Utilizing superRCA technology for Measurable Residual Disease monitoring in Dried Blood Spots

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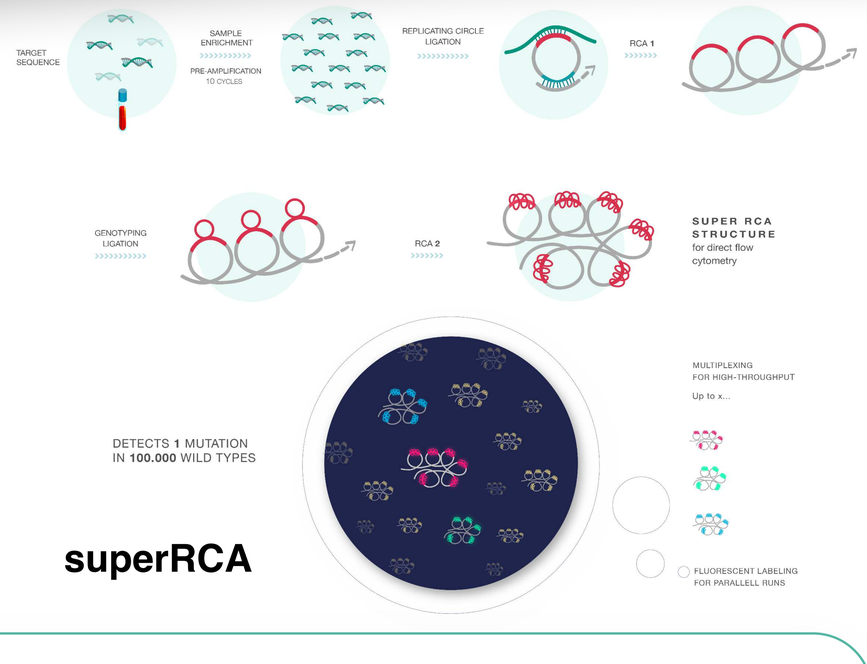
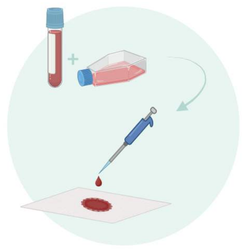
Introduction

- Measurable Residual Disease (MRD) refers to cancer cells that remain after treatment and is critical for monitoring relapse and treatment efficacy in leukemia.
- Traditional MRD testing in leukemia is often invasive, infrequent, and hospital-dependent.
- Dried Blood Spots (DBS) provide a minimally invasive, remote alternative for more frequent molecular MRD monitoring.
- This study evaluates the feasibility of combining DBS with the superRCA technology to achieve sensitivity comparable to fresh blood biopsies.



Method

1.



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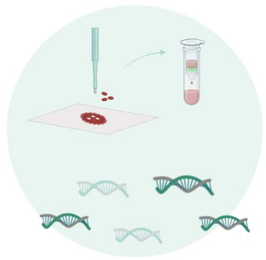

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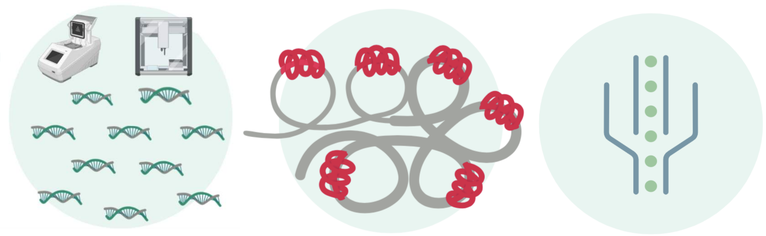


DBS Sample Preparation

- Blood samples collected from healthy donors
- Blood spiked with 1%, 0.05%, or 0.01% AML cell line Kasumi-1 (*TP53* p.R248Q).
- Samplefacts (30 uL) & Capitainer (50uL) blood cards used for DBS preparation
- DBS dried at room temperature for 7 days

DNA Extraction

- DNA extracted (QIAamp DNA Mini Kit, Qiagen)
- Samplefacts: One or two whole spots (30 uL x2) or 3x(3x3 mm) punches
- Capitainer: One (or two) whole disk removal (50 uL)

superRCA Assay & flow cytometry readout

- Leukocyte counts determined
- superRCA *TP53* p.R248Q assay used for mutation detection in spike-in samples and healthy donor samples
- Wildtype and mutant superRCA particles quantified by flow cytometry Mutant Allele Frequency (MAF) calculated
- Sensitivity of the assay assessed for different concentrations of mutant allele spike-in (1%, 0.05%, 0.01%).

