Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Cell-free DNA and Neuromediators in Detecting Aggressive Variant Prostate Cancer

Jost von Hardenberg[1], Thomas Stefan Worst[1], Niklas Westhoff[1], Philipp Erben[1], Stefan Fuxius[2], Markus Müller[3], Christian Bolenz[1*], Christel Weiß[4] and Elmar Heinrich[5#]

1 Department of Urology, University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany
2 Outpatient Oncology Practice Heidelberg, Heidelberg, Germany
3 Department of Urology, Hospital Ludwigshafen, Ludwigshafen, Germany
4 Department of Medical Statistics and Biomathematics, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany
5 Department of Urology, University Hospital Goettingen, Germany;
*present address: Department of Urology, University Hospital Ulm, Germany; #present address: Department of Urology, Hospital Barmherzige Brueder Salzburg, Austria

Content of the supplementary material:

Corresponding author:

Dr. med. Jost von Hardenberg

Department of Urology, University Medical Center Mannheim, University of Heidelberg,

Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany

Tel. +49-621-383-3245; Fax. +49-621-383-1452

Email: jost.vonhardenberg@medma.uni-heidelberg.de

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Methods: Further details on study cohort

Supplemental table S1 Characterization of study cohort

Patient characteristics (N = 23)		N (%)
Age (years)	mean ± standard deviation	69.6 ± 5.9
ECOG performance status	0	11 (47.8 %)
	1	11 (47.8 %)
	2	1 (4.4 %)
Charlson comorbidity index	< 2	19 (82.6 %)
	≥ 2	4 (17.4 %)
First diagnosis –study entry (months)	median (range)	60 (22 – 150)
Gleason score in biopsy/surgical	6	2 (9.5 %)
	7	7 (33.3 %)
	8	6 (28.6 %)
	9	4 (19 %)
	10	2 (9.5 %)
Site of metastatic disease*	Bone	20 (87 %)
	Visceral metastasis	6 (26 %)
	Lymph node metastasis	18 (78.3 %)
Clinical manifestation of NED[#]		19 (82.6 %)
Prior local treatment	Radical prostatectomy	12 (52.2 %)
	EBRT	15 (65.2 %)
Prior systemic treatment*	Androgen deprivation therapy	23 (100%)
	Docetaxel	23 (100 %)
	Abiraterone acetate	14 (61 %)
	Enzalutamide	12 (52.2 %)
Blood based marker	Hemoglobin (Hb), g/dl	11.3 (8.9 – 14.5)
	Alkaline phosphatase (AP), U/I	152 (43 – 1348)
	Lactat dehydrogenase (LDH), U/I	362 (168 – 1189)

Data are number (%) unless stated otherwise. ECOG=Eastern Cooperative Oncology Group ; [#] Epstein et al., 2014 * patients could have more than one

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Methods: Further details on cell-free DNA sequencing

Cell-free DNA was quantified using the Agilent Fragment Analyzer and the High Sensitivity Large Fragment Analysis Kit (AATI, USA).

The GATC LIQUID ONCOPANEL uses multiple amplicons to interrogate the following mutations/hotspots in 50 oncogenes, tumor suppressors and drug resistance markers (sample P_1 – P_8): ABL1 , AKT1 , ALK , APC , ATM , BRAF , CDH1 , CDKN2A , CSF1R , CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL. Single molecular droplet PCR was used to amplify the target genes. Subsequently the PCR products were sequenced on the Illumina MiSeq platform using paired end sequencing with a read length of 2 x 150 bp and an approximately target coverage of 10.000x. For data analysis, the base quality of each sequence read was inspected. Low quality calls were removed before proceeding with further processing. Using a sliding window approach, bases with low quality were removed from the 3′ and 5′ ends. Reads were removed if the average phred quality was below 25. Finally only mate pairs (forward and reverse read) were used for the next analysis step. Mapping to the reference sequence/database (human genome 19) was done using BWA [1] with default parameters. Percentage of reads in categories on target were calculated based on the number of reads mapping to entire reference. Single nucleotide variants (SNV) were detected using LoFreq [2]. Insertions and deletions (InDel) were detected using Platypus software [3]. Variants found in plasma that were annotated as single nucleotide polymorphisms (SNPs) in the dbSNP database were excluded from further analysis.

Despite allele frequencies below 1 % have been reported for somatic alterations in cfDNA [4], a lower frequency limit for calls of 1 % was used. To identify cancerous somatic mutations in the absence of germline controls, we focused on genes known to have tumor-promoting point mutations at COSMIC annotated sites [5]. We excluded identical variants identified in

more than 2 patients, as these were deemed most likely as technical artifacts. All COSMIC mutations were screened for driver mutations in cancerhotspots.org [6].

The GATC LIQUID ONCOPANEL ALL-IN-ONE covers the entire exons of 597 protein coding genes. SNPs and InDels can be detected with sensitivity down to 1%. The analysis also covers the detection of copy number variantions (CNV) using the software package CNVkit [7]. The analysis is used to gain additional inside into the potential change of genetic alterations at a later timepoint of three patients (P_2_2 and P_5_2 before the 3rd cycle of CAB and P_4_2 before the 6th cycle of CAB). Compared to the LIQUID ONCOPANEL a different target enrichment protocol is used. The target genes are enriched using the Agilent SureSelect hybridisation technology [8]. The target enrichment system captures genomic targets using long 120 nt RNA baits. The hybridisation-based strategy facilitates the deduction of PCR duplications in the assay using molecular barcodes. Ultra-high coverage sequencing was performed on Illumina's MiSeq or HiSeq 2500 with a 75-bp or 125-bp paired-end mode, respectively.

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Results: Further details on cell-free DNA sequencing results

Supplemental table S2 DNA amount (ng) extracted from each blood sample and DNA amount used for library preparation

	P_1	P_2	P_2_2	P_3	P_4	P_4_2	P_5	P_5_2	P_6	P_7	P_8
DNA (ng) extracted	80.6	74.7	251.94	128.2	96.5	29.58	118.1	2788	417.1	639.2	65.4
DNA (ng) used for library preparation	38.68	35.86	185.3	61.52	46.34	22	56.71	200	55.61	51.13	31.39

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Supplemental table S3 Summary of variants detected in each sample (single nucleotide variants (SNV) and insertions and deletions (InDels)).

