Signature® Technology Platform for the Rapid Multiplex Detection of Mutations in Solid Tumor Specimens

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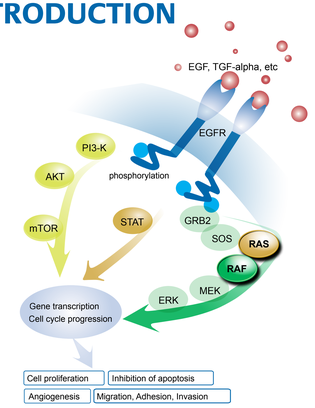
SUMMARY

- Advances in cancer identification and treatment have fundamentally improved patient care based on the underlying genetic signature of the cancer or individual.
- Several classes of anti-EGFR inhibitors require assessment of EGFR, KRAS or BRAF mutations to determine course of therapy.
- The Signature® Technology Platform enables the rapid and sensitive detection of specific mutations in flexible, multiplex gene panels that support individualized tumor profiling.†

INTRODUCTION



The EGFR signaling cascade (Figure 1) is a primary target of new cancer therapies. The assessment of KRAS and/or EGFR mutations are formal guidelines in identifying colorectal cancer (CRC) and lung cancer patients most likely to benefit from EGFR-targeted therapy. Additionally, BRAF and RAS mutation testing is included in the 2009 Revised ATA Thyroid Cancer Guidelines to improve preoperative FNA diagnosis of thyroid cancer. The Signature® Platform Technology offers rapid and sensitive detection of common mutations associated with cancer and response to treatment.

MATERIALS AND METHODS

The detection of codon variants was accomplished following isolation of DNA, PCR amplification using biotin-modified primers, hybridization with bead-bound, mutation-specific capture probes, and detection on a Luminex platform (Figure 2A). This workflow was applied to the detection of specific mutations in the Signature® KRAS/BRAF Mutations Panel along with prototype panels for BRAF, NRAS, HRAS and EGFR (Figure 2B). An endogenous control gene, EC, was included in each gene panel. Assay development and optimization incorporated cancer-derived cell lines or plasmid constructs. The analytical sensitivity for each DNA mutation was assessed by dilution of mutant cell line DNA or plasmids into a background of wild-type (WT) genomic DNA. Clinical specimens were obtained as residual, de-identified samples according to approved procedures.

A. Signature® Platform Technology Workflow

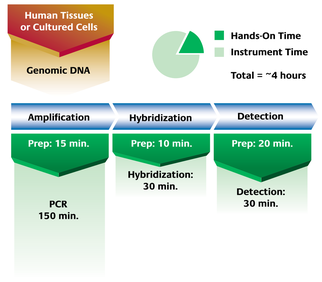


Figure 2. Signature® Technology Workflow and Mutation Panel Design. A) A schematic of amplification, hybridization and detection showing total and hands-on (prep) time. Following DNA isolation, biotin-modified PCR products were generated in specific panels and hybridized to bead-bound, codon-specific probes. All beads were detected using a Luminex system. **B)** The Signature® KRAS/BRAF Mutations* Panel were tested along with prototype panels for HRAS/NRAS, EGFR and BRAF V600E/K601E. Each sub-panel was tested in a separate PCR and included an endogenous control gene, EC.

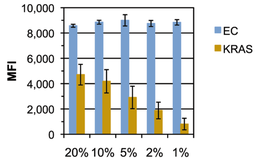
B. Signature® KRAS/BRAF Mutations* and Prototype Gene Panels†

Signature® KRAS/BRAF Mutations*		Prototype Panels†			
KRAS 7	BRAF/KRAS+5	BRAF	NRAS and HRAS		EGFR
G12V	BRAF V600E	V600E	Q61R	Q61L	T790M
G12D	G13S	K601E	Q61L	Q61R	Ex19-Del
G12S	G13C		Q61K	G12V	L858R
G12R	G13R				
G12C	G13V				
G12A	G13A				
G13D					

RESULTS

KRAS and BRAF

A. KRAS 7 Analytical sensitivity



B. BRAF/KRAS+5 Analytical Sensitivity and Specificity

	G13S	G13R	G13C	G13V	G13A	EC	V600E
AGC plasmid in HCT1116	5290	241	406	241	148	10986	203
CGC plasmid in HCT1116	407	5817	293	306	128	11644	121
TGC plasmid in HCT1116	165	95	4152	205	117	10861	59
GTC plasmid in HCT1116	279	125	488	7545	321	11159	167
GCC plasmid in HCT1116	274	0	280	144	4858	10611	181
HCT1116 100%	106	64	36	131	104	9224	131
HT29 100%	157	150	109	91	73	9406	10272
HT29 in HCT1116 10%	50	124	134	158	71	9458	6477
HT29 in HCT1116 5%	127	133	114	91	125	10265	4555
HT29 in HCT1116 1%	124	100	129	145	119	9652	2048

