

Supplementary figures and tables

Title: Anchored multiplex PCR for targeted next-generation sequencing

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Figure S1. Primer design strategy for gene fusion detection (targeted RNA-Seq).

Primer design strategy



Possible types of extension products using gDNA & cDNA as templates



■ Pre-ligated adapter

Figure S2. A clinical *ROS1* FISH lung adenocarcinoma case was reported as equivocal due to an unusual probe pattern showing tandem copies of *ROS1*, one seemingly normal while the neighboring copy shows more of the red *ROS1* 3' probe than the green *ROS1* 5' probe (white arrows, white scale bar = 10 μ m). The reported equivocal FISH result was discordant with the finding on the AMP gene rearrangement assay which definitively found a *CD74-ROS1* fusion.

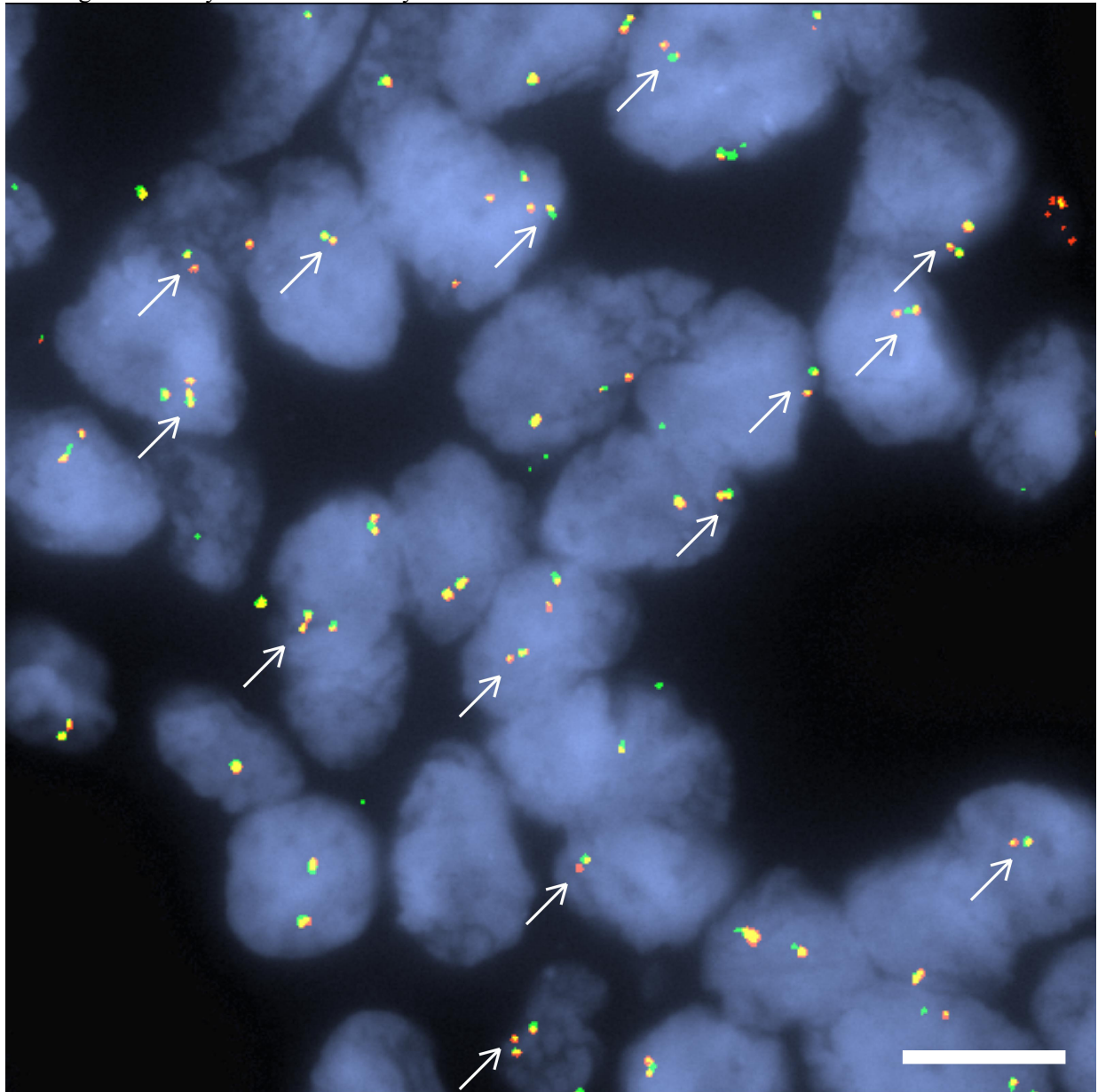


Figure S3. A clinical *ALK* FISH lung adenocarcinoma case showing an unusual FISH pattern with individual 5' (green only, white arrows) probe signals that represent a region upstream of the *ALK* gene (ISCN 2005 nomenclature: nuc ish (5'ALK x 2, 3'ALK x 1)(5'ALK con 3'ALK x 1)) (white scale bar = 10 μ m). The AMP gene rearrangement assay did not detect any typical *ALK* fusion gene transcripts (data not shown), suggesting that the finding may be related to an atypical structural change. Similarly, a clinical *ROS1* FISH case showing an unusual FISH pattern with individual 5' probe signals also did not show any typical *ROS1* fusion gene transcripts by our assay (data not shown).

