Ultra-sensitive flow cytometry assay for JAK2 V617F mutation for MRD analysis

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INTRODUCTION

Assay sensitivity threshold plays a critical role in MRD-negative classifications. According to ELN guidelines, negative MRD results do not necessarily indicate disease eradication but rather represent disease below the assay's threshold in the tested sample. The JAK2 V617F mutation is the most prevalent mutation in BCR-ABL1-negative MPN patients. A highly sensitive assay for monitoring patients post-treatment is fundamental for detecting the mutation at low frequencies that current methodologies, such as qPCR or ddPCR, cannot detect. The superRCA assay is an ultra-sensitive, novel molecular test that utilizes flow cytometry for rapid and precise detection of DNA sequence variants present at very low frequencies in DNA samples.

OBJECTIVE

Establish a highly sensitive assay to detect JAK2 V617F mutation from liquid biopsy for MRD analysis

METHOD

The superRCA JAK2 V617F mutation assay was designed using the superRCA assay platform. As described in the schematic figure 1 (A-C) the sequence of interest, JAK2 V617V here, is first enriched by targeted PCR amplification from a DNA sample and converted to DNA circles, which are then subjected to rolling-circle amplification (RCA). Padlock probes specific for mutant or wild-type sequences are then used to probe the repeated sequences of the RCA products with exceptional specificity, followed by RCA of the circularized probes (Figure 1A). The large DNA clusters that result from each starting DNA circle are referred to as superRCA products. The high number of superRCA products can be conveniently analyzed by flow cytometry (Figure 1B). The ratio of mutant to wild type is reported as mutant allele frequency (MAF%) after flow cytometry acquisition.

In this study, a corresponding WHO 1st International Reference Panel for Genomic JAK2 V617F (NIBSC code: 16/120) was used for assay verification and comparison using the automated liquid handling, Opentrons platform (Figure 1C). The freeze-dried genomic DNAs with different MAF% at 0, 0.03, 1.00,10.8, 29.6 were tested in our assay validation.

The superRCA Assay Work-Flow

Figure 1A. Technology

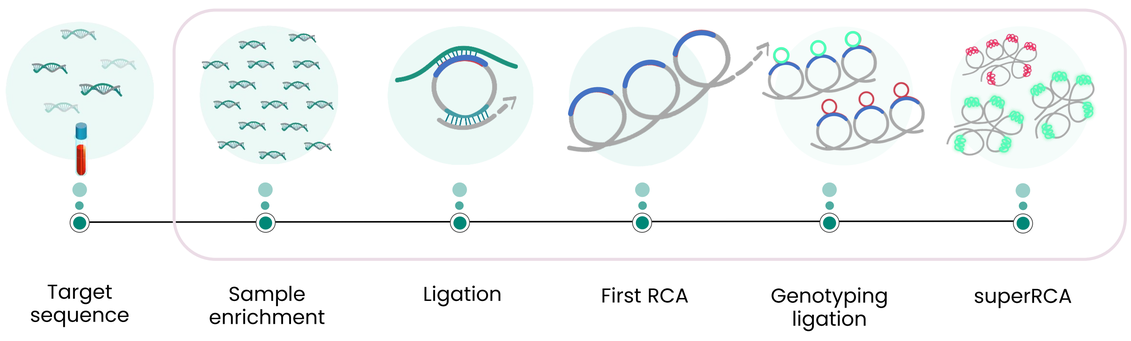
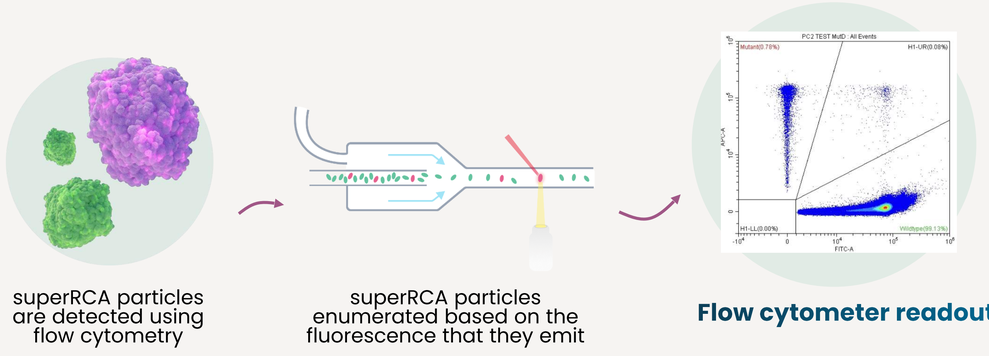


