2594. A deep sampling and single molecule digital counting assay for rare molecule detection

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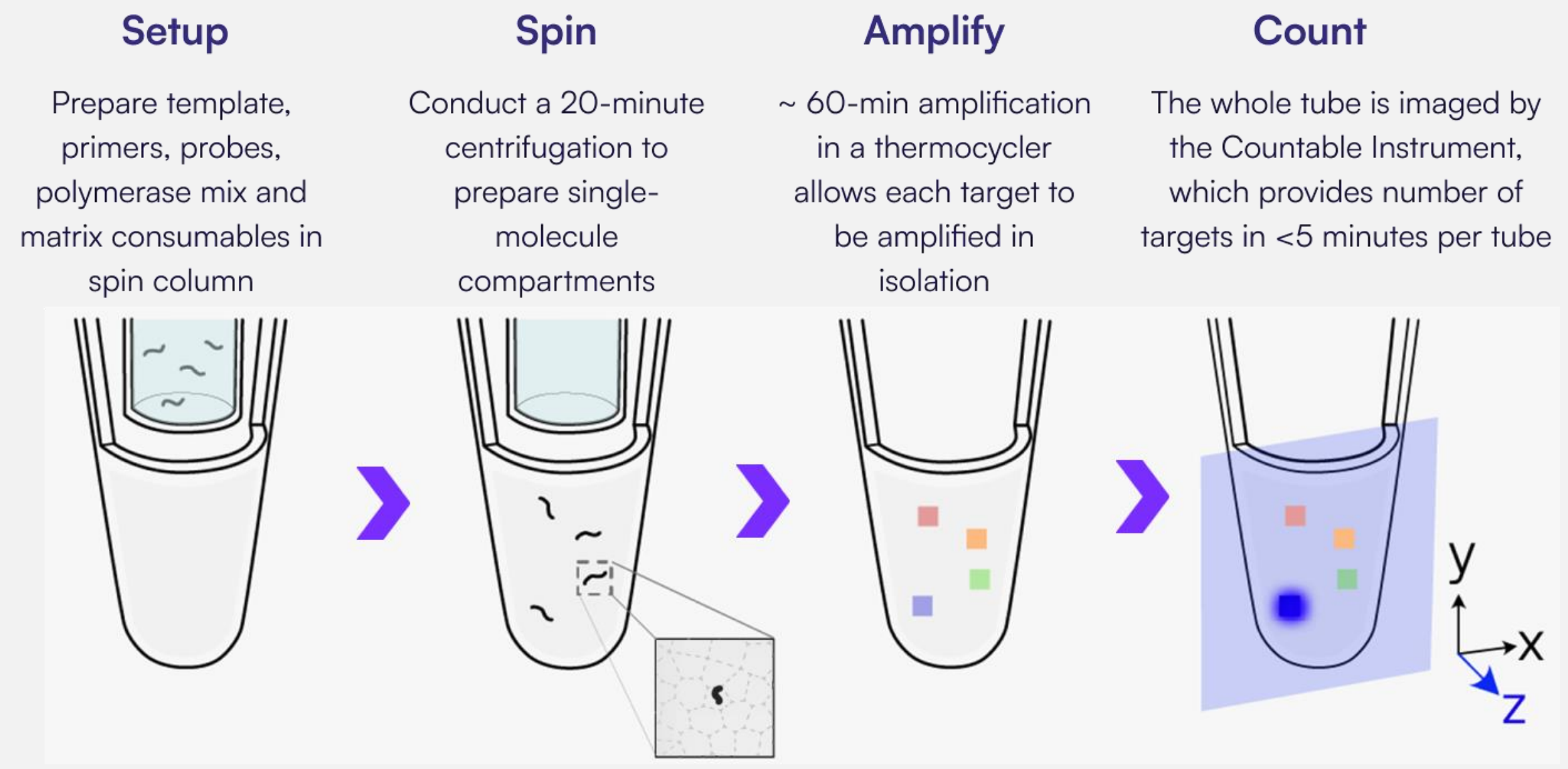
Introduction

Rare molecule detection remains a key challenge in the growing field of liquid biopsy. However, current analytical platforms have limitations on both DNA input capacity and dynamic range of counting. Countable uses a ‘deep-sampling’ PCR approach that can amplify high amounts of DNA input (>1 µg) allowing for the detection of rare molecules. Countable PCR (formerly UltraPCR) enables deep sampling by partitioning large DNA inputs into millions of single-molecule compartments for direct digital counting.