Variant category	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
Total SNV	70	148	108	74	109	122	112	70
Known SNV	16	32	24	23	27	31	31	20
Unknown SNV	54	116	84	51	82	91	81	50
Total InDel	11	8	7	9	7	11	5	5
Known InDel	0	0	0	0	0	0	0	0
Unkown InDel	11	8	7	9	7	11	5	5

Variant category	P_2_2	P_4_2	P_5_2
Total SNV	25887	30474	28355
Known SNV	4122	4568	3978
Unknown SNV	21765	25906	24377
Total InDel	7318	10326	7716
Known InDel	322	532	370
Unkown InDel	6996	9794	7346

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Supplemental table S4 List of variants detected in hotspots of samples P_1 – P_8 in the COSMIC (version 71) and ClinVar (released in 4.2016) databases and samples P_2_2, P_4_2 and P_5_2) in the ClinVar (released 02. Oct 2017) database. Variants which have clinical significance state as "likely pathogenic", "pathogenic" and "drug response" are filtered from a complete list of variants.

Sample P_1

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
APC	p.T1493T	51.68	20449	chr5	112175770	rs41115 COSM3760869
APC	p.I1524T	1.97	18588	chr5	112175862	rs200803739
DERL3		56.3	7849	chr22	24176287	COSM1090
EGFR	p.Q787Q	99.28	39869	chr7	55249063	rs1050171 COSM1451600
ERBB2	p.L836L	19.08	15409	chr17	37881314	rs104886008
FLT3		47.71	16448	chr13	28610183	COSM3999060
GNA11	p.T257T	99.21	11591	chr19	3119239	COSM3756588
HRAS	p.H27H	43.98	6417	chr11	534242	rs12628 COSM249860 COSM3752426
NOTCH1	p.D1698D	49.97	17738	chr9	139397707	COSM1461158 COSM33747
PDGFRA	p.P567P	99.73	11870	chr4	55141055	COSM1430082
RET	p.L769L	99.75	12240	chr10	43613843	rs1800861
SMAD4	p.C363S	3.29	15271	chr18	48591924	COSM14123
TP53	p.I255V	1.02	11728	chr17	7577518	COSM44290
TP53	p.S227S	1.07	11727	chr17	7577600	COSM45614
TP53	p.P72R	99.56	12502	chr17	7579472	rs1042522 COSM250061 COSM3766190 COSM3766191 COSM3766192 COSM3766193
VHL	p.W88*	1.98	17820	chr3	10183795	COSM249280

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_2

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
APC	p.T1493T	99.31	10774	chr5	112175770	rs41115 COSM3760869
ATM	p.A1309T	50.12	11667	chr11	108155132	rs149711770 COSM22507
BRAF	p.L588L	6.07	5553	chr7	140453171	COSM1568585
CDH1	p.W409R	2.1	14960	chr16	68847303	rs587778176
EGFR	p.N771D	1.2	15408	chr7	55249013	rs727503020
EGFR	p.Q787Q	99.41	27076	chr7	55249063	rs1050171 COSM1451600
ERBB2	p.L836L	17.24	9768	chr17	37881314	rs104886008
FIP1L1	p.T434A	1.67	16217	chr4	55144546	COSM1430087
FLT3		99.83	21502	chr13	28610183	COSM3999060
GNAS	p.Q870R	1.29	5950	chr20	57484596	rs121913494 COSM1566191 COSM27896
HRAS	p.H27H	29.2	9023	chr11	534242	rs12628 COSM249860 COSM3752426
KDR	p.Q472H	47.61	9773	chr4	55972974	rs1870377 COSM149673
KDR	p.V297I	51.11	12871	chr4	55979558	rs2305948 COSM1131107
MET	p.R988H	2.35	33650	chr7	116411924	rs45607832
PDGFRA	p.P567P	99.7	12273	chr4	55141055	COSM1430082
PTEN		7.72	4925	chr10	89685257	COSM5922
PTEN	p.I135M	1.72	4353	chr10	89692921	COSM5086
PTEN	p.Y225C	3.26	5150	chr10	89717649	COSM5138
RB1	p.R556Q	3.06	11898	chr13	48955551	COSM1367250 COSM1367251
RB1	p.R579*	1.14	4315	chr13	49027168	rs121913305 COSM1756816 COSM892
RET	p.L769L	99.83	9594	chr10	43613843	rs1800861
RET		50.12	11933	chr10	43615505	rs2472737
SMAD4	p.D355N	2.22	7192	chr18	48591900	COSM24274
SMAD4	p.R515R	3.86	13005	chr18	48604723	COSM1389103
TP53	p.R290H	1.18	11872	chr17	7577069	rs55819519 COSM1386594 COSM44017
TP53	p.V274A	1.33	11872	chr17	7577117	COSM131453 COSM1640826 COSM3701290 COSM44393
TP53	p.S269G	1.75	11872	chr17	7577133	COSM43962
TP53	p.G245G	2.83	8885	chr17	7577546	COSM1610832 COSM1610833 COSM1610834 COSM3717636 COSM3717637 COSM44900
TP53	p.R196P	20.56	2053	chr17	7578262	COSM218532 COSM218533 COSM218534 COSM218535 COSM2744765 COSM3733288 COSM43814
TP53	p.P72R	99.61	7257	chr17	7579472	rs1042522 COSM250061 COSM3766190 COSM3766191 COSM3766192 COSM3766193

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_3

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
APC	p.T1493T	57.25	13291	chr5	112175770	rs41115 COSM3760869
APC	p.I1524T	1.31	12003	chr5	112175862	rs200803739
ATM	p.H2872R	5.32	9395	chr11	108218036	COSM200683
CDKN2A	p.L121L	12.64	6337	chr9	21970997	rs142371511
EGFR	p.G779S	12.09	10964	chr7	55249037	COSM25016
ERBB2	p.L836L	5.28	3012	chr17	37881314	rs104886008
FBXW7	p.H500R	3.47	3924	chr4	153247303	COSM1695080 COSM1695081 COSM1695082 COSM1695083 COSM1695084
FIP1L1	p.V584V	67.1	2544	chr4	55152040	COSM22413
FLT3		80.7	3440	chr13	28610183	COSM3999060
GNA11	p.T257T	51.31	6377	chr19	3119239	COSM3756588
HNF1A	p.A239V	3.89	6473	chr12	121431969	rs587778397
HRAS	p.H27H	99.66	2962	chr11	534242	rs12628 COSM249860 COSM3752426
KIT	p.M541L	42.47	1116	chr4	55593464	rs3822214 COSM28026
KIT	p.L862L	46.31	1164	chr4	55602765	COSM1325
NOTCH1	p.D1698D	99.48	29151	chr9	139397707	COSM1461158 COSM33747
PDGFRA	p.P567P	99.64	2533	chr4	55141055	COSM1430082
PTEN	p.Y16C	3.44	5778	chr10	89624273	COSM5133
PTEN	p.I122I	3.83	2770	chr10	89692882	COSM1349519
RET	p.L769L	55.86	16267	chr10	43613843	rs1800861
TP53	p.Y234C	3.75	1440	chr17	7577580	rs587780073 COSM10725 COSM1646849 COSM165072 COSM165073 COSM165074 COSM3388193
TP53	p.C229C	3.75	1440	chr17	7577594	COSM44598
TP53	p.P72R	11.28	4328	chr17	7579472	rs1042522 COSM250061 COSM3766190 COSM3766191 COSM3766192 COSM3766193
VHL	p.Y98H	2.07	3774	chr3	10183823	rs5030809
VHL	p.T133A	1.76	8944	chr3	10188254	COSM1417305