Figure 1B. Readout

Compatible with flow cytometers that have at least two lasers



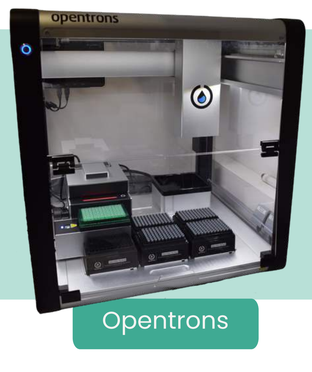
RESULTS

The assay sensitivity was determined using a standard 660 ng DNA input from whole blood, with a **Limit of Blank** (LoB) of **0.0012%** and a **Limit of Detection** (LoD) of **0.0037%**. The assay linearity ranges from 0.0037% to 10% (R2 ≥0.99). (Figure 2)

The analyzed data between the recorded MAF for the superRCA assay and the reference MAF for WHO genomic samples showed R2=0.9997. (Figure 3)

Figure 1C. Compatible with automated liquid handling platforms

Integrated Thermal Cycling



Opentrons

Automation-ready workflow

- Minimal hands-on time
- Plate based processing
- Scalable to high throughput

Figure 2

JAK2 V617F (c.1849 G>T) Standard Curve with 660 ng gDNA input. Pooled data from 3 technical replicates from automated run on Opentrons platform. Data acquired on BD FACSLyric instrument.

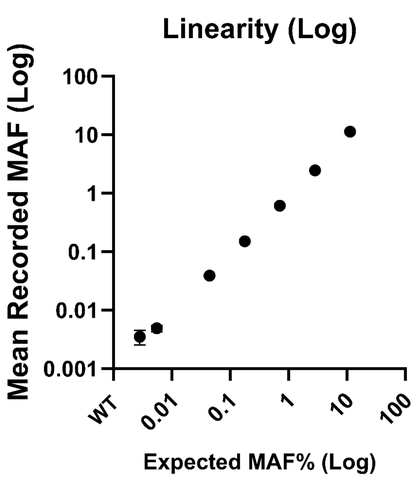
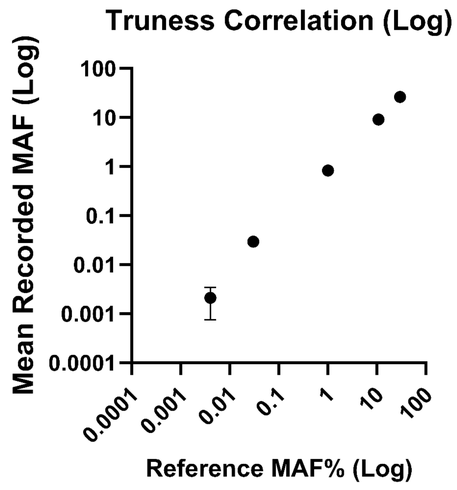


Figure 3

Trueness study for JAK2 V617F R2=0.9997. Data acquired on BC DxFLEX instrument.



CONCLUSION

The ultra-sensitivity of the superRCA technology for other mutations has previously been demonstrated and validated. Here for the first time, we present the superRCA assay sensitivity for the JAK2 V617F mutation. The data demonstrated the highly sensitive and robust DNA-based JAK2 V617F assay, which makes the assay highly suitable for MRD analysis.