Enspyre MRD: a novel enrichment approach for ultra-low variant detection with reduced sequencing requirements

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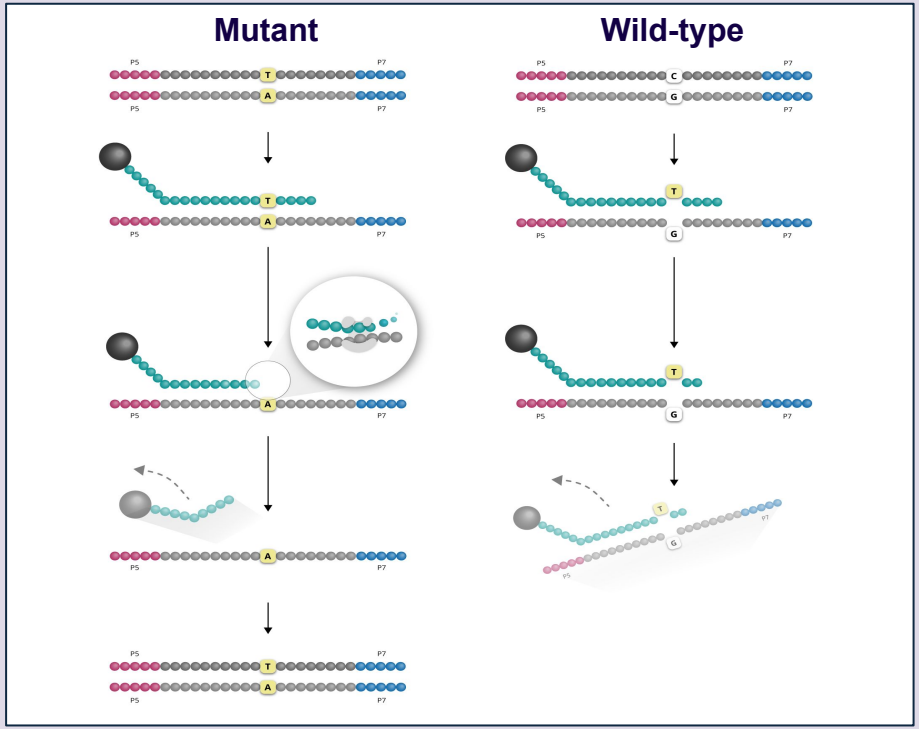
Technology overview

- Proprietary software designs probes targeting somatic variants without the need for *in vitro* testing
- Standard kits can be used for library preparation of extracted cell free DNA
- Variant-specific enrichment is achieved through pyrophosphorolysis, which only digests fully complementary probes
- Mutant molecules are released into the supernatant while wild-type molecules remain on the beads
- Here, we used contrived samples prepared from fragmented Genome in a Bottle (GIAB)[1] to model patient ctDNA
- Up to 2,000 probes were designed to match target variants and run through Enspyre



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[1]Zook JM, et al. Extensive sequencing of seven human genomes to characterize benchmark reference materials. Sci Data. 2016 Jun 7;3:160025.

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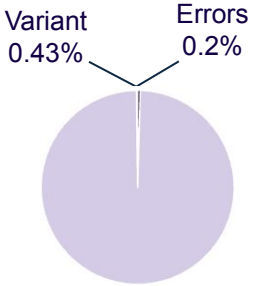
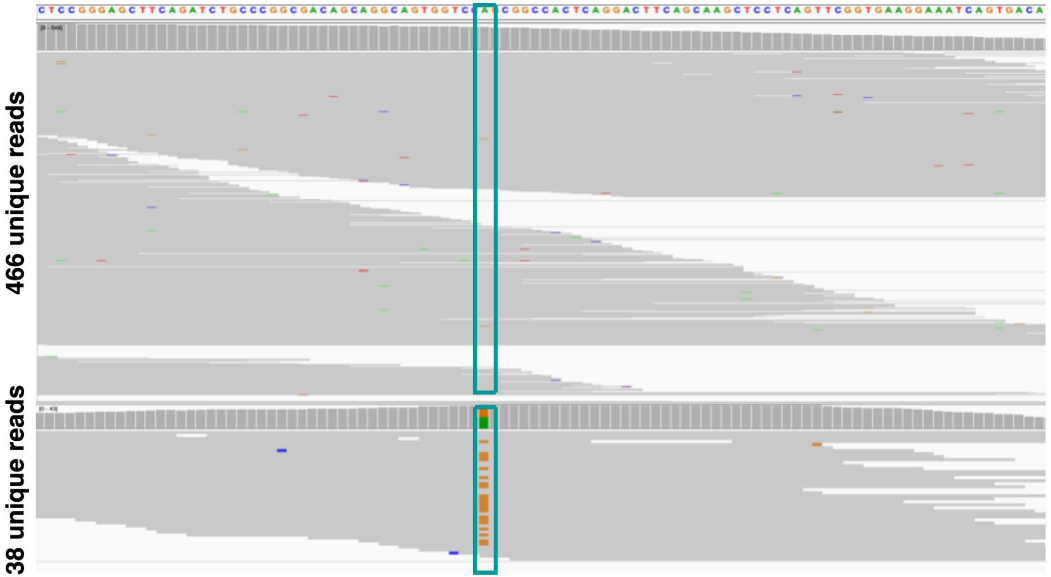
Selective enrichment for MRD detection

