Supplemental Table Legends

Supplementary Table 1. The 435 genes covered on the CANCERPLEX gene panel.

Supplementary Table 2. SNP detection. Pools of following reference samples from the Coriell Institute were sequenced in triplicates. Pools A-C: 20% NA19059/JPT59, 20% NA18595/CHB95, 20% NA19648/MXL48. 20% NA19701/ASW01, 20% NA19703/ASW03. Pools D-F: 20% NA18507/YRI07, 20% NA18595/CHB95, 20% NA19648/MXL48, 20% NA19701/ASW01, 20% NA19703/ASW03. The libraries were prepared in triplicates (A-C and D-F) and underwent CANCERPLEX testing. The true positive (TP), false positive (FP), and false negative rates were calculated. The sensitivity, positive predictive value (PPV) and accuracy were determined.

Supplementary Table 3. Indel detection performance Pools of following reference samples from Coriell Institute were sequenced in triplicates. Pools A-C: 20% NA19059/JPT59, 20% NA18595/CHB95, 20% NA19648/MXL48. 20% NA19701/ASW01, 20% NA19703/ASW03. Pools D-F: 20% NA18507/YRI07, 20% NA18595/CHB95, 20% NA19648/MXL48, 20% NA19701/ASW01, 20% NA19703/ASW03. The libraries were prepared in triplicates (A-C and D-F) and underwent CANCERPLEX testing. The true positive (TP), false positive (FP), and false negative rates were calculated. The sensitivity, positive predictive value (PPV) and accuracy were determined.

Supplementary Table 4. Concordance with Sanger sequencing for detecting SNVs. The concordance between Sanger sequencing and CANCERPLEX testing is excellent with a mean sensitivity of 100 and a positive predictive value (PPV) of 98.7. Six cell lines that had been previously validated using Sanger sequencing underwent CANCERPLEX testing and the true positive (TP), false positive (FP), and false negative rates were calculated. The sensitivity and positive PPV were determined.

Supplementary Table 5. Concordance with Sanger sequencing for detecting indels. CANCERPLEX testing accurately identified indels in all sixteen cell lines/FFPE tumor specimens that were tested. The chromosome, chromosome position, variant ID, and location, size and sequence of each gene variant is provided. The concordance between methods was determined.

Supplementary Table 6. Identification of low-allelic fraction variants. Reference DNA (Horizon) was used to quantify allelic fraction of known variants and compared to the expectation (ddPCR). Note that the standard deviation was found to be below 0.2.

Supplemental Table Legends (cont.)

Supplementary Table 8. SNV/indel limit of detection (LOD) as a function of sequencing depth. For SNVs with an allelic fraction of 0.1, high sensitivity detection (>98%) is achieved with 200X coverage. A series of diluted samples were sequenced in consecutive windows of sequencing depth, until 500X coverage was reached.

Supplementary Table 9. Detection of copy-number and translocation events is concordant with orthogonal methods. Detection of select gene amplifications and the EML4-ALK translocation using CANCERPLEX was highly concordant with results obtained by FISH, IHC, PCR or non-Illumina NGS. A series of cell lines and clinical FFPE specimens with known ERBB2, MYC or FGFR2 amplifications and several cell lines harboring the EML4-ALK translocation underwent CANCERPLEX testing. The concordance between methods was determined.

Supplementary Table 10. MSI detection using CANCERPLEX is 100% concordant with expected results. The MSS-status of fifteen clinical FFPE samples was determined using PCR with Bethesda markers and IHC to confirm the expression of MMR proteins. CANCERPLEX testing was performed on the fifteen characterized FFPE samples and the number of indels within the ROI was calculated. The concordance between methods was determined. Tumor mutation burden correlates with MSI-status.

Supplementary Table 11. Viral detection using CANCERPLEX is 100% concordant with expected results. The EBV/HPV-18 status of 6 FFPE samples and 4 cell lines was determined using PCR to amplify EBV/EPV-18 specific sequences. CANCERPLEX testing was performed on the 10 characterized samples and the percentage of total number of reads mapped to the viral genomes was calculated. The concordance between methods was determined.

