Poster ID: 434

Enspyre: A novel enrichment technology for selected DNA variants using pyrophosphorolysis

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Enspyre®

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Abstract

Enspyre (Enrichment by selective pyrophosphorolysis and release) is a novel target enrichment technology developed at Biofidelity with a broad spectrum of potential applications that allows for selective enrichment of specified variants via pyrophosphorolysis (PPL) prior to sequencing.

We present here proof-of-concept data of enrichment using Enspyre compared to a standard hybridization capture assay. A machine learning-based probe design algorithm developed at Biofidelity is used to design selective probes for Enspyre without the need for in vitro optimization. 98% of probes designed with this algorithm have a selectivity score† above 10, meaning mutant to wildtype molecule ratios following the assay increased at least 10-fold. Using these probes, Enspyre detects single variants with a 95% limit of detection (LoD95) of 0.5% variant allele fraction (VAF), with sensitivity comparable to other NGS-based variant detection assays but with far fewer sequencing reads. Enspyre can also be used to infer the input VAFs for variants. One of the potential practical uses of Enspyre is minimal residual disease (MRD)-tracking in oncology. In an MRD proof-of-concept experiment using 1,800 variant-specific probes and custom-made contrived samples, Enspyre accurately detected the presence of MRD down to 10 parts per million (ppm) with only 5 million reads per sample.

In summary, Enspyre can identify variants with a sequencing depth reduced by at least 95% compared to a standard hybridization capture assay. Additional benefits include lower data processing time and storage. In clinical diagnostic use, Enspyre offers the opportunity to implement high-depth liquid biopsy applications such as MRD on small benchtop NGS platforms, making tests more accessible to clinical laboratories while reducing costs, operational complexity, and turnaround times.

Introduction

Hybridization capture is a useful method, but sensitivity is limited in detection of rare variants

- Current methods require deep sequencing for rare variant detection and face challenges in distinguishing these variants from background noise
- Overall, this drives up cost and reduces efficiency – limiting its application, especially in areas such as clinical oncology

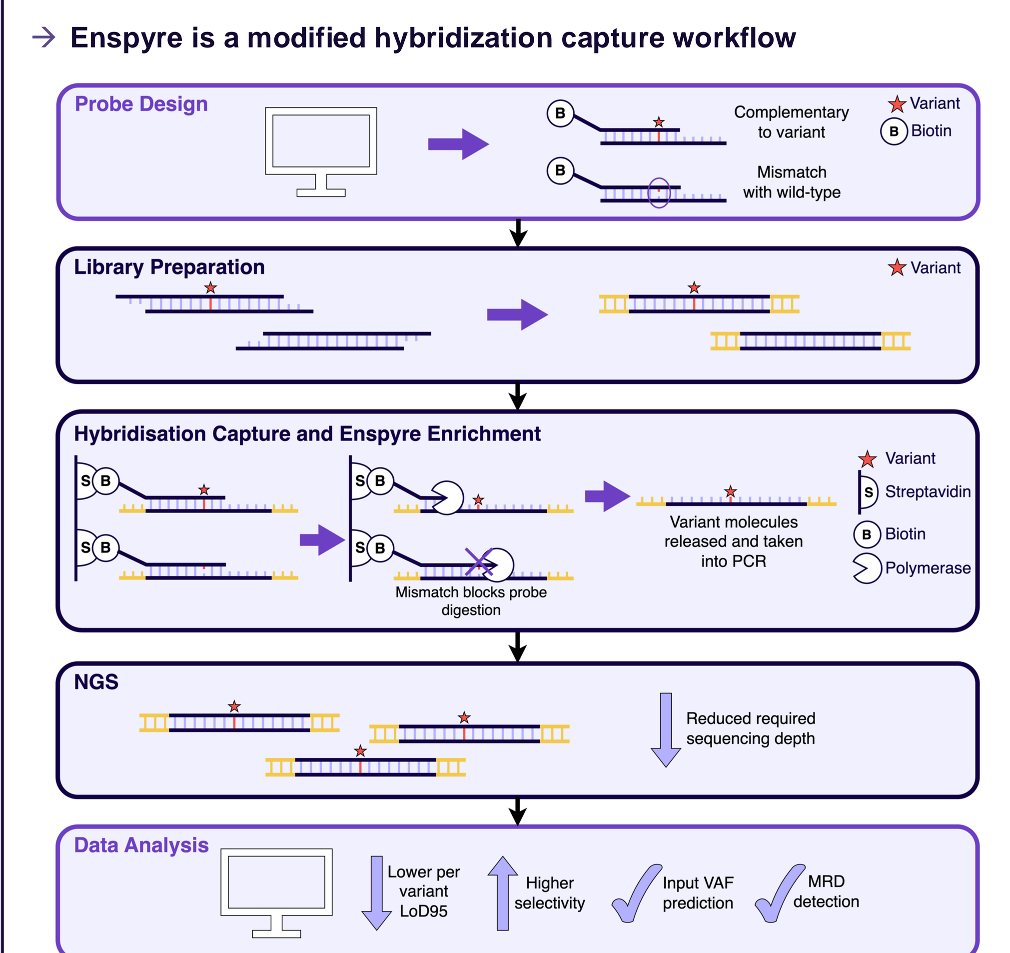
Enspyre is designed to address these limitations

