High-Definition Digital PCR (HDPCR™) Enables Sensitive Measurement of DNA and RNA Variants

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Introduction

Digital PCR (dPCR) is an emerging platform for detecting variants in cancer genomes due to its high sensitivity and fast time to result compared to massively parallel sequencing. However, translational oncology applications often require the measurement of more biomarkers than there are color channels available on dPCR platforms. One approach to address this is to split a sample across many wells and profile a subset of variants in each well. However, for input-limited samples, this results in fewer molecules being profiled in each well, resulting in a reduction in sensitivity and fewer samples processed per instrument run.

ChromaCode has developed a research use only (RUO) high-definition PCR (HDPCR™) assay on a digital PCR instrument for multiplexed detection of 14 DNA variants and 15 RNA fusion variants relevant in non-small cell lung cancer (NSCLC) samples. The assay is constructed using both amplitude modulation and multi-channel resilient signal coding methods.

Methods

DNA and RNA samples were contrived by diluting synthetic oligonucleotides in extracted nucleic acids from plasma and FFPE tissue. For the cell-free DNA contrived samples, we tested mutant allele fraction (MAF) in the range 0.25% to 20% and for FFPE contrived samples the MAF range was 1% to 40%. Samples were run with the HDPCR NSCLC RUO assay on the Applied Biosystems QuantStudio™ Absolute Q Digital PCR system, and data analysis was performed with proprietary analysis algorithms.

Resilient Coding Technology

ChromaCode's HDPCR[1,2] technology enables different variants to generate a signal at different intensity levels in single color channel, allowing for greater than N targets in N color channels. In contrast, resilient coding generates a signal in more than one color channel to create a form of error-detecting code.

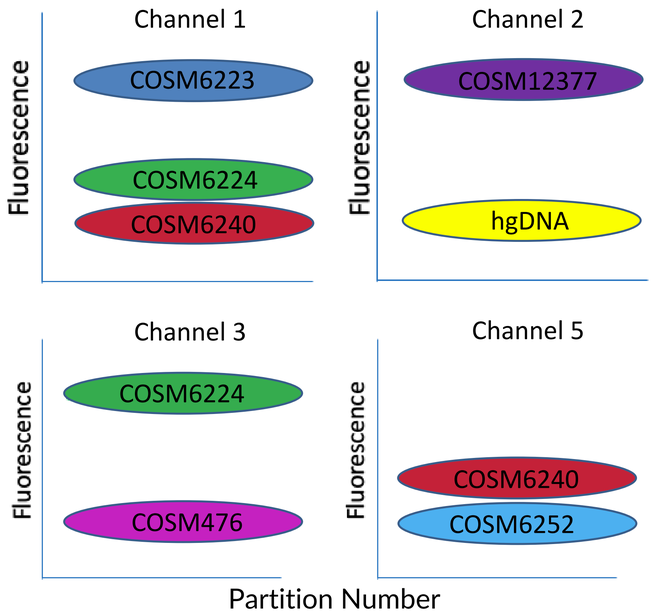

Figure 1. Resilient encoding strategy for molecular variants: The above four schematic plots show how multiple targets are encoded in a single channel using the HDPCR™ technology.

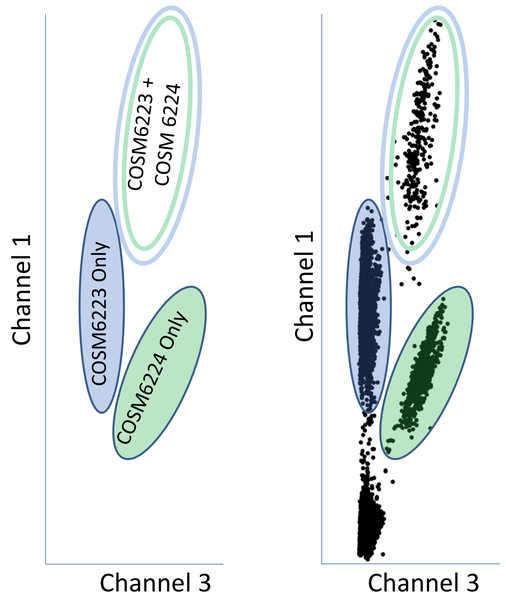

Figure 2. The above schematic plot (left) and the experimental data (right, 20% MAF, contrived with cfDNA background) show both a resilient encoding strategy and multiple levels of signals in HDPCR. COSM6224 is resiliently encoded in both channels 1 and 3. When both variants co-occur HDPCR is able resolve partitions that contain both and those that contain only one.

Results

The HDPCR NSCLC RUO assay demonstrated a limit of detection as low as 10 variant copies in a 10,000 haploid human genome copy DNA background. The tables below show results for samples from two different sources- cell-free DNA (contrived), FFPE extracted DNA (contrived). The assay demonstrates excellent performance across all categories in the wide range of mutant allele fractions tested.

Cell-free DNA		Detected	
		+	-
Spiked-in	+	259	0
	-	0	1117

FFPE DNA		Detected	
		+	-
Spiked-in	+	235	1
	-	0	1049

Cell-free RNA		Detected	
		+	-
Spiked-in	+	189	0
	-	6	881

FFPE RNA		Detected	
		+	-
Spiked-in	+	234	2
	-	9	1146

Table 1: Types of samples tested and their results

EGFR L858R COSM6224		Detected	
		+	-
Spiked-in	+	167	0
	-	0	211

EML4-ALK variant 1 COSMAB274722.1		Detected	
		+	-
Spiked-in	+	83	0
	-	3	406

KRAS G12C COSM516		Detected	
		+	-
Spiked-in	+	37	0
	-	0	70

MET exon 14 skipping		Detected	
		+	-
Spiked-in	+	86	1
	-	5	403

EGFR T790M COSM6240		Detected	
		+	-
Spiked-in	+	122	1
	-	0	254

KIF5B-RET (K15:R12) COSMAB795250.1		Detected	
		+	-
Spiked-in	+	86	0
	-	2	409

EGFR Exon20 H773dup COSM12377		Detected	
		+	-
Spiked-in	+	42	0
	-	0	326

MPRIP-NTRK1 (M21:N14) COSMKF724384.1		Detected	
		+	-
Spiked-in	+	87	0
	-	5	401

EGFR E746_A750del COSM6223		Detected	
		+	-
Spiked-in	+	126	0
	-	0	252

EZR-ROS1 (E10-R34) COSMAB795246.1		Detected	
		+	-
Spiked-in	+	81	1
	-	0	408

Table 2: Combined results of both contrived-cfDNA and contrived-FFPE samples with breakdown by representative DNA and RNA variants.

Conclusion

ChromaCode's HDPCR chemistry and resilient coding technology enables laboratories to maximize the amount of data that can be generated from a dPCR instrument. This prototype assay illustrates a prime application for comprehensive genomic variant detection that can be performed without the high-cost capital and long turnaround times associated with massively parallel sequencing.

References

1. Jacky, Lucien, et al. "Robust Multichannel Encoding for Highly Multiplexed Quantitative PCR." Analytical Chemistry 93.9 (2021): 4208-4216.
2. Jacky, Lucien, et al. "Virtual-Partition Digital PCR for High-Precision Chromosomal Counting Applications." Analytical Chemistry (2021).

[1] This protocol is not approved or cleared by the US FDA and is in development. For research use only, not for use in diagnostic procedures.