Analytical validation of MRD profiling with tumor informed whole-genome-ppmSeq

Youngmok Jung[1], Stephanie Ferguson[1], Sangmoon Lee[1], Brian Baek-Lok Oh[1], Ariel Jaimovich[2], Seungmin Nam[1], Dawoon Jung[1], Young Seok Ju[1]

[1] Inocras Inc., San Diego, CA, USA
[2] Ultima Genomics Inc., Fremont, CA, USA

Introduction

- Minimal residual disease (MRD) detection is critical for managing cancer, allowing for early identification of relapse and guiding treatment decisions.
- Most existing MRD assays rely on deep targeted sequencing of a limited number of somatic mutations, with a limit of detection (LOD) in the range of 100–1000 parts-per-million (PPM).
- In this study, we present analytical validation of MRDVision that combines CancerVision™ (Inocras) with ppmSeq™ (Ultima Genomics), integrating tumor whole-genome sequencing (WGS) with whole-genome circulating cell-free DNA (cfDNA) sequencing. By capturing a comprehensive range of cancer-related mutations and using an ultra-sensitive sequencing method, MRDVision significantly enhances the sensitivity of MRD detection, achieving an LOD of 2–15 PPM (2×10^{-6} to 15×10^{-5}).

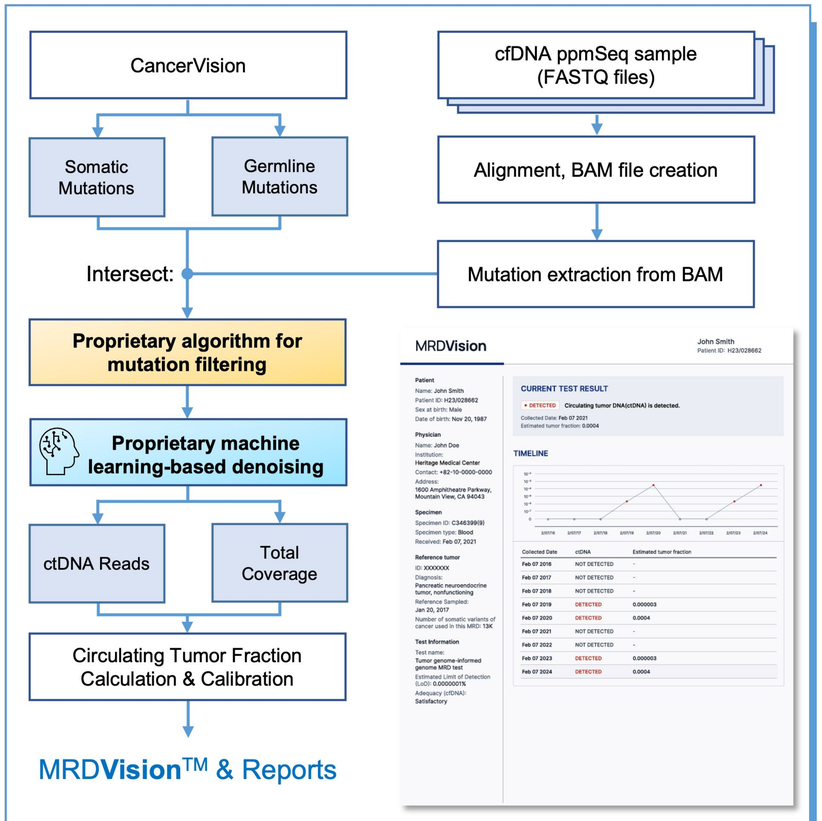
Why MRDVision?

- Robust and exceptional accuracy (ppm-level LOD)
- 50% lower cost than other MRD products on the market
- 2-week TAT from receipt of samples at the lab
- Minimum of 2 streck tubes of peripheral blood (10 mL each) for 100x coverage

Methods

- We used three tumor and matched-normal cell line pairs: HCC2218, HCC1395, and NCI-H2126 from the American Type Culture Collection.
- Each tumor DNA was diluted into 28 matched-normal DNAs at concentrations ranging from 10^{-2} to 10^{-7}, simulating 15 levels of circulating tumor DNA (ctDNA).
- These samples were sequenced using ppmSeq™ at a depth of 29–55x, yielding a mixed (duplex) rate of 25–39% and an absolute sequencing error rate of 5.5×10^{-7}.

Figure 1. MRDVision (RUO) Process



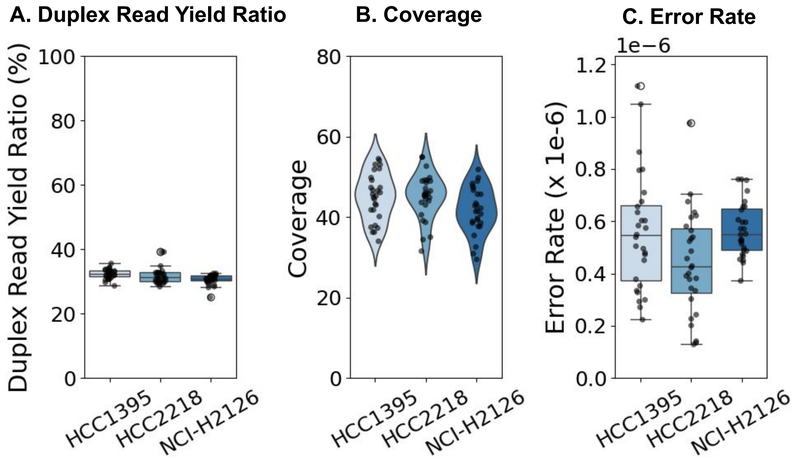
- Tumor informed: CancerVision provides whole-genome somatic mutations of a patient
- WGS based: Whole-genome sequencing of cfDNA using ppmSeq

