A Novel Enrichment Technology (Enspyre) Enables Ultra-Sensitive ctDNA Detection with 98% Reduction in Sequencing Requirements

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Background

Circulating tumor DNA (ctDNA) liquid biopsies show promise for minimal residual disease (MRD) detection, but clinical implementation is limited by high sequencing costs and the need for ultra-deep coverage to detect ctDNA at levels ≤100 parts per million (ppm). Current tumor-informed, wet-lab personalized assays require >500 million reads per sample, necessitating sample batching and high-throughput platforms, which limits accessibility and increases turnaround times.

Methods

We evaluated Enspyre (**En**richment by **s**elective **py**rophosphorolysis and **re**lease), a novel enrichment technology that enables selective enrichment of specific variant molecules rather than just target regions (Fig.1). Eight lung cancer patients underwent whole genome sequencing of FFPE tumor tissue (median coverage 118x) for personalized probe design targeting a median of 1,995 somatic variants. Patient plasma samples were diluted to create a concentration series (5-1000 ppm) using healthy donor plasma. Enspyre enrichment was performed on low-input cfDNA samples (median 7.67 ng) followed by sequencing on NextSeq 550 with only 9.6 million read pairs per sample. ctDNA detection and quantification were performed using a Bayesian MRD estimation model, with analyses conducted blinded to ground truth concentrations.

Summary of Results

Enspyre demonstrated exceptional analytical performance across 72 patient samples and 8 controls. The assay achieved 100% sensitivity at 10 ppm (6/6 samples detected) and 20% sensitivity at 5 ppm (1/5 samples detected) without molecular barcodes (Fig. 2). 100% specificity was maintained with no false positives in control samples (10/10 correctly called negative). Quantitative ctDNA estimates showed strong linear correlation with expected values ($r=0.90$, $p<2.2\times10^{-16}$), with estimates averaging 1.22-fold of target concentrations (Fig. 3). Performance was maintained despite low DNA inputs, with successful detection from as little as 0.84 ng cfDNA. Compared to standard hybrid capture methods, Enspyre achieved equivalent sensitivity with a 98% reduction in sequencing depth (10M vs 500M reads per sample estimated in non-selective hybridisation capture methods).

Graphical description of the Enspyre workflow

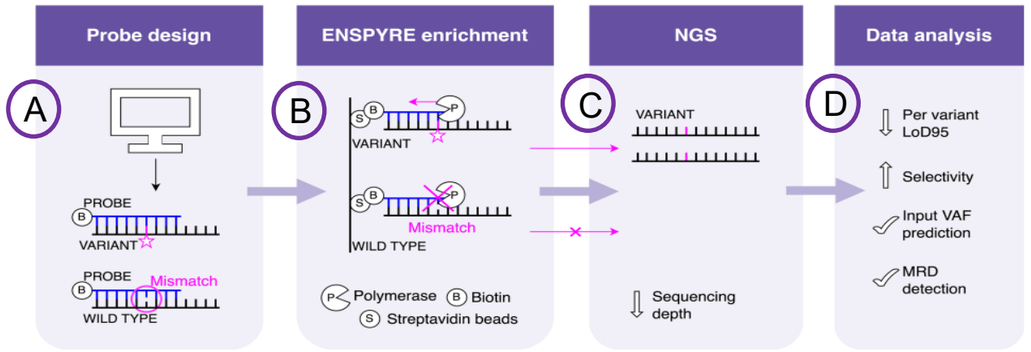

Figure 1: A) Probe design performed by WGS of the tumor and matched normal tissue (median coverage of 118x and 48x, respectively). From the variants called, approximately 2,000 variants were selected for the targeted panel. **B)** Hybridization capture of target regions by bait probes and pyrophosphorolysis digests only perfectly complementary sequence thus enriching the Mutant molecule. **C)** Libraries were sequenced on a NextSeq 550 instrument using a 2x150 cycle mid-output kit (Illumina) with a median of 9.6 million read pairs generated per sample. **D)** Duplex and single-strand molecules were aligned and counted. Consensus read bundles, for each given variant, were generated. The mutant molecule counts were then input into a Bayesian MRD estimation model, which uses the observed data together with priors on background error rates, probe performance and library preparation efficiency to estimate sample-level VAF and generate a binary (presence/absence) ctDNA call

