

## VARIANT*Plex* Core Solid Tumor

### Description

The VARIANT*Plex* Core Solid Tumor panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex* reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with VARIANT*Plex* HS/HGC protocol for Illumina® (RA-DOC-056).

VARIANT*Plex* Core Solid Tumor contains **1614** GSPs targeting **60** genes commonly mutated in solid tumors as well as microsatellite instability (**MSI**).

Description	Part number	Storage
VARIANT <i>Plex</i> Core Solid Tumor GSP1, 8 reactions	SA20122081	-20°C ± 10°C
VARIANT <i>Plex</i> Core Solid Tumor GSP2, 8 reactions	SA20122082	
PreSeq™ DNA QC Assay Standard, 32 µL	SA0597	
PreSeq™ DNA QC Assay 10X Primer Mix, 120 µL	SA0598	

### Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	32
B	First PCR	VARIANT <i>Plex</i> Core Solid Tumor GSP1	8
C	First PCR	10mM Tris-HCl pH 8.0	34
D	First PCR	Purified PCR1 eluate	32
E	Second PCR	VARIANT <i>Plex</i> Core Solid Tumor GSP2	8

## Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	60	10 sec	15
	4	63	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	
	3	60	10 sec	20 <sup>†</sup>
	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* Core Solid Tumor libraries should be sequenced to a minimum of **4.5M 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 (7.0, or greater). The VARIANT*Plex* Core Solid Tumor panel requires selection of the **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* Core 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](mailto:archer-tech@idtdna.com)

## Assay targets

Gene	Accession	Exon
<i>AKT1</i>	NM_005163	2,3,6,11
<i>ALK</i>	NM_004304	21,22,23,24,25
<i>APC</i>	NM_000038	16
<i>AR</i>	NM_000044	4,5,6,7,8
<i>ATRX</i>	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
<i>BRAF</i>	NM_004333	11,15
<i>CDK4</i>	NM_000075	2,3,4,5,6,7,8
<i>CDK6</i>	NM_001259	CNV Only
<i>CDKN2A</i>	NM_000077	2,3
<i>CDKN2A</i>	NM_058197	1
<i>CTNNB1</i>	NM_001904	3
<i>DDR2</i>	NM_006182	8,15,17
<i>EGFR</i>	NM_005228	3,7,12,15,18,19,20,21,22
<i>EGFR</i>	NM_201282	16
<i>EGFR</i>	NM_201283	10
<i>ERBB2</i>	NM_004448	8,10,17,19,20,21,22,24
<i>ERBB3</i>	NM_001005915	3

<b>Gene</b>	<b>Accession</b>	<b>Exon</b>
<i>ERBB3</i>	NM_001982	2,3,7,8,9,18,23,27,28
<i>ERBB4</i>	NM_005235	3,4,6,7,8,9,15,23
<i>ESR1</i>	NM_000125	5,6,7,8
<i>FBXW7</i>	NM_001013415	1
<i>FBXW7</i>	NM_001257069	4
<i>FBXW7</i>	NM_018315	1,2,3,4,5,6,7,8,9,10,11
<i>FGFR1</i>	NM_001174064	3
<i>FGFR1</i>	NM_001174067	2
<i>FGFR1</i>	NM_015850	2,4,5,8,13,14
<i>FGFR2</i>	NM_000141	2,5,7,9,12,13,14,15
<i>FGFR2</i>	NM_022970	8
<i>FGFR3</i>	NM_000142	3,7,9,13,14,16
<i>FOXL2</i>	NM_023067	1 (p.C134)
<i>GNA11</i>	NM_002067	5
<i>GNAQ</i>	NM_002072	4,5
<i>GNAS</i>	NM_000516	6,7,8,9
<i>H3F3A</i>	NM_002107	2
<i>HIST1H3B</i>	NM_003537	1
<i>HRAS</i>	NM_005343	2,3

<b>Gene</b>	<b>Accession</b>	<b>Exon</b>
<i>IDH1</i>	NM_005896	3,4
<i>IDH2</i>	NM_002168	4
<i>JAK2</i>	NM_004972	6,12,14,23
<i>KIT</i>	NM_000222	2,8,9,10,11,12,13,14,15,17,18
<i>KRAS</i>	NM_004985	2,3,4,5
<i>MAP2K1</i>	NM_002755	2,3,6
<i>MAP2K2</i>	NM_030662	2,6
<i>MET</i>	NM_000245	2,11,14,15,16,19,20,21
<i>MTOR</i>	NM_004958	30,39,40,44,45,47,48,50
<i>MYC</i>	NM_002467	1,2,3
<i>NOTCH1</i>	NM_017617	24,25,26,27,28,29,34,c.*7668+371 to c.*7668+378
<i>NOTCH2</i>	NM_024408	24,25,26,27,28,29,34
<i>NOTCH3</i>	NM_000435	24,25,26,27,28,29,33
<i>NOTCH4</i>	NM_004557	26,27,28,29,30
<i>NRAS</i>	NM_002524	2,3,4,5
<i>NTRK1</i>	NM_002529	14,15
<i>NTRK2</i>	NM_006180	18,19
<i>NTRK3</i>	NM_002530	16,17
<i>PDGFRA</i>	NM_006206	7,10,11,12,14,15,16,18,23

Gene	Accession	Exon
<i>PIK3CA</i>	NM_006218	2,3,5,7,8,9,10,14,19,21
<i>POLD1</i>	NM_001308632	14
<i>POLD1</i>	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
<i>POLE</i>	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
<i>PTEN</i>	NM_000314	1,2,3,4,5,6,7,8,9
<i>RAF1</i>	NM_002880	3,7,10,14
<i>RB1</i>	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
<i>RET</i>	NM_020630	10,11,13,14,15,16
<i>RICTOR</i>	NM_152756	CNV only
<i>ROS1</i>	NM_002944	36,37,38,39,40,41,42
<i>SMAD4</i>	NM_005359	2,3,4,5,6,7,8,9,10,11,12
<i>SMO</i>	NM_005631	1,2,3,4,5,6,7,8,9,10,11,12
<i>TERT</i>	NM_198253	3,6,10
<i>TERT</i>	NM_198253	Promoter (chr5:1295148-1295374)
<i>TP53</i>	NM_000546	1,2,3,4,5,6,7,8,9,10,11
<i>TP53</i>	NM_001276696	10
<i>VHL</i>	NM_000551	1,2,3

### Genes targeted for CNV

<i>AKT1</i>	<i>CDK6</i>	<i>FGFR1</i>	<i>KRAS</i>	<i>PIK3CA</i>
<i>ALK</i>	<i>EGFR</i>	<i>FGFR2</i>	<i>MET</i>	<i>PTEN</i>
<i>AR</i>	<i>ERBB2</i>	<i>FGFR3</i>	<i>MYC</i>	<i>RAF1</i>
<i>BRAF</i>	<i>ERBB3</i>	<i>JAK2</i>	<i>NRAS</i>	<i>RET</i>
<i>CDK4</i>	<i>ESR1</i>	<i>KIT</i>	<i>PDGFRA</i>	<i>RICTOR</i>
				<i>TERT</i>

Please contact [archer-tech@idtdna.com](mailto: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](mailto:archer-tech@idtdna.com) for further details.

## Limitations of use

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## Revision History

Document Number	Date	Description of change
RA-DOC-029/REV01	June 2023	Initial release
RA-DOC-029/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps.  Added MSI pipeline information to the “Archer Analysis settings” section.  Updated branding.