- Enspyre enables enrichment of specific variants, minimizing background noise and allowing accurate rare variant detection at a fraction of the sequencing depth and cost

Here we present the Enspyre workflow, mechanism, and proof-of-concept of a potential application in MRD detection

Overview of Enspyre Workflow

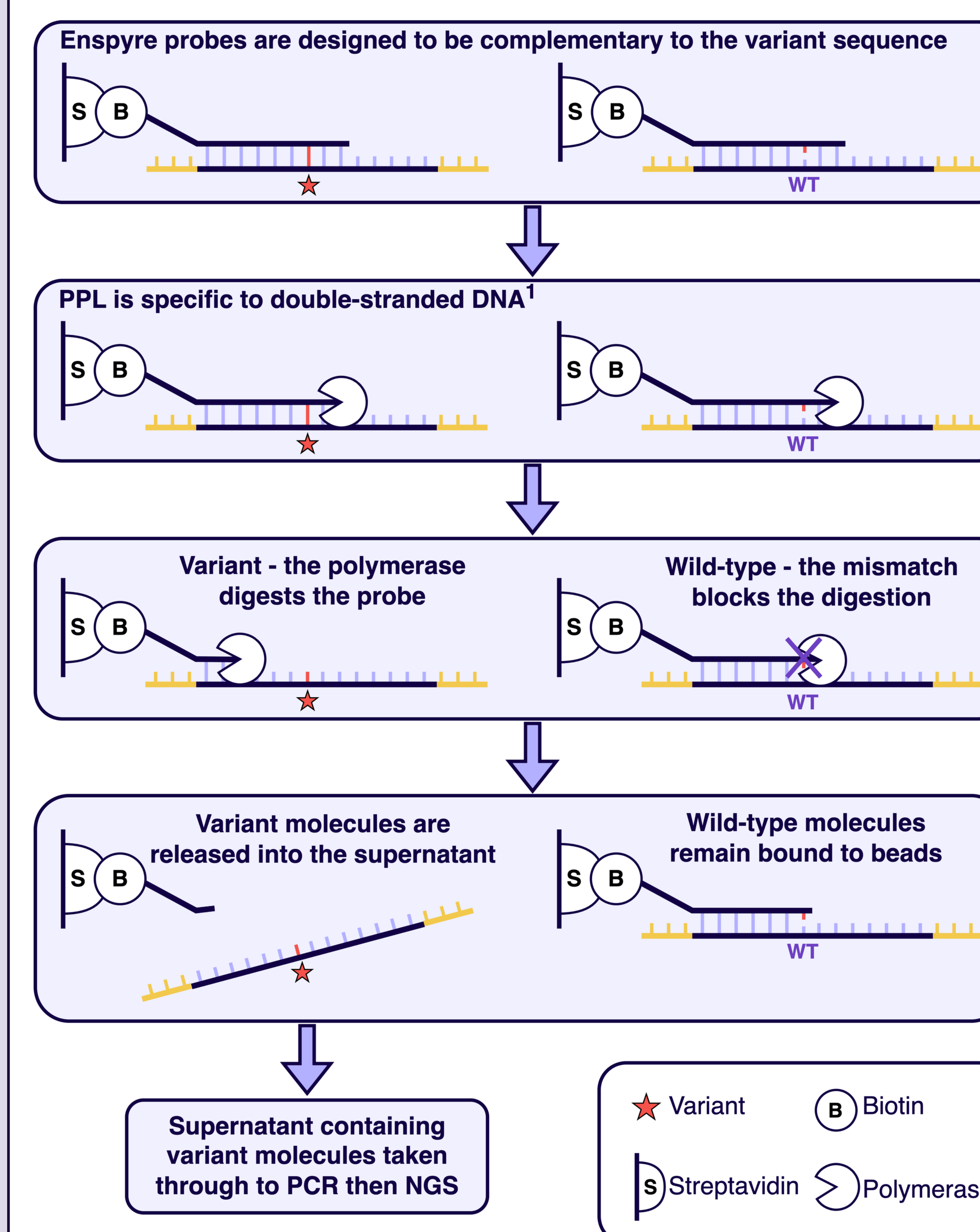
→ **Enspyre is a modified hybridization capture workflow**