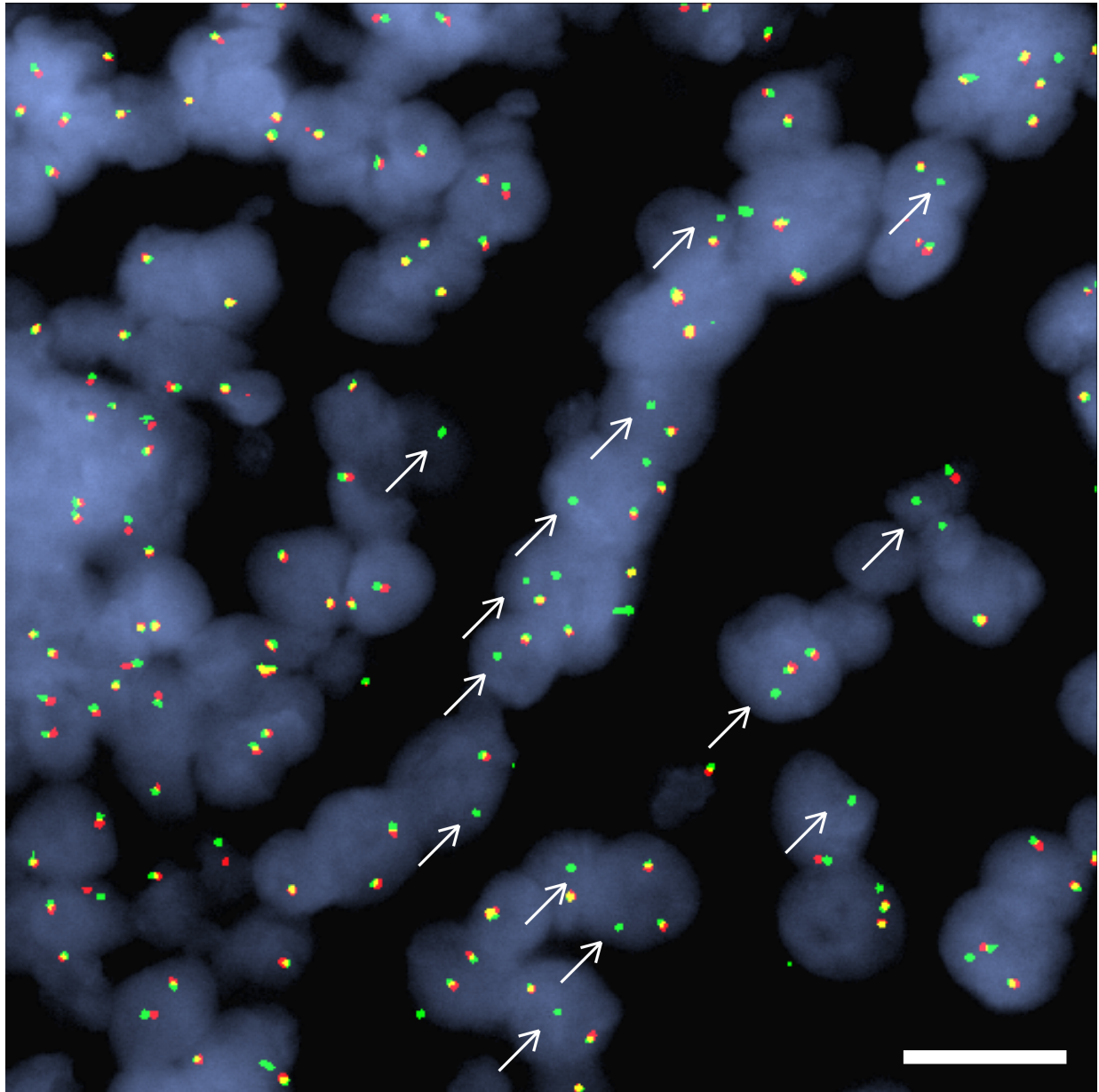


Figure S4. Six months AMP translocation (*ALK*, *RET*, *ROS1*, *PPARG*, *NTRK1*) testing of 262 clinical (tables 1-2) and 405 research (tables 3-5) specimens (each row = 1 case) in chronological order. All total nucleic acid samples were derived from clinical formalin-fixed paraffin embedded material and tested with our validated clinical assay. 1 = RNA Concentration by Qubit (red $\leq 10 \text{ ng uL}^{-1}$, yellow and green $> 10 \text{ ng uL}^{-1}$, range = 0 to over limit); 2 = GAPDH RNA Relative qPCR (red $\geq \text{Ct } 35$, yellow and green $< \text{Ct } 35$, range = 18.5 to undetected); 3 = AMP Library Yield (red = 2.3×10^2 , yellow = 1.3×10^{10} (50 percentile), green = 2.6×10^{10} total molecules); 4 = failure (red), negative (light green), positive (dark green). Note that the early assay failures were due to testing of older archival material and SPRI cleanup technical errors. The failure rate for both clinical and research testing in the latter 3 months is 3% (9 of 313 cases). Clinical fusion transcripts detected (number of cases): *EML4-ALK* (7), *KIF5B-RET* (3), *CD74-ROS1* (2), *CCDC6-RET* (2), and *TRIM24-RET* (1). Research fusion transcripts detected (number of cases): *SLC34A2-ROS1* (3), *CCDC6-RET* (3), *CD74-ROS1* (2), *KIF5B-RET* (2), *EML4-ALK* (1), *SQSTM1-ALK* (1), *EZR-ROS1* (1), and *TMP3-ROS1* (1).

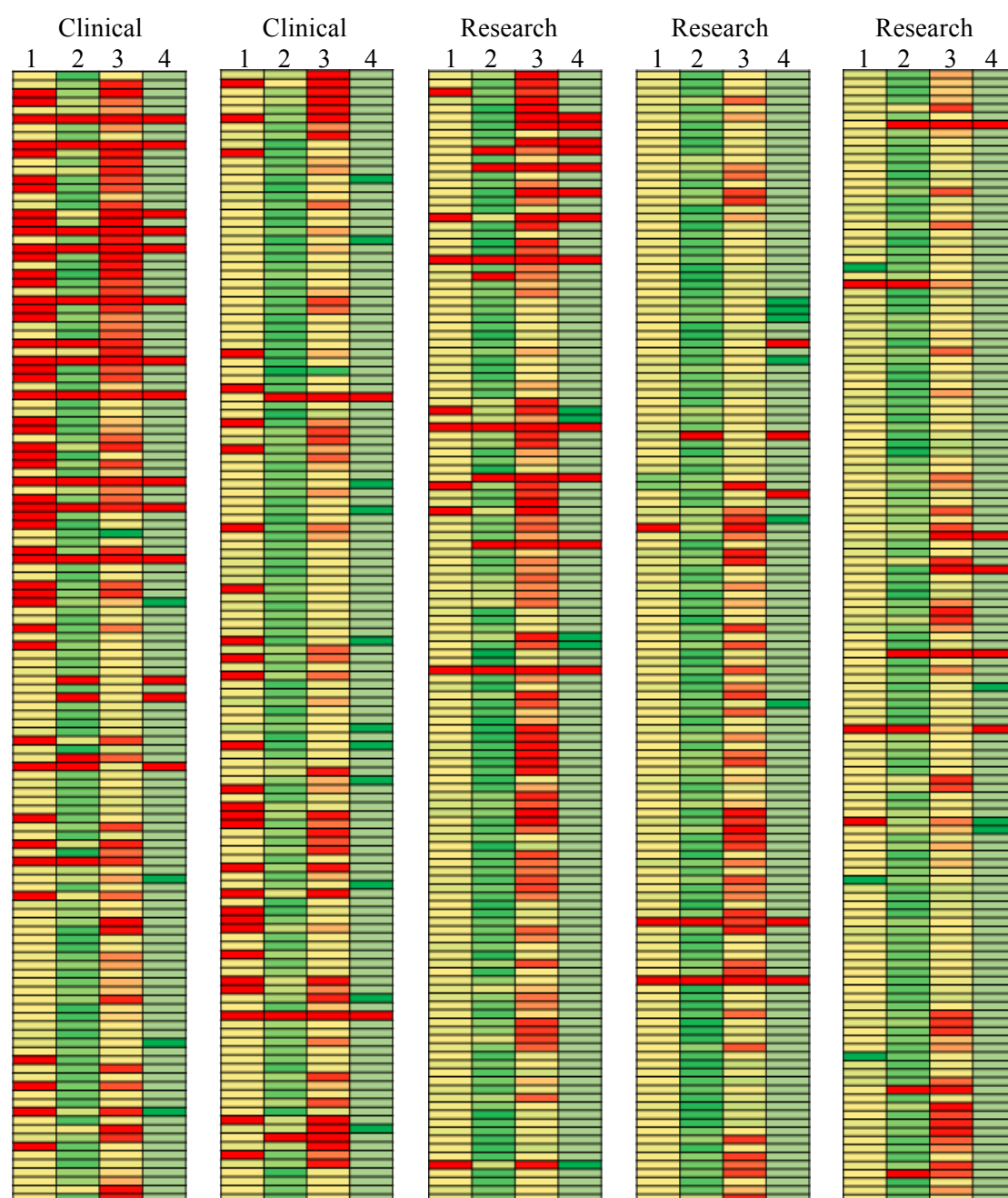


Figure S5. Duplication rate increases and library complexity decreases with low amount of input DNA. Input DNA in parentheses represent total double-stranded DNA split into two AMP reactions for the 96-amplicon cancer panel.