Supplementary Table 12. **LOD for *EML4-ALK* translocation as a function of sequencing depth** The minimum tumor content required to detect the translocation event in 100% cases was ≥20%, with coverage of about 300x (at least 4 split reads). For 10% tumor purity, accuracy was 93%. The H2228 cell line, which expresses the *EML4-ALK* gene fusion, was diluted to generate a series of samples with an allelic fraction ranging from 0.1 to 0.5. Each sample was sequenced up to six times and the number of chimeric reads, mean coverage and coverage of intron 19 of the ALK gene was determined.

Supplementary Table 13. Description of cell lines, tumor FFPE and DNA samples used to evaluate the performance of CANCERPLEX to detect all the different genomic aberrations.

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Supplemental Table Legends (cont.)

Supplementary Table 14. Selection of CANCERPLEX targets. The frequency of a given gene being mutated across each of 31 different cancer types.

Supplementary Table 15. CANCERPLEX attains high sequencing uniformity and depth of coverage across regions of interest (ROI). Ten clinical FFPE tumor specimens underwent testing and key parameters were determined, including depth of sequencing (mean coverage depth), coverage across ROI (uniformity of coverage), percentage of PCR duplicates, percentage of sequenced bases that were mapped to ROI (sequence mapped to ROI), ROI with no sequenced bases (zero coverage), target coverage at 100x and 500x.

Supplemental Table 1.

ABL1	BCL11A	CD276	CIC	EPHA7	FH	HSP90AA1	KLF5	MDM2	NFKBIA	PIK3C2B	PTEN	RPS6KA2	SPEN	TP53
ABL2	BCL11B	CD40	CREBBP	EPHB1	FLCN	HSP90AB1	KLF6	MDM4	NKX2-1	PIK3C2G	PTGS2	RPS6KB1	SPOP	TP63
ACVR1B	BCL2	CD79A	CRKL	EPHB6	FLI1	ICK	KLHL6	MED12	NOTCH1	PIK3CA	PTK2	RPTOR	SRC	TPR
ACVR2A	BCL2L1	CD79B	CRTC1	ERBB2	FLT1	IDH1	KMT2A	MEF2B	NOTCH2	PIK3CB	PTPN11	RUNX1	SSTR1	TPX2
ADGRA2	BCL6	CD80	CSF1R	ERBB3	FLT3	IDH2	KMT2C	MEN1	NOTCH3	PIK3CD	PTPRD	RUNX1T1	SSTR2	TRIM33
AKT1	BCL9	CD86	CTCF	ERBB4	FLT4	IGF1R	KMT2D	MET	NOTCH4	PIK3CG	PTPRT	RYR1	SSTR3	TRRAP
AKT2	BCOR	CDC73	CTLA4	ERG	FOXA1	IGF2	KRAS	MITF	NRAS	PIK3R1	PXDNL	SCN8A	SSTR5	TSC1
AKT3	BCORL1	CDH1	CTNNA1	ERRFI1	FOXO1	IGF2R	LIG3	MLH1	NRG1	PIK3R2	QKI	SDHA	STAG2	TSC2
ALK	BIRC2	CDH11	CTNNA2	ESR1	FOXP1	IKBKB	LMO1	MN1	NSD1	PIK3R3	RAC1	SDHB	STAT3	TSHR
AMER1	BIRC3	CDH2	CTNNB1	ETS1	FRS2	IKBKE	LPP	MRE11A	NTRK1	PLAG1	RAD17	SDHC	STAT4	TYMS
APC	BLM	CDH5	CUL3	ETV1	GATA2	IKZF1	LYN	MSH2	NTRK2	PLCG1	RAD50	SDHD	STK11	U2AF1
AR	BMPR1A	CDK1	CUX1	ETV4	GATA3	IL2RG	LZTR1	MSH6	NTRK3	PLCG2	RAD51	SETD2	STK36	UBR5
ARAF	BRAF	CDK12	CYLD	ETV5	GATA4	IL4R	MAGI2	MST1R	NUP214	PLK1	RAD51C	SETDB1	SUFU	USP9X
ARID1A	BRCA1	CDK2	DAXX	ETV6	GATA6	INHBA	MAGI3	MTOR	PAK3	PMS1	RAD51D	SF3B1	TAF1	VHL
ARID1B	BRCA2	CDK4	DCLRE1C	EZH2	GLI1	INPP4B	MALT1	MTR	PALB2	PMS2	RAF1	SGK1	TCF7L1	VTCN1
ARID2	BRD4	CDK5	DDR1	FANCA	GNA11	IRF2	MAML2	MTRR	PARK2	POLD1	RALGDS	SH2D1A	TCF7L2	WEE1
ARID5B	BRIP1	CDK6	DDR2	FANCB	GNA13	IRF4	MAP2K1	MUTYH	PARP1	POLE	RASA1	SLIT2	TEK	WISP3
ASXL1	BTG1	CDK7	DDX5	FANCC	GNAQ	IRS2	MAP2K2	MYC	PAX3	POLE4	RB1	SLIT3	TERT	WRN
ATM	BTK	CDK8	DICER1	FANCD2	GNAS	JAK1	MAP2K4	MYCL	PAX7	POLR2A	RBM10	SMAD2	TET2	WT1
ATR	BUB1B	CDK9	DNMT3A	FANCE	GRIN2A	JAK2	MAP3K1	MYCN	PAX8	PPARG	REL	SMAD3	TFE3	XPC
ATRX	CARD11	CDKN1A	DOT1L	FANCF	GRM3	JUN	MAP3K7	MYD88	PBRM1	PPP2R1A	RET	SMAD4	TGFB2	XPO1
AURKA	CBFB	CDKN1B	EGFR	FANCG	GSK3B	KDM5A	MAPK1	NBN	PBX1	PPP6C	RHEB	SMARCA4	TGFBR2	XRCC2
AURKB	CBL	CDKN2A	EML4	FANCL	HDAC1	KDM5C	MAPK3	NCOA1	PDCD1	PRDM1	RHOA	SMARCB1	TMPRSS2	XRCC5
AURKC	CCND1	CDKN2B	EMSY	FAT1	HDAC2	KDM6A	MAPK4	NCOA2	PDCD1LG2	PREX2	RICTOR	SMO	TNFAIP3	XRCC6
AXIN1	CCND2	CDKN2C	EP300	FBXW7	HGF	KDR	MAPK6	NF1	PDGFRA	PRKAR1A	RNASEL	SOS1	TNFRSF14	ZFHX3
AXIN2	CCND3	CHD2	EPCAM	FGFR1	HIF1A	KEAP1	MAPK7	NF2	PDGFRB	PRKCI	RNF2	SOX10	TNFRSF4	ZNF217
AXL	CCNE1	CHD4	EPHA2	FGFR2	HNF1A	KIAA1804	MAPK8	NFE2L2	PDK1	PRKDC	RNF43	SOX11	TNK2	ZNF384
BAP1	CCR4	CHEK1	EPHA3	FGFR3	HNF4A	KISS1R	MBD1	NFKB1	PGAP3	PRSS1	ROS1	SOX2	TOP1	ZNF521
BARD1	CD274	CHEK2	EPHA5	FGFR4	HRAS	KIT	MCL1	NFKB2	PIK3C2A	PTCH1	RPS6	SOX9	TOP2A	ZNF703

