Rarity, an innovative Mutation Detection technology: Bridging Molecular Biology and Flow Cytometry

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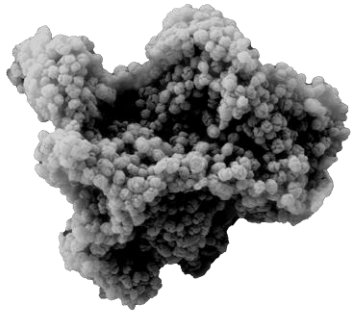


DNA mutation detection on flow cytometry using superRCA

Going with the Flow

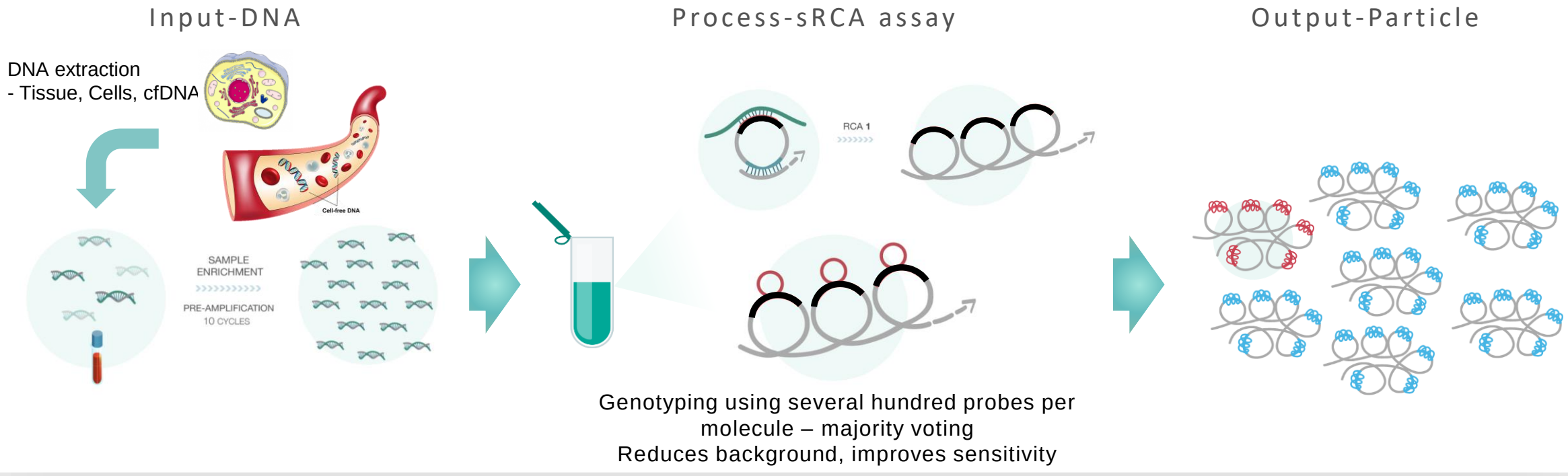
Agenda

- The superRCA Technology
  - How can we analyze DNA using flow cytometry
- Workflow
- Performance and Utility
- Recent posters and presentations
- Liquid Biopsy of Solid tumor mutations

Technology - Rarity superRCA: molecular on flow

Combines 2 x RCA and padlock probes that transform nucleic acid sequences into particles

