

NanOnco Plus Panel v3.0

Background

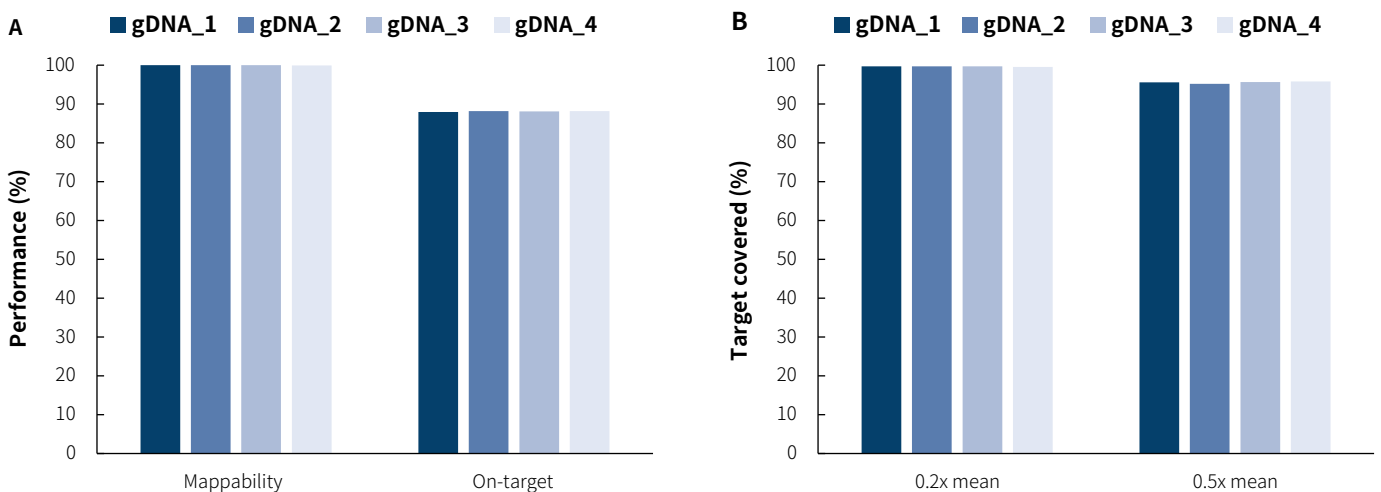
Large pan-cancer panels usually contain hundreds of important tumor-associated genes covering about 1–3 Mb of target regions. If the target region is small enough, it is easy to achieve high sequencing depth, which is beneficial for somatic variant analysis; if the target region is large enough, it can 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

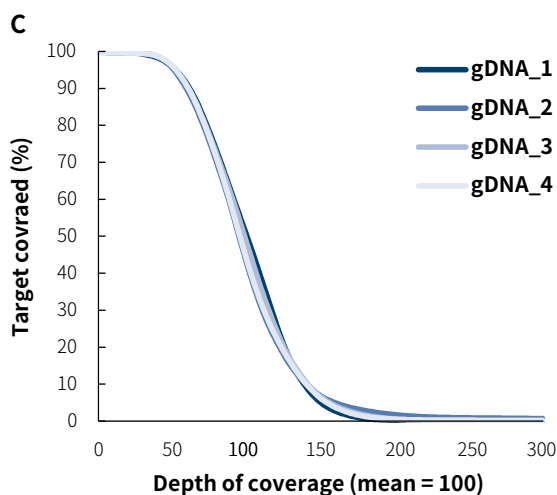


Fig 1. Capture performance of NanOnco Plus Panel v3.0 in different gDNA libraries. Libraries were prepared using NadPrep EZ DNA Library Preparation Kit coupled with NadPrep UDI Adapter (for Illumina®), and sequenced on Illumina Novaseq 6000 with PE150. The BWA was used for alignment to the reference 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 Multiplex 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 Allele 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

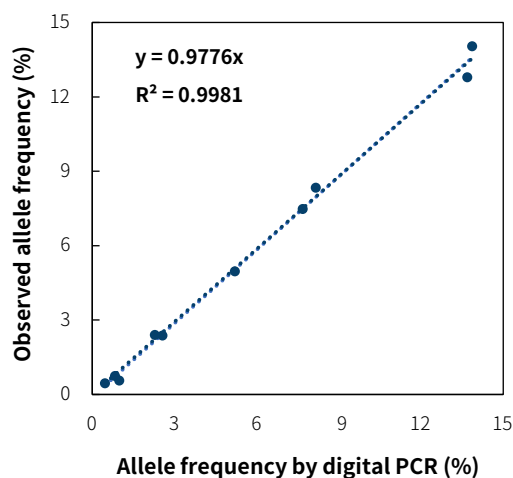


Fig 2. Consistency of the allele frequencies in NanOnco Plus Panel v3.0 capture data with the nominal frequencies of the standards. Libraries were prepared using NadPrep EZ DNA Library Preparation Kit coupled with NadPrep Universal Stubby Adapter (UDI), and sequenced on Illumina Novaseq 6000 with PE150. 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 Preparation 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 Multiplex 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
BMPRI1A	BRAF	BRCA1	BRC2	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	CTNNA1	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	ERRF1	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	HMG2	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	MYO1D	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	RFW2	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	STAT5A	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	XRCC3	YAP1	YES1	ZBTB2	ZFH3	ZNF217	ZNF423	ZNF703				

Selected intronic regions

ALK intron 18 - 19	BCL2 3'UTR	BCR intron 8,13 - 14	BRAF intron 7-10	BRCA1 intron 2,7 - 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					

Microsatellite markers

BAT-25	BAT-26	BAT-40	BAT-RII	NR-21	NR-22	NR-24	NR-27	MONO-27	D2S123	D5S346	D17S250	D17S261	D17S520	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.

Nanodigmbio Pte. Ltd. reserves all rights.