Supplemental Table 2.

Pool, mean coverage	Mutation allelic fraction	True positives (TP)	False positives (FP)	False negatives (FN)	Sensitivity (%)	PPV (%)	Accuracy (%)
	0.1	122	8	7	93.85	94.6	99.9995
	0.2	98	2	7	98	93.3	99.9997
	0.3	88	0	7	100	92.6	99.9998
A	0.4	67	2	7	97.10	90.5	99.9997
341x	0.5	60	0	7	100	89.5	99.9998
	0.6	49	0	7	100	87.5	99.9998
	0.7	38	0	7	100	8.44	99.9998
	0.8	68	0	7	100	90.6	99.9998
	0.1	127	3	4	97.69	96.9	99.9998
	0.2	100	0	4	100	96.1	99.9999
	0.3	88	0	4	100	95.6	99.9999
B	0.4	68	1	4	98.55	94.4	99.9998
486x	0.5	60	0	4	100	93.6	99.9999
	0.6	49	0	4	100	92.4	99.9999
	0.7	38	0	4	100	90.4	99.9999
	0.8	68	0	4	100	94.4	99.9999
	0.1	126	4	6	96.92	95.4	99.9996
	0.2	100	0	6	100	94.3	99.9998
	0.3	88	0	6	100	93.6	99.9998
C	0.4	68	1	6	98.55	91.9	99.9998
492x	0.5	60	0	6	100	90.9	99.9998
	0.6	49	0	6	100	89	99.9998
	0.7	38	0	6	100	86.3	99.9998
	0.8	68	0	6	100	91.9	99.9998
	0.1	101	0	1	100	99	99.9999
	0.2	61	0	1	100	98.3	99.9999
	0.3	63	0	1	100	98.4	99.9999
D	0.4	44	0	1	100	97.8	99.9999
599x	0.5	26	0	1	100	96.3	99.9999
	0.6	33	0	1	100	97	99.9999
	0.7	29	0	1	100	96.7	99.9999
	0.8	43	0	1	100	97.7	99.9999
	0.1	88	1	1	98.88	98.9	99.9999
	0.2	58	0	1	100	98.3	99.9999
	0.3	53	0	1	100	98.1	99.9999
E	0.4	40	0	1	100	97.6	99.9999
539x	0.5	26	0	1	100	96.3	99.9999
	0.6	31	0	1	100	96.9	99.9999
	0.7	25	0	1	100	96.1	99.9999
	0.8	36	0	1	100	97.3	99.9999
	0.1	124	4	4	96.88	96.9	99.9997
	0.2	81	1	4	98.78	95.3	99.9998
	0.3	77	1	4	98.72	95	99.9998
F	0.4	65	0	4	100	94.2	99.9999
543x	0.5	34	0	4	100	89.5	99.9999
	0.6	43	0	4	100	91.5	99.9999
	0.7	36	0	4	100	90	99.9999
	0.8	57	0	4	100	93.4	99.9999
				Mean	**99.46**	**94.1**	**99.9998**
				SD	1.21	3.5	0.00011

