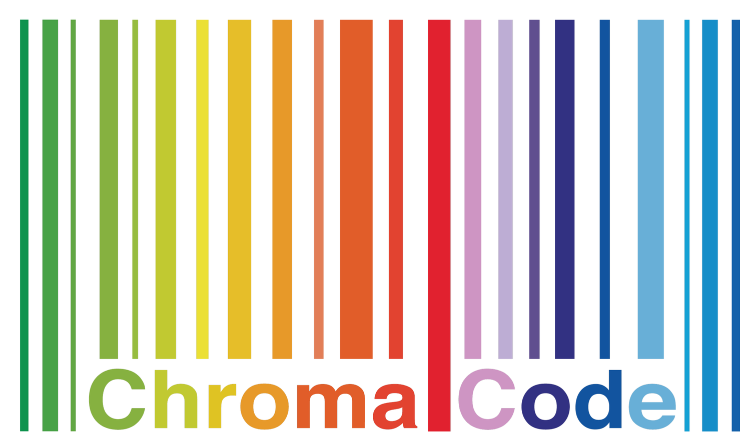

High-Definition PCR (HDPCR™) Detection of Precision Biomarkers in Non-Small Cell Lung Cancer Samples

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Introduction

Based on a 2020 ACS CAN survey of cancer patients and survivors, only 33% had received biomarker testing. Of those patients not tested, more than 25% of those reported it was because of high cost or insurance issues. A low cost and more accessible alternative to NGS is necessary so all cancer patients can have access to biomarker testing. Based on its high sensitivity, faster turnaround, and familiar workflow, digital PCR (dPCR) is an emerging platform for detecting genomic variants. dPCR is traditionally limited in multiplexing capabilities by the number of color channels on the instrument, limiting practical applications in complex fields like oncology. ChromaCode has leveraged their High Definition PCR (HDPCR™) technology to increase the multiplexing of dPCR instruments by ten-fold[1,2].

ChromaCode has developed a research use only (RUO) dPCR assay, 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 encoding methods. Amplitude modulation 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 encoding generates a signal in more than one color channel to create a form of error detecting code. The combination of these two techniques allows for unique fluorescence signatures to be associated with partition populations inhabited by specific targets as seen in Figure 1.

