# Technical Sheet – LightMix® Digital EGFR L858R assay

## Summary Information

### Assay kit information

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|---|---|
| Product Cat.-No: | 20-3008-32 |
| Assay type: | Detection Kit for EGFR L858R mutation |
| Coverage: | EGFR L858R mutations c.2573T>G and c2573_2574delinsGT |
| Probe fluorophores: | FAM/HEX |
| Probe quenchers: | BHQ2 |
| Primers/probes supplied as: | Air-dried oligo mix |
| Amplicon length: | 84 bp |
| MIQE context sequence: | GACCGTCGCTTGGTGCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAAAAC ACCGCAGCATGTCAAGATCACAGATTTTGGGC[T/G]GGCCAAACTGCTGGGTG CGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAGGTGGCTTTA GGTCAGCCAGCATT |
| Positive control: | 4-6% plasmid with COSM6224 sequence insert in genomic DNA (K562 cell line) background. |

### Gene information

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|---|---|
| Gene name: | Epidermal Growth Factor Receptor |
| Gene symbol: | EGFR |
| Species: | Human |
| COSMIC ID for mutation: | COSM6224 and COSM12429 |

### Verification information

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| 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 COSM6224 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 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.

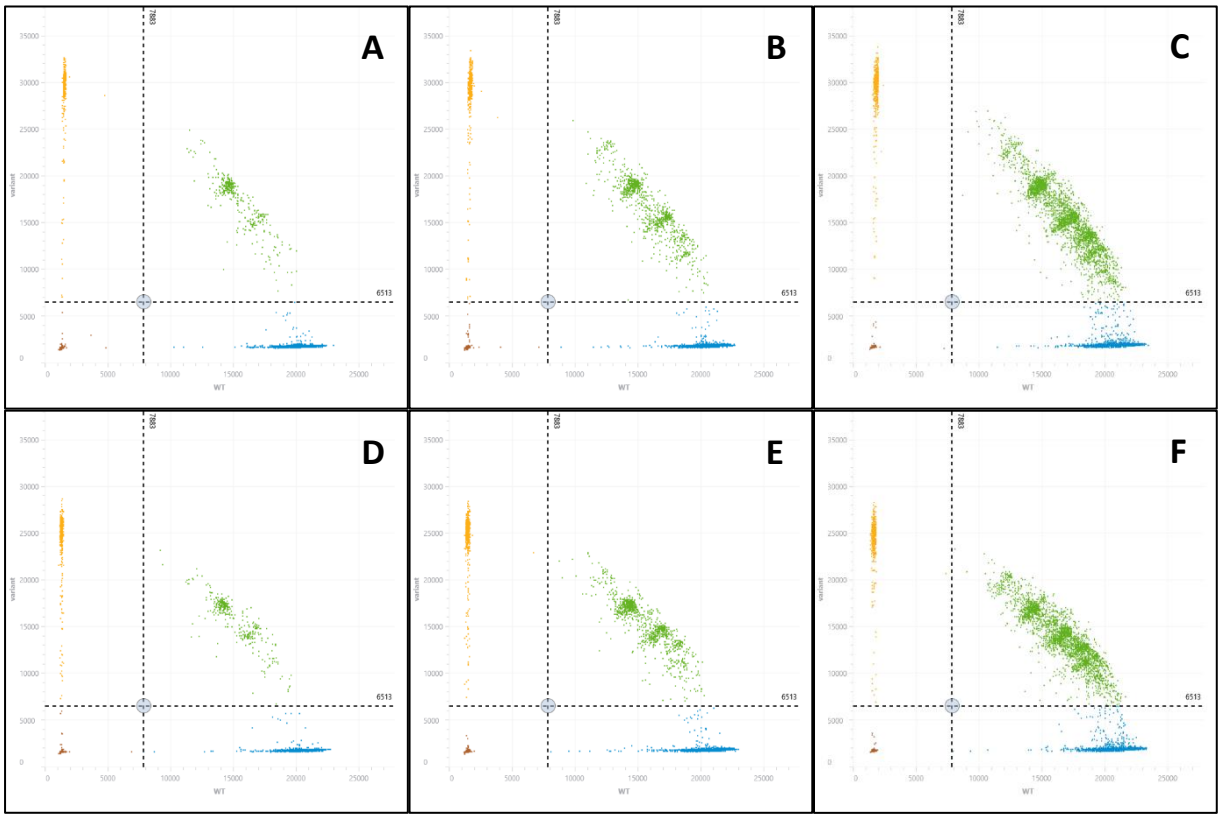


***Figure 1.*** *2D scatter plot of samples with 5% T>G (**A, B, C**) or delinsGT (**D, E, F**) mutant template in genomic WT background with a total loading of: **A, D)** 0.5cpp, **B, E)** 1cpp and **C, F)** 2cpp. Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive 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 0.002% and a lower end of the CI95% for the 0.05% mutant sample above this LOB.

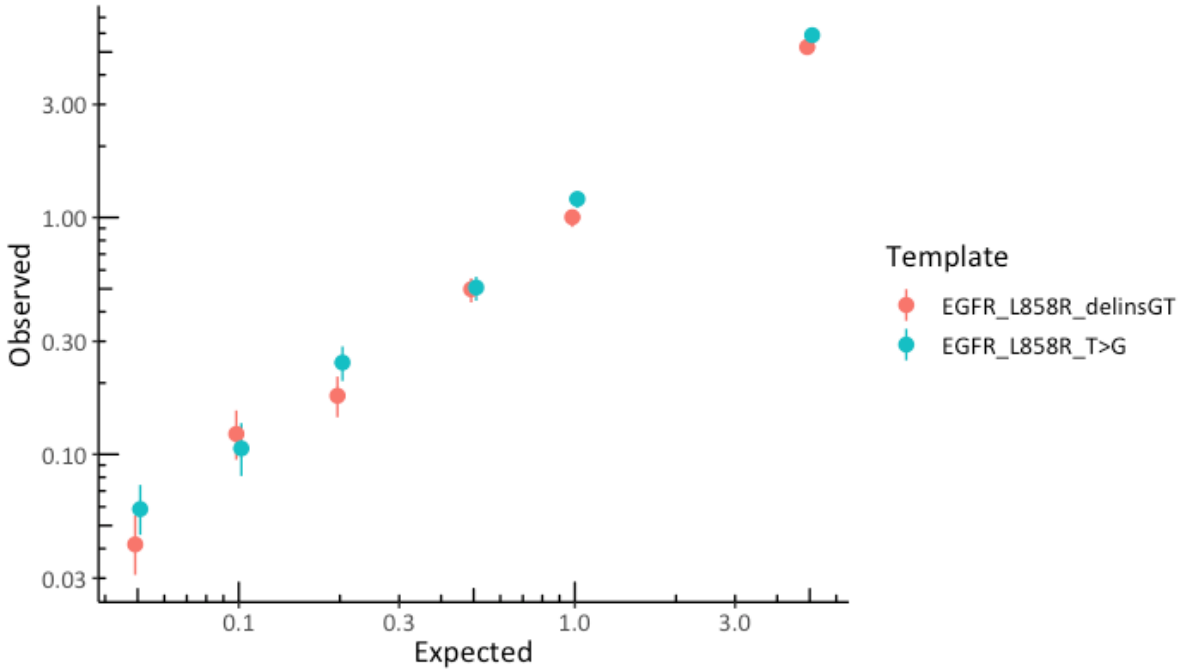


***Figure 2.*** *Percentage mutant detected in the sample with 0.05% to 5% mutant in a WT genomic background (error bars = CI95%, axis in log-scale)*

