10x Genomics released their Single Cell Fixed RNA Profiling pipeline (FRP) in late 2022. FRP uses synthetic linear probes to quantify levels of gene expression in single nuclei extracted from FFPE samples in droplets by directly hybridizing probes to mRNA transcripts without the need to perform reverse transcription. Since its release, the FRP assay has been internally tested with positive results. We now aim to build upon this technology and expand its use for detection of mutations alongside transcript level measurements in fixed samples.

To do so, we designed customized probes specific to preselected somatic mutations, like KRAS G12C, that can be used together with the commercially available probes that are used for assessing levels of gene expression. Furthermore, we aim to improve the specificity of hybridization and ligation, using padlock probes instead of linear probes. Padlock probes are a unique class of probing molecules known for their high specificity and multiplexity. We will design padlock probes specific to somatic mutations of interest and compare their performance in several human cell lines of known mutation status including H358 (NSCLC), PC9 (NSCLC) and K562 (CML). During initial development, we focused on KRAS mutations, which have been well characterized in the proposed cell lines through exome sequencing, RNA sequencing, single cell gene expression and long read sequencing. Furthermore, the proposed method could be further expanded to target larger genomic regions and enable the detection of insertions, deletions and fusion genes. Last but not least, given the similarities between the FRP kit and the Viscum spatial assays, we envision the possibility to apply our approach to in situ detection, thus allowing the interrogation of mutations and gene expression with single-cell and spatial resolution.

Due to the mismatch tolerant nature of the ligase used in the FRP pipeline, we got too many false positive readouts with our initial test. To combat this, we came up a new chemistry strategy. The key innovative piece of this idea is a direct gap filling chemistry for DNA padlock/linear probe binding to RNA template. The testing results showed excellent accuracy in mutation detection, almost eliminating the false signals.  
This design could be valuable for many advanced single cell and spatial genomics methods we are using in gRED, including Optical Pooled Screening, 10x Genomics Visum, 10xGenomics Xenium, some in house spatial single cell RNA and protein profiling in CTG.