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_4

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
APC	p.S1389S	1.29	18850	chr5	112175458	COSM1432363
APC	p.T1493T	47.39	12125	chr5	112175770	rs41115 COSM3760869
ATM	p.D2708D	2.12	7086	chr11	108205809	rs587781990
BRAF	p.E586E	2.18	5777	chr7	140453177	COSM1118
DERL3		40.67	6634	chr22	24176287	COSM1090
EGFR	p.Q787Q	50.71	27657	chr7	55249063	rs1050171 COSM1451600
EGFR	p.E868G	2.77	13489	chr7	55259545	COSM28607
FLT3		54.34	12468	chr13	28610183	COSM3999060
HRAS		50.62	5917	chr11	534332	rs41294870
IDH1	p.G105G	55.3	9655	chr2	209113192	COSM1741220
KDR	p.Q472H	50.29	5429	chr4	55972974	rs1870377 COSM149673
NOTCH1	p.D1698D	41.18	11175	chr9	139397707	COSM1461158 COSM33747
PDGFRA	p.P567P	99.77	11001	chr4	55141055	COSM1430082
PIK3CA	p.I391M	99.77	8757	chr3	178927410	rs2230461 COSM328028
PTEN	p.I135M	2.05	5085	chr10	89692921	COSM5086
RET	p.D631D	1.75	8121	chr10	43609941	rs55846256
RET	p.L769L	99.71	7329	chr10	43613843	rs1800861
RET	p.N777S	1.83	7328	chr10	43613866	rs377767415
RET		47.91	11711	chr10	43615505	rs2472737
SMAD4	p.C363S	1.67	9928	chr18	48591924	COSM14123
TP53	p.R273R	2.64	10119	chr17	7577119	COSM45283
TP53	p.G262G	1.15	10119	chr17	7577152	COSM45166
TP53	p.H233R	2.3	10831	chr17	7577583	COSM45669

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_5

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
APC	p.N1118D	1.01	7227	chr5	112174643	rs140493115
APC	p.T1493T	98.93	9521	chr5	112175770	rs41115 COSM3760869
APC	p.I1524T	2.44	9647	chr5	112175862	rs200803739
ATM	p.F858L	30.05	1308	chr11	108138003	rs1800056 COSM21826
BRAF	p.F610L	3.9	1461	chr7	140453107	COSM1448587
EGFR	p.Q787Q	40.66	27598	chr7	55249063	rs1050171 COSM1451600
EGFR	p.H870Y	4.47	13602	chr7	55259550	COSM53292
ERBB2	p.L836L	14.02	12192	chr17	37881314	rs104886008
FLT3		61.4	9879	chr13	28610183	COSM3999060
GNA11	p.T257T	51.67	10522	chr19	3119239	COSM3756588
HRAS	p.H27H	99.84	7914	chr11	534242	rs12628 COSM249860 COSM3752426
KIT	p.K546K	35	4517	chr4	55593481	COSM21983
KIT	p.Q556R	14.97	2197	chr4	55593601	COSM133766
KIT	p.E562G	6.32	2263	chr4	55593619	COSM1430149
PDGFRA	p.P567P	99.51	4306	chr4	55141055	COSM1430082
PTEN	p.H118R	8.93	1815	chr10	89692869	COSM23651
PTEN	p.G132S	1.32	1815	chr10	89692910	COSM5032
PTPN11	p.T52T	2.98	5373	chr12	112888140	COSM1358882
RET	p.L769L	99.83	9281	chr10	43613843	rs1800861
TP53		3.47	5972	chr17	7573914	rs77881630
TP53	p.G334G	3.05	5969	chr17	7574025	rs786203531
TP53	p.G262G	1.04	11296	chr17	7577152	COSM45166
TP53	p.M237I	2.01	11837	chr17	7577570	rs587782664 COSM11063 COSM1640834 COSM301402 COSM301403 COSM301404 COSM3378444
TP53	p.Y236H	1.59	11837	chr17	7577575	rs587782289 COSM3701292 COSM3701293 COSM3701294 COSM3701295 COSM3701296 COSM44326
TP53	p.V225A	1.15	11835	chr17	7577607	COSM44723
TP53	p.A138V	1.99	10883	chr17	7578517	rs750600586 COSM288784 COSM288785 COSM288786 COSM288787 COSM3421938 COSM3421939 COSM43818
TP53	p.P72R	57.16	9202	chr17	7579472	rs1042522 COSM250061 COSM3766190 COSM3766191 COSM3766192 COSM3766193

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_6

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
ABL1	p.F336L	5.95	15791	chr9	133748288	COSM49074
APC	p.S1400L	1.4	22461	chr5	112175490	COSM171089
APC	p.T1493T	99.2	12064	chr5	112175770	rs41115 COSM3760869
APC	p.I1524T	3.34	13303	chr5	112175862	rs200803739
APC	p.N1533D	2.6	7665	chr5	112175888	rs730881226
DERL3		68.43	6335	chr22	24176287	COSM1090
EGFR	p.A702A	2.86	16265	chr7	55241658	COSM1451570
EGFR	p.H870R	4.16	23551	chr7	55259551	COSM33725
ERBB2	p.L836L	17.27	8454	chr17	37881314	rs104886008
FIP1L1	p.V584V	9.46	16155	chr4	55152040	COSM22413
FLT3		99.75	17071	chr13	28610183	COSM3999060
GNA11	p.T257T	31.24	8080	chr19	3119239	COSM3756588
HRAS		53.61	12019	chr11	534332	rs41294870
KDR	p.Q472H	58.96	9195	chr4	55972974	rs1870377 COSM149673
KDR	p.V297I	41.32	8080	chr4	55979558	rs2305948 COSM1131107
NOTCH1	p.D1698D	69.71	9682	chr9	139397707	COSM1461158 COSM33747
PDGFRA	p.P567P	99.7	11837	chr4	55141055	COSM1430082
PTEN	p.W111R	2.43	14719	chr10	89692847	rs398123321 COSM5121
PTEN	p.G132G	1.44	14722	chr10	89692912	COSM1349530
PTPN11	p.F71F	2.87	6627	chr12	112888197	COSM1561834
RET	p.L769L	99.52	6433	chr10	43613843	rs1800861
SMAD4	p.I314I	5.7	7031	chr18	48586273	COSM1389047
SMAD4	p.C363S	2.69	6219	chr18	48591924	COSM14123
STK11	p.W332*	2.51	3470	chr19	1223059	COSM18652
TP53	p.N345D	3.89	5347	chr17	7573994	COSM4070032 COSM4070033
TP53	p.N263D	1.48	9177	chr17	7577151	rs72661119 COSM45752
TP53	p.L252H	2.55	10077	chr17	7577526	COSM45091
TP53	p.S241P	1.94	10077	chr17	7577560	COSM3356966 COSM3356967 COSM44578 COSM984905 COSM984907 COSM984908
TP53	p.S227S	1.22	10075	chr17	7577600	COSM45614
TP53	p.P72R	53.43	5660	chr17	7579472	rs1042522 COSM250061 COSM3766190 COSM3766191 COSM3766192 COSM3766193