Results

Tabe 1. Summary of Tumor cell-lines

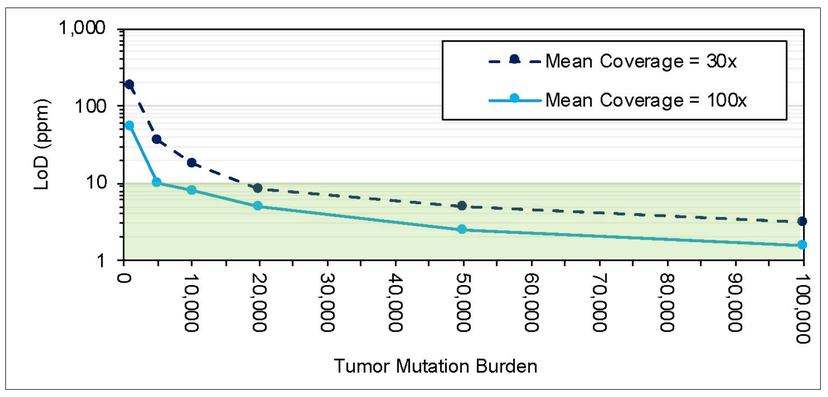
Cell line	Cancer type	Tumor depth	Normal Depth	Number of SNV
HCC1395	Breast	54.1	29.4	47,514
HCC2218	Breast	72.7	38.2	28,429
NCI-H2126	Lung	137.5	31.2	142,709

Figure 2. cfDNA ppmSeq Sequencing QC



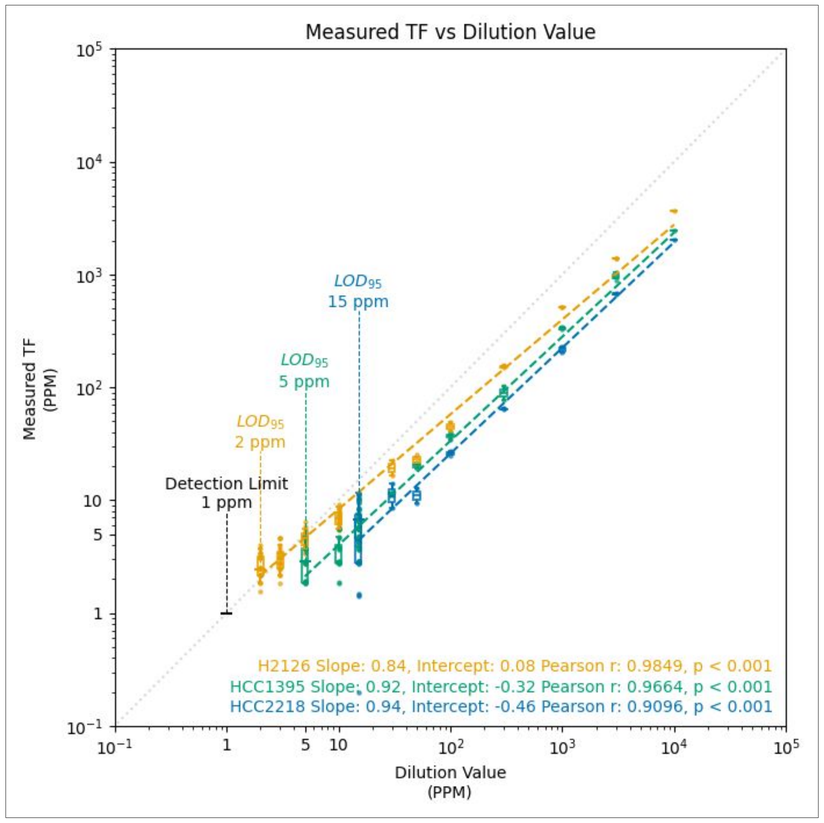
- **What is Duplex (Mixed) reads?:** ppmSeq™ employs emulsion PCR (ePCR) to amplify both strands of double-stranded DNA (dsDNA), producing beads that carry a mixture of top and bottom amplicons. This dual-strand amplification enables the identification of artifacts—arising from sample preparation or sequencing—by simultaneously examining both the forward and reverse strands from the same DNA molecule.
- **Duplex Read Yield Ratio:** the proportion of mixed reads generated by ppmSeq™.
- **Error rates:** Error rates are expressed in PPM. The background error rate is determined by counting reads that match synthetic false somatic mutations—mutations that are not present in the cell line and thus serve as a baseline for sequencing errors.

Figure 3. Simulated LOD across varying tumor mutation burden



- **Limit of Detection (LOD)** are simulated by using tumor mutation burden and coverage to determine the minimum required reads via a binomial probability model with a specified background error rate, specificity threshold, and selecting the smallest tumor fraction from simulation data that meets the LOD sensitivity criteria.

Figure 4. Measured tumor fraction vs Dilution value



- **Detection Limit** is the limit of blank observed from the negative blank samples.
- **LOD95** is the minimum concentration at which 95% of readings would be positively detected.

Table 2. Sensitivity & Specificity on LOD

Cell Line	LOD (ppm)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HCC1395	5	100	95	95.2	100
HCC2128	15	100	95	95.2	100
NCI-H2126	2	95	95	95	95

Conclusion: MRDVision leverages whole-genome somatic mutations and ultra-sensitive sequencing to deliver robust, cost-effective MRD detection with ppm-level sensitivity, achieving an LOD of 2–15 PPM.