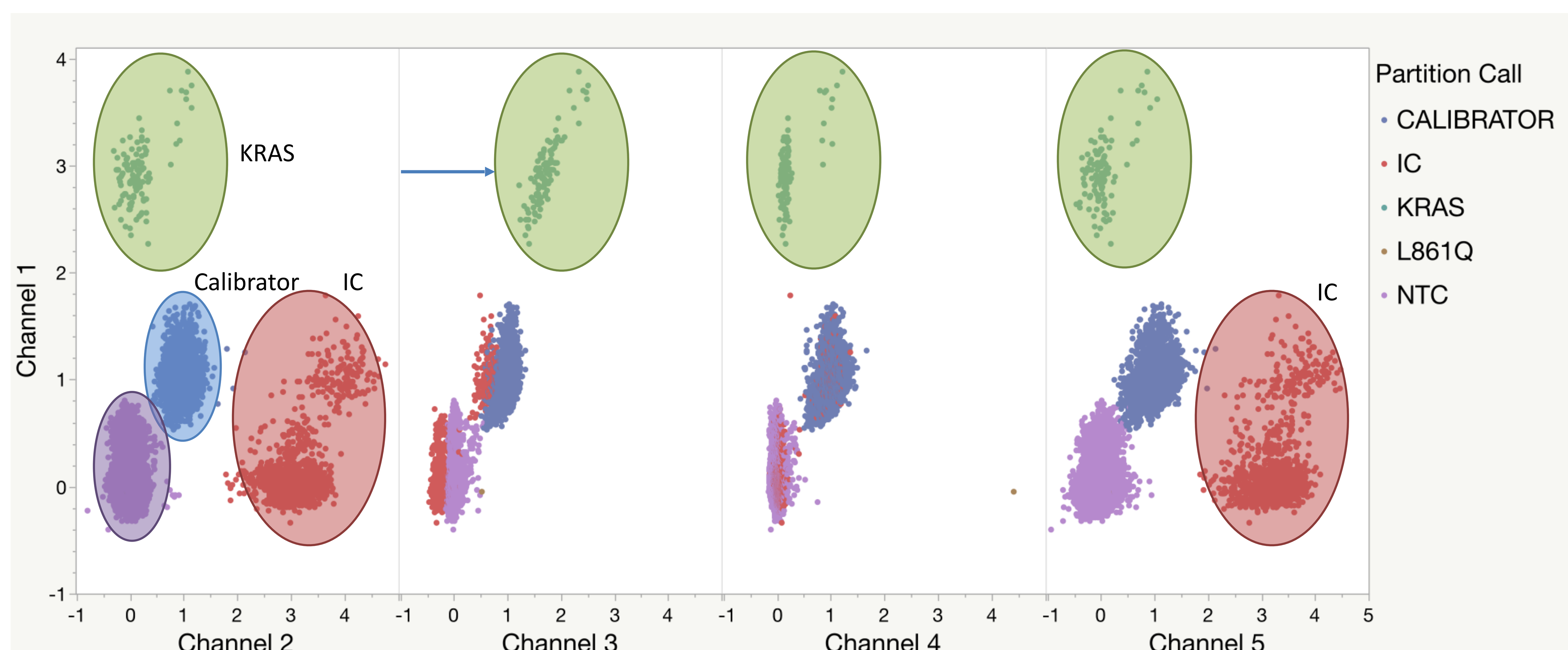

Figure 1. Example of amplitude modulation and resilient coding, where the KRAS target is at a normalized fluorescence value of 2 in Channel 3, a normalized fluorescence value of 0 in Channel 1, and a 0 in Channels 2, 4, and 5. The redundance of the KRAS target in multiple channels allows for increased target specificity.

Methods

The ChromaCode HDPCR NSCLC RUO assay is composed of three wells, two for DNA detection and one for RNA fusion detection. Each well contains a calibrator, internal control and five reportables. All tests were performed on a Qiagen QIAcuity™ instrument utilizing proprietary ChromaCode Cloud™ algorithms to enable automated calling. All extractions were completed using a custom Promega sequential DNA, RNA extraction. A cohort of contrived and clinical samples were run to characterize the limit of detection of the assay. Negative samples utilized to create contrived samples were pooled, and the concentration was measured by Qubit™. Twenty (20) or 10 ng of human gDNA were spiked with plasmids containing the target of interest at decreasing mutant allele fraction (MAF) levels. Twenty (20) ng of RNA extracted from negative FFPE samples was spiked with in vitro transcripts for the target of interest at decreasing copy number.

Clinical samples were extracted using the same method and quantitated by Qubit. The comparator method for the Clinical samples was the Oncomine™ Precision Assay GX. 20 ng of each clinical sample was input into each well of the assay.

Figure 2. Overview of HDPCR workflow, yielding a 24 hour turn-around-time

This assay is for research use only. Not to be used in diagnostic procedures.

Results

The MAF detected for DNA samples ranged from 0.3% to 1.2% with 20 ng of DNA sample input and from 1.9% to 3.8% with 10 ng of DNA sample input. The lowest copy number tested in with 20 ng of RNA input ranged from 42 to 75 positive partitions (Table 1).

Twenty-five (25) FFPE samples were evaluated using the HDPCR NSCLC RUO assay. In total four samples were invalid, three due to low Internal Control counts and one due to an instrument failure. Of the 21 samples that gave a valid result, 18 were concordant with the comparator NGS result with MAFs ranging from 0.3% to 61.5% (Table 2). Three false negatives were observed. One false positive for T790M (11 positive partitions, 0.2% MAF) was observed in "Sample 1" where S768I and L858R were correctly identified in the same sample.