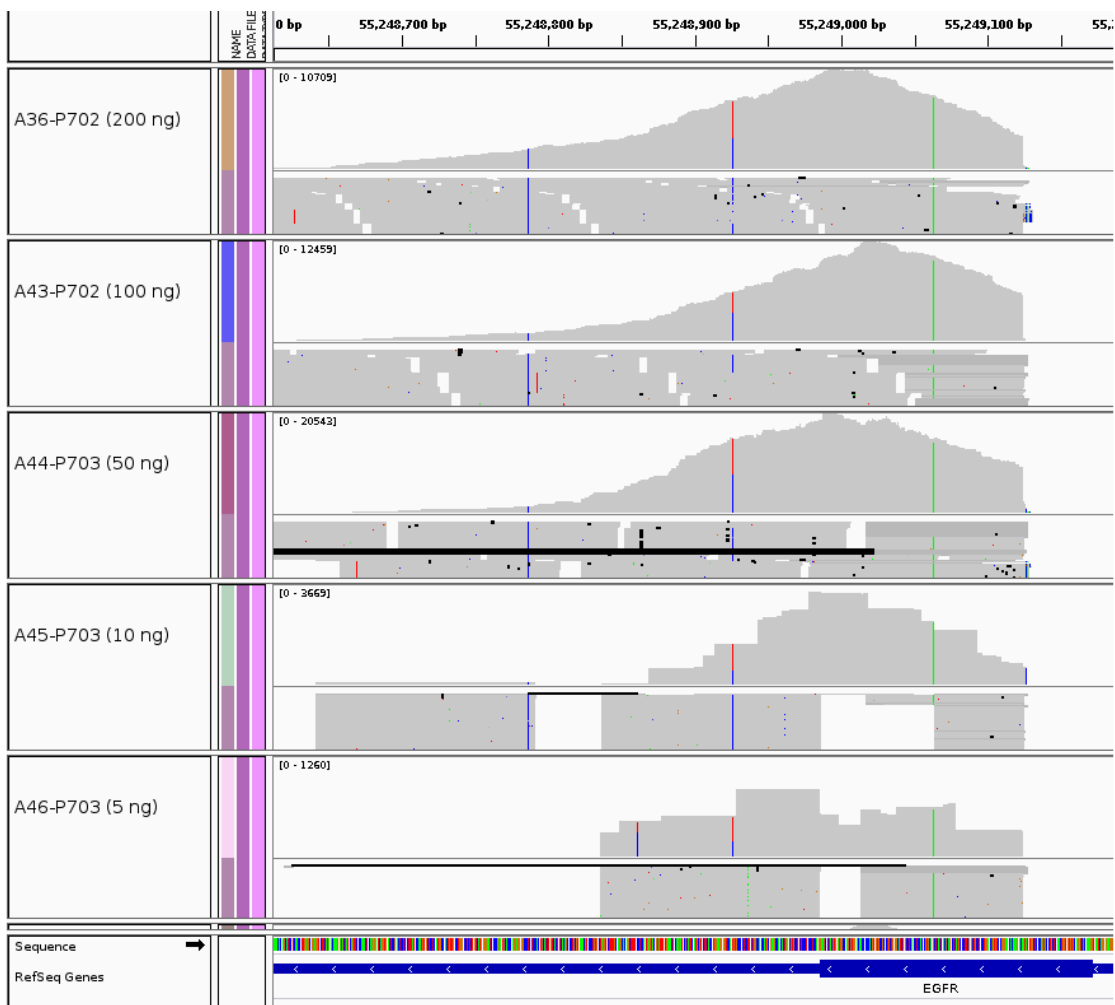


Figure S6. Anchored Multiplex PCR Library Construction Workflow. cDNA synthesis not required if using fragmented genomic DNA from FFPE material. ER = End repair, AddA = Adenylation, SPRI = Solid Phase Reversible Immobilization.

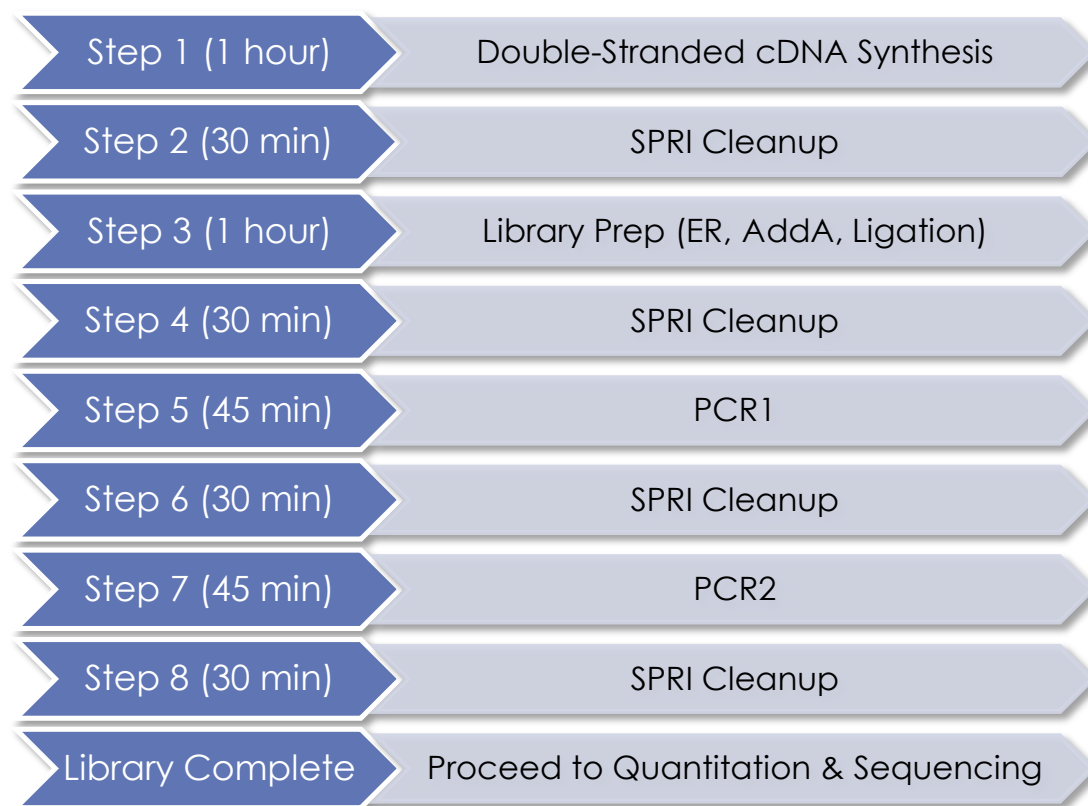


Table S1. List of samples with fusions detected using the targeted RNA-seq assay.

1) List of samples with fusions detected by the 14-gene targeted RNA-seq panel

Sample ID	No. fusion reads (primary and alternative splicing)	Fusion (gene, strand, Exon, transcript ID)	Tissue type	Note
1-252	1396	CHTOP+Exon5 (NM_015607)---NTRK1+Exon10 (NM_002529)	GBM	Not reported previously
	51	CHTOP+Exon5 (NM_015607)---NTRK1+Exon11 (NM_002529)		Not reported previously
2-3303	701	ARHGEF2-Exon21 (NM_004723)---NTRK1+Exon10 (NM_002529)	GBM	Not reported previously
1-521	4585	CHTOP+Exon5 (NM_015607)---NTRK1+Exon10 (NM_002529)	GBM	Not reported previously
	595	CHTOP+Exon5 (NM_015607)---NTRK1+Exon11 (NM_002529)		Not reported previously
3-3318	2682	PPL-Exon21 (NM_002705)---NTRK1+Exon11 (NM_002529)	Thyroid	Not reported previously
	30	PPL-Exon21 (NM_002705)---NTRK1+Exon10 (NM_002529)		Not reported previously
	13	PPL-Exon11 (NM_002705)---NTRK1+Exon13 (NM_002529)		Not reported previously
1-286	1173	FGFR3-Exon2 (NM_000142)---TACC3-Exon9 (NM_006342)	GBM	
2-3060	671	ETV6+Exon5 (NM_001987)---NTRK3-Exon15 (NM_002530)	Breast	

2) List of samples with fusions detected in the validation study using the 23-plex targeted RNA-seq