Supplemental Table 3.

Pool, mean coverage	Mutation allelic fraction	True positives (TP)	False positives (FP)	False negatives (FN)	Sensitivity (%)	PPV (%)	Accuracy (%)
A	0.1	11	0	0	100	100	100
341x	0.2	11	0	0	100	100	100
B	0.1	11	0	0	100	100	100
486x	0.2	11	0	0	100	100	100
C	0.1	11	0	0	100	100	100
492x	0.2	11	0	0	100	100	100
D	0.1	5	0	0	100	100	100
599x	0.2	5	0	0	100	100	100
E	0.1	5	0	0	100	100	100
539x	0.2	5	0	0	100	100	100
F	0.1	5	0	0	100	100	100
543x	0.2	5	0	0	100	100	100
				Mean	100	100	100

Supplemental Table 4.

Sample	True positives (TP)	False positives (FP)	False negatives (FN)	Sensitivity (%)	PPV (%)
HCC1143/HCC1143	4	0	0	100	100
HCC1954/PC	12	1	0	100	92.3
1EU2MALD/1EU	5	0	0	100	100
9N8G9AU1/9N8G	4	0	0	100	100
LZZ5UACW/LZZ	7	0	0	100	100
LQHWEAF9/LQH	5	0	0	100	100
Mean				100	98.7

Supplemental Table 5.

Sample	Chromosome	Position	Variant ID	Variant	Correctly detected by CANCERPLEX
P.T.610/610	17	37880997	rs397516979	ERBB2 c.2236_2237insTGT	yes
P.T.613/613	7	55242465	rs727504233	EGFR c.2236_2250delGAATTAAGAGAAGCA	yes
Clinical sample 28	7	55242464	rs121913421	EGFR c.2235_2249delGGAATTAAGAGAAGC	yes
Clinical sample 29	7	55242464	rs121913421	EGFR c.2235_2249delGGAATTAAGAGAAGC	yes
Clinical sample 30	7	55242465	rs727504233	EGFR c.2236_2250delGAATTAAGAGAAGCA	yes
Clinical sample 31	7	55242465	rs727504233	EGFR c.2236_2250delGAATTAAGAGAAGCA	yes
Clinical sample 32	7	55242466	rs121913425	EGFRc.2237_2251delAATTAAGAGAAGCAA	yes
Clinical sample 33	7	55242465	rs121913436	EGFRc.2236_2244delGAATTAAGA	yes
Clinical sample 34	7	55242464	rs121913421	EGFR c.2235_2249delGGAATTAAGAGAAGC	yes
Clinical sample 35	17	37880995	N/A	ERBB2 c.2324_2325insATACGTGATGGA	yes
Clinical sample 36	11	108205734	N/A	ATM c.8050dupC	yes
Clinical sample 37	7	55242469	rs121913438	EGFR c.2240_2257delTAAGAGAAGCAACATCTC	yes
Clinical sample 38	12	25398283	N/A	KRAS c.30_35dupAGCTGG	yes
Clinical sample 6	7	55249002	rs727503012	EGFR c.2300_2301insCAGCGTGGA	yes
GM14094	17	41246333	rs80359874	BRCA1 c.1175_1214delTGTTAGGTTCTGATGACTCACATGATGGGGAGTCTGAATC	yes
GM14096	17	41244056	rs80357877;rs80357910	BRCA1 c.3481_3491delGAAGATACTAG	yes

Supplemental Table 6.

