

## **Flow cytometer-enabled multiplex superRCA mutation assay in a single portion of circulating tumor dna (ctdna) sample**

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### **Abstract**

#### Background & Objectives

Digital PCR is constrained by limited fluorescence channels, restricting multiplex mutation detection within the same DNA sample. In circulating tumor DNA (ctDNA)-based minimal residual disease (MRD) analysis, where plasma-derived ctDNA is scarce, sample splitting for multiple reactions reduces DNA input per assay, compromising sensitivity.

The SuperRCA mutation assay is an ultra-sensitive molecular test that utilizes flow cytometry for readout. Flow cytometry enables high multiplexing capacity by detecting emissions at different wavelengths. Tandem dyes, which leverage fluorescence resonance energy transfer (FRET), allow a single excitation wavelength to produce multiple emissions. Labeling SuperRCA products with tandem dyes could facilitate the simultaneous detection of multiple mutations in a single reaction, enhancing efficiency and sensitivity.

#### Methods

A multiplex panel targeting six KRAS codon 12 variants—G12D, G12A, G12S, G12R, G12V, and G12C—was designed. A corresponding multiplex genotyping probe set, including probes for wild-type (WT) and each variant, was tested using single-positive control samples from Horizon Discovery.

#### Results

First, single-mutation probes were used to generate a 6×6 detection matrix, confirming that signals were exclusively observed in mutation-positive samples. Next, the 6-plex genotyping probe set was applied to individual mutant samples, demonstrating that variant allele frequency (VAF) measurements were consistent with single-mutation probe conditions. Finally, SuperRCA products were labeled with a tandem dye mixture, enabling the detection of all six KRAS variants within a single reaction.

#### Conclusion

This study demonstrates the feasibility of detecting six KRAS codon 12 variants from a single DNA sample without compromising sensitivity. When labeled with tandem dyes, SuperRCA products allowed flow cytometry to distinguish seven distinct populations, corresponding to WT and six KRAS variants. This highly multiplexed approach enhances the efficiency of ctDNA-based MRD analysis, offering a streamlined and sensitive method for mutation detection.

#### **Do you have any conflicts of interest?**

No, I do not have a conflict of interest.