Technical Sheet – LightMix® Digital KRAS G12C assay

Summary Information

Assay kit information

Product Cat.-No:	20-3006-32
Assay type:	Detection Kit for KRAS G12C mutation
Coverage:	KRAS G12C mutation c.34G>T
Probe fluorophores:	FAM/HEX
Probe quenchers:	BHQ1
Primers/probes supplied as:	Air-dried oligo mix
Amplicon length:	72bp
MIQE context sequence:	ATAGTCACATTTTCATTATTTTTATTATAAGGCCTGCTGAAAATGACTGAATATA AACTTGTGGTAGTTGGAGCT[G/T]GTGGCGTAGGCAAGAGTGCCTTGACGATA CAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAG
Positive control:	4-6% plasmid with COSM516 sequence insert in human genomic DNA background.

Gene information

Gene name:	KRAS proto-oncogene, GTPase
Gene symbol:	KRAS
Species:	Human
COSMIC ID for mutation:	COSM516

Verification information

Instrument:	Digital LightCycler
MasterMix:	Digital LightCycler 5x DNA Master
Restriction enzyme:	MseI
Wild type template:	Human genomic DNA from blood (buffy coat)
Sequence variant template:	Plasmid (with COSM516 sequence insert)
Annealing temperature:	58˚C
Cycling protocol:	

Step	Temperature (°C)	Time (sec)	Cycles
UNG activation	50	120	1
Denaturation	95	120	1
Amplification: *denaturation*	95	10	40
Amplification: *annealing/extension*	58	20	
Cooling	40	30	1

Stability

The stability of the reconstituted oligo mix has been tested for up to 60 days (stored at 4 °C to 8 °C) and showed < 10 % variability for 0.5 % and 5 % mutant samples (0.5 % and 5 % mutant in a background of 2cpp wild-type genomic DNA) in detected mutant concentration.

Template Input

Template input was varied between 0.5 and 2 copies per partition (cpp) to validate assay performance for different template loadings.