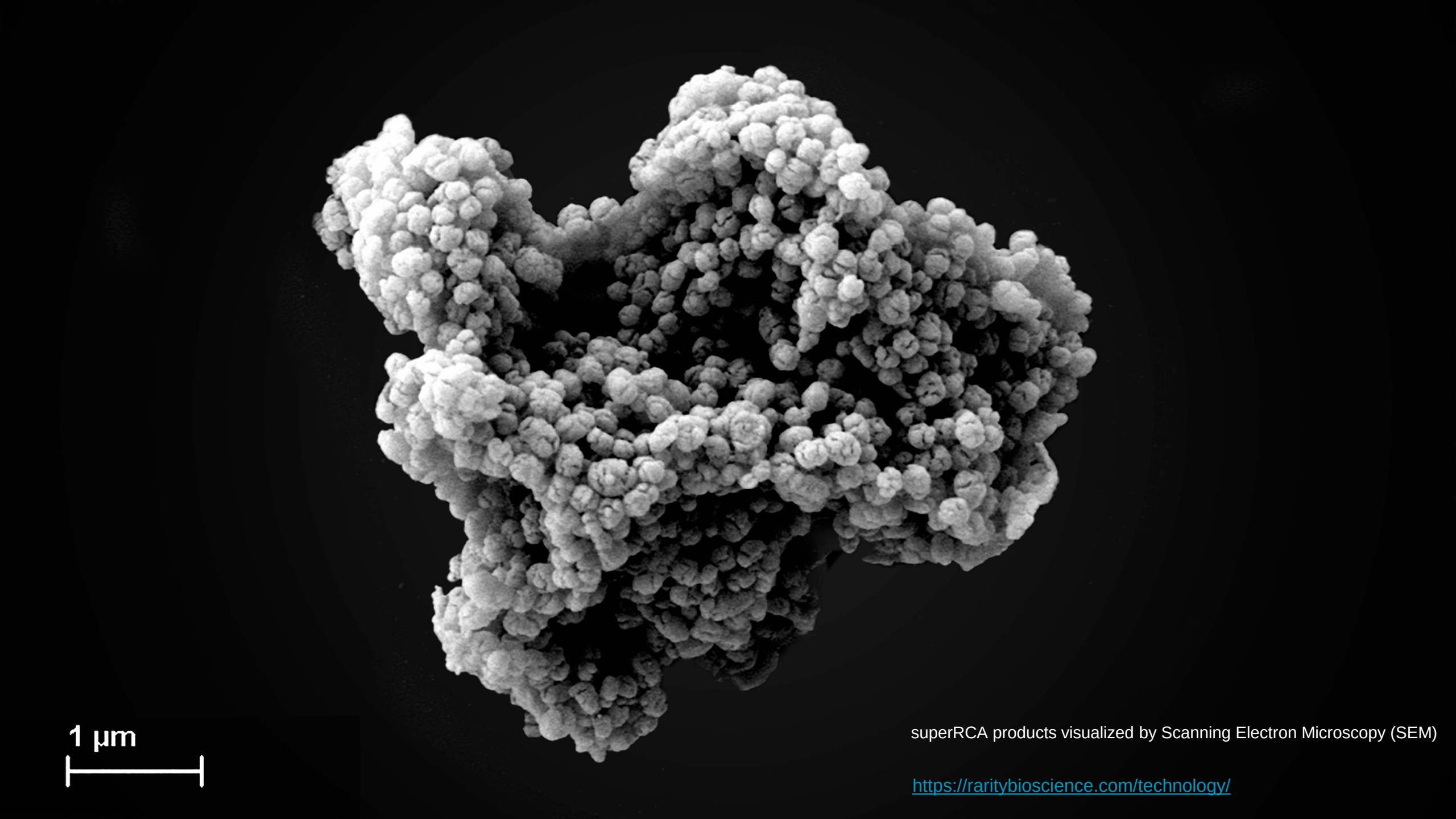
UPPSALA UNIVERSITET



Genotyping using several hundred probes per molecule – majority voting
Reduces background, improves sensitivity

https://raritybioscience.com/technology/

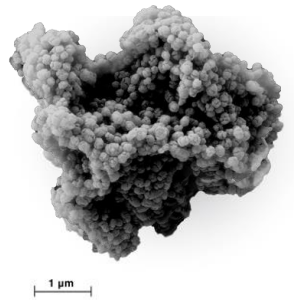
Informed approach:The probes designed for the mutations of discovered by NGS



superRCA products visualized by Scanning Electron Microscopy (SEM)

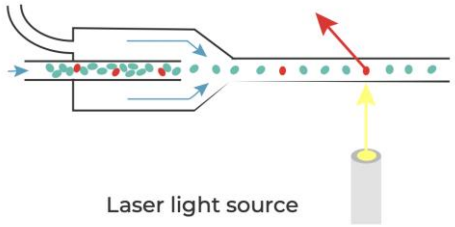
https://raritybioscience.com/technology/