Sample ID	No. fusion reads (primary and alternative splicing)	Fusion (gene, strand, Exon, transcript ID)	Tissue type	Note
2-3216	364	SDC4-Exon5 (NM_002999)---ROS1-Exon34 (NM_002944)	Lung	
2-2096	180	CCDC6-Exon1 (NM_005436)---RET+Exon12 (NM_020630)	Thyroid	
1-748	69	EML4+Exon6 (NM_019063)---ALK-Exon20 (NM_004304)	Lung	
2-2829	143	CCDC6-Exon1 (NM_005436)---RET+Exon12 (NM_020630)	Thyroid	
2-3710	124	EZR-Exon9 (NM_003379)---ROS1-Exon34 (NM_002944)	Lung	
s2-3385	388	EML4+Exon2 (NM_019063)---ALK-Exon20 (NM_004304)	Lung	
2-3711	75	SLC34A2+Exon13 (NM_006424)---ROS1-Exon34 (NM_002944)	Lung	

2-3794	19	SLC34A2+Exon13 (NM_006424)---ROS1-Exon33 (NM_002944)	
	1580	EZR-Exon9 (NM_003379)---ROS1-Exon32 (NM_002944)	Lung
	1203	EZR-Exon9 (NM_003379)---ROS1-Exon34 (NM_002944)	
2-4035	47	EZR-Exon9 (NM_003379)---ROS1-Exon33 (NM_002944)	
	302	EML4+Exon6 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
	108	EML4+Exon5 (NM_019063)---ALK-Exon20 (NM_004304)	
2-2395	1273	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
2-4374	1129	EZR-Exon9 (NM_003379)---ROS1-Exon34 (NM_002944)	Lung
	250	EZR-Exon9 (NM_003379)---ROS1-Exon35 (NM_002944)	
2-4310	95	SLC34A2+Exon4 (NM_006424)---ROS1-Exon33 (NM_002944)	Lung
	258	SLC34A2+Exon4 (NM_006424)---ROS1-Exon32 (NM_002944)	
2-3506	801	EML4+Exon6 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
	246	EML4+Exon5 (NM_019063)---ALK-Exon20 (NM_004304)	
9-1084	1199	KIF5B-Exon15 (NM_004521)---RET+Exon12 (NM_020630)	Lung
2-3106	443	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
2-4446	259	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung
2-4445	477	TPM3-Exon8 (NM_152263)---ROS1-Exon35 (NM_002944)	Lung
9-0577	3479	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
	1816	EML4+Exon14 (NM_019063)---ALK-Exon20 (NM_004304)	
	692	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
0-111	889	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
0-380	3148	NCOA4+Exon7 (NM_005437)---RET+Exon12 (NM_020630)	PTC
1-1708	2376	SLC34A2+Exon13 (NM_006424)---ROS1-Exon32 (NM_002944)	Lung
	1120	SLC34A2+Exon13 (NM_006424)---ROS1-Exon34 (NM_002944)	
	23	SLC34A2+Exon12 (NM_006424)---ROS1-Exon32 (NM_002944)	
1-2143	853	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung
1-2424	10312	EML4+Exon18 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
	215	EML4+Exon19 (NM_019063)---ALK-Exon19 (NM_004304)	
1-2674	1200	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung

1-805	830	EML4+Exon6 (NM_019063)---ALK-Exon20 (NM_004304)	PTC
2-1246	11	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
2-2019	6901	EML4+Exon6 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
2-2096	7851	CCDC6-Exon1 (NM_005436)---RET+Exon12 (NM_020630)	PTC
2-2646	8410	CREB3L2-Exon2 (NM_194071)---PPARG+Exon2 (NM_005037)	PTC
	52	CREB3L2-Exon1 (NM_194071)---PPARG+Exon2 (NM_005037)	
2-294	629	EZR-Exon9 (NM_003379)---ROS1-Exon34 (NM_002944)	Lung
	85	EZR-Exon9 (NM_003379)---ROS1-Exon35 (NM_002944)	
	39	EZR-Exon9 (NM_003379)---ROS1-Exon33 (NM_002944)	
2-3270	37741	PAX8-Exon8 (NM_003466)---PPARG+Exon2 (NM_005037)	PTC
	134	PAX8-Exon7 (NM_003466)---PPARG+Exon2 (NM_005037)	
2-3460	833	CCDC6-Exon1 (NM_005436)---RET+Exon12 (NM_020630)	PTC
2-3499	2133	KIF5B-Exon16 (NM_004521)---RET+Exon12 (NM_020630)	Lung
	619	KIF5B-Exon15 (NM_004521)---RET+Exon13 (NM_020630)	
	18	KIF5B-Exon16 (NM_004521)---RET+Exon11 (NM_020630)	
2-3923	90	CREB3L2-Exon2 (NM_194071)---PPARG+Exon2 (NM_005037)	PTC
2-4310	834	SLC34A2+Exon4 (NM_006424)---ROS1-Exon32 (NM_002944)	Lung
	323	SLC34A2+Exon4 (NM_006424)---ROS1-Exon33 (NM_002944)	-
2-492	7709	CCDC6-Exon1 (NM_005436)---RET+Exon12 (NM_020630)	PTC
	14	CCDC6-Exon1 (NM_005436)---RET+Exon13 (NM_020630)	
2-844	543	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
	67	EML4+Exon11 (NM_019063)---ALK-Exon20 (NM_004304)	
	22	EML4+Exon13 (NM_019063)---ALK-Exon22 (NM_004304)	
2-904	6123	SLC34A2+Exon4 (NM_006424)---ROS1-Exon32 (NM_002944)	Lung
	1031	SLC34A2+Exon4 (NM_006424)---ROS1-Exon34 (NM_002944)	
	665	SLC34A2+Exon4 (NM_006424)---ROS1-Exon31 (NM_002944)	
3-1164	5582	EML4+Exon20 (NM_019063)---ALK-Exon20 (NM_004304)	Lung
	169	EML4+Exon21 (NM_019063)---ALK-Exon20 (NM_004304)	
3-1172	3269	KIF5B-Exon15 (NM_004521)---RET+Exon12 (NM_020630)	Lung

3-1174	4496	EML4+Exon6 (NM_019063)---ALK-Exon20 (NM_004304)	Lung	
3-1250	518	SLC34A2+Exon13 (NM_006424)---ROS1-Exon34 (NM_002944)	Lung	
3-1617	8460	SLC34A2+Exon4 (NM_006424)---ROS1-Exon32 (NM_002944)	Lung	
	947	SLC34A2+Exon4 (NM_006424)---ROS1-Exon34 (NM_002944)		
	845	SLC34A2+Exon4 (NM_006424)---ROS1-Exon31 (NM_002944)		
3-1618	1371	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung	
	264	EML4+Exon14 (NM_019063)---ALK-Exon20 (NM_004304)		
3-1620	5640	EML4+Exon13 (NM_019063)---ALK-Exon20 (NM_004304)	Lung	
	40	EML4+Exon12 (NM_019063)---ALK-Exon20 (NM_004304)		
	23	EML4+Exon12 (NM_019063)---ALK-Exon22 (NM_004304)		
3-1873	1882	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung	
3-1875	2126	SDC4-Exon5 (NM_002999)---ROS1-Exon34 (NM_002944)	Lung	
3-1877	2152	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung	
3-1878	29378	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung	
	483	CD74-Exon6 (NM_004355)---ROS1-Exon33 (NM_002944)		
	36	CD74-Exon5 (NM_004355)---ROS1-Exon33 (NM_002944)		
3-1879	6802	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung	
3-1880	20299	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung	
3-1881	22950	CD74-Exon6 (NM_004355)---ROS1-Exon34 (NM_002944)	Lung	
	395	CD74-Exon5 (NM_004355)---ROS1-Exon34 (NM_002944)		
	250	CD74-Exon6 (NM_004355)---ROS1-Exon35 (NM_002944)		
3-2770	425	KIF5B-Exon16 (NM_004521)---RET+Exon12 (NM_020630)	Thyroid	
	67	KIF5B-Exon15 (NM_004521)---RET+Exon13 (NM_020630)		
9-401	5 *	MSN+Exon9 (NM_002444)---ROS1-Exon34 (NM_002944)	Lung	Not reported previously
9-465	42 *	EZR-Exon9 (NM_003379)---ROS1-Exon32 (NM_002944)	Lung	

* Ion Torrent 314-chip data

Table S2. Novel fusions involving potential therapeutically important targets (highlighted in red) were identified from routinely assayed formalin-fix and paraffin-embedded clinical samples.