Setup
Prepare template, primers, probes, polymerase mix and matrix consumables in spin column

Spin
Conduct a 20-minute centrifugation to prepare single-molecule compartments

Amplify
~ 60-min amplification in a thermocycler allows each target to be amplified in isolation

Count
The whole tube is imaged by the Countable Instrument, which provides number of targets in <5 minutes per tube

Figure 1. Overview of the Countable PCR deep-sampling and single-molecule workflow.

In contrast to conventional digital PCR (dPCR) platforms with limited partition numbers and high multi-molecule occupancy, Countable PCR enables single-molecule digital counting without Poisson correction.

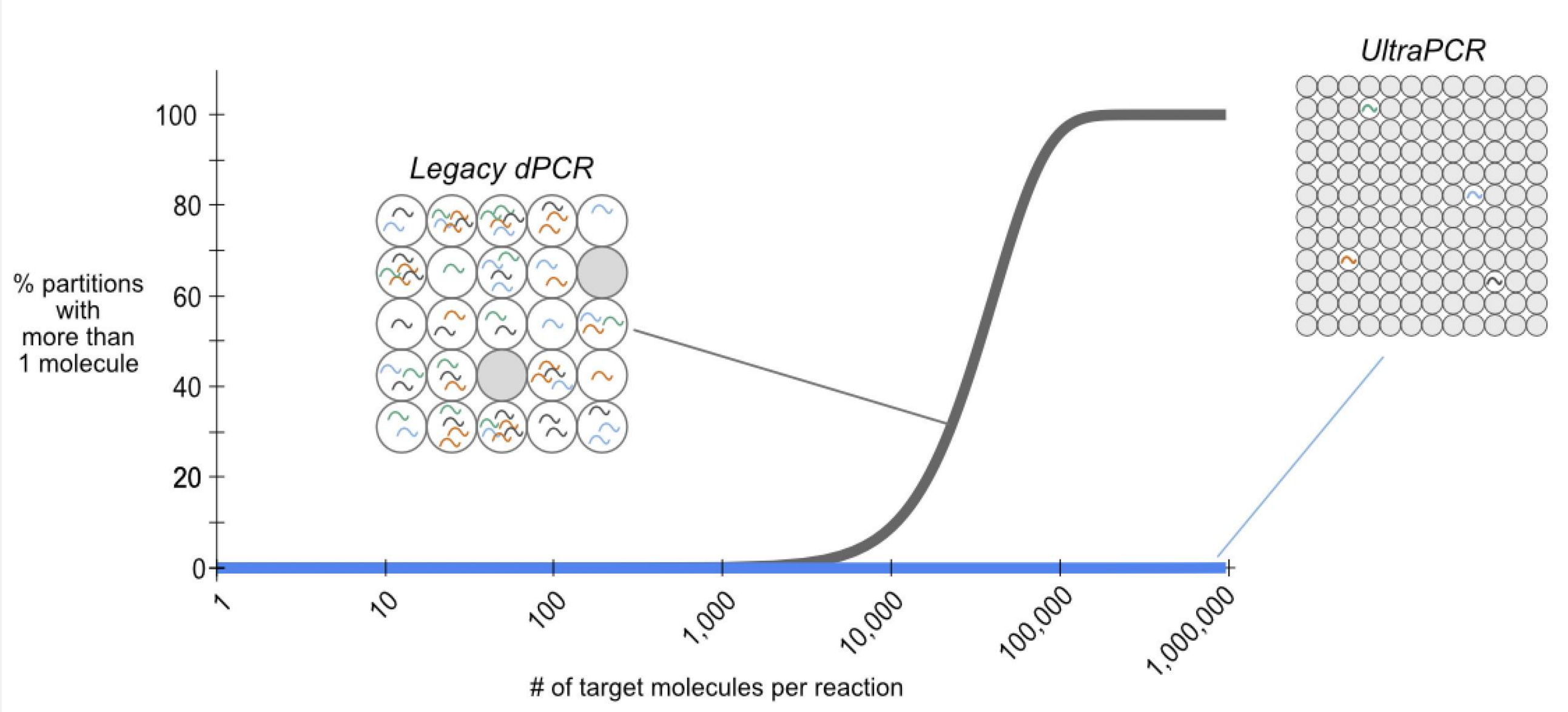


Figure 2. Legacy dPCR vs. Countable PCR partition occupancy.

Methods

A multiplexed digital PCR assay targeting KIT D816V was analytically evaluated. Fifteen samples were analyzed using Labcorp’s validated, commercially available dPCR method with 26k partition plates run in triplicate. Reactions were performed using 100 ng DNA input per replicate. Samples were independently tested by Countable using Countable PCR at their Palo Alto laboratory and compared to Labcorp results; a subset of samples was additionally tested using an early access Countable PCR instrument at Labcorp.

Assay sensitivity was assessed using contrived samples spanning expected KIT D816V variant allele frequencies of 0.015%, 0.03%, and 0.06%. Variant allele frequency was calculated from mutant-positive versus total partition counts. Additional sensitivity testing was performed using a patient blood sample spiked with a synthetic IDT gBlock control to generate defined variant frequencies, evaluated at 300 ng and 1 µg DNA input.

- **Countable PCR enables deep sampling and direct single-molecule digital counting without Poisson correction.**
- **Countable PCR showed 100% concordance and strong linearity versus a commercially available dPCR method ($r^2 > 0.999$).**
- **Consistent detection of KIT D816V was demonstrated down to 0.015% VAF with minimal background signal.**
- **High partition number and flexible DNA input support robust rare-variant detection in a single reaction.**

Results

Across all experiments, input DNA ranging from 150 to 1,425 ng per sample was analyzed. Results generated using Countable PCR at Countable showed complete concordance with those obtained using the commercially available dPCR method, with 100% agreement observed across all samples. Similarly, results generated using an early access Countable PCR instrument at Labcorp were fully concordant with the commercial dPCR method for the subset of samples tested. Quantitative agreement was high across platforms, with correlation coefficients (r^2) of 0.9993 for comparisons between the commercial dPCR method and Countable PCR, and 0.9998 for comparisons between the commercial dPCR method and Labcorp Countable PCR. Assay sensitivity and background performance were further evaluated using synthetic and contrived samples. Analysis of a synthetic template demonstrated 99.95% variant detection relative to the expected value, with 5 variant-positive partitions observed among 10,026 wild-type partitions, indicating minimal background signal generation.