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_7

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
APC	p.N1118D	1.58	4437	chr5	112174643	rs140493115
APC	p.T1493T	54.66	6023	chr5	112175770	rs41115 COSM3760869
APC	p.J1524T	5.53	5375	chr5	112175862	rs200803739
ATM	p.D840V	31.78	4062	chr11	108137950	rs786202605
DERL3		67.86	893	chr22	24176287	COSM1090
EGFR	p.Q787Q	54.65	7239	chr7	55249063	rs1050171 COSM1451600
ERBB2	p.L836L	8.88	5009	chr17	37881314	rs104886008
FGFR1	p.P293P	4.03	5033	chr8	38282177	COSM3900039 COSM3900040 COSM3900041 COSM3900042 COSM3900043 COSM3900044
FLT3		99.72	6421	chr13	28610183	COSM3999060
GNA11	p.T257T	98.46	6030	chr19	3119239	COSM3756588
HRAS	p.H27H	72.2	3097	chr11	534242	rs12628 COSM249860 COSM3752426
KIT	p.M541L	27.58	3742	chr4	55593464	rs3822214 COSM28026
KIT	p.N566D	2.99	6153	chr4	55593630	COSM1273
KIT	p.V654A	5.4	4447	chr4	55594258	COSM12706
KIT	p.L862L	47.12	2617	chr4	55602765	COSM1325
NOTCH1	p.Q2440*	4.96	2197	chr9	139390873	COSM13061 COSM1731624
NOTCH1	p.D1698D	42.72	5285	chr9	139397707	COSM1461158 COSM33747
PDGFRA	p.P567P	99.88	1626	chr4	55141055	COSM1430082
PIK3CA	p.T1025A	4.14	2585	chr3	178952018	rs397517202 COSM1041519 COSM303901 COSM771
PTEN	p.H123R	2.71	5508	chr10	89692884	rs121909222 COSM1349520
RET	p.L769L	99.44	4856	chr10	43613843	rs1800861
RET		52.14	7317	chr10	43615505	rs2472737
TP53	p.R335R	11.19	2270	chr17	7574022	COSM1386567 COSM1386568
TP53	p.L289F	4.44	9850	chr17	7577073	COSM1480056 COSM45446
TP53		6.47	1856	chr17	7577468	COSM45002
TP53	p.C238*	5.73	1866	chr17	7577567	COSM45677
TP53		6.16	1866	chr17	7577573	COSM44132
TP53	p.P177T	72.02	2337	chr17	7578401	COSM43669
TP53	p.M1?	5.46	2417	chr17	7578532	rs28934873 COSM1640867 COSM1640868 COSM1640869 COSM1640870 COSM1640871 COSM3712458 COSM43723
TP53	p.P72R	94.25	4293	chr17	7579472	rs1042522 COSM250061 COSM3766190 COSM3766191 COSM3766192 COSM3766193

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_8

Gene	AA Change	Mutation Frequency (%)	Coverage Depth (x)	Ref ID	Position	Database ID
APC	p.R876*	6.8	15696	chr5	112173917	rs121913333 COSM18852
APC	p.E1309*	8.08	8642	chr5	112175216	COSM18775
APC	p.T1493T	99.35	9948	chr5	112175770	rs41115 COSM3760869
CSF1R	p.P966L	5.33	11920	chr5	149433654	COSM3852700 COSM3852701
EGFR	p.R705G	2.01	6030	chr7	55241665	COSM1238071
EGFR	p.Q787Q	62.91	14141	chr7	55249063	rs1050171 COSM1451600
FBXW7	p.R278R	1.99	3411	chr4	153258981	COSM1427874 COSM1427875 COSM1427876 COSM1427877 COSM1427878
FLT3		29.89	17159	chr13	28610183	COSM3999060
GNA11	p.T257T	99.19	4566	chr19	3119239	COSM3756588
HRAS	p.H27H	22.32	7084	chr11	534242	rs12628 COSM249860 COSM3752426
KDR	p.G948E	2.75	4356	chr4	55961097	COSM138433
NOTCH1	p.D1698D	28.84	4736	chr9	139397707	COSM1461158 COSM33747
PDGFRA	p.P567P	99.64	15109	chr4	55141055	COSM1430082
RET	p.L769L	99.58	5942	chr10	43613843	rs1800861
SMAD4	p.C363S	4.63	11133	chr18	48591924	COSM14123
TP53	p.S269G	2.55	6619	chr17	7577133	COSM43962
TP53	p.S227S	1.16	8610	chr17	7577600	COSM45614
TP53	p.C141Y	22.09	8384	chr17	7578508	rs587781288 COSM131470 COSM131471 COSM131472 COSM131473 COSM3378366 COSM3378367 COSM43708
TP53	p.P72R	99.57	4912	chr17	7579472	rs1042522 COSM250061 COSM3766190 COSM3766191 COSM3766192 COSM3766193
VHL	p.R177R	2.07	9664	chr3	10191538	COSM1417337

