Rapid, Multi-Gene Mutation Detection Panel for Metastatic Colorectal Cancer

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Introduction

We have developed a real-time PCR assay for the detection of common metastatic colorectal cancer (mCRC) mutations in 5 oncogenes, including KRAS and NRAS (codon 12, 13, 59, 61, 117, 146); BRAF (codon 600), PIK3CA (codon 542, 545, 1047), and AKT1 (codon 17), using human genomic DNA isolated from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer samples. The purpose of this study was to validate performance of EntroGen's CRC Mutation Detection Panel by evaluating limit of detection (LoD) and accuracy. We also determined concordance between the prevalence of KRAS, NRAS, BRAF, PIK3CA, and AKT mutations in CRC patients reported in the literature and results attained using the CRC Panel.

Methods

For LoD assessment, broad-range dilutions of mutant DNA (isolated from FFPE specimens or gBlocks Gene Fragments-based controls) at an internal control (IC) Ct of 29 were made using wild-type human colon FFPE DNA to yield 10%, 5%, 2.5%, 1%, and 0.5% mutant allelic burden over wild-type background. The assay was performed 3 times in duplicates on an ABI 7500 Fast instrument. Based on the results, narrow-range serial dilutions (in 0.5% increments) were made from the lowest detectable broad-range dilution to establish a more precise LoD. Assay accuracy was tested using 120 FFPE DNA samples isolated from patient CRC tumor specimens. The DNA samples were screened for relevant mutations (i.e., KRAS, NRAS, BRAF, PIK3CA, or AKT) with a commercial mutation detection kit to determine mutation status. Concordance between the CRC Panel and the commercial kit was measured as the overall percent agreement, percent positive agreement, and percent negative agreement for all valid samples (within an IC Ct range of 26-29). For a comparison of the prevalence of CRC mutations reported in the literature and detection with the CRC Panel, the assay was applied to 686 DNA samples extracted from FFPE CRC tumors.

Results

The panel detects 78% (39/50) of mutations at or below a LoD of 2.5%. 92% (46/50) of mutations are detected at or below a LoD of 5% (Figure 1).

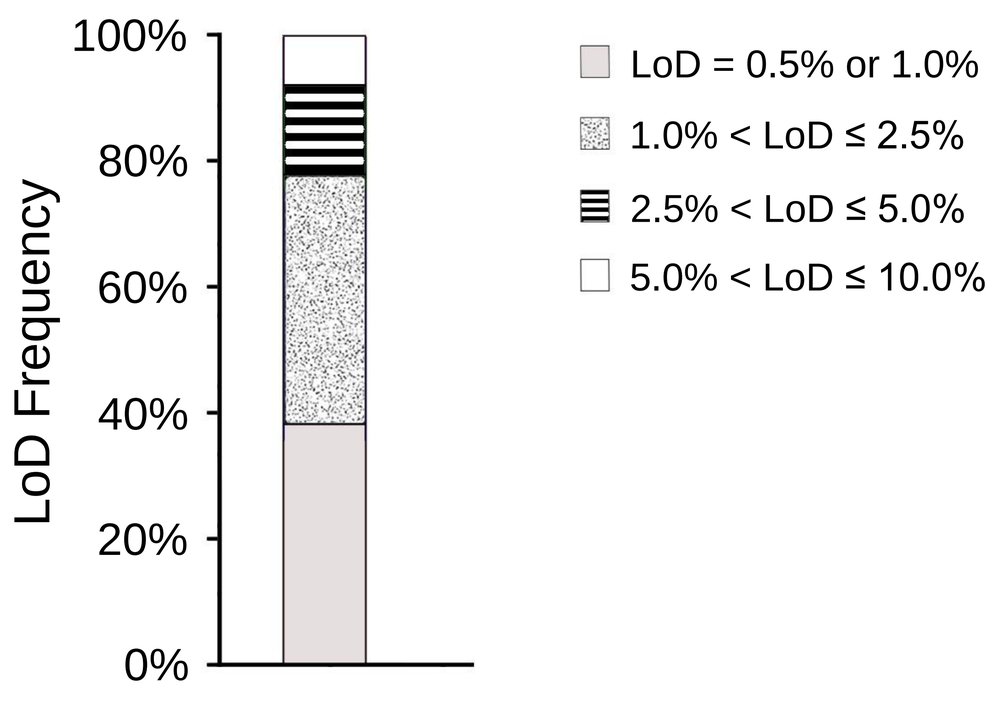

Figure 1. LoD summary for mutations detected by the Colorectal Cancer Mutation Detection Panel.