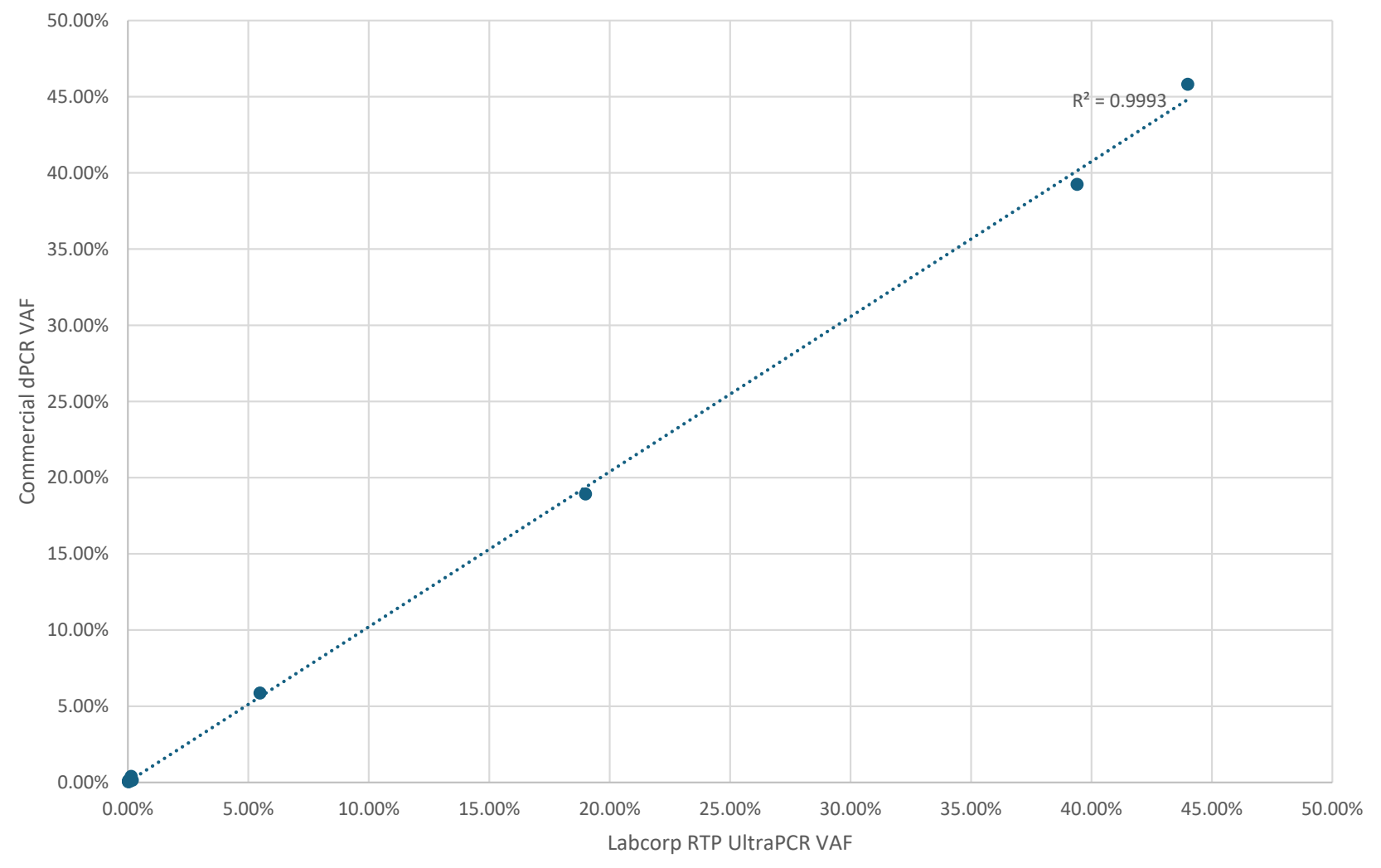


Figure 3A. Commercial dPCR platform vs. Countable VAF.

