Ultra-sensitive multiplex detection of IDH1/2 variants using a superRCA assay

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INTRODUCTION

The superRCA mutation assay is a DNA-based ultra-sensitive molecular test that utilizes flow cytometry for the 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. Labelling superRCA products with tandem dyes could facilitate the simultaneous detection of multiple mutations in a single reaction, enhancing efficiency and sensitivity.

OBJECTIVE

Increase the efficiency of mutation analysis by combining multiplex superRCA and flow cytometric readout for single-reaction detection of multiple IDH1/2 variants.

METHOD

A multiplex panel targeting 5 IDH1 variants including R132C, R132H, R132G, R132S, R132L and 4 IDH2 variants including R140Q, R140L, R172K and R172M, was designed (assay workflow, **Figure 1A**). Mutation-specific probes labeled with distinct fluorophores generate different fluorescent emission signals upon hybridization to superRCA products (representative flow cytometry readout, **Figure 1B**). A corresponding multiplex genotyping probe set, including probes for wild-type (WT) and each variant (synthetic DNA, IDT), was tested using single-positive control samples (Becton Dickinson).

superRCA WORKFLOW

Figure 1A. Assay

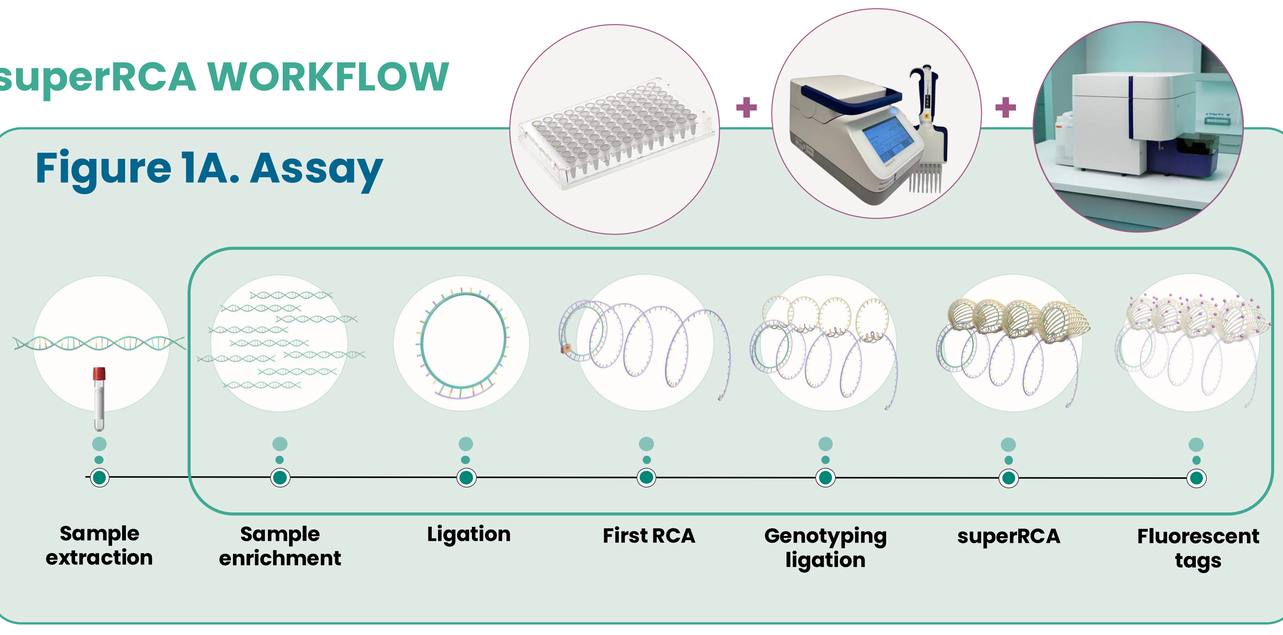
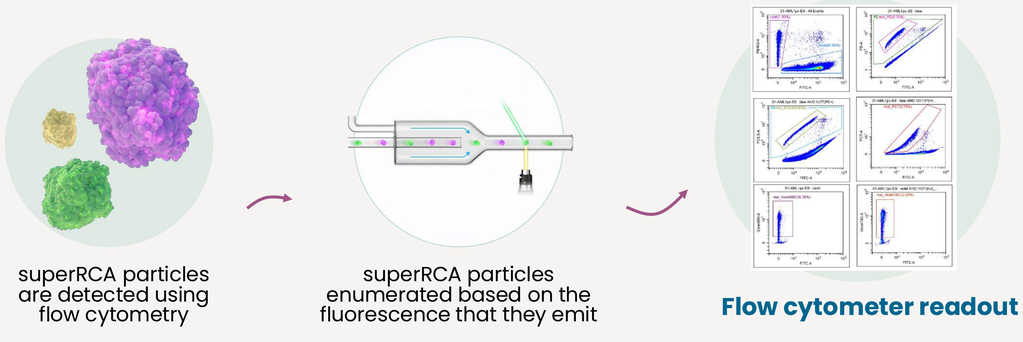


Figure 1B. Readout

Compatible with flow cytometers that have at least two lasers



RESULTS

First, single-mutation probes were used to generate a 5×5 detection matrix for IDH1 and a 4×4 detection matrix for IDH2, confirming that signals were exclusively observed in mutation-positive samples. Next, the 9-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 labelled with a tandem dye mixture, enabling the detection of all 9 IDH1/2 variants within a single portion DNA input.

Figure 2. superRCA Multiplex IDH1/2 configuration

A.