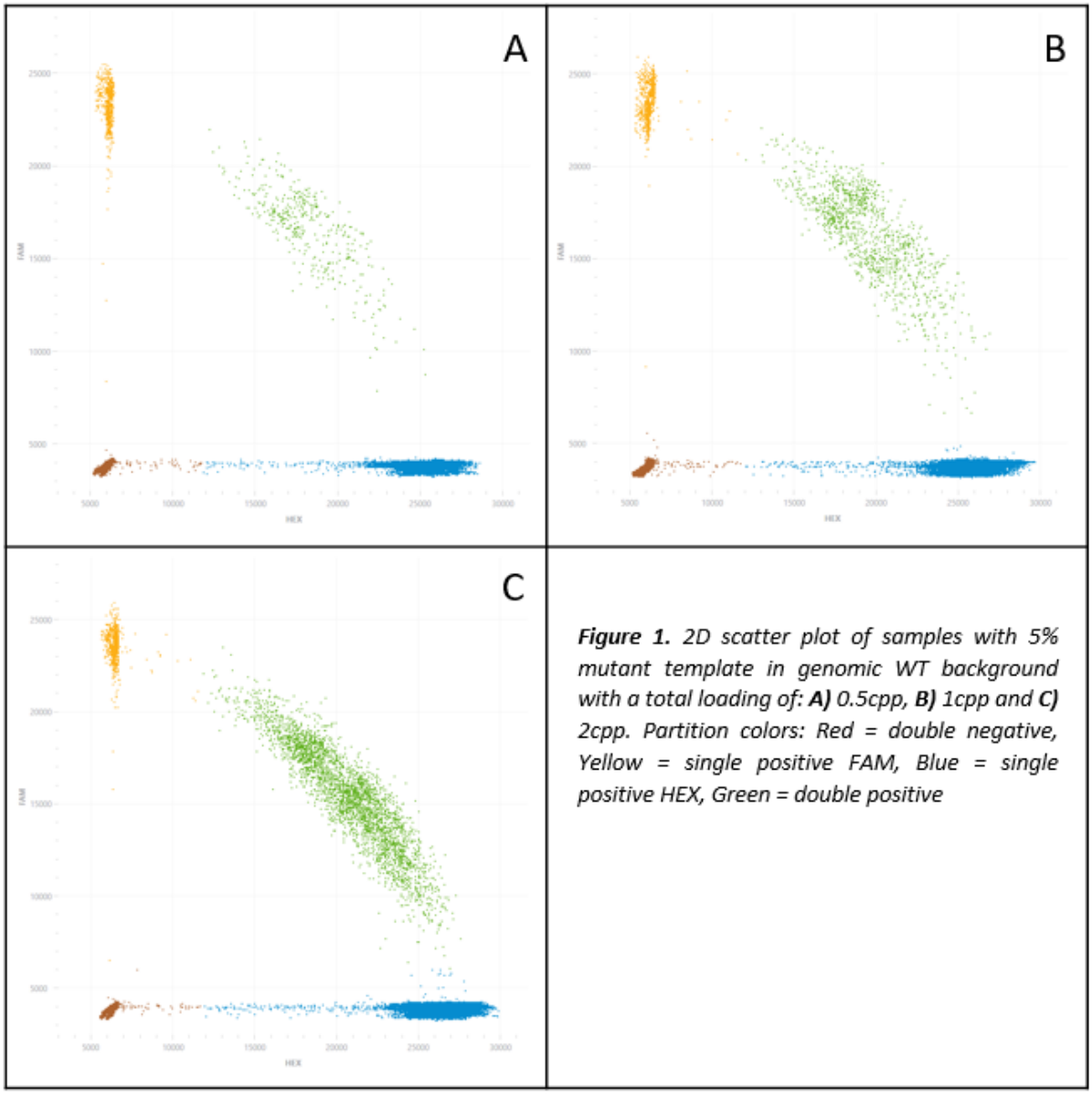

Figure 1. *2D scatter plot of samples with 5% mutant template in genomic WT background with a total loading of:* ***A)*** *0.5cpp,* ***B)*** *1cpp and* ***C)*** *2cpp. Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive HEX, Green = double positive*

Sensitivity

Varying amount of synthetic mutant DNA was spiked into a 2cpp background of wild-type genomic DNA. The contrived samples ranged from 0.05 % to 5 % mutant spike-in. Samples with only genomic DNA (0 % mutant) were also included as control. Analytical sensitivity estimated to be 0.05 % mutant.