Result

DNA Yield

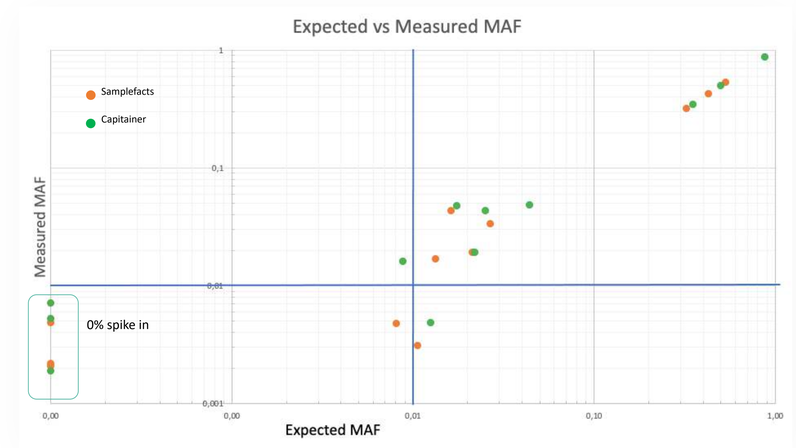
- Samplefacts: 13.53 - 49.2 ng from one spot
- Capitainer: 44.1 - 67.2 ng from one disc

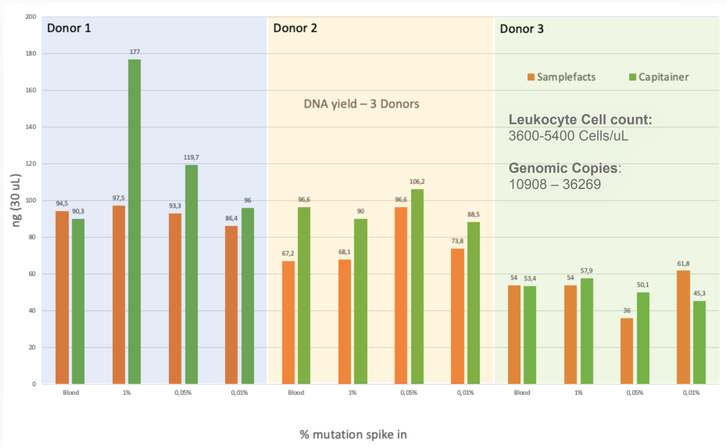
Mutation detection

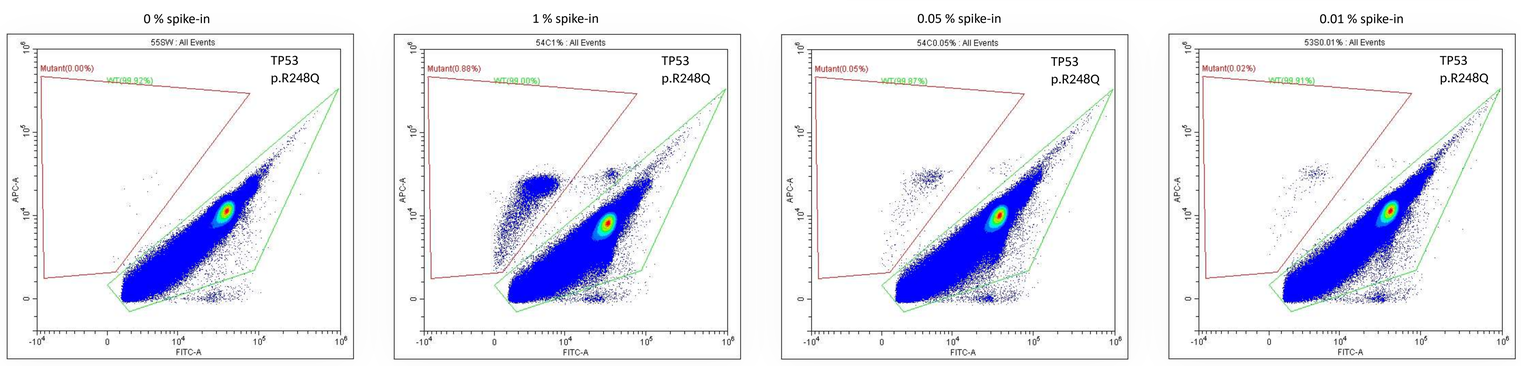
- DNA yield allowed 36 – 239 ng per reaction
- superRCA successfully detected 12/12 reactions for both 1% and 0.05% samples, and 5/12 reactions of the 0.01% samples
- All healthy donor samples had a MAF between 0.0019-0.0072%, which was below the Limit of detection (0.01%)
- No inhibition or severe DNA degradation observed from DBS

Impact of DNA Input in PCR

- Increased DNA input reduced background variation







Conclusion

- DBS provides enough DNA yield and combined with superRCA enables MRD detection
- Whole spot analysis maximized DNA yield
- No significant DNA degradation or reaction inhibition was observed
- Further optimization required for reducing background for the *TP53* p.R248Q target and increase the DNA yield to improve Limit of detection (target LoD 0.005% with superRCA *NPM1* assay)