5' gene-exon	3' gene-exon	Tumor	In-frame fusion	In exon-exon fusion	Confirmation
<i>CHTOP</i> -exon 5	<i>NTRK1</i> -exon 10	Glioblastoma	Yes	Yes	RT-PCR
<i>ARHGEF2</i> -exon 21	<i>NTRK1</i> -exon 10	Glioblastoma	Yes	Yes	FISH
<i>MSN</i> -exon 9	<i>ROS1</i> -exon 34	Lung adenocarcinoma	Yes	Yes	RT-PCR
<i>RUFY2</i> -exon 9	<i>RET</i> -exon 12	Lung adenocarcinoma	Yes	Yes	FISH
<i>FGFR2</i> -exon 17	<i>CREB5</i> -exon 7	Liver cholangiocarcinoma	Yes	Yes	RT-PCR
<i>TRIM4</i> -exon 5	<i>BRAF</i> -exon 10	Lung mucinous adenocarcinoma	Yes	Yes	RT-PCR
<i>VAMP2</i> -exon 4	<i>NRG1</i> -exon 4	Lung mucinous adenocarcinoma	Yes	Yes	RT-PCR
<i>TPM3</i> -exon 8	<i>NTRK1</i> -exon 10	Lung mucinous adenocarcinoma	Yes	Yes	RT-PCR
<i>PPL</i> -exon 21	<i>NTRK1</i> -exon 11	Thyroid carcinoma	Yes	Yes	FISH

CDNA sequences:

CHTOP-NTRK1:

CGAGGTCGTGGAGGTCCTGGGAGAGGGGGCCTAGGGCGTGAGCTATGGGTCGTGGCGGAATCG
GTGGTAGAGACACTAACAGCACATCTGGAGACCCGGTGGAGAAGAAGGACGAAACACCTTTTGG
GGTCTCGGTGGCTGTGGGCCTGGCCGTCTTTGCCTGCCTCTTCCTTTCTACG

ARHGEF2-NTRK1:

GCAGGAAGTGGGGAGCCCCGAAGAGCGGCTGCAAGACAGCAGTGACCCTGACACTGGCAGCGA
GGAGGAAGGTAGCAGCCGTCTGTCTCCGCCCCACAGTCCACGAGACACTAACAGCACATCTGGA
GACCCGGTGGAGAAGAAGGACGAAACACCTTTTGGGGTCTCGGTGGCTGTGGGCCTGGCCGTCTT
TGCCTGCCTCTTCCTTTCTACGCTGCTCCTTGTGCTCAACAAATGTGG

MSN-ROS1

GGAGAGGCTGAAGCAGATCGAGGAACAGACTAAGAAGGCTCAGCAAGATGATTTTTGGATACCA
GAAACAAGTTTCATACTTACTA

RUFY2-RET

AATAAATTGATTTTAAATGAAAACACAGCAGCACCTAGAGGAGGATCCAAAGTGGGAATTCCCTC
GGAAGAACTTGTTCTTGGAATACTCTAGGAGAAGGCGAATTTGGAAAAGTGGTCAAGGCAAC
GGCCTTCCATCTGAAAGGCAG

FGFR2-CREB5

GTTGGTAGAAGACTTGGATCGAATTCTCACTCTCACAACCAATGAGAGGTTGAAGGCTGCATTGA
CTCACCACCCTGCTGCCATGTCAAATGGGAACATGAACACCATGGGACACATGATGGAGATGAT
GGGCTCCCGGCAGGACCAGAC

TRIM4-BRAF:

GAAGTATTCTCTTGAAGCTGTAAAGGTGAAGACAGTGTGCCAGATACCATTGATGAAGGAAATG
CTAAAGCGATTCCAAGGATCAACCACAGGTTTGTCTGCTACCCCCCTGCCTCATTACCTGGCTC
ACTAATAACGTGAAAGCCTTACAGAAATCTCC

VAMP2-NRG1:

TGGTGGAAAAACCTCAAGATGATGATCATCTTGGGAGTGATTTGCGCCATCATCCTCATCATCAT
CATAGAGATCATCACTGGTATGCCAGCCTCAACTGAAGGAGCATATGTTCTTCAGAGTCTCCCAT
TAGAATATCAGTATCCACAGAAGGAGCAAATACTT
CTTCATC

TPM3-NTRK1:

CTGAGTTTGCTGAGAGATCGGTAGCCAAGCTGGAAAAGACAATTGATGACCTGGAAGACACTAA
CAGCACATCTGGAGACCCGGTGGAGAAGAAGGACGAAACACCTTTTGGGGTCTCGGTGGCTGTG
GGCCTGGCCGTC

PPL-NTRK1:

GCGGGAGGAGCTGGAGGCACTGAGGCGGCAGAAAGGGCGCCCGGGAGGCAGAGGTCTCGGTGGC
TGTGGGCCTGGCCGTCTTTGCCTGCCTCTTCCTTTCTACGCTGCTCCTTGTGCTCAACAAATGTGG
ACGGAGAAACAAGTTTGGG

Table S3. Single nucleotide variation, insertion and deletion, and amplification detection concordance between SNaPshot, PCR sizing and FISH and the 168-

	SampleID	Previous results_(SNaPshot/sizing/FISH)	AMP 168-amplicon confirmation results	AMP 168-amplicon additional calls (dbSNP137 variants are filtered)
Run.1				
	1 3-603	HER2 amplification	HER2 amplification	FGFR3:p.T394M
	2 3-663	PDGFR amplification	PDGFR amplification	-
	3 3-850	EGFR-c.2369C>T EGFR-exon19del	EGFR:c.2237_2251del, p.T790M	TP53:c.94_96G
	4 3-875	EGFR-c.2236_2250del EGFR-exon19del	EGFR:c.2236_2250del	-
	5 3-1220	EGFR INS Exon 20-Indel - exon20	EGFR:c.2308ins_CCAGCGTGG	TP53:p.S215G
	6 3-1337	EGFR amplification	EGFR amplification	-
	7 3-1430	EGFR-c.2236_2250del EGFR-exon19del	EGFR:c.2236_2250del	APC:p.Q1444X; TP53:p.S241F
	8 3-1546	MET amplification coEGFR	MET amplification coEGFR	EGFR:p.L858R; TP53:p.R248P; FGFR3:p.V306I
	9 3-1706	EGFR amplification	EGFR amplification	-
	10 3-1862	EGFR INS Exon 20-Indel	EGFR:c.2310ins_GGGTTT	TP53:p.R342P
	11 3-1851	EGFR 2236_2250 del	EGFR:c.2236_2250del	IDH2:c.436_del (7%)
	12 3-1957	ERBB2 (HER2) INS Exon 20-Indel	ERBB2:c.2324ins_ATACGTGATGGC	CDH1:p.G62S;
	13 3-1931	EGFR amplification	EGFR amplification	-
	14 3-2017	EGFR-exon19del	EGFR:c.c.2253_2276del	-
	15 3-2131	ERBB2 (HER2) INS Exon 20-Indel	ERBB2:c.2331ins_GGCTCCCCA	-
Run.2				
	16 3-2092	EGFR-exon19del	EGFR:c.2239_2247del	-
	17 3-2085	HER2 amplification	HER2 amplification	KRAS:p.G12D
	18 3-2222	BRAF 1799 T-A, TP53 742 C-T	BRAF:p.V600E; TP53:p.R248W;	-
	19 3-2208	KRAS 35G-T	KRAS:p.G12V	SMAD4:p.Q250*
	20 3-2211	KRAS 35 G-A, APC 4348 C-T	KRAS:p.G12D; APC:p.R1450X	APC:p.R876*; SMAD4:p.R100K; PTEN:c.809_811del
	21 3-2215	KRAS 34 G-T	KRAS:p.G12C;	-
	22 3-2258	PIK3CA 1633 G-C	PIK3CA:p.E545Q	FBXW7:p.R347L
	23 3-2261	KRAS 35 G-T	KRAS:p.G12V (5%, supported by one strand only)	-
	24 3-2212	WT	-	-