Reference variant	Coverage	Number of reads with variant	Reference AF	Predicted AF	SD
EGFR L858R	2728x	135	5.0%	4.95%	0.04%
EGFR del E746-A750	1774x	60	5.0%	3.38%	1.14%
EGFR T790M	1884x	89	5.0%	4.72%	0.20%
EGFR V769-D770insASV	589x	21	5.0%	3.40%	1.13%
KRAS G12D	1439x	60	6.3%	4.17%	1.51%
NRAS Q61K	1682x	99	6.3%	5.89%	0.29%
NRAS A59T	1719x	112	6.3%	6.52%	0.15%
PIK3CA E545K	2074x	131	6.3%	6.32%	0.01%

Supplemental Table 7.

Replicate 1	Replicate 2	Variants in run 1 (unique)	Variants in run 2 (unique)	Variants in both runs	Concordance (%)
Sample 1 - Run 1	Sample 1 - Run 2	24	14	265	93.3
Sample 1 - Run 3	Sample 1 - Run 4	22	20	267	97.7
Sample 1 - Run 5	Sample 1 - Run 6	16	24	263	92.9
				Mean	93

Replicate 1	Replicate 2	Variants in run 1 (unique)	Variants in run 2 (unique)	Variants in both runs	Concordance (%)
Sample 2 - Run 1	Sample 2 - Run 2	15	10	282	95.8
Sample 2 - Run 3	Sample 2 - Run 4	28	12	280	93.3
Sample 2 - Run 5	Sample 2 - Run 6	24	13	284	93.9
				Mean	93.63

Replicate 1	Replicate 2	Variants in run 1 (unique)	Variants in run 2 (unique)	Variants in both runs	Concordance (%)
Sample 3 - Run 1	Sample 3 - Run 2	19	22	281	93.2
Sample 3 - Run 3	Sample 3 - Run 4	15	21	285	94.1
Sample 3 - Run 5	Sample 3 - Run 6	18	21	285	93.6
				Mean	93.63

Day 1	Day 2	Variants in run 1 (unique)	Variants in run 2 (unique)	Variants in both runs	Concordance (%)
Sample 1 - Run 1	Sample 1 - Run 2	21	23	268	92.4
Sample 2 - Run 1	Sample 2 - Run 2	11	21	286	94.7
Sample 3 - Run 1	Sample 3 - Run 2	16	23	277	93.4
				Mean	93.5

Operator 1	Operator 2	Variants in run 1 (unique)	Variants in run 2 (unique)	Variants in both runs	Concordance (%)
Sample 1 - Run 1	Sample 1 - Run 2	20	25	269	92.3
Sample 2 - Run 1	Sample 2 - Run 2	15	17	280	94.6
Sample 3 - Run 1	Sample 3 - Run 2	20	22	278	93
				Mean	93.3

Supplemental Table 8.

Mutation allelic fraction	X≤50	50<X≤100	100<X≤150	150<X≤200	200<X≤300	300<X≤500
0.1	5	22.1	40.9	51.4	98.5	100
0.2	7.5	41.4	88.1	98.3	100	100
0.3	6.7	81.5	96.9	100	100	100
0.4	6	86.7	100	100	100	100
0.5	50	100	100	100	100	100
0.6	50	100	100	100	100	100
0.7	100	100	100	100	100	100
0.8	100	100	100	100	100	100
0.9	100	100	100	100	100	100
1	100	100	100	100	100	100

Supplemental Table 9.