Variant Enrichment via Pyrophosphorolysis

→ **PPL is the direct reverse of the DNA polymerase reaction, specifically digesting only perfectly matched double-stranded DNA**[1]

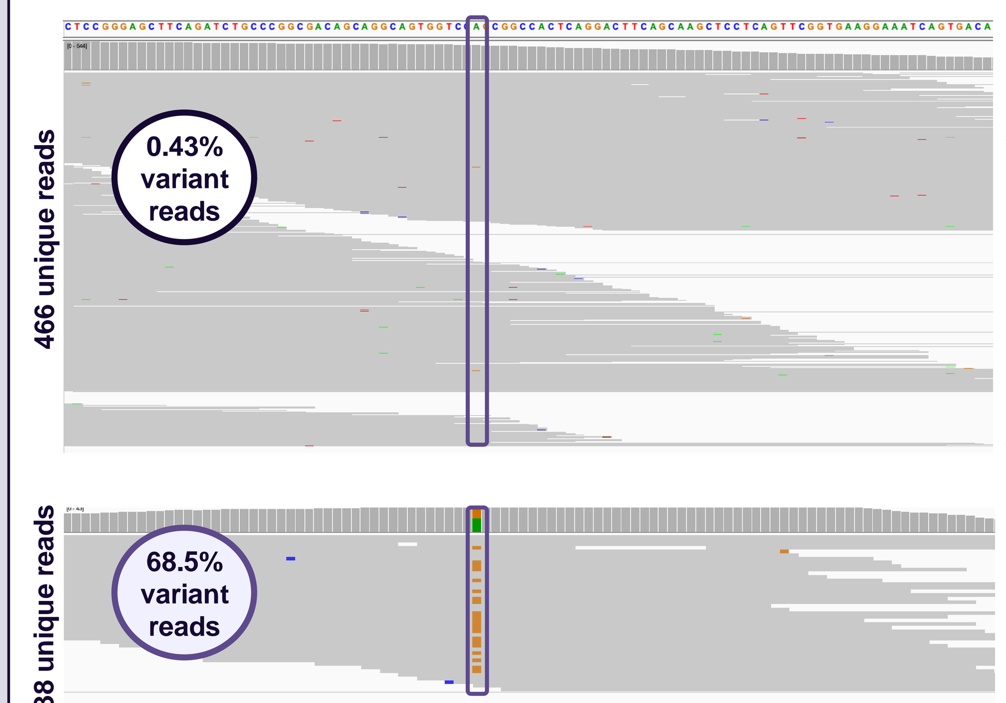
→ Probes have full complementarity to the variant sequence. Only variants completely matched to their probes are released after probe digestion.



