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Application note: Absolute quantification of KRAS G12S mutation from cell-free DNA (cfDNA) using molecular beacons in Optolane's 2-channel Dr. PCR 10k system.

Jirawas Sornkom and Seung Shick Shin

Lung cancer is one of the leading causes of cancer-related mortality worldwide. The most frequently mutated oncogenes in lung adenocarcinoma are the RAS family GTPases and EGFR[1]. Oncogenic KRAS mutations are well established negative predictors of response to anti-EGFR therapies, because the mutations result in a constitutively activation of downstream effectors, such as the RAF/MEK/ERK pathways[2]. Therefore, patients eligible for anti-EGFR treatments are recommended to be tested for KRAS mutations, presenting in approximately 29.7% of all non-small cell lung cancer (NSCLC) cases[3] before starting such therapies.

The KRAS G12S single nucleotide mutation results in a missense mutation and amino acid substitution at the position 12 of KRAS protein from a glycine (G) to a serine (S). KRAS G12S mutation is found is approximately 3.2% of NSCLC cases and 4.7% of all human cancer cases[4]. In this application note, we demonstrate the use of molecular beacons to specifically and effectively quantify KRAS G12S mutation in cell-free DNA samples using Optolane's 2-channel Dr. PCR 10k system.

Absolute quantification of KRAS G12S mutation

We used molecular beacon probes ("EasyBeacon", Pentabase Aps) targeting wild-type KRAS and the G12S mutant. Asymmetric PCR was used to enhance the generation of single-stranded amplicons complimentary to molecular beacon probes. Cell-free DNA from the wild-type (HEK 293) and mutant (A549) cell lines were harvested using QIAGEN circulating nucleic acid extraction kit. In this study, 0.25 μM forward and 0.5 μM reverse primers as well as molecular beacon probes (0.5 μM for each of the WT and G12S) were mixed with cfDNA and 2x Dr. PCR master mix. The data analysis was performed by LOAA analyzer.

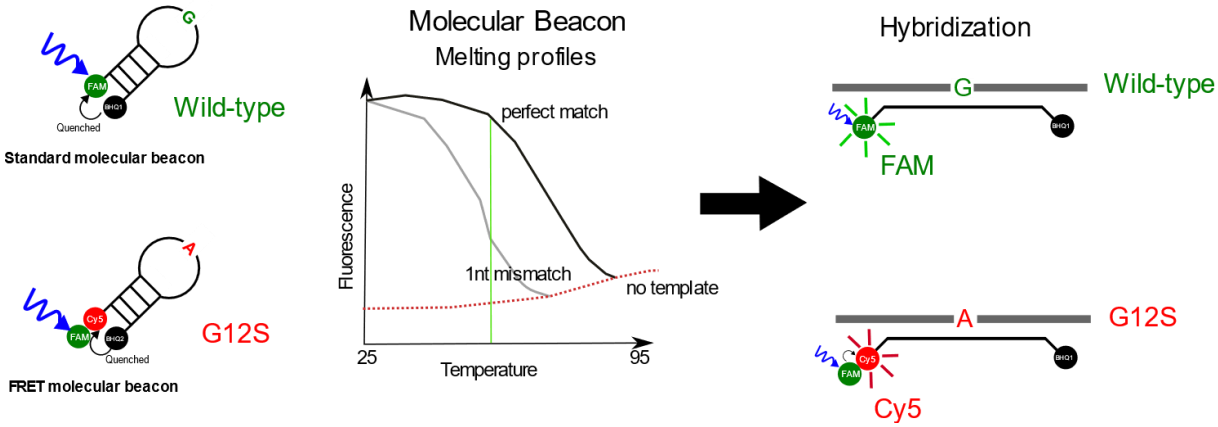

Figure 1: Schematics of molecular beacons and the melting profiles when the probes bind to perfectly matched and 1nt mismatched targets. Because the binding efficiency of molecular beacons depends on the melting temperature, at optimized condition only probes specific for their perfectly matched template are amplified in each dPCR well.

To demonstrate the robustness of the assay, cfDNA containing KRAS G12S mutation was linearly diluted into the wild-type cfDNA, generating samples containing 100%, 75% 50%, 25% and 0% KRAS G12S mutant. The samples were then analyzed using 2-channel 10k Dr. PCR cartridges. Example of scatter plots generated from this experiment reveal the discrimination of wells containing no amplification (G-/R-), wild-type KRAS (G+/R-) or G12S KRAS (G-/R+) (Figure 2). The linearity and accuracy of the assay are demonstrated in figure 3.