Target	ng Input (DNA)	MAF
EGFR Exon 19 deletions	20	1.18 %
EGFR S768I	20	1.08 %
EGFR L858R	20	1.22 %
BRAF V600E	20	0.47 %
EGFR T790M	20	0.33 %

Target	ng Input (DNA)	MAF
EGFR Exon 20 insertions	10	3.87 %
ERBB2 (HER2) Exon 20	10	2.50 %
EGFR G719X	10	2.14 %
KRAS G12C	10	2.06 %
EGFR L861Q	10	1.91 %

Target	ng Input (RNA)	Lowest Positive Partitions Detected
ROS Fusions	20	61
RET fusions	20	55
NTRK fusions 1/2/3	20	59
ALK fusions	20	75
MET	20	42

Table 1. Limit of detection for each well of the HDPCR NSCLC Assay

Sample (NGS Result)	HDPCR Reported Target	Result	Reportable Partitions	IC Partitions	MAF
Sample 1 (L858R, S768I)	S768I	True Positive	2567	6059	42.4 %
	L858R	True Positive	3457	6059	57.1 %
	T790M	False Positive	11	6059	0.2 %
Sample 2 (S768I)	S768I	True Positive	513	2817	18.2 %
Sample 3 (L858R)	L858R	True Positive	1032	3250	31.8 %
Sample 4 (L858R)	L858R	True Positive	8	1498	0.05 %
Sample 5 (BRAF V600E)	Invalid	Invalid (Instrument Failure)	NA	NA	NA
Sample 6 (BRAF V600E)	BRAF	True Positive	336	926	36.3 %
Sample 7 (Exon19 Deletion)	Invalid	Invalid (Internal Control Failure)	NA	340	NA
Sample 8 (Exon19 Deletion)	Invalid	Invalid (Internal Control Failure)	NA	90	NA
Sample 9 (T790M)	No Target Detected	False Negative	0	705	NA
Sample 10 (T790M)	T790M	True Positive	3	821	0.04%
Sample 11 (G719A)	G719X	True Positive	3970	6459	61.5 %
Sample 12 (G719A)	G719X	True Positive	486	2845	17.1 %
Sample 13 (L861Q)	Invalid	Invalid (Internal Control Failure)	102	47	NA
Sample 14 (L861Q)	L861Q	True Positive	296	1489	19.9 %
Sample 15 (KRAS G12C)	KRAS	True Positive	533	1188	44.9 %
Sample 16 (HER2 Exon20 Insertion)	HER2 EX20 INS	True Positive	1566	5991	26.1 %
Sample 17 (ALK)	ALK	True Positive	47	NA	NA
Sample 18 (ALK)	ALK	True Positive	73	NA	NA
Sample 19 (ROS)	ROS	True Positive	187	NA	NA
Sample 20 (ROS)	ROS	True Positive	66	NA	NA
Sample 21 (RET)	RET	True Positive	214	NA	NA
Sample 22 (RET)	RET	True Positive	728	NA	NA
Sample 23 (NTRK)	No Target Detected	False Negative	10	NA	NA
Sample 24 (NTRK)	No Target Detected	False Negative	30	NA	NA
Sample 25 (MET)	MET	True Positive	1282	NA	NA

Table 2. Summary of NSCLC specimen performance on the HDPCR NSCLC Assay

Conclusion

The assay includes relevant research biomarkers in current National Comprehensive Cancer Network (NCCN) guidelines , including variants in EGFR, BRAF, KRAS, ERBB2, ALK, ROS1, RET, MET, and NTRK, is sensitive down to < 5% variant allele fractions, and has >95% concordance with sequencing-based assays. This assay leverages the proprietary bioinformatics suite, the ChromaCode Cloud, to report relevant variants with the click of a button and in 24 hours, allowing for an easily adopted solution to testing needs.

References

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