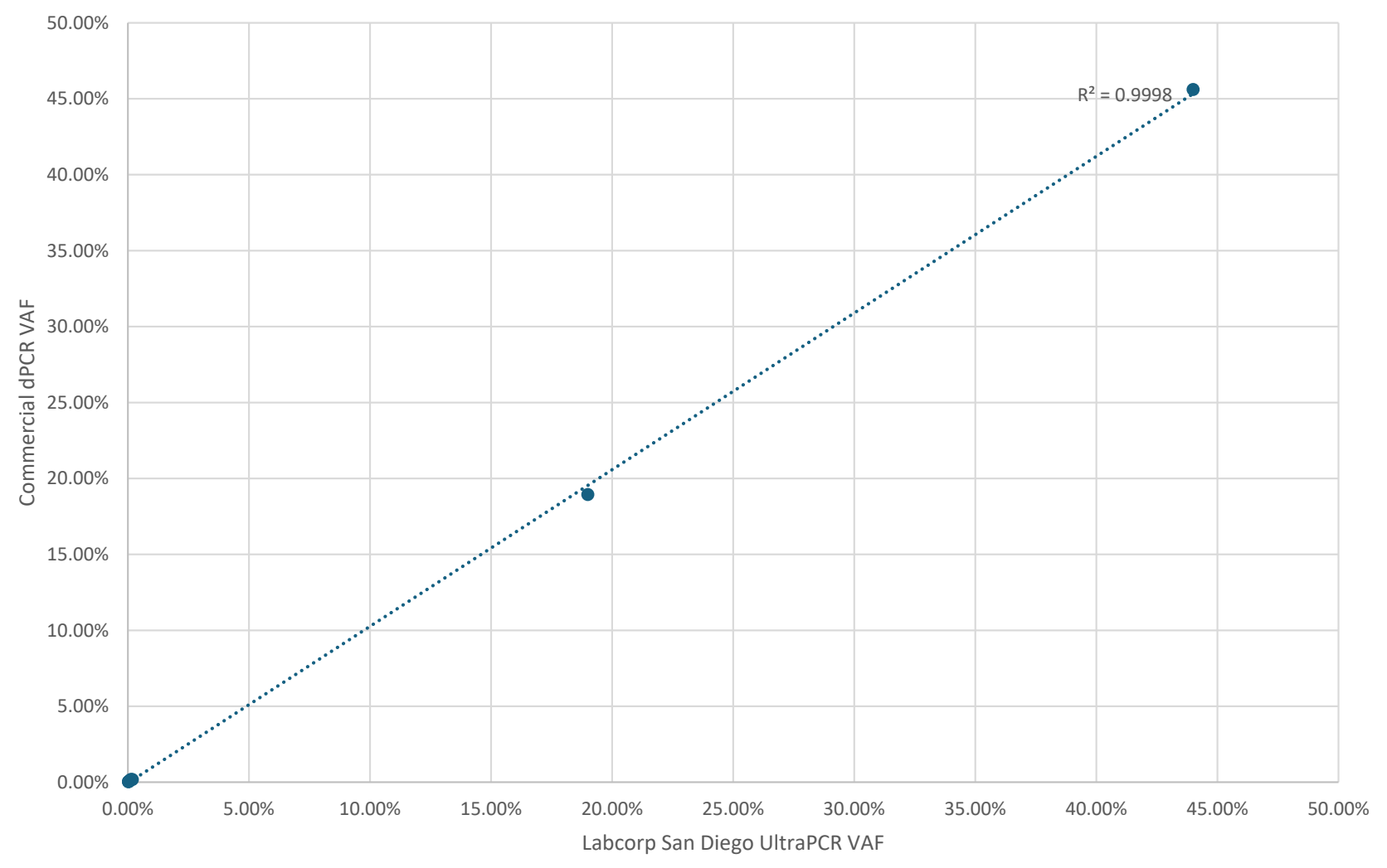


Figure 3B. Commercial dPCR vs. Labcorp VAF.

Ultra-low VAF performance was assessed using contrived samples spanning expected KIT D816V frequencies of 0.015% to 0.06%. Consistent mutant detection was observed across all replicates at each input level, with measured variant allele frequencies tracking expected values and variability consistent with stochastic sampling at low copy number.