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_2_2

Location	Gene	AA Change	Codon Change	Mutation Freq.	Depth	ClinVar ID	ClinVar Significance
chr7:87160618	ABCB1	p.S893A p.S829A	c.2677T>G c.2485T>G	54.5 %	1081x	166622	drug response
chr19:49458970	BAX	p.E41fs p.R24fs p.E24fs	c.121insG c.69insG c.70insG	1.1 %	476x	9511	pathogenic
chr7:141672604	TAS2R38	p.I296V	c.886A>G	44.3 %	2029x	2906	drug response
chr7:141673345	TAS2R38	p.A49P	c.145G>C	42.8 %	1653x	2904	drug response
chr17:7578262	TP53	p.R185P p.R64P p.R157P p.R103P p.R37P p.R196P	c.554G>C c.191G>C c.470G>C c.308G>C c.110G>C c.587G>C	54.4 %	1324x	231165	likely pathogenic
chr17:7579472	TP53	p.P33R p.P72R	c.98C>G c.215C>G	99.7 %	612x	12351	drug response
chr3:14187449	XPC	p.Q939K .	c.2815C>A c.*2268C>A	100.0 %	403x	190215	drug response

Sample P_4_2

Location	Gene	AA Change	Codon Change	Mutation Freq.	Depth	ClinVar ID	ClinVar Significance
chr7:87160618	ABCB1	p.S893A p.S829A	c.2677T>G c.2485T>G	100.0 %	468x	166622	drug response
chr19:41518221	CYP2B6	p.I328T p.I92T	c.983T>C c.275T>C	46.6 %	786x	225985	drug response
chr10:96702047	CYP2C9	p.R144C	c.430C>T	100.0 %	709x	8409	drug response
chr1:98165091	DPYD	p.M166V	c.496A>G	50.6 %	413x	100116	drug response

Location	Gene	AA Change	Codon Change	Mutation Freq.	Depth	ClinVar ID	ClinVar Significance
chr12:21331549	SLCO1B1	p.V174A	c.521T>C	46.0 %	544x	37346	drug response
chr7:141672604	TAS2R38	p.I296V	c.886A>G	48.8 %	814x	2906	drug response
chr7:141673345	TAS2R38	p.A49P	c.145G>C	49.5 %	721x	2904	drug response
chr3:14187449	XPC	p.Q939K .	c.2815C>A c.*2268C>A	99.3 %	269x	190215	drug response

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_5_2

Location	Gene	AA Change	Codon Change	Mutation Freq.	Depth	ClinVar ID	ClinVar Significance
chr5:131931451	AC116366.3	. . . .	c.*2341delA c.*1321delA c.*2025delA c.*2155delA	1.6 %	569x	408407	pathogenic
chr5:112175951	APC	p.T1556fs .	c.4666insA c.*3988insA	33.9 %	1105x	428112	pathogenic
chr19:49458970	BAX	p.E41fs p.R24fs p.E24fs	c.121insG c.69insG c.70insG	1.1 %	379x	9511	pathogenic
chr19:41515263	CYP2B6	p.K262R	c.785A>G	3.1 %	417x	120171	drug response
chr10:96702047	CYP2C9	p.R144C	c.430C>T	100.0 %	1021x	8409	drug response
chr11:67352689	GSTP1	. p.I105V	c.*137A>G c.313A>G	52.3 %	872x	37340	drug response
chr5:131931451	RAD50	p.K722fs . . p.?661fs	c.2165delA c.*1791delA c.*351delA c.1982delA	1.6 %	569x	408407	pathogenic
chr17:7579472	TP53	p.P33R p.P72R	c.98C>G c.215C>G	62.8 %	471x	12351	drug response
chr3:14187449	XPC	p.Q939K .	c.2815C>A c.*2268C>A	100.0 %	358x	190215	drug response

Supplemental table S5 Summary and detailed list of copy number variations (CNV) detected. Copy number events with minimum 100 x coverage are reported. Gene column lists the name of genes (HGNC convention), CN column contains copy number observed and Depth column displays the coverage depth at the location (Loci column).

Sample	Duplication event	Deletion event
P_2_2	50	38
P_4_2	11	41
P_5_2	34	23

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_2_2

Duplication events

Gene	CN	Depth	Loci
AR	23	8885.61	chrX:66764936-67021739
SAMD9L	4	2354.88	chr7:92760606-92765406
MET	4	2541.89	chr7:116312530-116340461
WWP1, NBN, RUNX1T1, ESRP1	3	773.89	chr8:87424027-95718352
USP25	3	677.13	chr21:17135058-17250927
USP16, BACH1	3	622.68	chr21:30396985-30717926
UGT1A8, UGT1A8, UGT1A10, UGT1A9, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A1	3	1631.86	chr2:234526630-234669815
TRIO	3	1035.43	chr5:14488893-14508559