For assay accuracy, a comparison of mutation status calls between the CRC Panel and the commercial kit shows 95.19%, 99.02%, 100%, 100%, 100%, and 99.10% overall percent agreement for reactions 1-6, respectively (Table 1).

CRC Reaction #	Invalid Samples (outside IC Ct range of 26-29)	Valid Samples (within IC Ct range of 26-29)	% Mutation Status Agreement
1	16	104	95.19% (99/104)
2	18	102	99.02% (101/102)
3	15	105	100% (105/105)
4	12	108	100% (108/108)
5	10	110	100% (110/110)
6	9	111	99.10% (110/111)

Table 1. Accuracy results summary.

Of the 686 CRC samples tested for assay concordance with the reported occurrence of mutations in patient samples from the literature, 35 (5.10%) did not meet the input requirement for the assay and gave an invalid result. Of the 651 valid samples, 391 (60.06%) presented a single mutation (Table 2), while 51 (7.83%) had double mutations occurring concurrently (data not shown). When grouped by oncogene, the incidence of observed single mutations was comparable to what has been described in the literature (Table 3).

CRC Mutation	Prevalence %
KRAS 12/13	224/651 = 34.41%
BRAF 600	37/651 = 5.68%
PIK3CA 1047	35/651= 5.38%
PIK3CA 542/545	34/651 = 5.22%
KRAS 146	25/651 = 3.84%
KRAS 61	12/651 = 1.84%
NRAS 61	8/651 = 1.23%
NRAS 12/13	7/651 = 1.08%
KRAS 117	4/651 = 0.61%
AKT1 E17K	4/651 = 0.61%
KRAS 59	1/651 = 0.15%

Table 2. Single CRC mutations detected with the CRC Mutation Detection Panel.

	Prevalence %	
Target	Literature	CRC Panel
KRAS	36-40[1]	40.85
PIK3CA	10-30[2]	10.60
BRAF	8-10[3]	5.58
NRAS	1-6[4]	2.31
AKT1	<1-6[5]	0.61

Table 3. Prevalence of CRC mutations reported in the literature compared with those detected by the CRC Mutation Detection Panel.

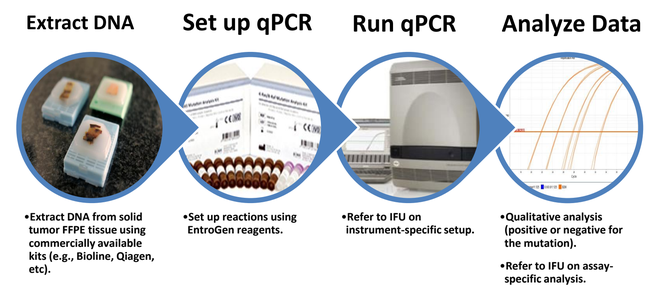

Figure 2. Colorectal Cancer Mutation Detection Panel assay workflow.

Conclusions

The CRC Mutation Detection Panel can accurately recognize single and double mutations in 5 different oncogenes with high sensitivity. Furthermore, the occurrence of the mutations detected is in line with what has been reported in the literature.

References

1. COSMIC; Faulkner et al. 2010; Neumann et al. 2009.
2. COSMIC; Samuels et al. 2004.
3. Varghese et al. 2015; Tejpar et al. 2010.
4. COSMIC; De Roock et al. 2010; Irahara et al. 2009; Vaughn et al. 2011.
5. COSMIC; Fumagalli et al. 2008; Kim et al. 2008.