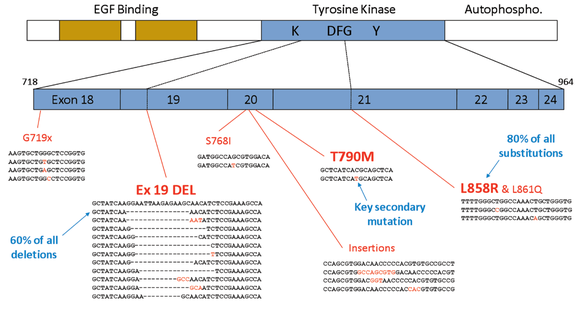
C. FFPE Specimen Testing

Sample	EC	G12A	G12C	G12D	G12R	G12S	G12V	G13D	G13A	G13C	G13R	G13S	G13V	V600E	EC	Signature call	LDT Call
Pooled Control	6088	8419	5221	8248	5298	5097	6089	5452	1447	3497	2793	1791	3690	6973	3429	Pass	
NTC	129	115	21	10	189	199	119	40	60	101	65	124	62	33	24	Pass	
101	7799	127	51	4928	181	98	127	50	20	33	69	60	123	19	6823	G12D	G12D
102	4122	95	43	50	125	102	1892	12	81	36	29	35	47	37	3091	G12V	G12V
103	5671	61	75	52	127	125	109	48	63	63	86	40	54	1293	1811	V600E	V600E
104	6154	1461	60	118	193	125	128	100	94	81	65	90	154	12	5539	G12A	G12A
105	5073	61	129	73	1008	165	132	115	53	56	54	27	95	73	6031	G12R	G12R
106	3522	65	52	40	140	186	118	66	63	23	779	77	51	41	2476	G13R	G13R
107	5264	121	61	94	154	163	75	58	91	89	89	33	149	53	5608	Neg	Neg
108	6216	37	76	80	172	1475	101	64	70	54	32	67	109	27	5981	G12S	G12S
109	104	132	47	86	181	190	172	84	38	87	51	35	97	0	69	ND*	G12V
110	7928	116	2843	45	223	247	56	71	36	73	86	63	65	66	5909	G12C	G12C
111	7087	89	75	124	131	113	124	70	26	88	62	16	219	7337	6348	V600E	V600E
112	8108	85	106	73	179	179	37	5503	6	55	55	26	187	71	7901	G13D	G13D

Figure 3. Signature® KRAS/BRAF Preliminary Sensitivity, Specificity and Detection of FFPE Samples. A) Analytical sensitivity of the Signature® KRAS Mutations 7 assay. The mean MFI values were calculated by testing genomic DNA isolated from FFPE blocks containing 3 KRAS mutant positive cells (G12C, G12V, or G13D) diluted in a background of KRAS WT cells at 20, 10, 5, 2, or 1%. The error bars show the respective standard deviations from the mean signal for each dilution tested. **B)** Signature® BRAF/KRAS+5 Mutations. The assay specifically detected 5 additional mutations in KRAS codon 13 and BRAF V600E with a preliminary analytical sensitivity of about 1%. The HCT1116 cell line is positive for KRAS G13D, HT29 is WT for KRAS and positive for BRAF V600E. **C)** Example of results using the combined assays (12 KRAS mutations and V600E) and 10 ng of DNA isolated from representative FFPE CRC specimens. The genotype of each sample determined using an independent clinically-validated, LDT is shown on the far right (partial results from an ongoing external comparator study with over 340 archived FFPE metastatic CRC specimens). NTC-No template control. *ND, not detected. As indicated in the signal for the EC, the submitted sample lacked detectable DNA.

EGFR

A. High-incidence EGFR Mutations



B. EGFR Analytical Sensitivity

	L858R	EX19-DEL	T790M	EC
NTC	146	27	439	111
DU-145	51	47	198	11861
HCT-116	79	110	147	12238
HT-29	81	45	197	12631
H1975				
100%	7617	38	10040	11505
20%	6789	97	7470	11969
10%	5973	57	6329	12427
5.0%	4935	18	4224	11615
2.5%	3567	80	2837	12257
1.0%	2604	83	1760	11767
0.5%	734	96	961	12440
H1650				
100%	103	12314	97	11632
20%	49	10307	190	12259
10%	80	9715	47	12237
5.0%	97	8066	201	12211
2.5%	83	6748	63	12278
1.0%	136	4934	35	12082
0.5%	67	4685	118	12695

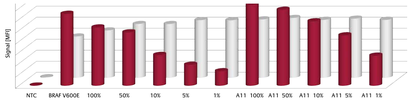
Figure 4. EGFR Targets with Preliminary Detection Sensitivity and Specificity. A) Schematic of the EGFR gene showing complexity of DNA variations and high-frequency mutations. **B)** Detection of EGFR targets in a titration series of two cell lines (H1975 is a double mutant) from 100% to 0.5% input. NTC and cell line negative controls are shown. The results indicate detection to at least 1% target mutation.

