

# Reporter Emission Multiplexing (REM): Increasing Multiplexing capacities in digital PCR using Population Specific Reporters

**Reporter Emission Multiplexing (REM) is a new patented\* assay technology for digital PCR. Per detection channel the multiplexing capacity can be multiplied, making it highly attractive for liquid biopsy sample analysis, where a high sensitivity, precision and specificity is required.**

In REM label free target-specific mediator probes are used for target detection. During PCR amplification they are cleaved in a base specific manner. The released mediator sequences are now free to activate a fluorophore labeled population specific reporter oligonucleotide (PSR). Due to the unique fluorogenic labels, different PSRs types have altered fluorescence intensities resulting in several distinguishable populations within the same detection channel. By this means, REM assays can directly increase multiplexing capacities in existing digital PCR devices. In addition, since signal generation and DNA detection are separated processes, PSRs can be used for many different target panels. Furthermore, mediator probes are highly sensitive towards SNP detection since only base specific cleavage will release a mediator sequence to activate a PSR type.<sup>2</sup>

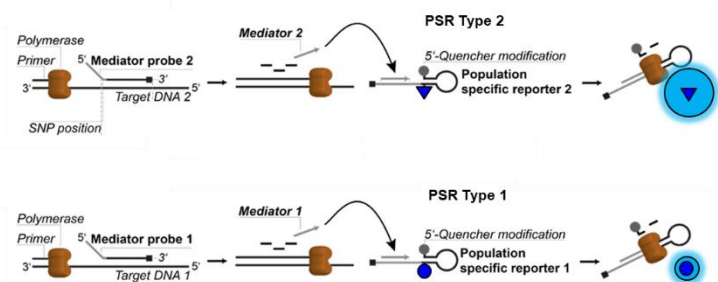
\*patent application pending

## Advantages

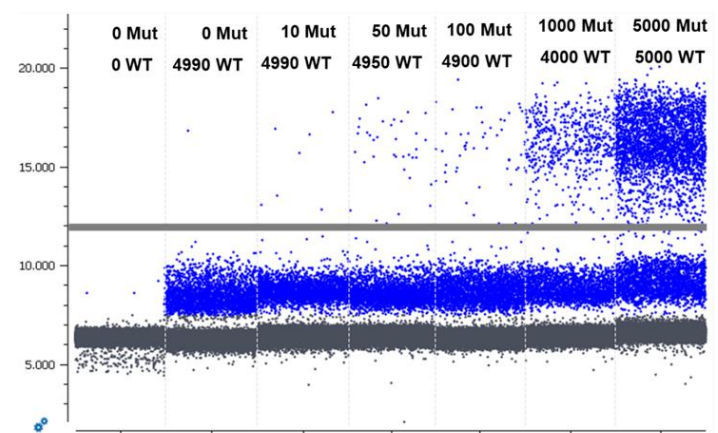
- Increasing multiplexing capacities in digital PCR
- Highly sensitive, specific and precise

## Application

- Multiplex digital PCR
- Liquid biopsy and oncology
- SNP detection



**Fig. 1:** Reporter emission multiplexing using population specific reporters, labeled with fluorophores of different intensities but similar emission spectrum.<sup>1</sup> During sample analysis this results in clearly distinguishable signal populations in the same detection channel, leading to an increase of multiplexing capacity of digital PCR devices.



**Fig. 2:** 2-Plex REM digital PCR: Precise quantification of KRAS-G12V mutant DNA (highest signal intensity over threshold) in access of KRAS wild type DNA in one detection channel.<sup>1</sup> Due to the base specific cleavage of mediator probes population specific reporter allow highly sensitive SNP detection.<sup>2</sup>

## References:

<sup>1</sup>Silvia Calabrese\*, Anja M. Markl\*, Maximilian Neugebauer, Stefanie J. Krauth, Nadine Borst, Felix von Stetten and Michael Lehnert. Reporter emission multiplexing in digital PCRs (REM-dPCRs): direct quantification of multiple target sequences per detection channel by population specific reporters. *Analyst*, **2023**, 148, 5243 – 5254 –open access-

<sup>2</sup> Franziska Schlenker, Elena Kipf, Max Deuter, Inga Höffkes, Michael Lehnert, Roland Zengerle, Felix von Stetten, Florian Scherer, Julius Wehrle, Nikolas von Bubnoff, Peter Jülg, Tobias Hutzenlaub and Nadine Borst. Stringent Base Specific and Optimization-Free Multiplex Mediator Probe ddPCR for the Quantification of Point Mutations in Circulating Tumor DNA. *Cancers*, **2021**, 13(22), 5742 –open access-