Validation of the OncoDEEP® kit comprehensive genomic panel on the MGI platform.

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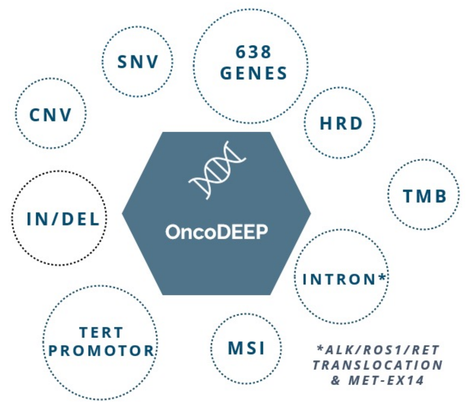
Introduction :

- OncoDEEP® kit is a CE-IVD Comprehensive Genomic Panel, based on Twist Bioscience technology and Illumina sequencing, allowing the study of both **DNA and RNA** combined with **Bio-IT analysis** and **clinical reporting**.
- Complete tumor characterization **through 638 genes, genomic signatures and fusions** that helps **reducing the costs** of testing and delivering **faster results** in the selection of appropriate cancer treatment options.
- Implementation in alternative sequencers to Illumina, such as **MGI**, makes the solution more **cost-effective**, which is crucial for CGP routine use while maintaining clinical standards.



Materials and Methods:

- Clinical samples, carrying known variants/fusion/genomic signatures previously detected with OncoDEEP® kit combined with Illumina sequencing, were selected (**Table 1**). Reference standards were also used: 6 from Horizon for variants & fusions and 3 from Seracare for tumor mutational burden (TMB). DNA inputs of 6ng, 50ng & 100ng were used and some samples were replicated. In total, 90 samples (54 clinical & 36 reference samples) were sequenced to evaluate the performance of MGI System.

Table 1: Samples used to compare OncoDEEP® panel based on Illumina or MGI sequencer

Samples	Aim	n
Clinical samples	Variant/fusion calling and MSI	34
Ovarian clinical samples*	HRD	20
HD678, HD789, HD798, HD799, HD803, HD832, GM24149	Variant/fusion calling	29
Seracare samples	TMB	7

*FFPE ovarian clinical samples coming from the Hospital 12 Octubre (Madrid, Spain)

- The OncoDEEP® kit allows the full characterization, from the DNA extraction to the final report, of the samples in less than 5 working days (**Figure 1**). After extraction, libraries were constructed (**3h**) and enriched (**hands on time: 4h**) based on Twist Biosciences Technology. Then the libraries were sequenced on a CE-IVD MGI DNBSEQ-G400 device. Finally, FastQ files were uploaded and analyzed through OncoDNA dedicated BioIT pipeline.

Figure 1: Full OncoDEEP® workflow from the wetlab part (DNA extraction to sequencing) to FASTQ files upload and secondary/tertiary analysis.

Results

- MGI generated **high quality data**, as highlighted by QC metrics (**Figure 2**). **Mean coverage was above the threshold** (<350x) (**A**) at recommended DNA inputs (100ng & 50 ng), while below but still informative at 6 ng**. Uniformity was highly stable** and high across the 3 DNA inputs (**B**). Additionally, all the OncoDEEP® panel was **correctly covered** at all 3 DNA inputs (**C**).

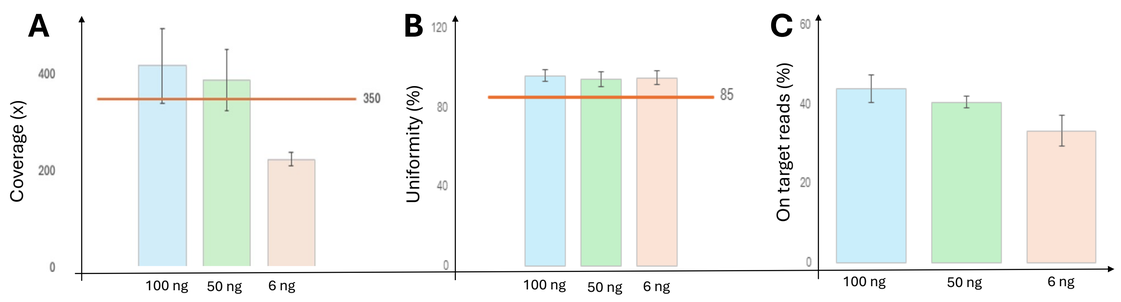


Figure 2: Quality metrics of NGS data generated *via* the OncoDEEP panel and sequenced on MGI device showing a good coverage (A), uniformity (B) and on target reads (C) at DNA inputs from 100 ng to 6 ng.