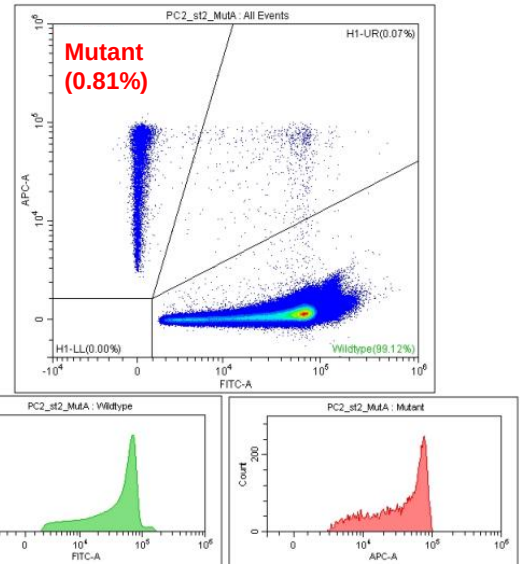
Technology – Readout on the CytoFLEX cytometer



superRCA particle

Fluorescence emitted from stained SuperRCA products





Population	Events	Mean FITC-A	Mean APC-A
All Events	924193	45049.6	633.5
Mutant	7458	172.3	43033.7
Wildtype	916097	45408.0	252.5

~1 Million events
Improves reliability and statistics

Standardized data acquisition

Use of current infrastructure

Simple data analysis

Workflow – Manual and auutomated workflows

Manual workflow

DNA extracted from blood/plasma

Accessory kit with enzymes, buffers etc

Mutation kit with specific probes

96 well Microplate

+

Multichannel Pipette

+

Thermocycler

TAT ~3h

The microplate is directly read on a flow cytometer ~3min/well

96 well Microplate

+

Liquid handler with thermocycler

Automated workflow

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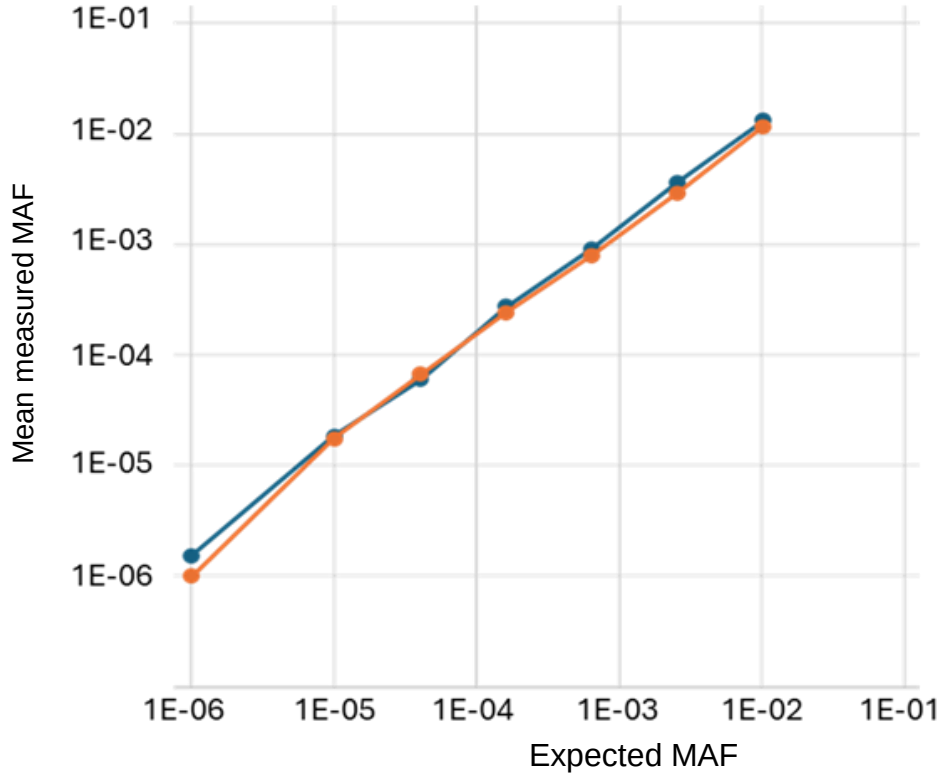
Performance and Utility – linear correlation and repeatability