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Gene	CN	Depth	Loci
TPMT, LINC00340, LINC00340, LOC729177, HIST1H3B, MAS1L, DDR1, DDR1, MIR4640, CCHCR1, BAG6, APOM, APOM, RXRB, SLC39A7, DAXX, BAK1, FANCE, CDKN1A, CCND3, VEGFA	3	667.45	chr6:18130734-43752544
TP53, AURKB	3	747.42	chr17:7569431-8113380
TACC1, KAT6A, PRKDC, LYN, CHD7	3	845.74	chr8:38693706-61720993
SMC3, SHOC2	3	609.57	chr10:107516149-112771695
SBDS, WBSCR17, PTPN12, MAGI2, MAGI2, MAGI2-AS3, HGF, GRM3, CROT, ABCB1, AKAP9, CDK6, SAMD9L	3	852.03	chr7:66453201-92760606
RET, MSMB, CCDC6, ARID5B, ZNF365, PRF1, VCL	3	801.11	chr10:43596188-75863741
POLD1, PPP2R1A, U2AF2	3	541.51	chr19:50921151-56185586
PIK3CG, NRCAM	3	993.01	chr7:106507984-107880675
PIK3C2B, MDM4, SLC45A3, IKBKE, TRAF5, NEK2, H3F3A, H3F3AP4, FH, SDCCAG8, AKT3, AKT3	3	708.09	chr1:204411487-244006639
PDZRN3, EPHA3, TFG, CBLB, ZBTB20, GSK3B	3	756.18	chr3:73673908-119812427
PDGFRA, KIT, KDR	3	1001.09	chr4:55124858-55991426
NTRK1, SPTA1, SDHC, DDR2, RXRG, ABL2, CDC73, NR5A2, PIK3C2B	3	947.03	chr1:156841260-204410834
NRG1	3	1900.66	chr8:32474285-32585654
NF1	3	799.9	chr17:29482852-29694459
MUC16	3	2348.23	chr19:9045634-9074374
MUC16	3	1933.13	chr19:9082306-9091845
MUC16	3	1885.88	chr19:9074374-9077477
MET, POT1, GRM8, SMO, KIAA1549, BRAF, TAS2R38, KEL, EZH2, RHEB, MLL3, XRCC2	3	1016.92	chr7:116371597-152357921
MED12L, IGSF10, IGSF10	3	2232.99	chr3:151154340-151166969
LMTK2, TRRAP	3	847.3	chr7:97816136-98540700
KLF6, IL2RA, GATA3, CUBN	3	751.86	chr10:3827265-17171923
KIF5B	3	794.39	chr10:32304301-32324597
IRF8, CDK10, ZNF276, FANCA, FANCA, MC1R, AFG3L1P	3	701.45	chr16:85944248-90067125
IL7R, RICTOR, DAB2, PRKAA1, FGF10	3	752.27	chr5:35856957-44662704
IGSF10, PPM1L, MECOM, TERC, MYNN, PRKCI, PIK3CA, SOX2-OT, SOX2, ATP11B, DCUN1D1, KLHL6, PSMD2, LOC100131635, BCL6, BCL6, TP63, XXYLT1	3	812.75	chr3:151166969-194858563
FGFR1, TACC1	3	2064.56	chr8:38326257-38693706
FGFR1	3	1831.2	chr8:38271216-38325054
FGF3, POLD3, C11orf30, DLG2, EED, TYR, FAT3, MRE11A, CEP57, YAP1, BIRC3, BIRC2, ATM, C11orf93, TIMM8B, SDHD, TEX12, MLL, PHLDB1, CBL, CHEK1	3	929.76	chr11:69625476-125525357
FGF23, FGF6, CHD4, CHD4, SCARNA11, ETV6, ETV6, RNU6-19, CDKN1B, SLCO1B1, KRAS	3	831.93	chr12:4479679-26453527
FAM46C, HSD3B1, NOTCH2	3	967.58	chr1:118165678-120548231
EPHB1, PIK3CB, FOXL2, ZBTB38, ATR, SLC9A9, MED12L	3	757.89	chr3:134644499-151143671
DMRT1, JAK2, CD274, PDCD1LG2, PTPRD	3	822.87	chr9:845380-8636771
CSMD3	3	753.29	chr8:113236859-114327060
CHD7, PREX2, NCOA2, ATP6V0D2, WWP1	3	975.32	chr8:61728782-87424027
CDC27, HOXB13	3	937.46	chr17:45198151-46805510

Gene	CN	Depth	Loci
CARD11, PMS2, RAC1, DNAH11, IL6, TBX20, ELMO1, AMPH, INHBA, INHBA, INHBA-AS1, IKZF1, EGFR, EGFR, EGFR-AS1	3	952.54	chr7:2946153-55273441
BRD4	3	2007.24	chr19:15355595-15365237

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Deletion events

Gene	CN	Depth	Loci
TRIO	1	281.86	chr5:14508559-14532057
TMEM127, RANBP2, ACOXL, BCL2L11	1	645.27	chr2:96930947-111921948
TERT, CLPTM1L	1	198.88	chr5:1293612-1342831
SPEN, EPHA2, SDHB, PADI6, PAX7, CDA, ARID1A	1	414.36	chr1:16265966-27099126
SMC1A, AMER1	1	768.52	chrX:53423100-63406025
SLC7A8, BCL2L2, BCL2L2-PABPN1, NFKBIA, NKX2-1, NKX2-8, FOXA1, SSTR1	1	289.82	chr14:23623318-38678682
SETBP1, SLC14A1, SMAD2, SMAD7, SMAD4	1	472.58	chr18:42643417-48581143
ROS1, DCBLD1, GOPC, GOPC, LAMA2, MAP7	1	509.73	chr6:117650568-136665275
PPP2R3B, CRLF2	1	779.23	chrX:320389-1331677
PDZRN3	1	295.85	chr3:73651338-73673908
NRG1, ZNF703	1	111.77	chr8:32621847-37556030
NOTCH3, BRD4	1	254.46	chr19:15271334-15355595
NKX3-1	1	364.79	chr8:23526272-23540539
MIR548AN, FGF14, BIVM-ERCC5, ERCC5, IRS2	1	540.33	chr13:100041547-110434960
IRF4, EXOC2	1	482.46	chr6:393040-637979
IL6ST, MAP3K1, SETD9, MIER3, MIER3, PDE4D, PIK3R1, DHFR, MSH3, MSH3, CHD1, APC, SRP19, SNCAIP, SNCAIP, MGC32805	1	514.04	chr5:55259036-121799417
IL6ST	1	662.37	chr5:55236788-55248242
IL2RG, MED12, BCYRN1, ZMYM3, BCYRN1, TAF1, BCYRN1, ATRX	1	434.31	chrX:70327458-76778023
IL2RB, RAC2, SOX10, PLA2G6, EP300	1	225.1	chr22:37515070-41513172
GAS6-AS1, GAS6, GAS6	1	299.42	chr13:114524894-114566870
FOXO1, RB1	1	106.98	chr13:41135019-48878197
FGFR1	1	551.06	chr8:38325154-38326257
FANCB, ZRSR2, NHS, DMD, BCOR	1	524.83	chrX:9751283-39933464
ERG, MX2, TMPRSS2	1	367.67	chr21:39870200-42866643
EPHA7, PRDM1	1	553.28	chr6:93952980-106546871
DSE, RFX6, ROS1	1	627.33	chr6:116575267-117645547
CYP2D6	1	186.53	chr22:42524386-43500291

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Gene	CN	Depth	Loci
CYLD, LOC643714, NUP93, CBFB, CTCF, CDH1, ZFHX3, PLCG2	1	610.43	chr16:50783491-81991771
CUL3, SP140, PSMD1	1	210.79	chr2:225434257-231921937
CRKL, LZTR1, MAPK1, BCR, SMARCB1	1	505.6	chr22:21272097-24176566
CDK12, ERBB2	1	335.85	chr17:37687506-37856717
BTK, PAK3, STAG2, BCORL1, GPC3, PHF6, ZIC3, G6PD, G6PD, IKBKG	1	342.11	chrX:100604718-153775114
BRD4, TPM4, BABAM1, JAK3, PIK3R2, MEF2B, MEF2BNB-MEF2B	1	313.9	chr19:15383487-19257943
BMPR1A, PTEN, ACTA2, FAS	1	377.05	chr10:88683445-90750838
BCOR, DDX3X, KDM6A, RBM10, ARAF, GATA1, KDM5C, SMC1A	1	438.01	chrX:39933464-53423100
ATRX	1	459.02	chrX:76778584-76939892
ATRX	1	280.67	chrX:76939892-77041477
IL6ST	0	248.91	chr5:55248242-55256529