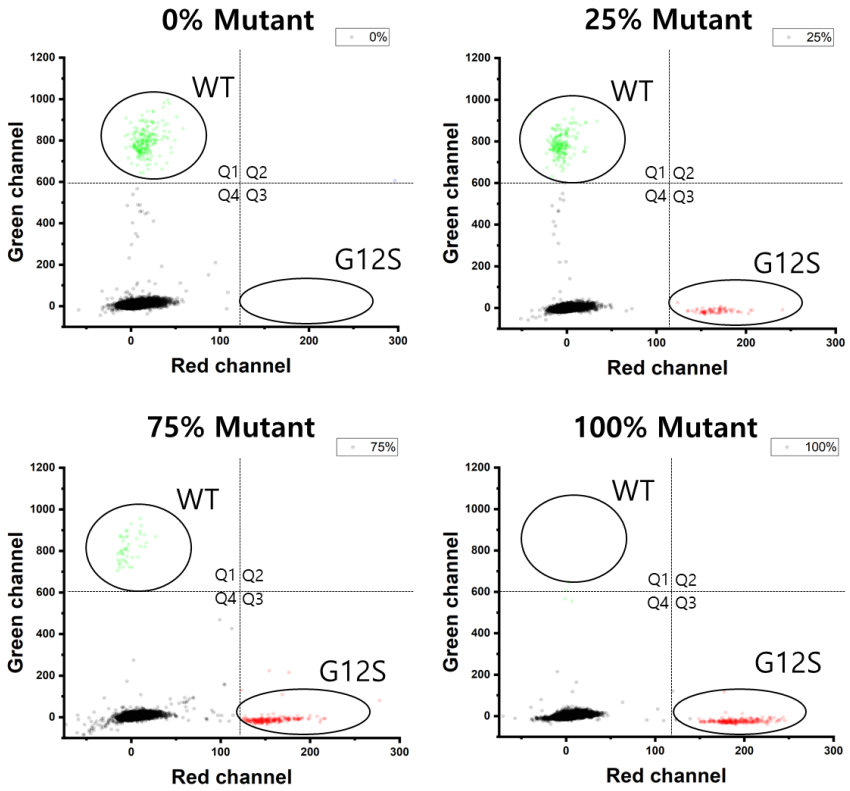

Figure 2 Duplex clustering profiles of cell-free DNA samples collected from KRAS G12S mutant (A549) lung cancer and wild-type (HEK 293) cell lines. Samples were mixed at various proportion of %mutant as indicated. Wells positive for wild-type KRAS are clustered in Q1 (FAM cluster). G12S mutant positive wells are clustered in Q3 (Cy5 cluster) and the wells with no amplification are clustered in Q4.

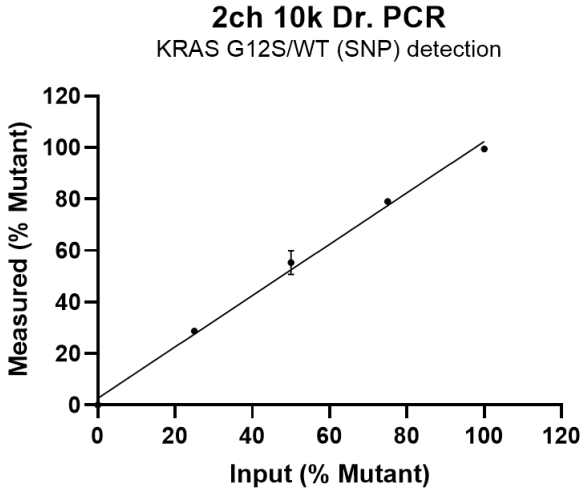

Figure 3: Linearity curve of KRAS G12S mutant detection using 2ch 10k cartridges

Conclusion

- 2-channel 10k Dr. PCR cartridges is capable to accurately and robustly perform absolute quantification of KRAS G12S in highly fragmented cell-free DNA materials
- 2-channel 10k Dr. PCR cartridges is compatible with molecular beacon probes and able to provide highly sensitive and specific amplification of targets with only 1 nucleotide difference

References

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