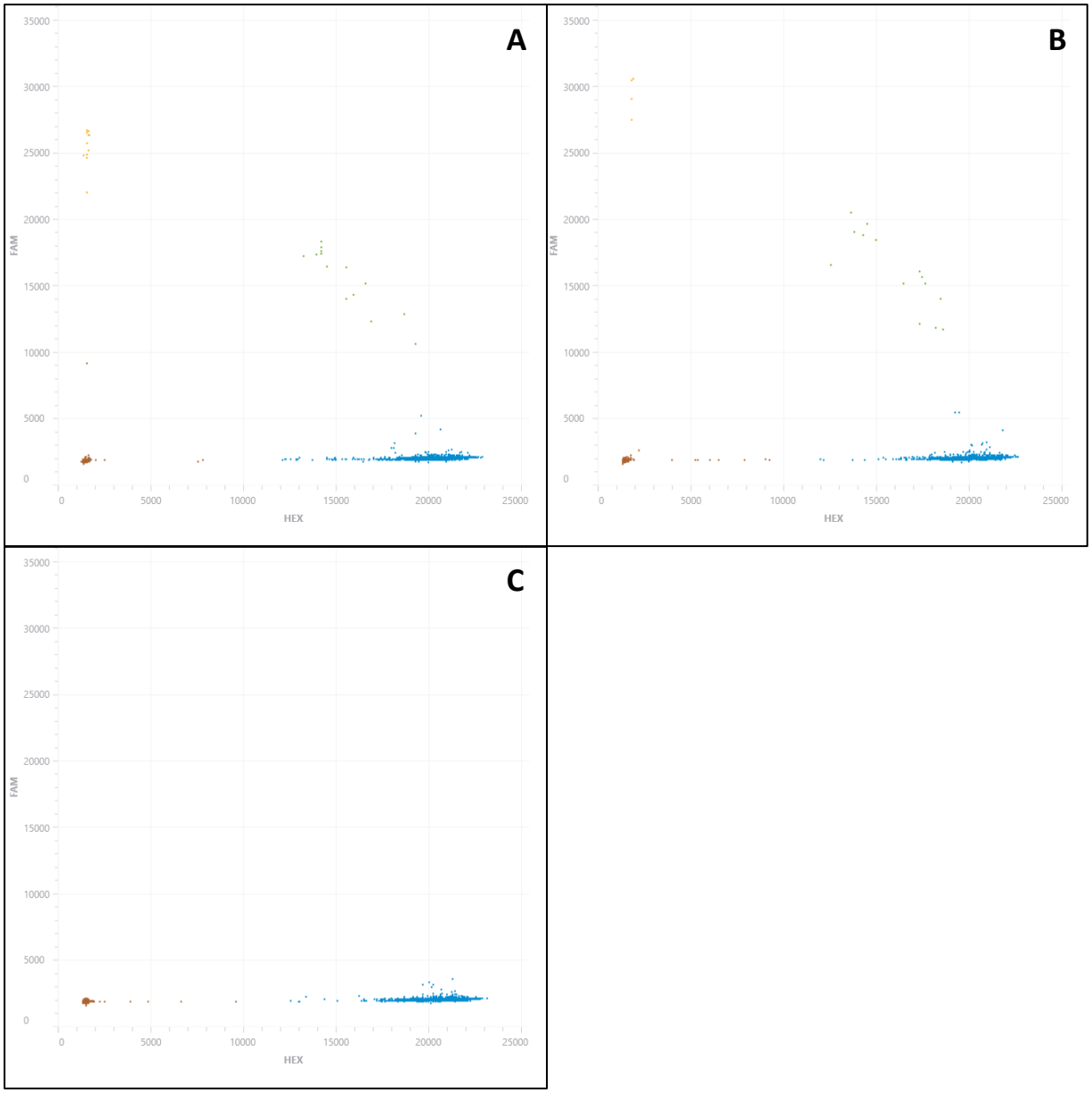


**Figure 3. A)** 2D scatter plot of a 0.1% delinsGT mutant template in a 1cpp WT background. **B)** 2D scatter plot of a 0.1% T>G mutant template in a 1cpp WT background. **C)** 2D scatter plot of WT sample (1cpp). Partition colors: Red = double negative, Yellow = single positive FAM, Blue = single positive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

## Version History

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