Repeatable with standard lab equipment and novice users

- **Mutation tested: cKIT D816V**

  660ng of input DNA

  Serial dilution and spiked

  Biological triplicates
- **2 different flow cytometers**

  2 different labs

  2 different operators

  2 different batches of reagents

Data generated by Dr. Steve Kussick, Phenopath/Quest

NOTE: This assay was developed as a private mutation for an MDS study on molecular MRD, i.e. not fully validated.

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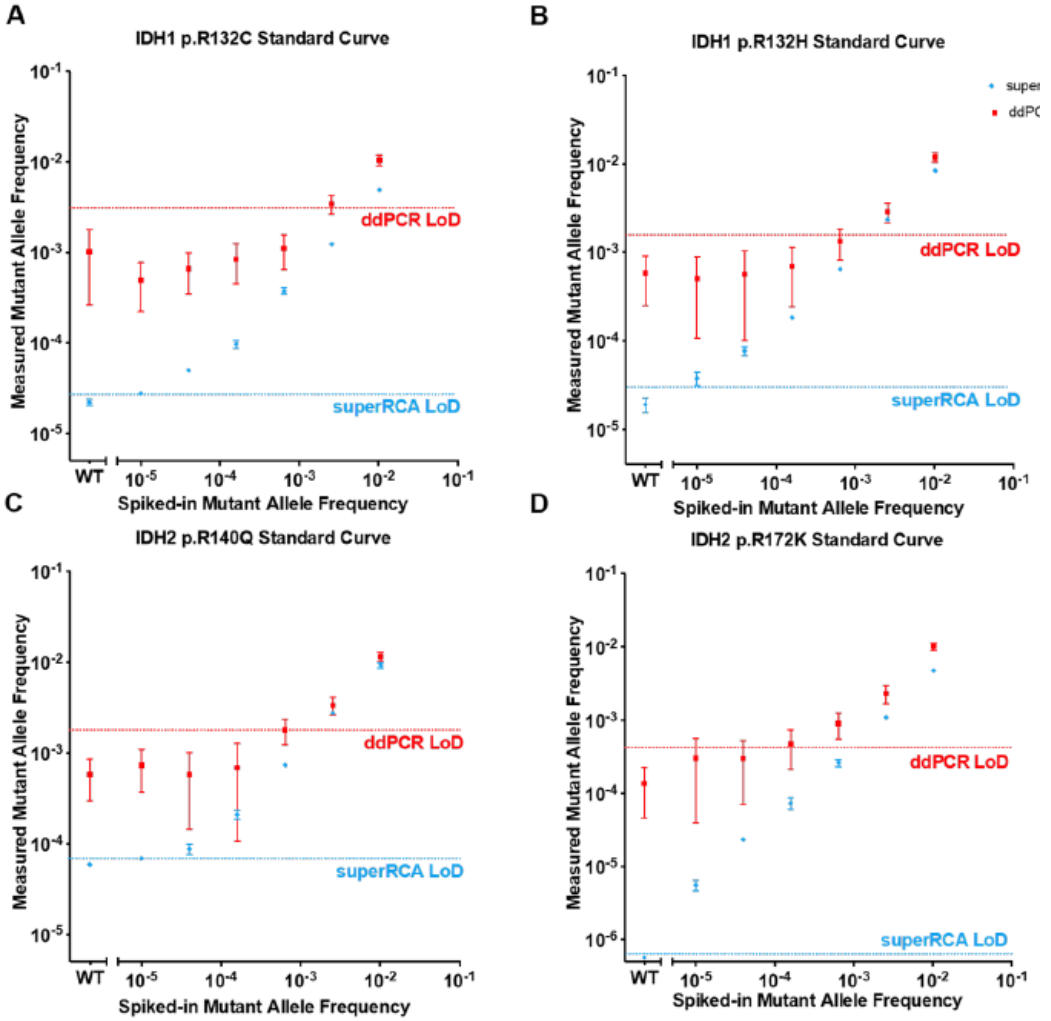
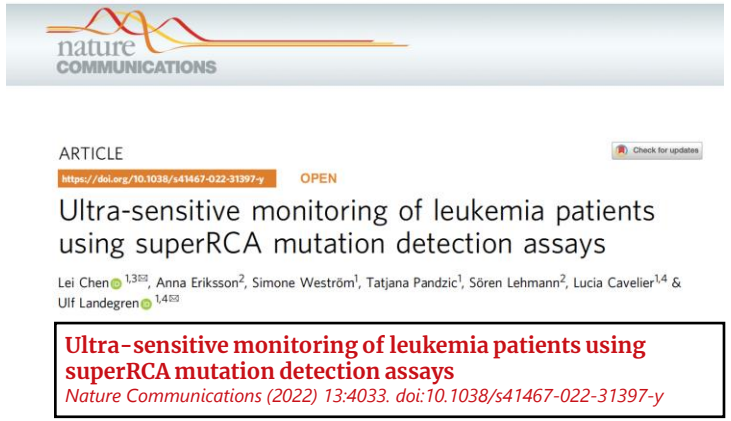
Performance and Utility – superRCA vs ddPCR



