Technical Sheet – LightMix® Digital EGFR L861Q assay

Summary Information

Assay kit information

Product Cat.-No:	20-3011-32
Assay type:	Detection Kit for EGFR L861Q mutation
Coverage:	EGFR L861Q mutation c.2582T>A
Probe fluorophores:	FAM/HEX
Probe quenchers:	BHQ2
Primers/probes supplied as:	Air-dried oligo mix
Amplicon length:	76 bp
MIQE context sequence:	TTGGTGCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAAAACACCGCAGCATGTCAAGATCACAGATTTTGGGCTGGCCAAAC[T/A]GCTGGGTGCGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAGGTGGCTTTAGGTCAGCCAGCATTTTCCTGACA
Positive control:	4-6% plasmid with COSM6213sequence insert in genomic DNA (K562 cell line) background.

Gene information

Gene name:	Epidermal Growth Factor Receptor
Gene symbol:	EGFR
Species:	Human
COSMIC ID for mutation:	COSM6213

Verification information

Instrument:	Digital LightCycler
MasterMix:	Digital LightCycler 5x DNA Master
Restriction enzyme:	MseI, HindIII
Wild type template:	Human genomic DNA from blood (buffy coat)
Sequence variant template:	Plasmid (with COSM6213sequence 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 in +2-8°C) and showed < 20% variability for 5% mutant samples (5% mutant in a background of 1cpp 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.

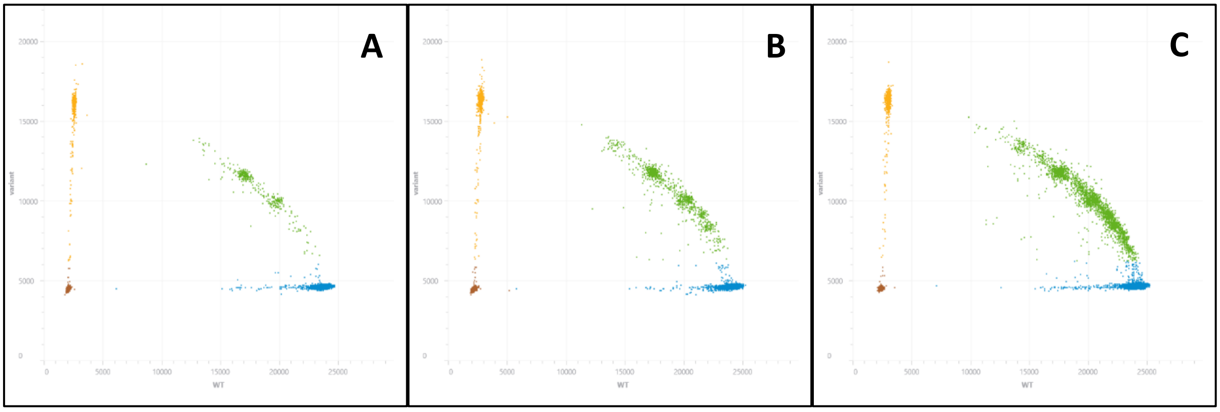

Figure 1. *2D scatter plot of samples with 5% mutant template in genomic WT background with a total loading of: **A)** 0.5cpp, **B)** 1cpp and **C)** 2cpp. Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive HEX, Green = double positive*

Sensitivity

Varying amounts of synthetic mutant DNA were spiked into a 1cpp background of wild-type genomic DNA. The contrived samples ranged from 0.05% to 5% mutant spike-in. Blank samples and samples with only genomic DNA (0% mutant) were also included as negative controls. Analytical sensitivity was estimated to be <0.1% mutant based on a limit of blank (LOB) of 0.004% and a lower end of the CI95% for the 0.05% mutant sample above this LOB.

