EntroGen's Colorectal Cancer Mutation Detection Panel enables simultaneous screening of 50 somatic mutations associated with colorectal cancer tumors

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Introduction

Colorectal cancer (CRC) represents the third most common form of cancer worldwide. Tumor-associated somatic mutations, commonly found in different oncogenes, may be prognostic or predictive markers for available treatment.

The Colorectal Cancer Mutation Detection Panel (cat. No. CRC-RT48) is the first available real-time PCR kit able to detect common mutations in the 5 oncogenes, KRAS and NRAS (codon 12, 13, 59, 61, 117), BRAF (codon 600), PIK3CA (codon 542, 545, 1047) and AKT1 (codon 17) in one run. Here we demonstrate that EntroGen's Colorectal Cancer Mutation Detection Panel can reliably detect these oncogenic mutations when applied to a series of human genomic DNA samples from CRC patients.

Product Application

The Colorectal Cancer Mutation Detection Panel contains all necessary reagents for the detection of 50 somatic mutations associated with CRC by real-time PCR. As an example, the Colorectal Cancer Mutation Detection Panel was applied to 686 DNA samples isolated from formalin fixed, paraffin embedded CRC tumors.

Table 1a. Single CRC mutations detected with the Colorectal Cancer Mutation Detection Panel.

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%

Out of 686 samples, 35 (5.10%) did not meet the input requirement for the assay and gave an invalid result. Out of the 651 valid samples, 391 (60.06%) presented a single mutation, while 51 (7.83%) had double mutations occurring concurrently. Examples of double mutant amplification curves are presented in Figure 2. The frequency of specific single and double mutations is shown in Table 1a and 1b respectively.

Table 1b. Concurrent CRC mutations detected with the Colorectal Cancer Mutation Detection Panel.

CRC Mutation	Double Mutants Prevalence %
KRAS 12/13; PIK3CA 542/545	24/651 = 3.69%
KRAS 12/13; PIK3CA 1047	13/651 = 2.00%
KRAS 61; PIK3CA 1047	4/651 = 0.61%
KRAS 12/13; KRAS 146	2/651 = 0.31%
BRAF 600; PIK3CA 1047	2/651 = 0.31%
BRAF 600; AKT1 E17K	2/651 = 0.31%
KRAS 61; KRAS 146	1/651 = 0.15%
KRAS 117; PIK3CA 1047	1/651 = 0.15%
KRAS 146; PIK3CA 1047	1/651 = 0.15%
NRAS 12/13; PIK3CA 542/545	1/651 = 0.15%

When grouped by oncogene, the incidence of observed single mutations was comparable to what has been reported in the literature (Table 2).

Table 2. Comparison of prevalence of CRC mutations reported in the literature and detected with the Colorectal Cancer Mutation Detection Panel.

	Prevalence %	
Oncogene	Literature	EntroGen 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

Figure 1. Workflow for the EntroGen's CRC Mutation Detection Panel.

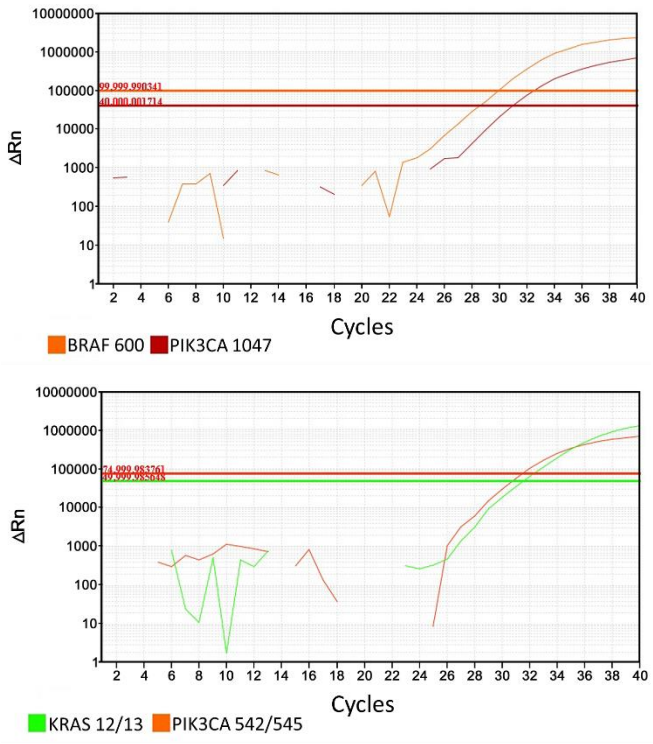

Figure 2. Double mutant FFPE DNA samples amplification with CRC Mutation Detection Panel. Only the positive reactions are shown and internal control curves are omitted for clarity.

Conclusions

Here we demonstrated that the CRC Mutation Detection Panel can accurately and efficiently recognize single and double mutations in 5 different oncogenes.

Moreover, the occurrence of the mutations detected is in line with what has been described in the literature. The CRC Mutation Detection Panel represents the first assay of its kind; screening 45 clinically relevant mutations in a single run. Compared to other comprehensive assays, such as NGS targeted gene panels, the CRC Mutation Detection Panel offers faster turnaround time, ease of use and simplified analysis (Figure 1). The CRC Mutation Detection Panel combines the high specificity and sensitivity of real-time PCR with a well-designed multiplex format for broader coverage than most targeted approaches.

References

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