Benchmark for AML targets IDH1 (p.R123C and p.R132H) and IDH2 (p.R149Q and p.R172K)

- Spiked samples show that ddPCR can detect down to ~10^{-3} ~10^{-4}
- SuperRCA remains linearly detectable down to 10^{-5} ~10^{-6}
- Significant and reliable sensitivity across multiple targets



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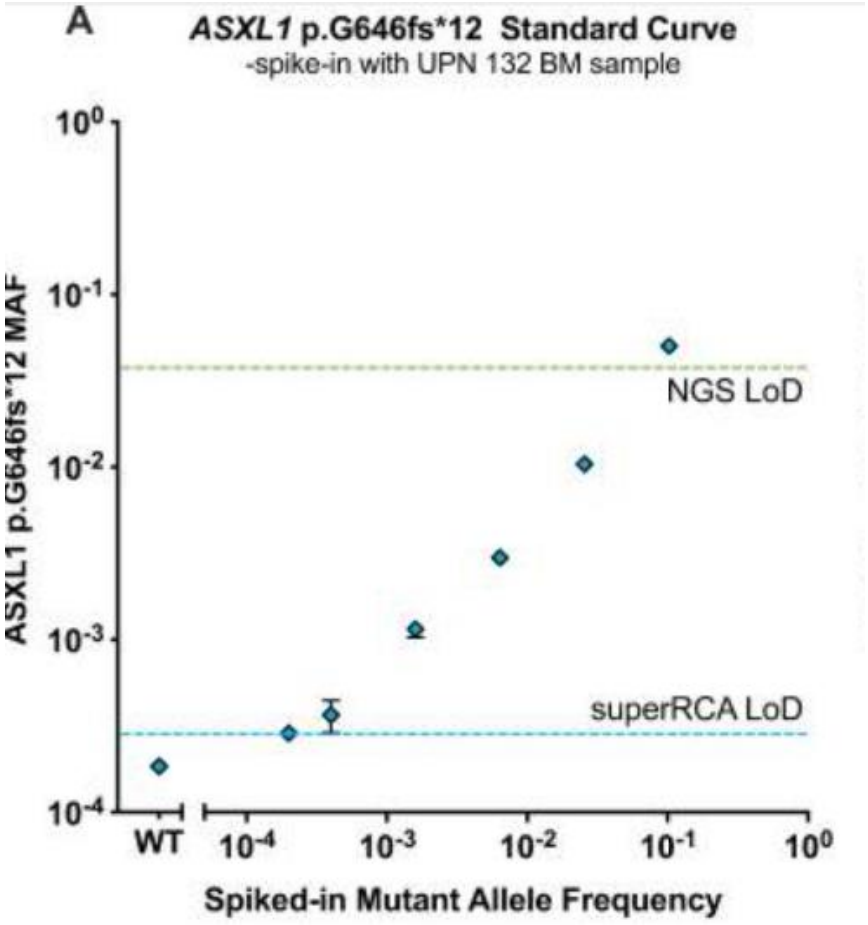
Performance and Utility – superRCA vs NGS

