PrimeraDx
The Multiplex PCR Company

ONCOLOGY   INFECTIOUS DISEASE   TRANSLATIONAL MEDICINE

SNP Detection | Copy Number Variation | Chromosomal Abnormalities | Gene Expression | miRNA | Pathogen Detection | Pathogen Quantitation | Methylation | Multimodal

Application Brief

NRAS/BRAF Point Mutation Analysis Panel

Unique All-In-One-Well Assay with High Specificity and Sensitivity

INTRODUCTION

The *NRAS* and *BRAF* genes are proto-oncogenes involved in nor¬mal cellular function of controlling cellular growth. However, *NRAS* and *BRAF* point mutations can lead to tumor development and proliferation and have been implicated in variety of human tumors and particularly in melanoma. *NRAS* and *BRAF* somatic mutations are found in 9-29% and 53-66% of melanomas, respectively. These mutations result in con¬tinuous cell growth and are potential targets for therapy. Information on the mutation status can help clinicians choose appropriate treatment. Here we have developed a single-well multiplex *NRAS/BRAF* Point Mutation Analysis Panel on the ICEPlex® system that can detect and discriminate 12 *NRAS* and 4 *BRAF* clinically important mutations as shown below:

CDS Mutation	Amino Acid
NRAS c.35 G>A	G12D
NRAS c.34 G>A	G12S
NRAS c.38 G>C	G13A
NRAS c.38 G>A	G13D
NRAS c.37 G>C	G13R
NRAS c.38 G>T	G13V
NRAS c.37 G>T	G13C
NRAS c.183 A>C (H1)	Q61H

CDS Mutation	Amino Acid
NRAS c.182 A>G (R1)	Q61R
NRAS c.182_183 AA>GG (R2)	Q61R
NRAS c.182 A>T	Q61L
NRAS c.181 C>A	Q61K
BRAF c.1799_1800 TG>AT	V600D
BRAF c.1799 T>A	V600E
c.1799_1800 TG>AA	V600E
c.1798_1799 GT>AA	V600K

MULTIPLE ANSWERS IN LESS THAN 4 HOURS
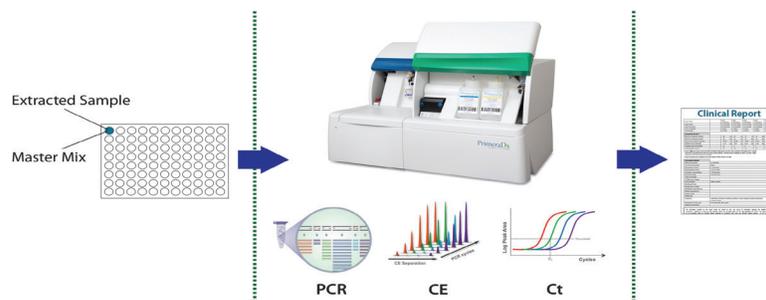
SUMMARY

- Delivers multiple answers - 16 *NRAS/BRAF* mutations in a single PCR reaction.

- Provides high quality by simultaneous detection of built-in controls such as: a set of calibration controls or size stan-dards, DNA quality and extraction controls.

- Requires minimum DNA input and addresses specimen size issue.

- Expedites sample turn-around time to less than 4 hours.

METHOD HIGHLIGHTS

- Primers were designed using PrimeraDx's unique strategy that can selectively amplify *NRAS/BRAF* point mutations. All primers were analyzed in silico for primer-primer interaction and cross-reactivity. One of the primers in each primer set was labeled with either FAM- or TYE- dye.
- PCR amplification conditions were optimized using proprietary PCR chemistry on the ICEPlex system.
- Multiplex PCR reactions were subjected to thermocycling on a standard 96-well PCR plate on the ICEPlex system.
- The fluorescently labeled amplicons for the different *NRAS/BRAF* mutations were injected, separated and detected in the capillary electrophoresis module of the ICEPlex system.
- ICEPlex system software plotted the fluorescent signals for different amplicons, generated amplification curves for all targets and controls, and calculated cycle thresholds (Cts).

TYPICAL DATA

As shown below, we were able to detect and discriminate 13 important mutations in *NRAS* and 3 important mutations in *BRAF* genes.
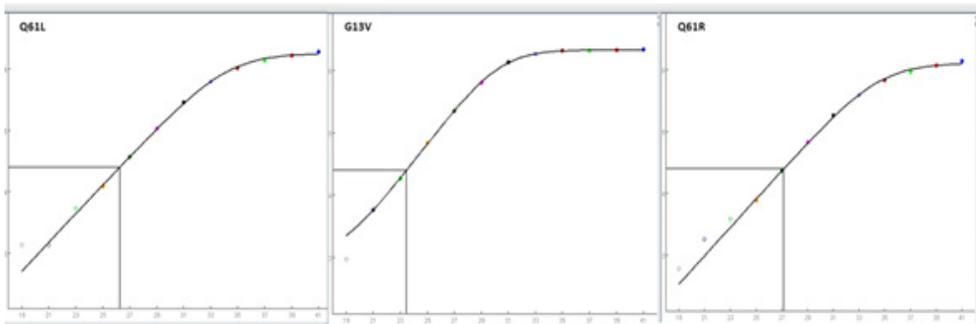
Figure 1 Representative amplification curves for 3 *NRAS/BRAF* targets on the ICEPlex system.

		G12D	G12S	G13A	G13C	G13D	G13R	G13V	Q61H1	Q61H2	Q61K	Q61L	Q61R1	Q61R2	V600D	V600E	V600K
Sample 1	Ct											26.2					
	Result	No	No	No	No	No	No	No	No	No	No	Detected	No	No	No	No	No
Sample 2	Ct							23.5									
	Result	No	No	No	No	No	No	Detected	No	No	No	No	No	No	No	No	No
Sample 3	Ct												27.2				
	Result	No	No	No	No	No	No	No	No	No	No	No	Detected	No	No	No	No

Figure 2. Representative results for three samples.

FOR MORE INFORMATION

For a list of publications and to find out more about how PrimeraDx can help your lab, please contact us at 508.618.2300 or visit www.primeradx.com.