Sample	Expected aberration	Validation by	Detected by CANCERPLEX
5360T/536	ERBB2 gain	FISH/IHC	yes
5943T/5943T	ERBB2 gain	FISH/IHC	yes
7259TY/7259TY	ERBB2 gain	FISH/IHC	yes
S12-39759/397	ERBB2 gain	FISH/IHC	yes
S0357971K/1K.E1	ERBB2 gain	FISH	no
HCC1954	ERBB2 gain	qPCR	yes
NCI-N87	ERBB2 gain	FISH	yes
SNU-16	FGFR2 gain	qPCR	yes
Clinical FFPE sample 1	ERBB2 gain	FISH/IHC	yes
Clinical FFPE sample 2	ERBB2 gain	FISH	yes
Clinical FFPE sample 3	ERBB2 gain	FISH	yes
Clinical FFPE sample 4	MYC gain	FISH/IHC	yes
Clinical FFPE sample 5	MYC gain	FISH	yes
NCI-H2228	CDKN2A/B loss	qPCR	yes
Hela	STK11 loss	qPCR	yes
NCI-N87	SMAD4 loss	qPCR	yes
S10-57398/510	ALK-EML4	FISH	yes
2898T/289	ALK-EML4	FISH	yes
NCI-H2228	ALK-EML4	FISH	yes
Clinical sample 39	EZR-ROS1	NGS	yes
Clinical sample 40	RET-ERC1	NGS	yes
Clinical sample 41	ALK-EML4	FISH	yes
Clinical sample 42	ALK-EML4	FISH	yes

Supplemental Table 10.

Sample	MSI status Detected by CANCERPLEX (number of indels)	Validation by IHC	Validation by PCR	Mutation burden
Clinical FFPE sample 7	MSI-H (90)	MSI-H	MSI-H	40
Clinical FFPE sample 8	MSI-H (213)	MSI-H	MSI-H	37
Clinical FFPE sample 9	MSI-H (135)	MSI-H	MSI-H	36
Clinical FFPE sample 10	MSI-H (139)	MSI-H	MSI-H	30
Clinical FFPE sample 11	MSI-H (65)	MSI-H	MSI-H	26
Clinical FFPE sample 12	MSI-H (142)	MSI-H	MSS	35
Clinical FFPE sample 13	MSS (4)	MSS	MSS	18
Clinical FFPE sample 14	MSS (5)	MSS	MSS	17
Clinical FFPE sample 15	MSS (9)	MSS	MSS	17
Clinical FFPE sample 16	MSS (1)	MSS	MSS	17
Clinical FFPE sample 17	MSS (0)	MSS	MSS	15
Clinical FFPE sample 18	MSS (2)	MSS	MSS	15
Clinical FFPE sample 19	MSS (1)	MSS	MSS	15
Clinical FFPE sample 20	MSS (0)	MSS	MSI-H	15
Clinical FFPE sample 21	MSS (12)	MSS	MSS	15

Supplemental Table 11.

Sample	Cancer type	Expected virus	Detected by CANCERPLEX	Detected by PCR
Clinical FFPE sample 22	Gastroesophageal cancer	EBV	yes	yes
Clinical FFPE sample 23	Gastroesophageal cancer	EBV	yes	yes
Clinical FFPE sample 24	Gastroesophageal cancer	EBV	yes	yes
Clinical FFPE sample 25	Gastroesophageal cancer	EBV	yes	yes
NA14094	cell line	EBV	yes	yes
GM14096	cell line	EBV	yes	yes
HeLa	cell line	HPV 18	yes	N/A
Clinical FFPE sample 26	Colorectal cancer	none, negative control	no	no
Clinical FFPE sample 27	Breast cancer, ER/PR-, Her2-	none, negative control	no	no
SNU-16	cell line	none, negative control	no	no