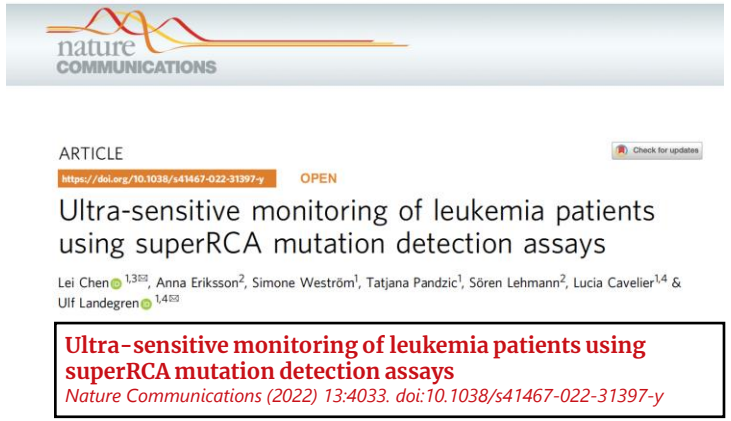


Detection in High GC regions

- ASXL1 p.G646fs*12 mutation is considered a marker of poor prognosis in both MDS and AML
- High GC rich region,

(GGAGGGGGGGG[-/G]TGGCCCGGGTG)

- Rarity assay performs well in high GC rich regions as compared to other technologies


nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-022-31397-y OPEN

Ultra-sensitive monitoring of leukemia patients using superRCA mutation detection assays

Lei Chen[1,3✉], Anna Eriksson[2], Simone Weström[1], Tatjana Pandzic[1], Sören Lehmann[2], Lucia Cavelier[1,4] & Ulf Landegren[1,4✉]

Ultra-sensitive monitoring of leukemia patients using superRCA mutation detection assays