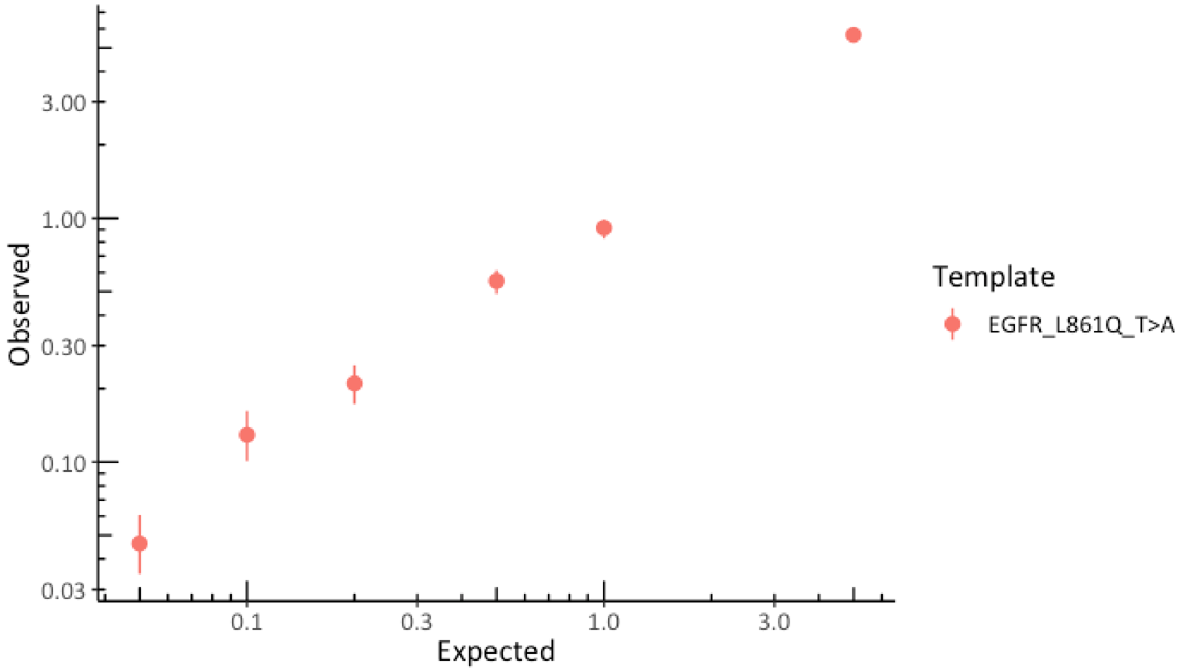

Figure 2. *Percentage mutant detected in the sample with 0.05% to 5% mutant in a WT genomic background (error bars = CI95%, axis in log-scale)*

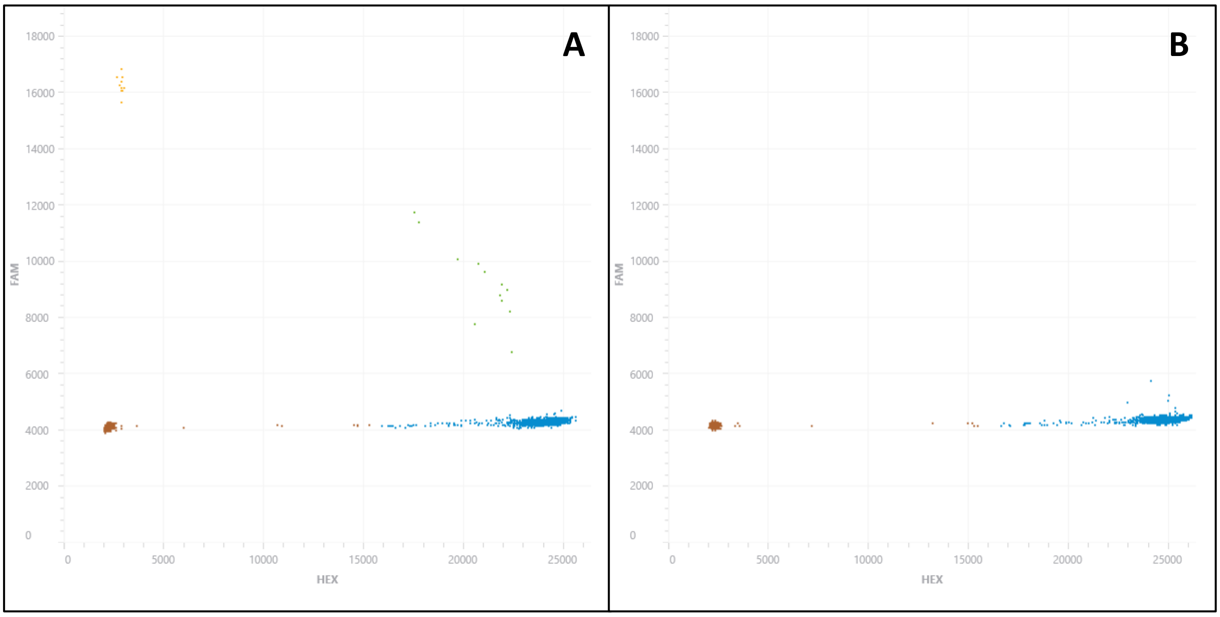

Figure 3. A) 2D scatter plot of a 0.1% mutant template in a 1cpp WT background. **B)** 2D scatter plot of WT sample (1cpp). Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive HEX, Green = double positive.

Version History

Tech Sheet ID	Change/ Event	Date
V1.0	Initial Release	2024-07-24