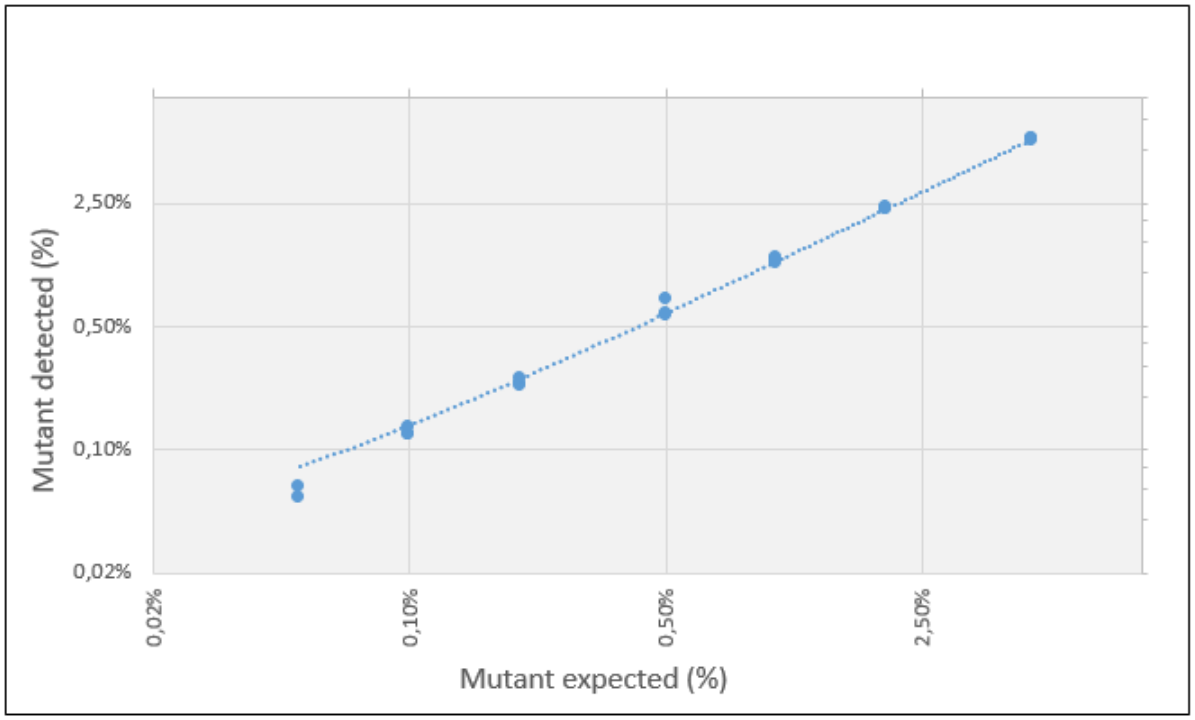

Figure 2. *Percentage mutant detected in the sample with 0.05 % to 5 % mutant in a WT genomic DNA background (axis in log-scale)*

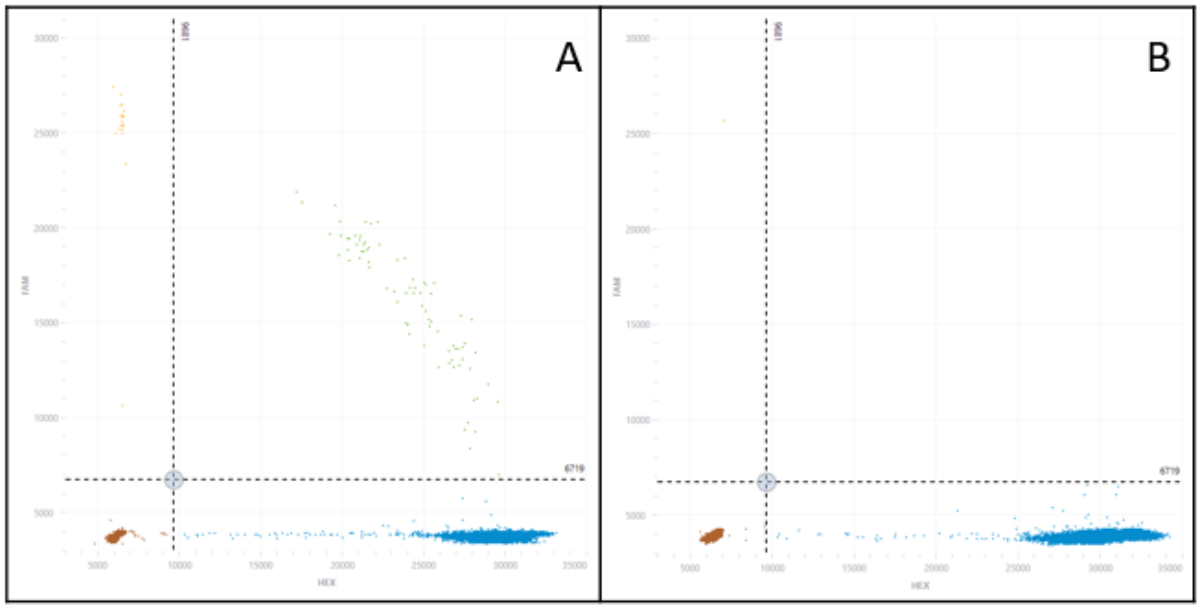

Figure 3. A) *2D scatter plot of a 0.05 % mutant template in a 2cpp WT genomic DNA background.* ***B)*** *2D scatter plot of a WT genomic DNA sample (2cpp). Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive HEX, Green = double positive*

