VARIANT*Plex*-HT Expanded Solid Tumor

Description

The VARIANT*Plex*-HT Expanded Solid Tumor panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex*-HT reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with VARIANT*Plex*-HT protocol for Illumina® (RA-DOC-058).

VARIANT*Plex*-HT Expanded Solid Tumor contains **2676** GSPs targeting **76** genes commonly mutated in solid tumors as well as microsatellite instability (**MSI**).

Description	Part number	Storage
VARIANT*Plex*-HT Expanded Solid Tumor GSP1, 24 reactions or VARIANT*Plex*-HT Expanded Solid Tumor GSP1, 96 reactions	SA20123241 or SA20123961	–20°C ± 10°C
VARIANT*Plex*-HT Expanded Solid Tumor GSP2, 24 reactions or VARIANT*Plex*-HT Expanded Solid Tumor GSP2, 96 reactions	SA20123242 or SA20123962	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	20
B	First PCR	VARIANT*Plex*-HT Expanded Solid Tumor GSP1	8
C	First PCR	10mM Tris-HCl pH 8.0	18
D	First PCR	Purified PCR1 eluate	16
E	Second PCR	VARIANT*Plex*-HT Expanded Solid Tumor GSP2	8

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	**1**	95	3 min	1
	2	95	30 sec	15
	3	60	10 sec	
	4	62	10 min (100% ramp rate)	
	5	72	3 min	1
	6	4	Hold	1
Second PCR reaction	**1**	95	3 min	1
	2	95	30 sec	20†
	3	60	10 sec	
	4	65	10 min (100% ramp rate)	
	5	72	3 min	1
	6	4	Hold	1

†The number of PCR2 cycles may be decreased if you regularly experience library yields greater than 200 nM.

Recommended reads and multiplexing

VARIANT*Plex*-HT Expanded Solid Tumor libraries should be sequenced to a minimum of **6M** reads. Starting read depth recommendations for standard profiling may be adjusted based on user needs.

Archer™ Analysis settings

Sequencing data should be processed using Archer Analysis (v7.0, or greater). The VARIANT*Plex*-HT Expanded Solid Tumor panel requires selection of ***SNV/Indel, Structural Variation, Copy Number Variation, and MSI*** pipelines, found under the ***DNA*** Input Type (see the Archer Analysis User Guide for more details on setting up your analysis). Selection of the DNA Target Coverage pipeline is optional.

Processing of VARIANT*Plex*-HT Expanded Solid Tumor libraries requires a one-time upload of the Panel GTF. When performing DNA Target Coverage analysis, users must also select a

Region of Interest BED file. Users may optionally add a Targeted Mutations VCF file for targeted SNV/Indel detection. Files can be obtained by contacting archer-tech@idtdna.com

Assay targets

Gene	Accession	Exon
AKT1	NM_005163	2,3,6,11
ALK	NM_004304	21,22,23,24,25
APC	NM_000038	16
AR	NM_000044	4,5,6,7,8
ATM	NM_000051	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63
ATRX	NM_000489	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35
BARD1	NM_000465	1,2,3,4,5,6,7,8,9,10,11
BRAF	NM_004333	11,15
BRCA1	NM_007294	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
BRCA1	NM_007300	13
BRCA2	NM_000059	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27
BRIP1	NM_032043	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
CDK12	NM_015083	1,2,3,4,5,6,7,8,9,10,11,12,13
CDK12	NM_016507	14
CDK4	NM_000075	2,3,4,5,6,7,8

Gene	Accession	Exon
CDK6	NM_001259	CNV Only
CDKN2A	NM_000077	2,3
CDKN2A	NM_058197	1
CHEK1	NM_001114121	1
CHEK1	NM_001274	3,4,5,6,7,8,9,10,11,12,13
CHEK1	NM_001330427	2
CHEK2	NM_001349956	4
CHEK2	NM_001005735	3
CHEK2	NM_007194	2,3,4,5,6,7,8,9,10,11,12,13,14,15
CTNNB1	NM_001904	3
DDR2	NM_006182	8,15,17
EGFR	NM_005228	3,7,12,15,18,19,20,21,22
EGFR	NM_201282	16
EGFR	NM_201283	10
ERBB2	NM_004448	8,10,17,19,20,21,22,24
ERBB3	NM_001005915	3
ERBB3	NM_001982	2,3,7,8,9,18,23,27,28
ERBB4	NM_005235	3,4,6,7,8,9,15,23
ESR1	NM_000125	5,6,7,8

Gene	Accession	Exon
FANCA	NM_000135	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31, 32,33,34,35,36,37,38,39,40,41,42,43
FANCA	NM_001018112	11
FANCL	NM_001114636	7
FANCL	NM_018062	1,2,3,4,5,6,8,9,10,11,12,13,14
FBXW7	NM_001013415	1
FBXW7	NM_001257069	4
FBXW7	NM_018315	1,2,3,4,5,6,7,8,9,10,11
FGFR1	NM_001174064	3
FGFR1	NM_001174067	2
FGFR1	NM_015850	2,4,5,8,13,14
FGFR2	NM_000141	2,5,7,9,12,13,14,15
FGFR2	NM_022970	8
FGFR3	NM_000142	3,7,9,13,14,16
FOXL2	NM_023067	1 (p.C134)
GNA11	NM_002067	5
GNAQ	NM_002072	4,5
GNAS	NM_000516	6,7,8,9
H3F3A	NM_002107	2
HIST1H3B	NM_003537	1