25 3-2216	WT	-	CDH1:p.A628V
26 3-2264	WT	-	-
27 3-2311	IDH1 395 G-A	IDH1:R132H	-
28 3-2335	KRAS 35G-A	KRAS:p.G12D	TP53:p.P250L
29 3-2347	PIK3CA 3140 A-G	PIK3CA:p.H1047R	-
30 3-2353	CTNNB1122 C-T	CTNNB1:p.T41I	-

Run.3

31 3-2354	KRAS 34 G-T	KRAS:p.G12C	-
32 3-2359	WT		BRAF:p.N581S; APC:c.8439_8442del
33 3-2321	PDGFRA amplification	PDGFRA amplification	-
34 3-2379	EGFR-c.2369C>T EGFR-exon19del	EGFR:c.2237_2251del, p.T790M;	-
35 3-2306	WT		TP53:p.V216L
36 3-2307	WT		-
37 3-2439	KRAS 34 G-T	KRAS:p.G12C;	-
38 3-2308	WT	-	TP53:p.S215N
39 3-2442	IDH1 394 C-G, TP53 817 C-T	IDH1:p.R132G; TP53:p.R273C	-
40 3-2346	WT	-	-
41 3-2438	WT	-	TP53:p.S166*
42 3-2350	WT		-
43 3-2466	KRAS 35 G-T	KRAS:p.G12V	CTNNB1:p.V79I;
44 3-2358	WT	-	DDR2:p.S737R
45 3-2556	Her2 amplification (low)	-	KRAS:p.G12D
46 3-2489	WT	-	-
47 3-2609	EGFR-exon19del	EGFR:c.2237_2254del	-
48 3-2808	EGFR-exon19del	EGFR:c.2240_2257del	-
49 3-2959	PIK3CA-c.1624G>A EGFR-exon20del	PIK3CA:p.E542K; EGFR:c.2354_2377del;	-

Run.4

50 3-1491	EGFR amplification EGFR-exon19del EGFR-c.2235_2249del	EGFR amplification	TP53:p.N239S
51 3-930	EGFR-c.2369C>T	EGFR:c.2235_2249del, p.T790M	TP53:p.Y220C

52 3-1071	EGFR-c.2236_2250del EGFR-exon19del	EGFR:c.2235_2249del;	-
53 3-1954	HER2 amplification	HER2 amplification	-
54 3-2153	EGFR-c.2369C>T EGFR-exon19del	EGFR:c.2237_2251del, p.T790M	TP53:c.95_96del
55 3-1220	EGFR Exon 20-Indel - exon20	EGFR:c.2237_2251del	None
56 3-2221	WT	-	-
57 3-2259	WT	-	-
58 3-2344	IDH2 419 G-A	IDH2:p.R140Q	-
59 3-2348	KRAS 38 G-A, APC 3340 C-T	KRAS:p.G13D; APC:p.R1114*	APC:p.Q1429*; TP53:p.E298X
60 3-2437	AKT1 49 G-A	AKT1:p.E17K (4%)	-
61 3-2436	WT	-	-
62 3-2448	WT	-	-
63 3-2449	WT	-	-

Table S4. AMP cost analysis (not including personnel and consumables)					
Reagents	Price (USD)	Total Volume (uL)	Volume per test	Volume per batch*	cost per batch (\$)
Random Hexamers	15	100	2	96	14
RNAse H	282	1000	1	48	14
RNAseOut	125	125	1	48	48
Second-strand Reaction Buffer, 10X	24	400	5	240	14
SuperScript® III RT	294	50	1	48	282
Agencourt AMPure Beads, 60mL	1104	60 000	206	9888	182
DNA polymerase I	282	500	4	192	108
dNTP Solution Mix, 25 mM	282	1000	2	96	27
End-Repair Mix	282	400	2	96	68
Klenow (3'-5' exo-)	305	200	0,5	24	37
Platinum® Taq DNA Polymerase	327	60	0,7	33,6	183
Taq DNA Polymerase	284	100	0,5	24	68
T4 DNA Ligase	282	1000	2	96	27
Barcode adaptors*					50
Primers*					50
PhiX Control DNA v3	138	10		2	28
Sodium Hydroxide Solution, 1N	71	100 000		40	0,03
MiSeq Reagent Kit, v2 (300cycles)	930				930
KAPA qPCR library quantification Kit	575				287,5
Total cost per Batch					2045,06

* Barcodes and primers costs are based on a rough calculation

* 48 samples per batch

Per sample	USD
Library prep	24,43
Sequencing (48 samples per Miseq run)	25,94
Sequencing (16 samples per Miseq run)	77,82

Table S5. Two targeted RNA-Seq AMP panels for gene fusion detection.

4-gene 33-plex panel		14-gene panel including all the primers in the 4-gene panel. Gene name labeled with -3' indicates sense primer.									
Gene	Target	Gene	Target	Gene	Target	Gene	Target	Gene	Target	Gene	Target
CTBP1*	Exon 6	B2M*	Exon 2	FGFR3	Exon 8	INSRR-3'	Exon 22	NTRK2	Exon 9	PDGFRA-3'	Exon 21
ALK	Exon 19	GAPDH*	Exon 6	FGFR3	Exon 9	MET	Exon 11	NTRK2	Exon 10	PDGFRA-3'	Exon 22
ALK	Exon 20	EGFR	Exon 14	FGFR3	Exon 10	MET	Exon 12	NTRK2	Exon 11	PDGFRA-3'	Exon 23
ALK	Exon 21	EGFR	Exon 15	FGFR3	Exon 11	MET	Exon 13	NTRK2	Exon 13		
ALK	Exon 22	EGFR	Exon 16	FGFR3	Exon 12	MET	Exon 14	NTRK2	Exon 14		
RET	Exon 8	EGFR	Exon 17	FGFR3-3'	Exon 16	MET	Exon 15	NTRK2-3'	Exon 16		
RET	Exon 9	EGFR	Exon 18	FGFR3-3'	Exon 17	MET	Exon 16	NTRK2-3'	Exon 17		
RET	Exon 10	EGFR-3'	Exon 23	FGFR3-3'	Exon 18	MET-3'	Exon 17	NTRK2-3'	Exon 18		
RET	Exon 11	EGFR-3'	Exon 24	INSR	Exon 13	MET-3'	Exon 18	NTRK2-3'	Exon 19		
RET	Exon 12	EGFR-3'	Exon 25	INSR	Exon 14	MUSK	Exon 8	NTRK2-3'	Exon 20		
RET	Exon 13	EGFR-3'	Exon 26	INSR	Exon 15	MUSK	Exon 9	NTRK3	Exon 11		
ROS1	Exon 31	EGFR-3'	Exon 27	INSR	Exon 16	MUSK	Exon 11	NTRK3	Exon 12		
ROS1	Exon 32	EGFR-3'	Exon 28	INSR	Exon 17	MUSK	Exon 12	NTRK3	Exon 13		
ROS1	Exon 33	FGFR1	Exon 7	INSR	Exon 18	MUSK	Exon 13	NTRK3	Exon 14		
ROS1	Exon 34	FGFR1	Exon 8	INSR-3'	Exon 21	MUSK	Exon 14	NTRK3	Exon 15		
ROS1	Exon 35	FGFR1	Exon 9	INSR-3'	Exon 22	NTRK1	Exon 8	NTRK3	Exon 16		
ROS1	Exon 36	FGFR1	Exon 10	INSRR	Exon 13	NTRK1	Exon 9	NTRK3-3'	Exon 18		
ROS1	Exon 37	FGFR1	Exon 11	INSRR	Exon 14	NTRK1	Exon 10	PDGFRA	Exon 9		
MUSK	Exon 9	FGFR1	Exon 12	INSRR	Exon 15	NTRK1	Exon 11	PDGFRA	Exon 10		
MUSK	Exon 11	FGFR1-3'	Exon 16	INSRR	Exon 16	NTRK1	Exon 12	PDGFRA	Exon 11		
MUSK	Exon 12	FGFR1-3'	Exon 17	INSRR	Exon 17	NTRK1	Exon 13	PDGFRA	Exon 13		
MUSK	Exon 13	FGFR1-3'	Exon 18	INSRR	Exon 18	NTRK1-3'	Exon 15	PDGFRA	Exon 14		
MUSK	Exon 14	FGFR3	Exon 7	INSRR-3'	Exon 21	NTRK1-3'	Exon 16	PDGFRA-3'	Exon 20		