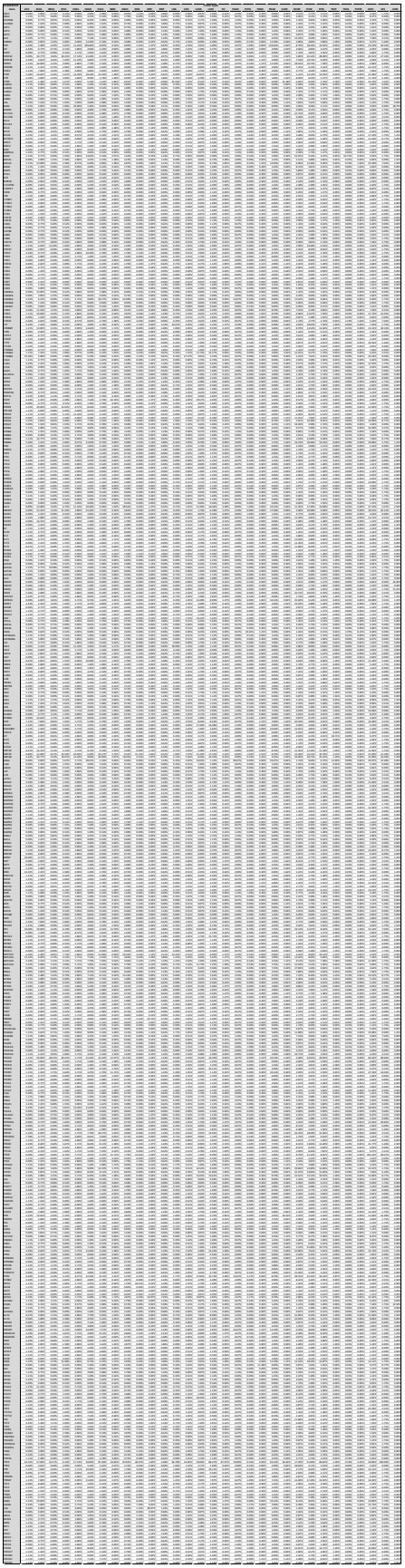
Supplemental Table 12.

Sequencing experiment	H2228 dilution, %	Mean coverage depth	*ALKintron 19 coverage depth*	Detected unique ALK-EML4 split reads	Reported by CANCERPLEX
1	10	168	239	1	no
2	10	211	288	4	yes
3	10	289	377	5	yes
4	10	589	702	5	yes
5	10	648	819	6	yes
6	10	693	866	7	yes
7	20	221	295	4	yes
8	20	361	446	11	yes
9	20	385	472	5	yes
10	20	550	672	12	yes
11	20	743	884	16	yes
12	20	757	890	22	yes
13	30	279	351	10	yes
14	30	301	366	10	yes
15	30	583	685	16	yes
16	30	650	758	15	yes
17	30	694	805	25	yes
18	50	592	658	23	yes
19	50	597	667	23	yes
20	50	743	808	33	yes
21	100	748	698	51	yes

Supplemental Table 13.