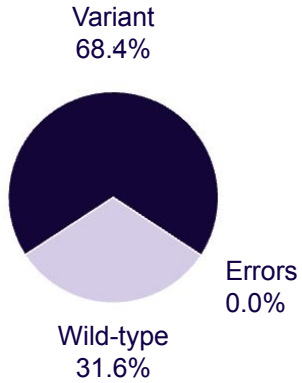
- Current next generation sequencing (NGS)-based liquid biopsy MRD tests either suffer from limited sensitivity or high costs due to the amount of sequencing data required
- Enspyre is a novel hybridisation and capture technique that selectively enriches target variants, thus reducing sequencing requirements while boosting detection sensitivity

Non-selective assay





Enspyre



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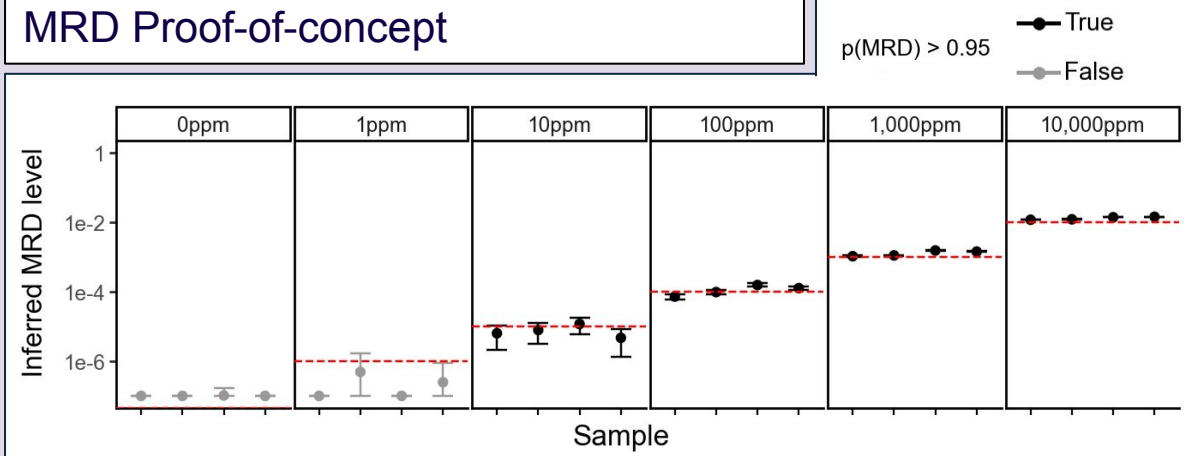
MRD Proof-of-concept



- Enspyre accurately calls MRD status down to 10 ppm in contrived samples, requiring only 20 ng DNA and 5M read pairs
- No false positives detected in blank samples
- Single variant detection <1% VAF for SNVs and INDELs

Benefits of Enspyre:

- Massively reduced NGS requirements compared to other hybridisation capture assays
- Option to use benchtop sequencer
- Simplified data handling & bioinformatics

Sample throughput across instruments	Enspyre		Standard hyb capture	
	Per run	Per year	Per run	Per year
MiSeq v3	2	200	0	0
MiSeq i100 Plus 100M	20	5,000	0	0
NextSeq 550 High Output	80	12,500	0	0
NovaSeq S4	1,600	166,400	16	1,664
NovaSeqX 25B	5,000	520,000	50	5,200

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Clinical potential

- Here, we demonstrated a proof-of-concept of MRD detection using Enspyre, achieving a sample-level sensitivity of 10 ppm
- Combined with tumour whole-genome sequencing for variant discovery, Enspyre offers gold-standard MRD detection at a fraction of the sequencing cost
- Being able to track thousands of variants with one assay, Enspyre's clinical applications cover the full patient journey, from therapy selection to recurrence detection and treatment response monitoring