*Internal control for RNA sample quality.

Table S6. 96-Amplicon Hotspot Mutation Plus Tumor Suppressor AMP Panel. Exon targets were designed to be fully covered for their coding regions.

Gene	Target exon or hotspots	Gene	Target exon or hotspots	Gene	Target exon or hotspots	Gene	Target exon or hotspots
<i>PTEN</i>	Exon 1	<i>CDKN2A</i>	Exon 1	<i>EGFR</i>	p.G719	<i>ERBB2</i>	p.M774
<i>PTEN</i>	Exon 2	<i>CDKN2A</i>	Exon 2	<i>EGFR</i>	p.E746	<i>GNA11</i>	p.Q209
<i>PTEN</i>	Exon 3	<i>CDKN2A</i>	Exon 3	<i>EGFR</i>	p.T790	<i>GNAS</i>	p.R201
<i>PTEN</i>	Exon 4	<i>NRAS</i>	p.Q61	<i>EGFR</i>	p.L858	<i>APC</i>	Exon 16
<i>PTEN</i>	Exon 5	<i>NRAS</i>	p.G12	<i>BRAF</i>	p.K601	<i>ALK</i>	Exon 20
<i>PTEN</i>	Exon 6	<i>IDH1</i>	p.R132	<i>BRAF</i>	p.V600	<i>ALK</i>	Exon 21
<i>PTEN</i>	Exon 7	<i>CTNMB1</i>	p.A5	<i>GNAQ</i>	p.Q209	<i>ALK</i>	Exon 22
<i>PTEN</i>	Exon 8	<i>CTNMB1</i>	p.T41	<i>NOTCH1</i>	p.P2515	<i>ALK</i>	Exon 23
<i>PTEN</i>	Exon 9	<i>PIK3CA</i>	p.R88	<i>NOTCH1</i>	p.L1679	<i>ALK</i>	Exon 24
<i>TP53</i>	Exon 1	<i>PIK3CA</i>	p.N345	<i>NOTCH1</i>	p.L1601	<i>ALK</i>	Exon 25
<i>TP53</i>	Exon 2	<i>PIK3CA</i>	p.C420	<i>NOTCH1</i>	p.L1575	<i>ALK</i>	Exon 26
<i>TP53</i>	Exon 3	<i>PIK3CA</i>	p.E545	<i>RET</i>	p.E632	<i>ALK</i>	Exon 27
<i>TP53</i>	Exon 4	<i>PIK3CA</i>	p.H1047	<i>RET</i>	p.M918	<i>ALK</i>	Exon 28
<i>TP53</i>	Exon 5	<i>FGFR3</i>	p.S249	<i>HRAS</i>	p.Q61	<i>ALK</i>	Exon 29
<i>TP53</i>	Exon 6	<i>FGFR3</i>	p.Y373	<i>HRAS</i>	p.G12	<i>MAP2K1</i>	Exon 2
<i>TP53</i>	Exon 7	<i>FGFR3</i>	p.K650	<i>KRAS</i>	p.A146	<i>MAP2K1</i>	Exon 2
<i>TP53</i>	Exon 8	<i>FGFR3</i>	p.G697	<i>KRAS</i>	p.Q61		
<i>TP53</i>	Exon 9	<i>KIT</i>	p.Y503	<i>KRAS</i>	p.G12		
<i>TP53</i>	Exon 10	<i>KIT</i>	p.W557	<i>AKT1</i>	p.E17		
<i>TP53</i>	Exon 11	<i>KIT</i>	p.D816	<i>IDH2</i>	p.R172		

Table S7. A 626-amplicon tumor suppressor AMP Panel. All coding exons were covered in a bi-template design with GSP1 and GSP2 primers targeting both the sense and anti-sense strands of exons longer than 200 bp in a 2-tube format.

Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon
<i>APC</i>	1	<i>ATM</i>	36	<i>BRCA1</i>	17	<i>MEN1</i>	9	<i>NF1</i>	45	<i>PIK3CA</i>	13	<i>SMARCB1</i>	3	<i>TSC2</i>	5	<i>VHL</i>	3
<i>APC</i>	2	<i>ATM</i>	37	<i>BRCA1</i>	18	<i>MEN1</i>	10	<i>NF1</i>	46	<i>PIK3CA</i>	14	<i>SMARCB1</i>	4	<i>TSC2</i>	6	<i>WT1</i>	1
<i>APC</i>	3	<i>ATM</i>	38	<i>BRCA1</i>	19	<i>NF1</i>	1	<i>NF1</i>	47	<i>PIK3CA</i>	15	<i>SMARCB1</i>	5	<i>TSC2</i>	7	<i>WT1</i>	2
<i>APC</i>	4	<i>ATM</i>	40	<i>BRCA1</i>	20	<i>NF1</i>	2	<i>NF1</i>	48	<i>PIK3CA</i>	16	<i>SMARCB1</i>	6	<i>TSC2</i>	8	<i>WT1</i>	3
<i>APC</i>	5	<i>ATM</i>	41	<i>BRCA1</i>	21	<i>NF1</i>	3	<i>NF1</i>	49	<i>PIK3CA</i>	17	<i>SMARCB1</i>	7	<i>TSC2</i>	9	<i>WT1</i>	5
<i>APC</i>	6	<i>ATM</i>	42	<i>BRCA1</i>	22	<i>NF1</i>	4	<i>NF1</i>	50	<i>PIK3CA</i>	18	<i>SMARCB1</i>	8	<i>TSC2</i>	10	<i>WT1</i>	6
<i>APC</i>	7	<i>ATM</i>	44	<i>BRCA1</i>	23	<i>NF1</i>	6	<i>NF1</i>	51	<i>PIK3CA</i>	19	<i>SMARCB1</i>	9	<i>TSC2</i>	11	<i>WT1</i>	7
<i>APC</i>	8	<i>ATM</i>	45	<i>BRCA2</i>	2	<i>NF1</i>	7	<i>NF1</i>	52	<i>PIK3CA</i>	20	<i>STK11</i>	1	<i>TSC2</i>	12	<i>WT1</i>	8
<i>APC</i>	9	<i>ATM</i>	46	<i>BRCA2</i>	3	<i>NF1</i>	8	<i>NF1</i>	53	<i>PIK3CA</i>	21	<i>STK11</i>	2	<i>TSC2</i>	13	<i>WT1</i>	9
<i>APC</i>	11	<i>ATM</i>	47	<i>BRCA2</i>	6	<i>NF1</i>	9	<i>NF1</i>	54	<i>RB1</i>	1	<i>STK11</i>	3	<i>TSC2</i>	14	<i>WT1</i>	10
<i>APC</i>	12	<i>ATM</i>	48	<i>BRCA2</i>	7	<i>NF1</i>	10	<i>NF1</i>	55	<i>RB1</i>	3	<i>STK11</i>	4	<i>TSC2</i>	15		
<i>APC</i>	13	<i>ATM</i>	49	<i>BRCA2</i>	9	<i>NF1</i>	11	<i>NF1</i>	56	<i>RB1</i>	5	<i>STK11</i>	5	<i>TSC2</i>	16		
<i>ATM</i>	4	<i>ATM</i>	50	<i>BRCA2</i>	10	<i>NF1</i>	12	<i>NF1</i>	57	<i>RB1</i>	6	<i>STK11</i>	6	<i>TSC2</i>	17		
<i>ATM</i>	5	<i>ATM</i>	51	<i>BRCA2</i>	11	<i>NF1</i>	13	<i>NF1</i>	58	<i>RB1</i>	7	<i>STK11</i>	7	<i>TSC2</i>	18		
<i>ATM</i>	6	<i>ATM</i>	52	<i>BRCA2</i>	12	<i>NF1</i>	14	<i>NF2</i>	1	<i>RB1</i>	8	<i>STK11</i>	8	<i>TSC2</i>	19		
<i>ATM</i>	7	<i>ATM</i>	53	<i>BRCA2</i>	13	<i>NF1</i>	16	<i>NF2</i>	2	<i>RB1</i>	9	<i>STK11</i>	9	<i>TSC2</i>	20		
<i>ATM</i>	8	<i>ATM</i>	54	<i>BRCA2</i>	14	<i>NF1</i>	17	<i>NF2</i>	3	<i>RB1</i>	10	<i>TSC1</i>	2	<i>TSC2</i>	21		
<i>ATM</i>	9	<i>ATM</i>	55	<i>BRCA2</i>	15	<i>NF1</i>	18	<i>NF2</i>	4	<i>RB1</i>	12	<i>TSC1</i>	3	<i>TSC2</i>	22		
<i>ATM</i>	10	<i>ATM</i>	56	<i>BRCA2</i>	16	<i>NF1</i>	19	<i>NF2</i>	5	<i>RB1</i>	13	<i>TSC1</i>	4	<i>TSC2</i>	23		
<i>ATM</i>	11	<i>ATM</i>	57	<i>BRCA2</i>	17	<i>NF1</i>	20	<i>NF2</i>	6	<i>RB1</i>	14	<i>TSC1</i>	5	<i>TSC2</i>	24		
<i>ATM</i>	12	<i>ATM</i>	58	<i>BRCA2</i>	18	<i>NF1</i>	21	<i>NF2</i>	7	<i>RB1</i>	15	<i>TSC1</i>	6	<i>TSC2</i>	25		
<i>ATM</i>	13	<i>ATM</i>	59	<i>BRCA2</i>	21	<i>NF1</i>	22	<i>NF2</i>	8	<i>RB1</i>	16	<i>TSC1</i>	7	<i>TSC2</i>	26		
<i>ATM</i>	14	<i>ATM</i>	60	<i>BRCA2</i>	22	<i>NF1</i>	23	<i>NF2</i>	9	<i>RB1</i>	18	<i>TSC1</i>	8	<i>TSC2</i>	27		
<i>ATM</i>	16	<i>ATM</i>	61	<i>BRCA2</i>	23	<i>NF1</i>	24	<i>NF2</i>	10	<i>RB1</i>	19	<i>TSC1</i>	9	<i>TSC2</i>	28		
<i>ATM</i>	17	<i>ATM</i>	62	<i>BRCA2</i>	24	<i>NF1</i>	25	<i>NF2</i>	11	<i>RB1</i>	22	<i>TSC1</i>	10	<i>TSC2</i>	29		
<i>ATM</i>	18	<i>ATM</i>	63	<i>BRCA2</i>	25	<i>NF1</i>	26	<i>NF2</i>	12	<i>RB1</i>	23	<i>TSC1</i>	11	<i>TSC2</i>	30		

Table S7. Continued.

Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon	Gene	Exon
<i>ATM</i>	19	<i>BRCA1</i>	2	<i>BRCA2</i>	26	<i>NF1</i>	27	<i>NF2</i>	13	<i>RB1</i>	24	<i>TSC1</i>	12	<i>TSC2</i>	31		
<i>ATM</i>	20	<i>BRCA1</i>	3	<i>BRCA2</i>	27	<i>NF1</i>	28	<i>NF2</i>	14	<i>RB1</i>	25	<i>TSC1</i>	13	<i>TSC2</i>	32		
<i>ATM</i>	21	<i>BRCA1</i>	4	<i>CDKN2A</i>	1	<i>NF1</i>	29	<i>NF2</i>	15	<i>RB1</i>	26	<i>TSC1</i>	14	<i>TSC2</i>	33		
<i>ATM</i>	22	<i>BRCA1</i>	5	<i>CDKN2A</i>	2	<i>NF1</i>	30	<i>NF2</i>	16	<i>RB1</i>	27	<i>TSC1</i>	15	<i>TSC2</i>	34		
<i>ATM</i>	23	<i>BRCA1</i>	6	<i>CDKN2A</i>	3	<i>NF1</i>	31	<i>PIK3CA</i>	2	<i>SMAD4</i>	2	<i>TSC1</i>	16	<i>TSC2</i>	35		
<i>ATM</i>	24	<i>BRCA1</i>	7			<i>NF1</i>	34	<i>PIK3CA</i>	3	<i>SMAD4</i>	3	<i>TSC1</i>	17	<i>TSC2</i>	36		
<i>ATM</i>	25	<i>BRCA1</i>	9	<i>IDH2</i>	4	<i>NF1</i>	35	<i>PIK3CA</i>	4	<i>SMAD4</i>	4	<i>TSC1</i>	18	<i>TSC2</i>	37		
<i>ATM</i>	26	<i>BRCA1</i>	10	<i>MEN1</i>	2	<i>NF1</i>	37	<i>PIK3CA</i>	5	<i>SMAD4</i>	5	<i>TSC1</i>	19	<i>TSC2</i>	38		
<i>ATM</i>	28	<i>BRCA1</i>	11	<i>MEN1</i>	3	<i>NF1</i>	38	<i>PIK3CA</i>	6	<i>SMAD4</i>	6	<i>TSC1</i>	20	<i>TSC2</i>	39		
<i>ATM</i>	29	<i>BRCA1</i>	12	<i>MEN1</i>	4	<i>NF1</i>	39	<i>PIK3CA</i>	7	<i>SMAD4</i>	7	<i>TSC1</i>	22	<i>TSC2</i>	40		
<i>ATM</i>	30	<i>BRCA1</i>	13	<i>MEN1</i>	5	<i>NF1</i>	40	<i>PIK3CA</i>	8	<i>SMAD4</i>	9	<i>TSC1</i>	23	<i>TSC2</i>	41		
<i>ATM</i>	31	<i>BRCA1</i>	14	<i>MEN1</i>	6	<i>NF1</i>	42	<i>PIK3CA</i>	9	<i>SMAD4</i>	12	<i>TSC2</i>	2	<i>TSC2</i>	42		
<i>ATM</i>	32	<i>BRCA1</i>	15	<i>MEN1</i>	7	<i>NF1</i>	43	<i>PIK3CA</i>	11	<i>SMARCB1</i>	1	<i>TSC2</i>	3	<i>VHL</i>	1		
<i>ATM</i>	34	<i>BRCA1</i>	16	<i>MEN1</i>	8	<i>NF1</i>	44	<i>PIK3CA</i>	12	<i>SMARCB1</i>	2	<i>TSC2</i>	4	<i>VHL</i>	2		