→ **Through enrichment of variants, signal from wild-type molecules is reduced**

→ **This enables a higher proportion of reads to be dedicated to variant molecules in NGS**

→ **Enspyre therefore reduces the read depth required for accurate detection of low-frequency variants**

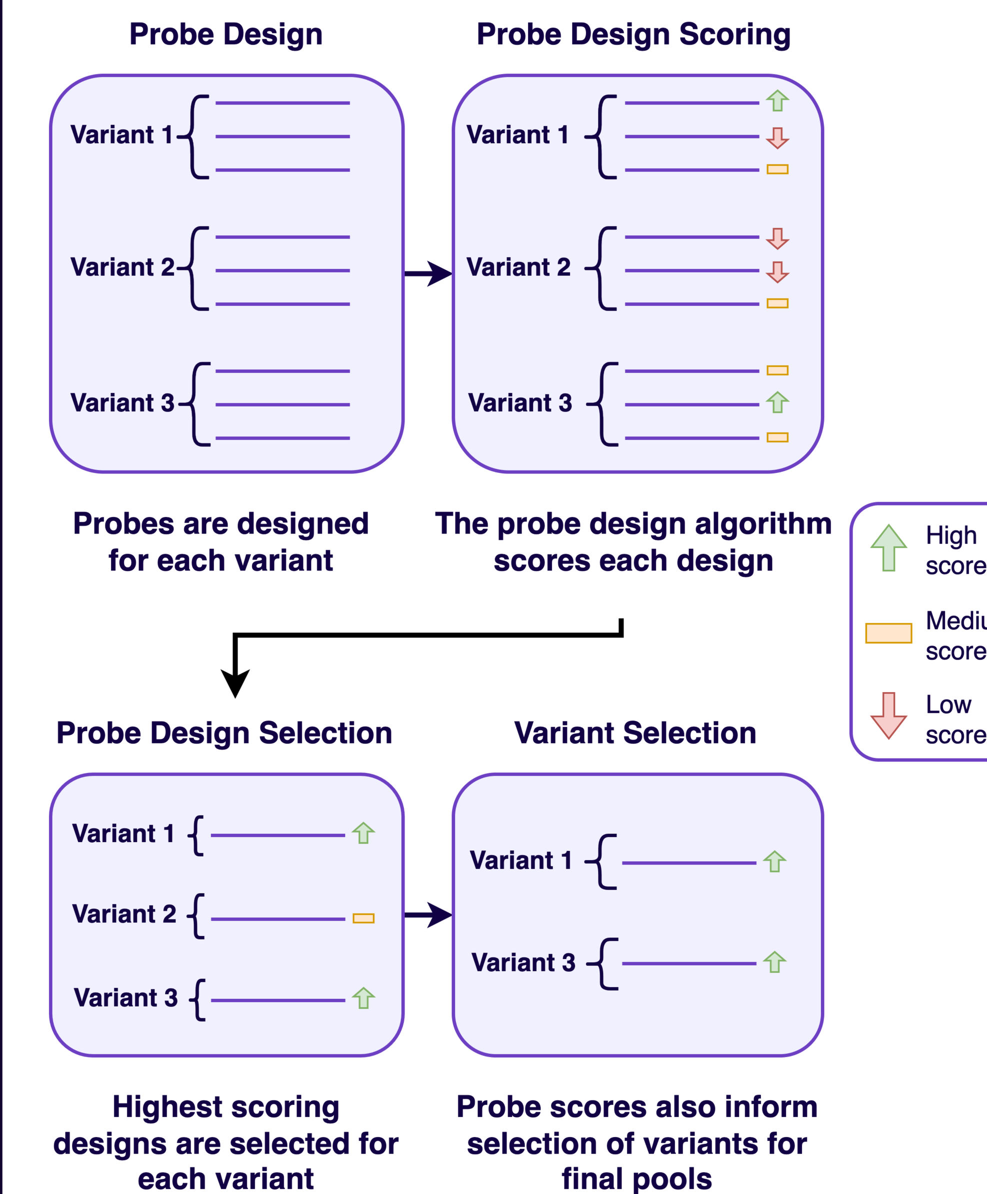


Enspyre reduces the sequencing depth required for variant detection. A screenshot of IGV view for variant rs1056171 (shown in orange) at 0.5% VAF, comparing the fraction of unique variant reads using a standard hybridization capture assay vs. Enspyre assay.

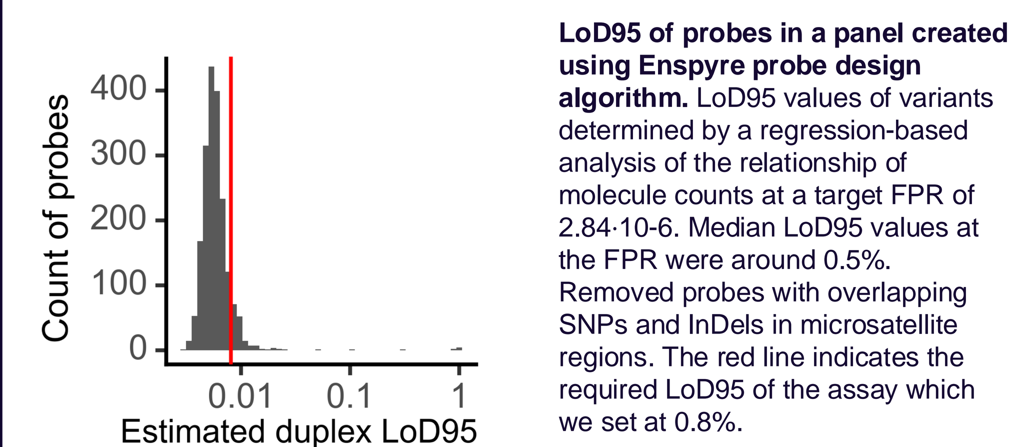
Enspyre Probe Design and Variant Selection

→ **Probe design is a critical aspect of successful variant enrichment**

→ **The Enspyre probe design algorithm enables creation of large variant-specific panels without the need for in vitro optimisation**



→ Here we generated data using 1,800 variant-specific probes in an overall panel size of ~4,000 probes



LoD95 of probes in a panel created using Enspyre probe design algorithm. LoD95 values of variants determined by a regression-based analysis of the relationship of molecule counts at a target FPR of 2.84·10-6. Median LoD95 values at the FPR were around 0.5%. Removed probes with overlapping SNPs and InDels in microsatellite regions. The red line indicates the required LoD95 of the assay which we set at 0.8%.

Selectivity† level	No. of probes (percent of pool) InDel	SNV
Non-selective: (0, 1]	4 (2.5%)	0 (0%)
Weakly selective: (1, 10]	13 (8%)	15 (1%)
Selective: (10, 50]	70 (44%)	984 (66%)
Strongly selective: > 50x	74 (46%)	484 (33%)
Total	161	1,483