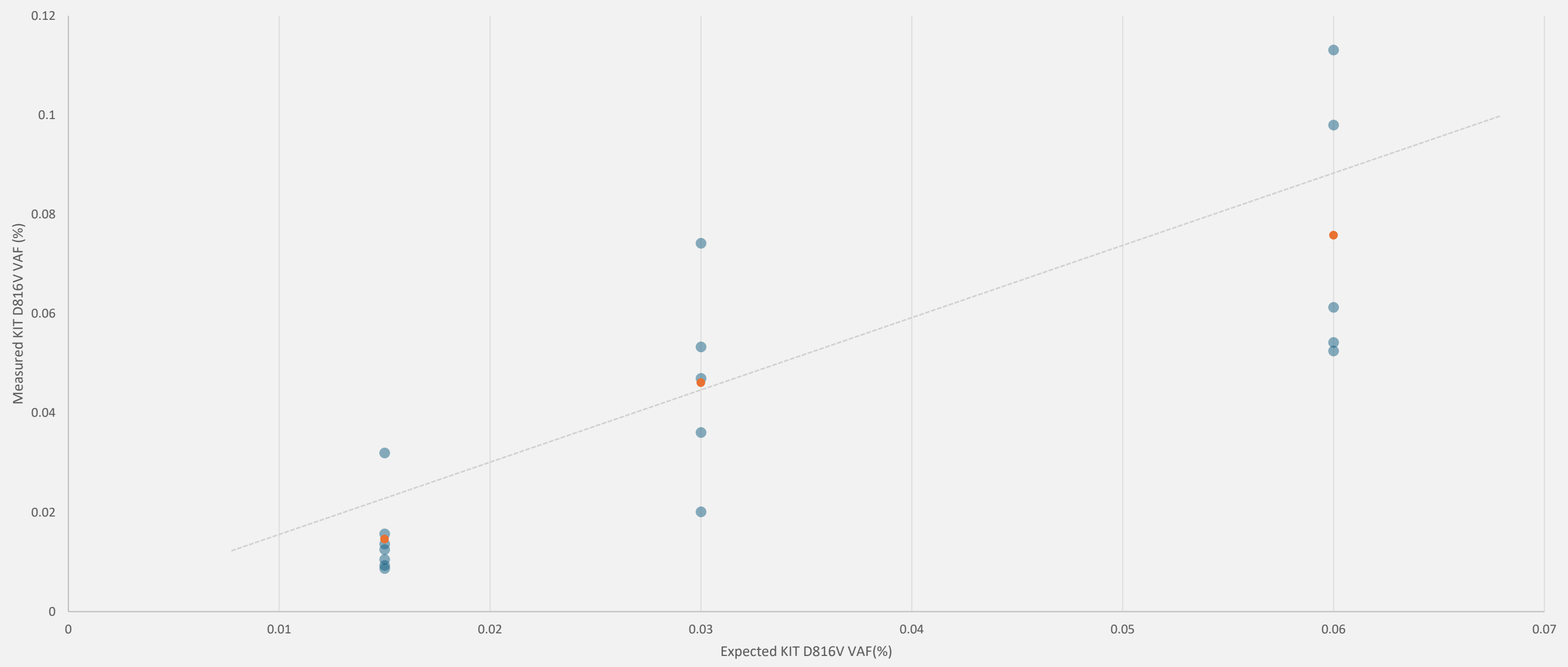


Figure 4. Consistent detection of KIT D816V at ultra-low VAFs.

Conclusions

Countable PCR enables highly sensitive detection of KIT D816V with quantitative performance comparable to a commercially available dPCR method across a wide range of DNA inputs. Results generated using Countable PCR demonstrated 100% concordance with the commercial dPCR platform and with an independent early access Countable PCR instrument, with strong linearity observed across platforms ($r^2 > 0.999$). Ultra-low VAF experiments further demonstrated consistent detection down to 0.015% VAF with minimal background signal, extending detectable sensitivity below the stated 0.03% limit of detection of the commercial dPCR method.

The Countable PCR platform achieves this performance through generation of >30 million partitions in a single, user-friendly reaction, enabling ultra-high sensitivity without the need for multiple wells or increased reaction complexity. The platform’s wide dynamic range and high DNA input capacity provide flexibility across clinical and analytical use cases, while direct counting of partition events eliminates the need for Poisson correction, enabling rapid and accurate variant quantification. Collectively, these results support Countable PCR as a next-generation digital nucleic acid analysis approach capable of robust, low-background detection at ultra-low VAFs.

Future Directions for Research:

- Future studies will expand evaluation across additional targets and sample cohorts.

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