Detection Rate for the tested Dilution Range

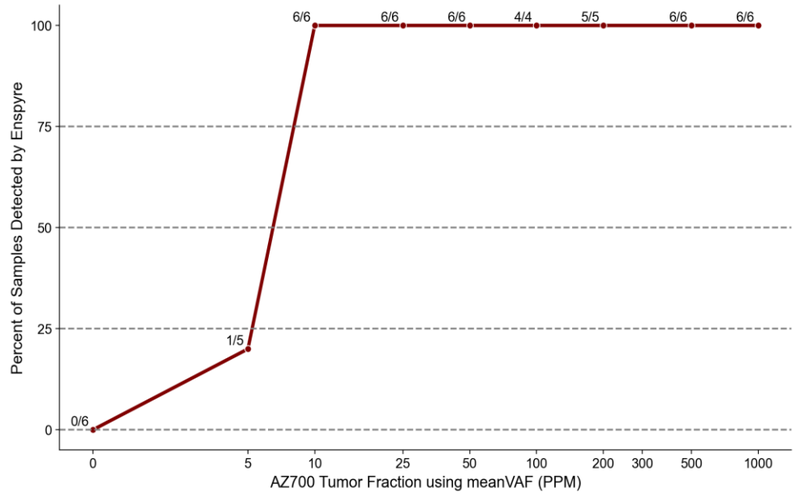

Figure 2: Enspyre ctDNA detection rate across different dilution types without the use of UMIs. The numbers of ctDNA-positive out of all QC-passing samples are annotated for each dilution level. *Two of the eight samples were removed from analysis due to insufficient normal tissue availability. **3 samples were marked as drop-outs post-sequencing through the use of in-sample QC probes and removed.

Correlation between Expected and Reported PPM

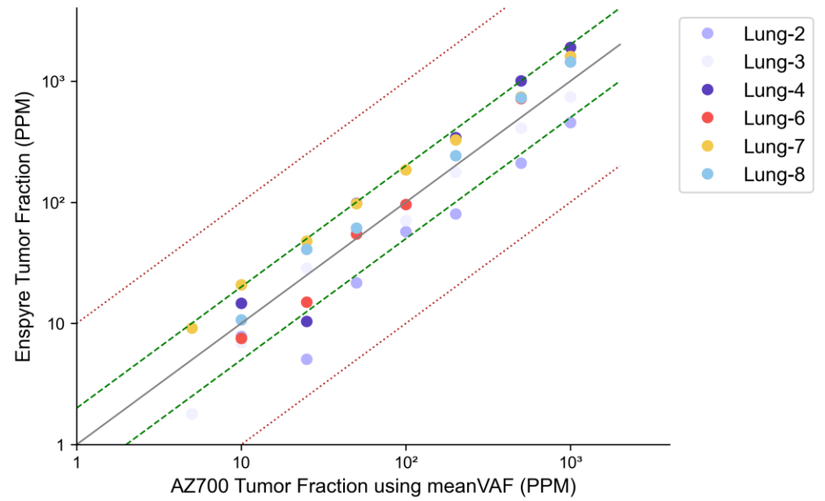

Figure 3: Enspyre ctDNA level estimates versus expected (ground-truth) ctDNA levels. Solid grey line represents perfect correlation, dashed green lines shows a 2-fold and dotted red line a 10-fold deviation from expected levels. *Two of the eight samples were removed from analysis due to insufficient normal tissue availability.

Conclusions

- Enspyre enables ultra-sensitive ctDNA detection at 10 ppm with dramatically reduced sequencing requirements, addressing key barriers to clinical implementation.
- The technology's ability to maintain performance with low DNA inputs and simplified workflows makes ctDNA testing accessible on benchtop sequencers, potentially enabling broader adoption in clinical trials and community oncology.
- Sample throughput increases from 1 to 40 samples per NextSeq run represent a 40-fold improvement in accessibility.
- Enspyre can democratize MRD. The 98% reduction in sequencing depth redefines the utility of mid-range sequencers for MRD. By maximizing the output of smaller footprints, we can enable transition from centralized lab infrastructure to localized, accessible diagnostic platforms.

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