Sample P_4_2

Duplication events

Gene	CN	Depth	Loci
ZFHX3, PLCG2, IRF8, CDK10, ZNF276, FANCA, FANCA	3	534.41	chr16:72845355-89815230
SUFU, NT5C2	3	446.59	chr10:104389962-104899389
SMAD3, AGPHD1, CHRNA3, NTRK3	3	516.6	chr15:67430211-88727397
RB1	3	426.07	chr13:48881579-73916817
PTEN	3	586.09	chr10:89653728-89725356
IRF2, FAT1	3	680.65	chr4:185309785-187538926
GEN1	3	326.65	chr2:17953755-17961953
GAS6-AS1, GAS6, GAS6	3	360.06	chr13:114523693-114566870
FGF9, TNFRSF19	3	522.15	chr13:22246039-24293953
CYLD, LOC643714, NUP93	3	572.54	chr16:50783491-56832608

Gene	CN	Depth	Loci
CHEK2, KREMEN1	3	529.32	chr22:29083739-29534936

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Deletion events

Gene	CN	Depth	Loci
UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A8, UGT1A10, UGT1A9, UGT1A7, UGT1A6, UGT1A5, UGT1A4, UGT1A3, UGT1A1	1	1068.58	chr2:234621920-234669815
TSHR	1	1129.79	chr14:81609170-81610810
TACC3	1	1368.54	chr4:1739020-1740219
SLC9A9, MED12L, MED12L, IGSF10, IGSF10, PPM1L, MECOM, TERC, MYNN, PRKCI	1	831.28	chr3:143566856-169952968
ROS1	1	1172.83	chr6:117646251-117650467
RARA	1	1213.58	chr17:38498978-38504761
RARA	1	1054.07	chr17:38487632-38497482
PCBP1	1	419.92	chr2:70314740-70315770
NTRK1	1	1587.23	chr1:156843893-156844968
NRG1	1	991.16	chr8:32474383-32585654
MYC, PSCA, RECQL4	1	306.92	chr8:128753222-145742885
MYC	1	570.19	chr8:128748664-128753222
MUC16	1	1236.99	chr19:9045634-9091243
MLL	1	1177.95	chr11:118373176-118378188
MET, POT1, GRM8, SMO	1	516.11	chr7:116339966-128852416
MET	1	1173.51	chr7:116312530-116339966
KIAA1549, BRAF	1	622.32	chr7:138596971-140481315
FGFR3	1	1103.42	chr4:1808412-1809605
FGFR2	1	1101.59	chr10:123239440-123243186
FGFR1, TACC1	1	1227.22	chr8:38306840-38693604
FGFR1	1	846.4	chr8:38300272-38306840
FGFR1	1	1357.28	chr8:38287180-38300272
FAT3	1	1018.43	chr11:92085341-92535107
CSMD3, RAD21, EXT1, FBXO32, LOC727677	1	468.07	chr8:113236859-128539549
CDK8	1	131.05	chr13:24293953-26829062
CDK6, SAMD9L	1	987.57	chr7:92462466-92765306
CD74	1	1210.65	chr5:149782254-149784270
C2orf44	1	1072.23	chr2:24260523-24262485
BRD4	1	1309.94	chr19:15355595-15365237
BRCA1	1	1059.84	chr17:41234286-41246705
BRAF, TAS2R38, KEL, EZH2, RHEB, MLL3, XRCC2, SHH	1	564.79	chr7:140493180-155604946
BRAF	1	969.9	chr7:140481315-140493180
BCR	1	1312.06	chr22:23631535-23634476

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Gene	CN	Depth	Loci
ALK	1	1214.98	chr2:29456870-29462840
AKT1	1	365.53	chr14:105238840-105259147
ABL1	1	1060.23	chr9:133710682-133723511
ZIC3, G6PD, G6PD, IKBKG	0	195.3	chrX:136648953-153775114
SRSF2, SRSF2, MIR636, SRSF2, MIR636, MFSD11, SRSF2, MFSD11	0	193.13	chr17:74732300-74733267
RARA	0	722.8	chr17:38497482-38498978
PPP2R3B, CRLF2, FANCB, ZRSR2, NHS, DMD, BCOR, DDX3X, KDM6A, RBM10, ARAF, GATA1, KDM5C, SMC1A, AMER1, IL2RG, MED12, BCYRN1, ZMYM3, BCYRN1, TAF1, BCYRN1, ATRX	0	301.89	chrX:320389-77041477
BTK	0	274.23	chrX:100604718-100645757

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Sample P_5_2

Duplication events

Gene	CN	Depth	Loci
AMER1, AR	4	1540.87	chrX:63412616-67021739
TRRAP, PIK3CG	3	1060.72	chr7:98602642-106515196
TACC3	3	848.7	chr4:1725009-1725732
TACC1, KAT6A	3	1659.56	chr8:38326357-41790966
STK11	3	452.13	chr19:1206777-1218552
SMAD4	3	733.03	chr18:48573290-48593692
SLC9A9, MED12L, MED12L, IGSF10, IGSF10	3	1385.54	chr3:143566856-151156520
SAMD9L	3	1730.32	chr7:92760606-92765306
ROS1	3	1941.94	chr6:117646151-117650366
RNU6-28, TP53BP1, B2M	3	697.1	chr15:43784304-45008533
RANBP2	3	1312.66	chr2:109379558-109384877
PIK3R1	3	777.07	chr5:67522390-67593554

Gene	CN	Depth	Loci
PCBP1	3	769.22	chr2:67241330-70316080
NSD1	3	1492.59	chr5:176561987-176563147
NRG1	3	1489.48	chr8:32474187-32599464
MYC	3	926.6	chr8:128748854-129192175
MUC16	3	2016.35	chr19:9045735-9074474
MET	3	1848.6	chr7:116312530-116340461
KAT6A, PRKDC	3	995.37	chr8:41844806-48686725
KAT6A	3	1040.64	chr8:41790966-41801431
IRF2, FAT1	3	1091.6	chr4:185309878-187631098
IL6ST, MAP3K1, SETD9, MIER3, MIER3, PDE4D	3	719.85	chr5:44388807-58408007
IGSF10	3	1935.37	chr3:151162887-151167169
HSP90AA1	3	762.92	chr14:102548103-102568206
FGFR1	3	1762.99	chr8:38286955-38325054
FANCD2, FANCD2OS, VHL, PPARG	3	811.64	chr3:10143003-12421566
EGFR	3	1112.38	chr7:55259359-55273441
DHFR, MSH3, MSH3	3	535.17	chr5:79950488-80109701
CDK12	3	1117.37	chr17:37618305-37627089
CD74, GABRA6, NPM1	3	710.64	chr5:149782155-170837452
CARD11, PMS2, RAC1, DNAH11, IL6, TBX20	3	676.36	chr7:2946153-35242521
APC	3	1345.96	chr5:112170864-112178936
ALK	3	1732.74	chr2:29456474-29462840
ABL1	3	1378.78	chr9:133711260-133738535