Gene	Accession	Exon
HRAS	NM_005343	2,3
IDH1	NM_005896	3,4
IDH2	NM_002168	4
JAK2	NM_004972	6,12,14,23
KIT	NM_000222	2,8,9,10,11,12,13,14,15,17,18
KRAS	NM_004985	2,3,4,5
MAP2K1	NM_002755	2,3,6
MAP2K2	NM_030662	2,6
MET	NM_000245	2,11,14,15,16,19,20,21
MTOR	NM_004958	30,39,40,44,45,47,48,50
MYC	NM_002467	1,2,3
NOTCH1	NM_017617	24,25,26,27,28,29,34,c.*7668+371 to c.*7668+378
NOTCH2	NM_024408	24,25,26,27,28,29,34
NOTCH3	NM_000435	24,25,26,27,28,29,33
NOTCH4	NM_004557	26,27,28,29,30
NRAS	NM_002524	2,3,4,5
NTRK1	NM_002529	14,15
NTRK2	NM_006180	18,19
NTRK3	NM_002530	16,17

Gene	Accession	Exon
PALB2	NM_024675	1,2,3,4,5,6,7,8,9,10,11,12,13
PDGFRA	NM_006206	7,10,11,12,14,15,16,18,23
PIK3CA	NM_006218	2,3,5,7,8,9,10,14,19,21
POLD1	NM_001308632	14
POLD1	NM_002691	2,3,4,5,6,7,8,9,10,11,12,13,14,16,17,18,19,20,21,22,23,24,25,26,27
POLE	NM_006231	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31, 32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49
PTEN	NM_000314	1,2,3,4,5,6,7,8,9
RAD51B	NM_001321809	12
RAD51B	NM_001321810	11
RAD51B	NM_001321814	11
RAD51B	NM_001321818	11
RAD51B	NM_001321819	11
RAD51B	NM_002877	2,3,4,5,6,7,8,9,10,11
RAD51B	NM_133509	11
RAD51B	NM_133510	11
RAD51C	NM_002876	1,2
RAD51C	NM_058216	3,4,5,6,7,8,9
RAD51D	NM_001142571	3
RAD51D	NM_002878	1,2,3,4,5,6,7,8,9,10

Gene	Accession	Exon
RAD54L	NM_003579	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18
RAF1	NM_002880	3,7,10,14
RB1	NM_000321	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27
RET	NM_020630	10,11,13,14,15,16
RICTOR	NM_152756	CNV Only
ROS1	NM_002944	36,37,38,39,40,41,42
SMAD4	NM_005359	2,3,4,5,6,7,8,9,10,11,12
SMO	NM_005631	1,2,3,4,5,6,7,8,9,10,11,12
STK11	NM_000455	1,2,3,4,5,6,7,8,9
TERT	NM_198253	3,6,10
TERT	NM_198253	Promoter (chr5:1295148-1295374)
TP53	NM_000546	1,2,3,4,5,6,7,8,9,10,11
TP53	NM_001276696	10
VHL	NM_000551	1,2,3

Genes targeted for CNV

AKT1	*BRCA2*	*EGFR*	*JAK2*	*PIK3CA*
ALK	*BRIP1*	*ERBB2*	*KIT*	*PTEN*
AR	*CDK12*	*ERBB3*	*KRAS*	*RAD51B*
ATM	*CDK4*	*ESR1*	*MET*	*RAF1*
BARD1	*CDK6*	*FGFR1*	*MYC*	*RET*
BRAF	*CHEK1*	*FGFR2*	*NRAS*	*RICTOR*

BRCA1	*CHEK2*	*FGFR3*	*PDGFRA*	*STK11*
				TERT

Please contact archer-tech@idtdna.com to inquire about enabling additional genes for CNV detection.

SNPs and sites targeted for sample tracking

rs560681	rs430046	rs987640	rs10776839	rs12393891
rs740598	rs8078417	rs6444724	rs6530357	chrX:4429309
rs1498553	rs9951171	rs6811238	rs5971553	chrX:11314433
rs10773760	rs576261	rs13182883	rs5953060	chrY:6738552
rs1058083	rs1109037	rs214955	rs6524626	chrY:19490214
rs4530059	rs1523537	rs321198	rs5940270	
rs1821380	rs221956	rs4606077	rs722847	

SNPs may be used in combination to uniquely tag and track samples over time. Contact archer-tech@idtdna.com for further details.

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Revision History

Document Number	Date	Description of change
RA-DOC-459/REV01	October 2023	Initial release.
RA-DOC-459/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.