*Research Use Only. Not For Use In Diagnostic Procedures.

†Preliminary Research Data. The performance characteristics of this assay have not yet been established.

BRAF V600E/K601E

A. BRAF V600E Analytical Sensitivity



B. BRAF V600E and K601E Co-detection

Wt: CTAGCTACAG**GTGAAA**TCTCGATGGAG
V600E: CTAGCTACAG**AG**AAATCTCGATGGAG
K601E: CTAGCTACAGTG**AA**TCTCGATGGAG

Sample	V600E	K601E
NTC	15	70
HT-29 V600E	1631	111
p.K601E	12	2934

Figure 5. BRAF V600E Preliminary Analytical Specificity and Sensitivity and variant co-detection A) Representative data with genomic DNA purified either from B-CPAP, a papillary thyroid cancer-derived cell line (DSMZ) homozygous (HOM) for V600E mutation, or from a fresh-frozen papillary thyroid cancer biopsy sample (A11), respectively. In both cases, a preliminary sensitivity equivalent to at least 1% mutant was achieved. **B)** Feasibility assessment of multiplexed detection of V600E cell line DNA or K601E plasmid in a background of WT DNA, each at 10 ng input.

NRAS and HRAS

A. HRAS Analytical Sensitivity and Specificity

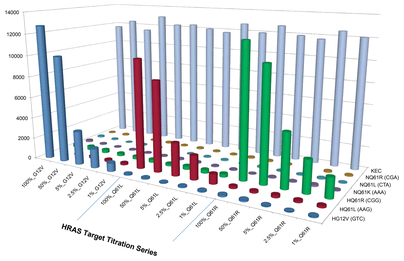


Figure 6. HRAS and NRAS Mutation Panels for Sensitive Detection of Flash Frozen and Fine Needle Aspirate (FNA) Specimens. A) Representative data for the detection of HRAS or NRAS codon 61 variants in flash frozen or fine needle aspirate (FNA) specimens. All sample positives were confirmed with the reference method. *ND, not detected; the sample DNA yield was below a calling threshold. **B)** Representative titration of cell line gDNA (10 ng) or plasmid (3,300 copies) with specific HRAS mutations. A preliminary sensitivity equivalent to 1% mutant was achieved.

B. Thyroid Flash Frozen and FNA Specimens

Source	Sample	HRAS G12V	HRAS Q61L	HRAS Q61R	NRAS Q61R	NRAS Q61K	NRAS Q61L	Control	Call
Controls	Pooled Positive	7115	6725	6645	5369	5409	6620	11432	PASS
	NTC	120	47	132	63	89	78	104	PASS
Flash Frozen	Follicular Carcinoma	83	160	205	153	136	90	10263	NEG
	Medullary Carcinoma	150	134	9686	155	223	54	10416	HRAS Q61R
	Follicular Adenoma	159	88	160	5140	81	359	9844	NRAS Q61R
	Anaplastic Carcinoma	126	132	383	80	145	80	9555	NEG
	Follicular Adenoma	101	157	197	140	87	75	10544	NEG
Fine Needle Aspirate	FNA-035P	107	104	94	33	120	72	7509	NEG
	FNA-036P	82	80	75	72	8502	53	10172	NRAS Q61K
	FNA-037P	78	60	64	58	109	39	8561	NEG
	FNA-034P	116	111	150	71	62	80	1546	ND*
	FNA-012P	93	55	87	68	69	43	9927	NEG

CONCLUSION

- The Signature® Technology Platform* enables the specific and sensitive detection† of codon variants within multiple gene targets associated with targeted EGFR cancer therapies.
- This highly multiplexed approach can be scaled to accommodate modular mutation panels, and enable flexibility in variant detection.
- This simple and rapid workflow is amenable to processing 96 samples for one panel or 24 samples across all panels in a single-plate within 5 hours.†
- The limit of detection was 1% input of each mutant DNA with both cell line and clinical samples representing a range of specimen sources.†
- Personalized medicine is the forefront of new cancer therapies. Determination of the unique genetic characteristics of the tumor or the individual with cancer can contribute to improved patient care.