Validation sample	Sample type	Genetic aberration	Source
HCC1143/HCC1143	cell line	SNPs, INDELs	ATCC
HCC1954/PC	cell line	ERBB2 gain	ATCC
NCI-N87	cell line	ERBB2 gain	ATCC
H2228/H2228	cell line	ALK rearrangement	ATCC
SNU-16	cell line	FGFR2 gain	ATCC
HeLa	cell line	HPV-18	ATCC
NA14094	DNA from EBV-transformed cell line	EBV, BRCA1 indel	Coriell Institute for Medical Research
GM14096	DNA from EBV-transformed cell line	EBV, BRCA1 indel	Coriell Institute for Medical Research
NA19059/JPT59	DNA from EBV-transformed cell line	SNPs, INDELs	Coriell Institute for Medical Research
NA18595/CHB95	DNA from EBV-transformed cell line	SNPs, INDELs	Coriell Institute for Medical Research
NA19648/MXL48	DNA from EBV-transformed cell line	SNPs, INDELs	Coriell Institute for Medical Research
NA19701/ASW01	DNA from EBV-transformed cell line	SNPs, INDELs	Coriell Institute for Medical Research
NA19703/ASW03	DNA from EBV-transformed cell line	SNPs, INDELs	Coriell Institute for Medical Research
S10-57398/510	FFPE Tumor Patient Sample	ALK rearrangement	University Of Massachusetts Cancer Center Tissue and Tumor Bank
2898T/289	FFPE Tumor Patient Sample	ALK rearrangement	University Of Massachusetts Cancer Center Tissue and Tumor Bank
5360T/536	FFPE Tumor Patient Sample	ERBB2 gain	University Of Massachusetts Cancer Center Tissue and Tumor Bank
5943T/5943T	FFPE Tumor Patient Sample	ERBB2 gain	University Of Massachusetts Cancer Center Tissue and Tumor Bank
7259TY/7259TY	FFPE Tumor Patient Sample	ERBB2 gain	University Of Massachusetts Cancer Center Tissue and Tumor Bank
P.T.610/610	FFPE Tumor Patient Sample	ERBB2 exon 20 indel [insertion, ch17:37880997-37880997, Ref:-, Mut:TGT] [NM_004448]	University Of Massachusetts Cancer Center Tissue and Tumor Bank
P.T.613/613	FFPE Tumor Patient Sample	EGFR exon 19 indel [deletion, chr7:55242466-55242480, Ref:GAATTAAGAGAAGCA, Mut:-] [NM_005228]	University Of Massachusetts Cancer Center Tissue and Tumor Bank
S0357971K/1K.E1	FFPE Tumor Patient Sample	ERBB2 gain	University Of Massachusetts Cancer Center Tissue and Tumor Bank
S12-39759/397	FFPE Tumor Patient Sample	ERBB2 gain	University Of Massachusetts Cancer Center Tissue and Tumor Bank
LZZ5UACW/LZZ	FFPE Tumor Patient Sample	SNPs, INDELs	BioServe
1EU2MALD/1EU	FFPE Tumor Patient Sample	SNPs, INDELs	BioServe
9N8G9AU1/9N8G	FFPE Tumor Patient Sample	SNPs, INDELs	BioServe
LQHWEAF9/LQH	FFPE Tumor Patient Sample	SNPs, INDELs	BioServe
HD780	DNA Reference Standard	SNPs, INDELs	Horizon Discovery
Clinical sample 1	FFPE Tumor Patient Sample	ERBB2 gain	internal
Clinical sample 2	FFPE Tumor Patient Sample	ERBB2 gain	internal
Clinical sample 3	FFPE Tumor Patient Sample	ERBB2 gain	internal
Clinical sample 4	FFPE Tumor Patient Sample	MYC gain	internal
Clinical sample 5	FFPE Tumor Patient Sample	MYC gain	internal
Clinical sample 6	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 7	FFPE Tumor Patient Sample	MSI-H, negative control for viral detection	internal
Clinical sample 8	FFPE Tumor Patient Sample	MSI-H	internal
Clinical sample 9	FFPE Tumor Patient Sample	MSI-H	internal
Clinical sample 10	FFPE Tumor Patient Sample	MSI-H	internal
Clinical sample 11	FFPE Tumor Patient Sample	MSI-H	internal
Clinical sample 12	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 13	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 14	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 15	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 16	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 17	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 18	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 19	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 20	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 21	FFPE Tumor Patient Sample	MSS	internal
Clinical sample 22	FFPE Tumor Patient Sample	EBV	internal
Clinical sample 23	FFPE Tumor Patient Sample	EBV	internal
Clinical sample 24	FFPE Tumor Patient Sample	EBV	internal
Clinical sample 25	FFPE Tumor Patient Sample	EBV	internal
Clinical sample 26	FFPE Tumor Patient Sample	negative control for viral detection	internal
Clinical sample 27	FFPE Tumor Patient Sample	negative control for viral detection	internal
Clinical sample 28	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 29	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 30	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 31	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 32	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 33	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 34	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 35	FFPE Tumor Patient Sample	ERBB2 indel	internal
Clinical sample 36	FFPE Tumor Patient Sample	ATM indel	internal
Clinical sample 37	FFPE Tumor Patient Sample	EGFR indel	internal
Clinical sample 38	FFPE Tumor Patient Sample	KRAS indel	internal
Clinical sample 39	FFPE Tumor Patient Sample	ROS1 rearrangement	internal
Clinical sample 40	FFPE Tumor Patient Sample	RET rearrangement	internal
Clinical sample 41	FFPE Tumor Patient Sample	ALK rearrangement	internal
Clinical sample 42	FFPE Tumor Patient Sample	ALK rearrangement	internal

Supplemental Table 14.



Supplemental Table 15.

FFPE Sample	Mean Coverage Depth	Uniformity of Coverage (Pct>0.2*mean)	PCR duplication	Sequence mapped to ROI	Target Coverage at 100X	Target Coverage at 500X
1	648x	98.29%	5%	83%	98.86%	95.90%
2	693x	98.25%	6%	84%	99.08%	96.60%
3	543x	98.63%	2%	76%	99.25%	96.25%
4	743x	98.30%	7%	83%	99.22%	96.93%
5	757x	98.34%	6%	84%	99.08%	96.75%
6	694x	98.37%	6%	80%	98.96%	96.32%
7	742x	98.27%	7%	79%	98.95%	96.44%
8	526x	98.38%	5%	76%	99.13%	95.92%
9	590x	98.26%	4%	78%	98.95%	95.87%
10	599x	98.48%	7%	77%	99.19%	96.38%