSF2*	*STAG2*	*STAT3*	*STAT4*	*STAT5B*	*STK11*	*STK19*
STK40	*SUFU*	*SUZ12*	*SYK*	*TAF1*	*TAP1*	*TAP2*	*TBX3*	*TCEB1*	*TCF3*	*TCF7L2*	*TEK*	*TERC*	*TERT*	*TET1*	*TET2*
TFE3	*TGFBR1*	*TGFBR2*	*TIPARP*	*TMEM127*	*TMPRSS2*	*TNFAIP3*	*TNFRSF14*	*TOP1*	*TOP2A*	*TP53*	*TP53BP1*	*TP63*	*TRAF2*	*TRAF7*	*TSC1*
TSC2	*TSHR*	*TSPAN31*	*TTF1*	*TYRO3*	*U2AF1*	*UPF1*	*VEGFA*	*VHL*	*VTCN1*	*WHSC1*	*WHSC1L1*	*WISP3*	*WRN*	*WT1*	*WWTR1*
XIAP	*XPO1*	*XRCC2*	*WWTR1*	*XRCC3*	*YAP1*	*YES1*	*ZBTB2*	*ZFHX3*	*ZNF217*	*ZNF423*	*ZNF703*				

Selected intronic regions

ALK intron 18 - 19	*BCL2* 3'UTR	*BCR* intron 8,13 - 14	*BRAF* intron 7-10	*BRCA1* intron2,7 - 8,12,16,19 - 20	*BRCA2* intron 2	*CD74* intron 6-8	*EGFR* intron 7,15,24-27	*ETV4* intron 5 - 6	*ETV5* intron 6 - 7	*ETV6* intron 5 - 6
EWSR1 intron 6-13	*EZR* intron 9-12	*FGFR1* intron 1,5,17	*FGFR2* intron 1,17	*FGFR3* intron 17	*FLI1* intron 3-8	*KIT* intron 16	*KMT2A* intron 6-11	*MET* intron 1,14	*MSH2* intron 5	*MYB* intron 14
MYC intron 1	*NOTCH2* intron 26	*NTRK1* intron 8-10	*NTRK2* intron 12,15	*NTRK3* intron 13 - 14	*NUTM1* intron 1	*PDGFB* intron 1	*PDGFRA* intron 7,9,11	*RAF1* intron 4-9	*RARA* intron 2	*RET* intron 7-11
ROS1 intron 31-35	*RSPO2* Upstream,5'UTR, exon 1 - 2, intron 1		*SDC4* intron 2	*SLC34A2* intron 4	*TMPRSS2* intron 1-3					

Microstatellite markers

BAT-25	*BAT-26*	*BAT-40*	*BAT-RII*	*NR-21*	*NR-22*	*NR-24*	*NR-27*	*MONO-27*	*D2S123*	*D5S346*	*D17S250*	*D17S261*	*D17S20*	*D18S34*

Medicine related sites SNPs

rs1801133	rs1801268	rs67376798	rs1801160	rs3918290	rs72549303	rs17376848	rs59086055	rs55886062	rs1801159	rs1801158
rs56038477	rs78060119	rs75017182	rs72549306	rs1801266	rs115232898	rs2297595	rs72549309	rs1801265	rs396991	rs4673993
rs10929302	rs3064744	rs4148323	rs1142345	rs1800584	rs1800460	rs1800462	rs186364861	rs116855232	rs147390019	rs45445694
rs25487	rs1051266	rs1135840	rs75467367	rs74478221	rs766507177	rs28371735	rs1135835	rs1135833	rs1058172	rs59421388
rs28371725	rs5030867	rs16947	rs5030656	rs72549352	rs35742686	rs3892097	rs5030655	rs1058164	rs61736512	rs28371706
rs28371704	rs28371703	rs201377835	rs1065852	rs769258						

Ordering Information

Product	Catalog #
NanOnco Plus Panel v3.0, 16 rxn	1001112F
NanOnco Plus Panel v3.0, 96 rxn	1001111F

For research use only. Not for use in diagnostic procedures.

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