Closely-related mutations

Samples with 5 % synthetic mutant template representing the various closely-related mutations were spiked into a 2cpp background of wild-type genomic DNA. This assay showed minimum cross-reactivity to closely related mutation KRAS G12R and G12S; with <25% fluorescence intensity generated in comparison to the intended target G12C. Manual clustering was used to ensure all cross-reactive species were thresholded as negative and true positives were called correctly. For the application of manual clustering, refer to the "Clustering and Data Analysis Guide" to choose the appropriate settings and determine where to place the manual threshold correctly.

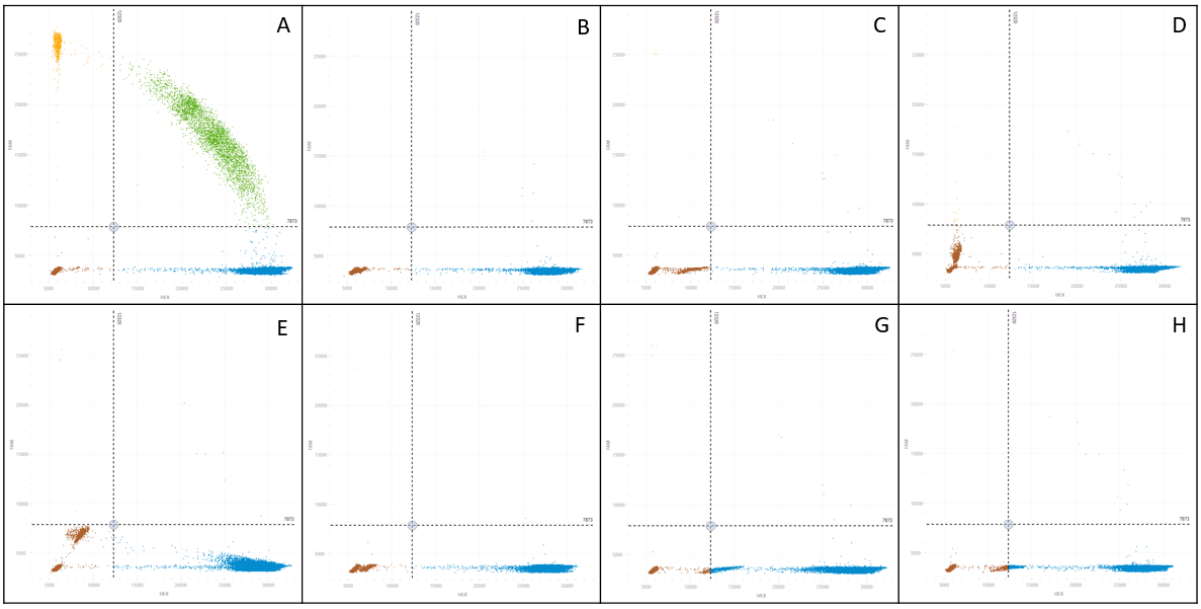

Figure 4. *2D scatter plots of samples with 5% mutant template representing the various closely-related mutations in a 2cpp WT genomic DNA background.* ***A)*** *KRAS G12C,* ***B)*** *KRAS G12A,* ***C)*** *KRAS G12D,* ***D)*** *KRAS G12R,* ***E)*** *KRAS G12S,* ***F)*** *KRAS G12V,* ***G)*** *KRAS G13D and* ***H)*** *KRAS G13C. Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive HEX, Green = double positive*

Version History

Tech Sheet ID	Change/ Event	Date
V1.0	Initial Release	2024-06-11