IDH1

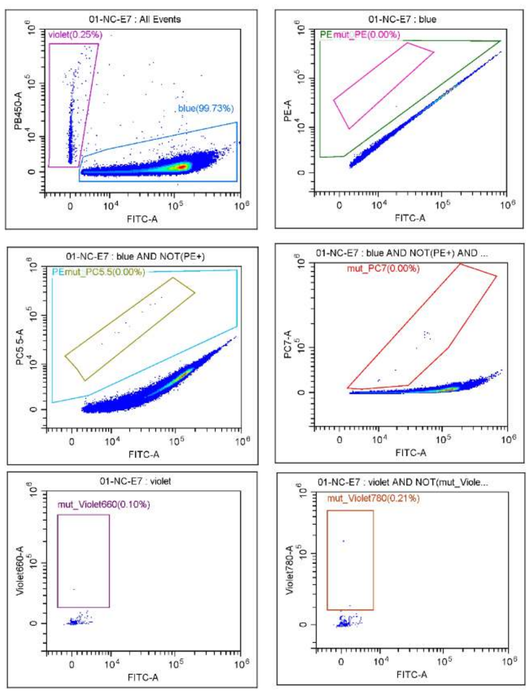
Channels	Target	VAF (%) AML1 PC
PE	R132S	0.7239
PC5.5	R132H	0.9387
PC7	R132C	0.7525
Violet660	R132G	0.8363
Violet780	R132L	0.8000

IDH2

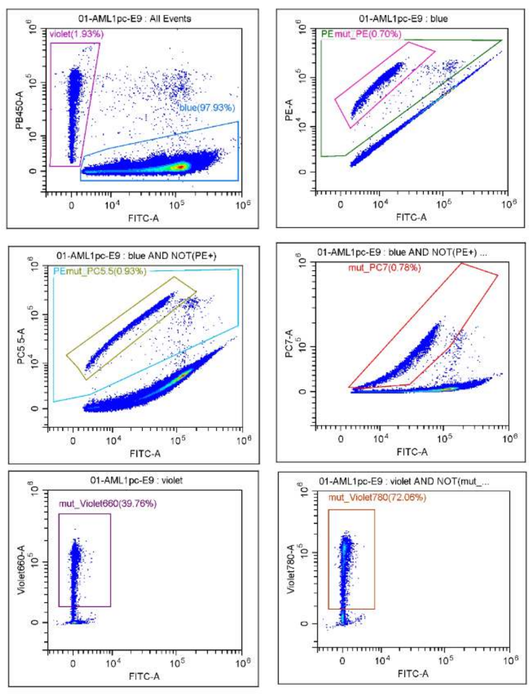
Channels	Target	VAF (%) AML1 PC
PE	R140L	0.7347
PC5.5	R140W	0.8010
PC7	R140Q	0.7841
Violet660	R172S	n/a
Violet780	R172K	1.0019

Figure 3A: Summary of channels and targets for each gene and mutation, including the VAF (%) in the AML sample. **Figure 3B-E:** Representative flow cytometry plots from samples negative (3B and 3D) or positive (3C and 3E) for IDH1/2 mutations.

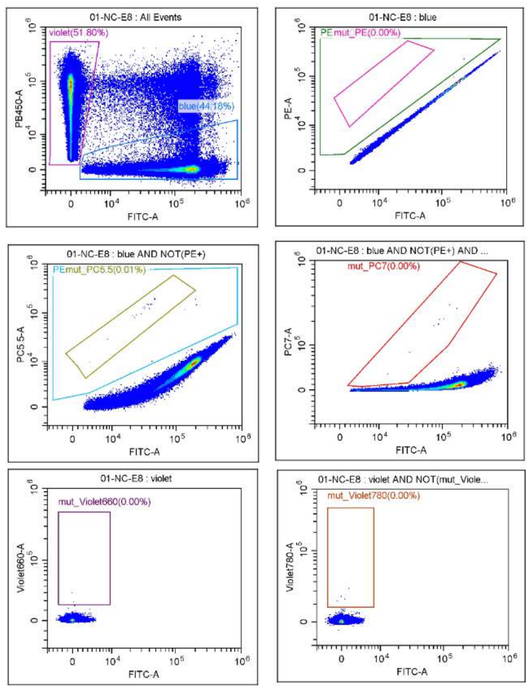
B. IDH1 Neg Ctrl



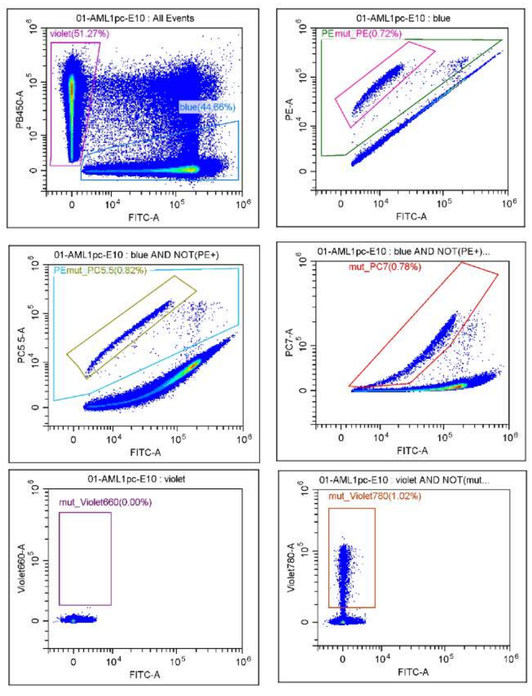
C. IDH1 Pos Ctrl



D. IDH1 Neg Ctrl



E. IDH1 Pos Ctrl



CONCLUSION

superRCA enabled sensitive detection of nine IDH1/2 variants from a single DNA sample, distinguishing 12 populations by flow cytometry and streamlining multiplex DNA-based MRD analysis.