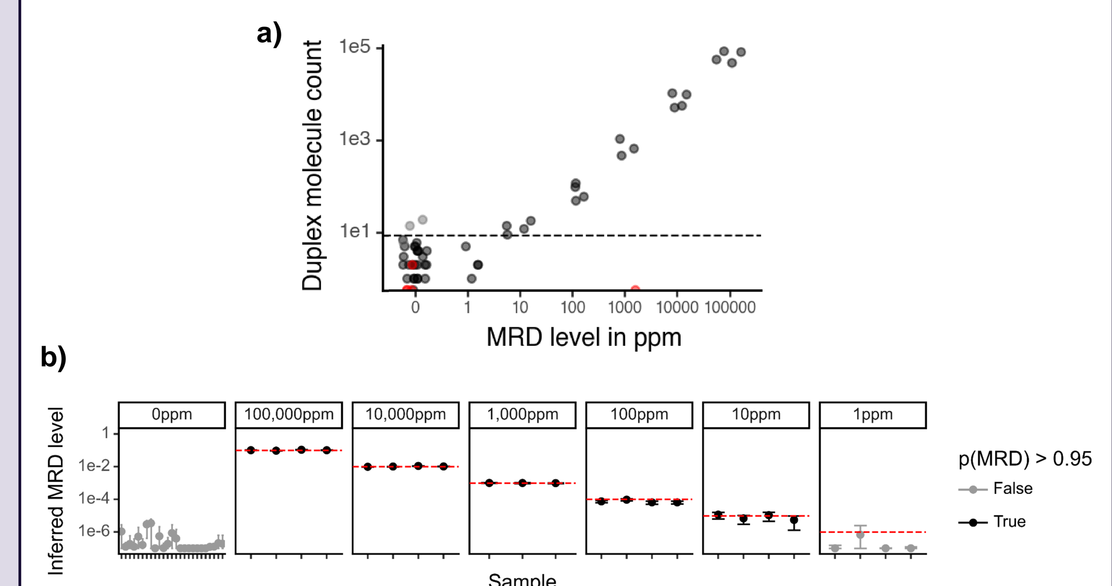
Summary of estimated selectivity values for InDels and SNVs across probe panel. Removed probes with overlapping SNPs and InDels in microsatellite regions.

†Selectivity of a probe is defined here as the ratio of the rate of variant molecule return to the rate of wild-type molecule return.

MRD Detection Using Enspyre

Conducted MRD detection proof-of-concept using Genome in a Bottle (GIAB) DNA[2]

- Targeted variants heterozygous in HG002 and absent in HG003
- Designed and selected 1,800 probes for these variants using Enspyre probe design algorithm
- Diluted HG002 in HG003, creating a dilution series from 100,000 ppm to 1 ppm. HG003 used as blank samples
- Ran samples through Enspyre then NGS (Illumina NextSeq, mid-output kit)
- In-house MRD analysis of resulting data



MRD detection using Enspyre. a) Analysis of non-InDel variants. There is a relationship between the number of duplex variant molecules and the MRD level. Red dots: QC-failed samples, light grey dots: cross-contaminated samples. Cross-contamination was determined through the analysis of linked GIAB SNPs. Blank samples (0 ppm) were below the background noise level of 8 molecules (dashed horizontal line). Molecule counts above the background level were observed for samples above 10 ppm and increased continuously with the MRD input. b) Model-based analysis of all samples demonstrate the ability of Enspyre to correctly identify MRD down to 10 ppm without false-positive calls in the blank samples. Red lines correspond to the known input VAF for each condition.

Discussion

Enspyre addresses limitations in traditional hybridisation capture methods

- Through variant enrichment, **Enspyre can achieve a per-variant LoD95 of 0.5% VAF with just 5 million reads per sample**, compared to other commercially available assays that can require up to 400 million reads per sample[3]
- This enables high-sensitivity detection of rare variants using benchtop NGS platforms
- Smaller data output reduces costs of data storage and reduces analysis time
- Using Enspyre probe design algorithm, large variant-specific panels can be designed without need for further optimisation

Potential applications of Enspyre

- The efficiency and cost reduction of Enspyre makes it especially attractive for clinical applications such as MRD detection and tracking in clinical oncology
- Here we demonstrated a proof-of-concept of MRD detection using Enspyre, achieving a **sample-level sensitivity of 10 ppm**
- Beyond oncology, Enspyre could benefit any field where selective enrichment is required, for example: forensics, infectious disease surveillance, or genotyping in agriculture

Enspyre is a powerful new tool that can transform variant enrichment and detection in NGS-based assays

References

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