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Deletion events

Gene	CN	Depth	Loci
ZBTB20, GSK3B	1	354.23	chr3:114362858-119721099
WNK2	1	213.56	chr9:96009876-96026189
RARA, TOP2A, SMARCE1, STAT3	1	492.26	chr17:38501470-40500580
PLCG2, IRF8, CDK10, ZNF276, FANCA, FANCA, MC1R, AFG3L1P	1	456.93	chr16:81892554-90067125
PIK3CD	1	130.99	chr1:9780233-9781424
PAX7	1	308.53	chr1:18958044-19071475
NOTCH3, BRD4	1	173.82	chr19:15303084-15355194
NKX2-1, NKX2-8, FOXA1	1	158.43	chr14:36738455-38061141
MLL2, PRPF40B, PRPF40B, FMNL3, ACVRL1	1	409.16	chr12:49448786-52308996
MLL2	1	503.17	chr12:49422933-49436085
LINC00299, MYCNOS, MYCNOS, MYCN, MYCN	1	342.85	chr2:8178544-16083046
KIF5B, RET, MSMB, CCDC6, ARID5B, ZNF365, PRF1	1	476.97	chr10:32304301-72360688
JAK1, FUBP1	1	357	chr1:65348948-78426223
IRF4	1	151.88	chr6:393040-393480
HNF1B, CISD3, PCGF2, PCGF2	1	336.14	chr17:36097944-36896819

Gene	CN	Depth	Loci
FLT1, HSPH1, BRCA2	1	482.5	chr13:29041610-32944616
CDK12, ERBB2, ERBB2, MIR4728, RARA	1	466.49	chr17:37627089-38487436
CDC27	1	572.95	chr17:45198151-45266714
RBM10	0	352.43	chrX:47040977-47046155
RBM10	0	162.01	chrX:47030239-47040977
MED12, BCYRN1, ZMYM3	0	346.52	chrX:70349294-70473128
CYP2A6	0	213.17	chr19:41349571-41356359
ATRX	0	304.42	chrX:76829565-76972883

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Supplemental table S6 Target specificity. High quality reads (HQ). On-target rates (reads mapped to target +/- 100bp extension) allow evaluating the panels-workflow efficiency.

Read category	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
Total reads	6,567,716	4,675,504	3,371,650	4,159,126	3,633,368	5,541,610	4,952,776	8,869,582
HQ Reads	5,799,816 (88.3%)	4,045,716 (86.5%)	2,922,420 (86.7%)	3,678,052 (88.4%)	3,102,264 (85.4%)	4,596,942 (83.0%)	3,792,754 (76.6%)	7,229,128 (81.5%)
Mapped Reads	5,785,280 (99.75%)	4,037,910 (99.81%)	2,911,166 (99.61%)	3,671,479 (99.82%)	3,095,259 (99.77%)	4,549,477 (98.97%)	3,627,672 (95.65%)	7,087,137 (98.04%)
On target	5,615,324 (97.06%)	3,977,703 (98.51%)	2,890,628 (99.29%)	3,582,728 (97.58%)	3,025,766 (97.75%)	4,388,872 (96.47%)	2,049,305 (56.49%)	3,343,847 (47.18%)

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Read category	P_2_2	P_4_2	P_5_2
Total reads	44,930,192	49,604,726	38,999,996
HQ Reads	43,274,822 (96.3%)	47,668,180 (96.1%)	37,476,322 (96.1%)
Mapped Reads	43,173,135 (99.77%)	47,387,448 (99.41%)	37,423,253 (99.86%)
On target	34,727,250 (82.75%)	36,378,255 (79.05%)	30,122,462 (82.57%)

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Supplemental table S7 Coverage distribution of the target region (29,208 bp). Avg average coverage. P_2_2, P_4_2 and P_5_2 are shown excluding duplicated fragments.

	Target coverage		Coverage uniformity		% of target covered with at least		
Sample	Total bases	Average (x)	0.2x avg	%	2x	500x	1000x
P_1	375.76 MB	12864.86	2573x	96	100.0	100.0	99.0
P_2	262.68 MB	8993.27	1799x	95	100.0	99.8	98.9
P_3	182.47 MB	6247.12	1249x	75	99.8	93.8	80.4
P_4	240.12 MB	8221.15	1644x	96	100.0	99.3	98.9
P_5	203.51 MB	6967.72	1394x	85	100.0	94.9	88.3
P_6	298.04 MB	10204.15	2041x	96	100.0	99.5	99.2
P_7	136.48 MB	4672.64	935x	91	100.0	96.5	90.4
P_8	218.53 MB	7482.00	1496x	98	100.0	99.9	98.9

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

	Target coverage		% of target covered with at least				
Sample	Total bases	Average (x)	2x	50x	100x	300x	500x
P_2_2	3.21 GB	1104.73	99.9	97.9	96.2	87.8	76.2
P_4_2	2.08 GB	714.70	99.9	98.0	96.2	86.7	69.9
P_5_2	2.63 GB	904.22	99.9	98.1	96.2	85.6	70.0

Oncology Research and Treatment

Research Article

Oncol Res Treat :
DOI: 10.1159/000490618

Supplemental Material

Further References

1 Li H, Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754–60.

2 Wilm A, Aw PPK, Bertrand D, Yeo GHT, Ong SH, Wong CH, et al.: LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res 2012;40:11189–201.

3 Rimmer A, Phan H, Mathieson I, Iqbal Z, Twigg SRF, WGS500 Consortium AOM, et al.: Integrating mapping-, assembly- and haplotype-based approaches for calling variants in clinical sequencing applications. Nat Genet 2014;46:912–8.

4 Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, et al.: Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci 2005;102:16368–16373.

5 Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al.: COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45:D777–D783.

6 Chang MT, Asthana S, Gao SP, Lee BH, Chapman JS, Kandoth C, et al.: Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. Nat Biotechnol 2015;34:155–163.

7 Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V, Korbel JO: DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinformatics 2012;28:i333–i339.

8 Barber LJ, Mansukhani S, Herman B, Arezi B, Gerlinger M: Ultra-sensitive Cancer Liquid Biopsy Analysis with the Agilent SureSelectXT HS Target Enrichment Workflow. 2017.Available from: https://www.agilent.com/cs/library/applications/5991-8464EN.PDF