

# Virtual Fluorescence Color Channels

## Increasing Multiplexing Capacities in Digital PCR for Precise Quantification of Point Mutations

**Virtual digital multiplexing is a new technique to increase the capacities in digital PCR devices beyond the number of fluorescence detection channels. It is based on the combination of mediator probe PCR and selective photo bleaching.**

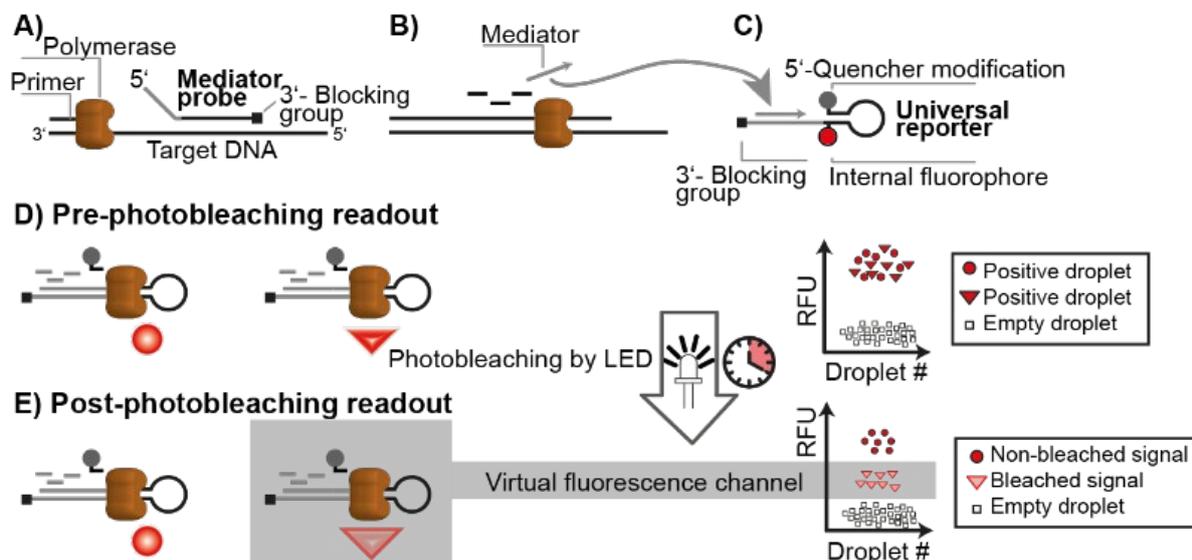
In Mediator Probe PCR, target sequences are detected using label free mediator probes. These are cleaved base specifically during PCR and so release a mediator sequence.

Only a correctly cleaved mediator will generate a fluorescence signal at a universal reporter, which enables SNP-detection. Signals of universal reporters generated by different fluorophores, which emit light within the same spectral range, can furthermore be distinguished by photo bleaching. This creates virtual fluorescence color channels, which increase multiplexing capacities over the number of detection channels of digital PCR devices (see figure below).

This was successfully demonstrated by a limit of detection (LOD) experiment for the quantification of cancer-associated *KRAS* point mutations that confirmed the high sensitivity and linearity of this novel multiplexing method (Fig. 2).

### References

<sup>1</sup> F. Schlenker et al., Virtual fluorescence color channels by selective photo-bleaching in digital PCR applied to the quantification of *KRAS* point mutations. *Anal. Chem.*, 2021, 93, 30, 10538-10545



**Fig. 1** Reaction mechanism of the mediator probe technology in combination with photobleaching. (A-C) Primer and MP annealing to the target and subsequent mediator cleavage induces the annealing of the cleaved mediator to the UR. D) The mediator elongation activates the respective UR, pre-photobleaching. E) After photobleaching by a white light LED, a second droplet population is visible by a decreased fluorescence signal generated from the bleaching sensitive fluorophore. Adapted with permission from American Chemical Society<sup>1</sup>

### Features

- Increased multiplexing capacities
- Highly specific
- Quantitative detection

### Service + Competence

- Assay design & optimization
- Kit development
- Microfluidic platform integration

### Applications

- Diagnostics of cancerous mutations
- Therapy monitoring