Nature Communications (2022) 13:4033. doi:10.1038/s41467-022-31397-y

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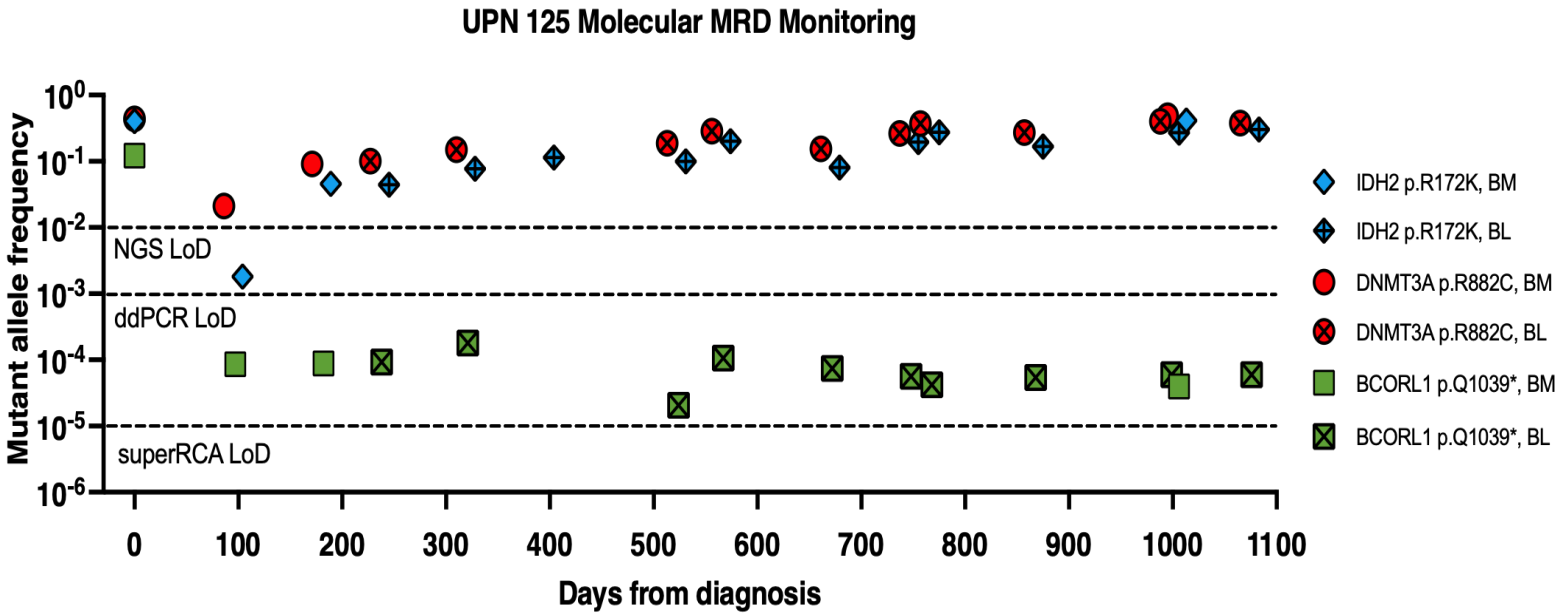
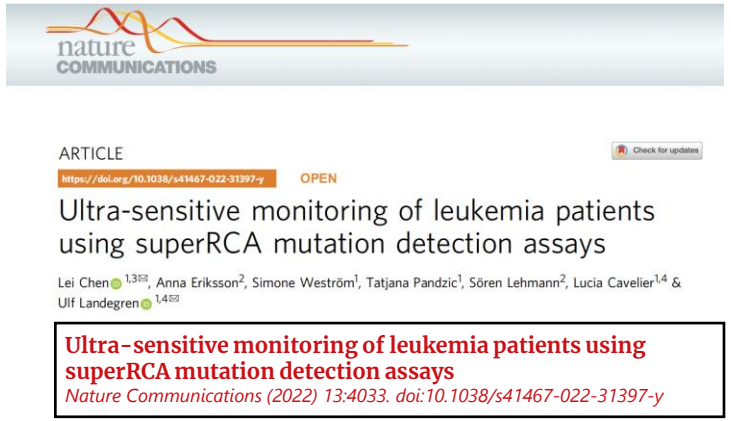
Performance and Utility – superRCA in blood and Bone marrow



Detection in Blood and Bone marrow

- Three AML patients for whom consecutive samples were available were analyzed at several time points for 3 mutations
- The mutations were detected in blood and bone marrow at the same time points



