JN MEDSYS

APPLICATION NOTE

Rare mutation detection using Clarity™ Digital PCR System

I. Overview

Mutation of the BRAF oncogene has shown to be associated with a myriad of human cancers such as malignant melanomas, papillary thyroid and colorectal cancers[1]. So far, more than 40 different BRAF mutations have been reported and at least 90% of these substitution correspond to the V600E rare mutation. Detection of V600E allele with high sensitivity is therefore critical as it serves as a biomarker for targeted cancer therapy using BRAF inhibitors which improve clinical response and survival rates[2]. Here, we describe the use of JN Medsys Clarity™ digital PCR system as a useful non-invasive tool for BRAF V600E rare mutant detection.

II. Clarity™ digital PCR system detected less than 0.001% of V600E mutant allele

BRAF V600E mutant fractions were prepared by mixing purified HT-29 cell line DNA with wild-type human DNA (Promega). These fractions were then analyzed using a duplex Taqman assay. Each reaction mixture was sub-divided into approximately 10000 partitions using the Clarity™ tube-strip. This was followed by PCR and data detection using the Clarity™ reader. As shown in Table 1, Clarity™ detected as low as 0.0007% of mutant DNA. When the data were plotted against the expected mutant fraction, the results showed a strong linear relationship with an R^2 value of 0.9989 (Figure 1).

Table 1. Results of BRAF V600E mutant detection using Clarity™ digital PCR system. All experiments were performed in

Table 2. List of primers and probes used in the duplex assay. Underlined nucleotide in mutant probe sequence corresponds to the mutation site.

Expected Mutant Fraction (%)	Mutant (FAM Assay)		Wildtype (VIC Assay)		Measured Mutant Fraction (%)
	Measured Concentration (copies/µL)	Relative Uncertainty (%)	Measured Concentration (copies/µL)	Relative Uncertainty (%)	
1	19.24	3.33	2533	1.51	0.7540
0.1	2.6	5.22	2670	3.57	0.0973
0.01	0.21	1.13	2661	1.00	0.0079
0.001	0.02	86.6	2700	1.00	0.00074
0	0	-	2566	0.67	0

Oligonucleotide	Sequence (5′-3′)
Forward primer	TACTGTTTTCCTTTACTTACTACACCTCAGA
Reverse primer	ATCCAGACAACTGTTCAAACTGATG
Mutant probe (FAM)	TAGCTACAG<u>A</u>GAAATC
Wildtype probe (VIC)	CTAGCTACAGTGAAATC

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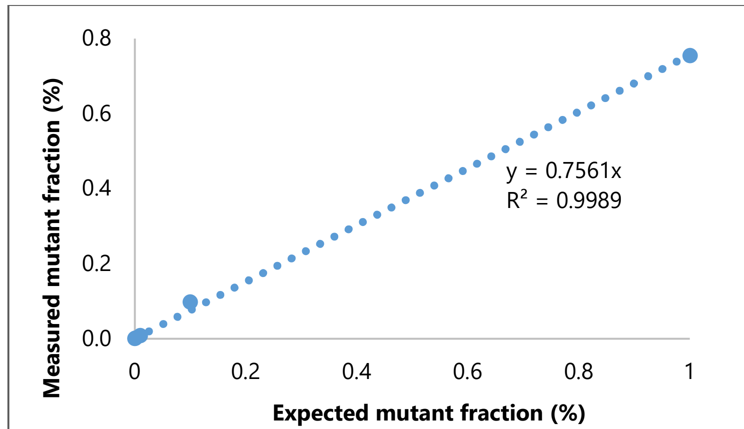

Figure 1. Plot of measured V600E mutant fraction against its expected mutant fraction.

III. Conclusion

Detection of rare mutant target from a background of wild-type allele requires a high degree of sensitivity. Conventional methodologies such as real-time PCR typically could only detect down to 1% mutant DNA with an optimized assay. The availability of new approaches with higher sensitivities are thus desirable for detecting extremely low levels of mutant. In this study, we demonstrated that Clarity™ digital PCR enabled detection of very low level of BRAF V600E mutant, which is 1000 times more sensitive than real-time PCR. This approach of detecting rare mutant allele is anticipated to play a valuable role in supporting cancer diagnosis, prognosis and therapeutic options.

References

1. Cantwell-Dorris ER, O'Leary JJ and Sheils OM (2011) BRAF V600E: implications for carcinogenesis and molecular therapy. Mol Cancer Ther. 10(3):385-94
2. Kwong LN, Davies MA. (2014) Targeted therapy for melanoma: rational combinatorial approaches. Oncogene. 33(1):1-9

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