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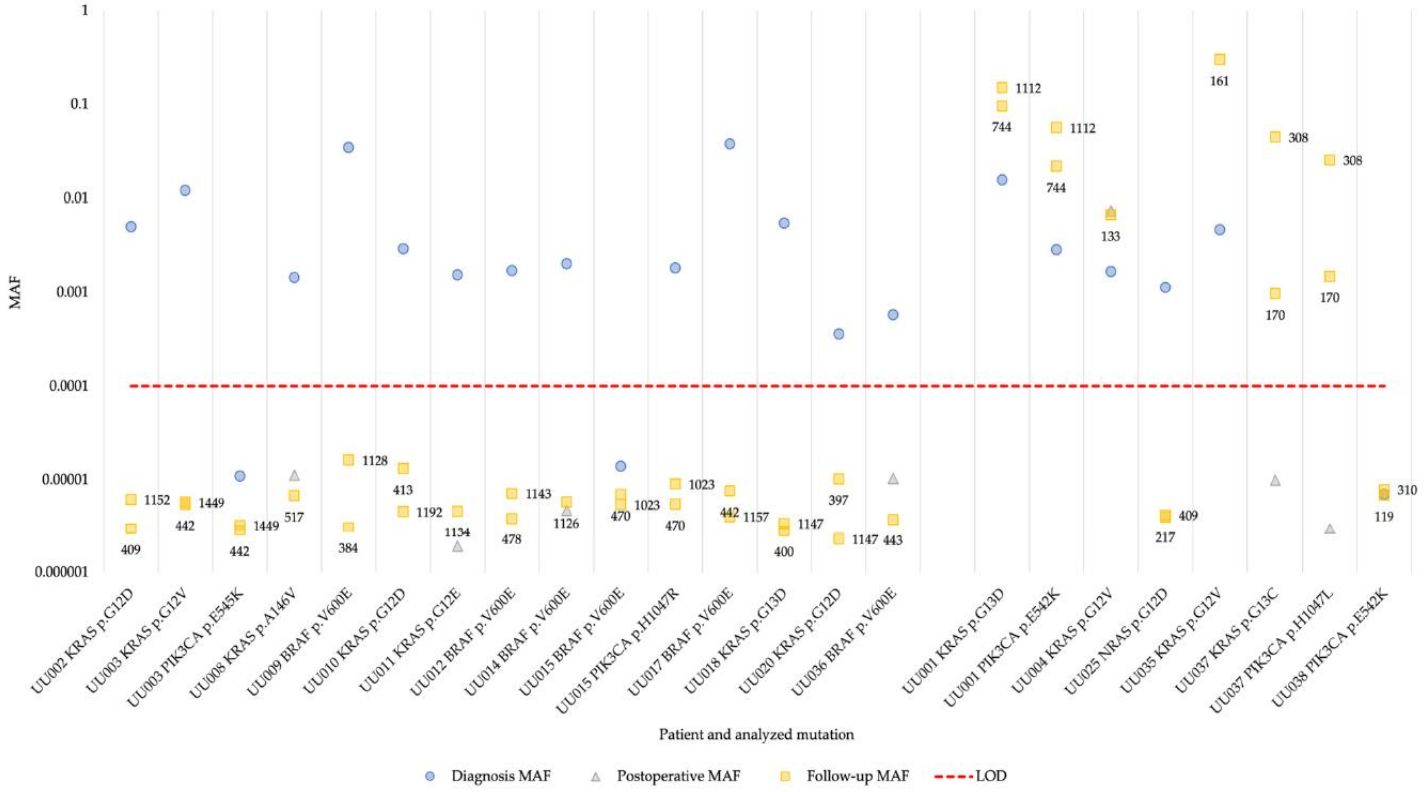
Liquid Biopsy of Solid tumor mutations



The MAF levels fall from diagnosis to Follow-up samples

44 different mutant variants were detected cfDNA plasma samples with limited DNA amounts

- 25 CRC patients was analyzed using a multiplex superRCA mutation detection assay.
- When analyzing cfDNA from plasma (1.8 ml) with a typical input of 33 ng, the practical detection limit was ~10^{-4} or 0.01% mutant allele frequency (MAF).
- ddPCR could detect only at a MAF of 0.1%, hence missing 11 patients who had lower MAFs, which were detected by the rarity assay.

cancers MDPI

Article

Sensitive and Specific Analyses of Colorectal Cancer Recurrence through Multiplex superRCA Mutation Detection in Blood Plasma

Emma Sandberg [1], Luís Nunes [1], Per-Henrik Edqvist [1], Lucy Mathot [1], Lei Chen [1,2], Tomas Edgren [2], Shahed Al Nassralla [1], Bengt Glimelius [1], Ulf Landegren [1,*] and Tobias Sjöblom [1,*]

Cancers 2024, 16, 549. https://doi.org/10.3390/cancers16030549

Conclusions:

- **SuperRCA is a highly sensitive assay to detect mutations, uses a majority voting system thereby reduces background, improves sensitivity**
- **Flow cytometry enables the acquisition of ~1 million particles, improving the reliability and CV**
- **Studies have shown that superRCA is 10-100 folds more sensitive than other methodologies**
- **The improved sensitivity allows use with DNA from blood as well as bone marrow**
- **SuperRCA can be used for Liquid biopsy, detecting 40+ mutations from plasma of CRC patients**

THANK YOU FOR YOUR TIME AND ATTENTION!