- MGI device showed **a good reproducibility** across sequencing (**Figure 3**), as highlighted by HD832 Horizon sample performed in quadruplicates. All variants were detected in all replicates at similar VAF, despite one BRCA2 variant showing a higher VAF in one replicate.

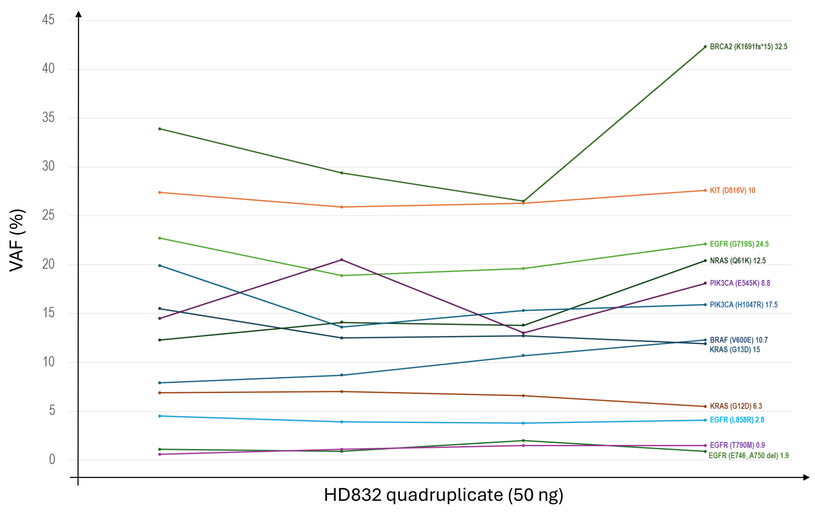


Figure 3: Quadruplicates of HD832 control sample generated via OncoDEEP panel and sequenced on MGI device showing the high reproductibility across all 4 sequencing with the detection of all variants

- MGI device detected most of the variants in both clinical and control samples, even at low VAF, at DNA input as low as 6ng and in degraded sample (**Table 2**). All variants were detected in both HD798 and HD832, **showing performance comparable to Illumina**. In both HD799 and HD803, one variant was missed on both Illumina and MGI devices at 6ng (counted <5x). Interestingly, **two variants** at low VAF were missed on Illumina device but **detected on MGI** thanks to a higher mean coverage. All fusions present were detected at all DNA inputs, except one at 6 ng, allowing the **identification of the partner and the fusion variant**.

Table 2: Results of HD798, HD799, HD803 and HD832 showing the high quality of MGI sequencing to call variant at low VAF, low DNA input and degraded samples, comparable to Illumina using the OncoDEEP® panel.

Gene	Variant (VAF%)⌀	HD798 (mild)				HD799 (moderate)				HD803 (severe)				HD832					
		Illumina		MGI		Illumina		MGI		Illumina		MGI		Illumina			MGI		
		50ng *	6ng	50ng *	6ng	50ng *	6ng	50ng *	6ng	50ng *	6ng	50ng *	6ng	100ng	50ng **	6ng	100ng	50ng **	6ng
BRAF	V600E (10.5/**10.7**)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
KIT	D816V (10)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
EGFR	E746_A750 del (2/**1.9**)	✓	✓	✓	✓	✓	✗▲	✓	✓	✓	✗▲	✓	✗▲	✓	✓	✓	✓■	✓◎◎	✓■
	L858R (3/**2.8**)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	T790M (1/**0.9**)	✓	✓■	✓◎◎	✓■	✓	✗▲	✓	✗▲	✓	✗▲	✓	✓	✓■	✓◎◎	✓	✓	✓◎	✓■
	G719S (24.5)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
KRAS	G13D (15)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	G12D (6/**6.3**)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
NRAS	Q61K (12.5)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
PIK3CA	H1047R (17.5)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	E545K (9/**8.8**)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

⌀ VAF in green is specific for Reference HD832; * Duplicated; ** Quadruplicate; ▲Detected but discarded due to failing QC cut-off; ■ Detected but not reported due to VAF <1%; ◎ 1 duplicate detected but not reported due to VAF <1%; ◎◎ 2 duplicates detected but not reported due to VAF <1%

- Illumina and MGI sequencing showed comparable results concerning all genomic signatures (**Figure 4**). **TMB** was **highly comparable** between the two platforms (R^2=0,9894) (**A**), with all control samples showing expecting results and 100% of concordant clinical results. **MSI was highly stable**, with a R^2=0,9987 and 100% of concordance (8 positive and 26 negative clinical samples) (**B**). Finally, **HRD showed 100% of concordance** of the final results (11 positive and 9 negative) (**C**), with **all BRCA1/2 variants detected** and a **high correlation regarding the GS** (R^2=0,9273).

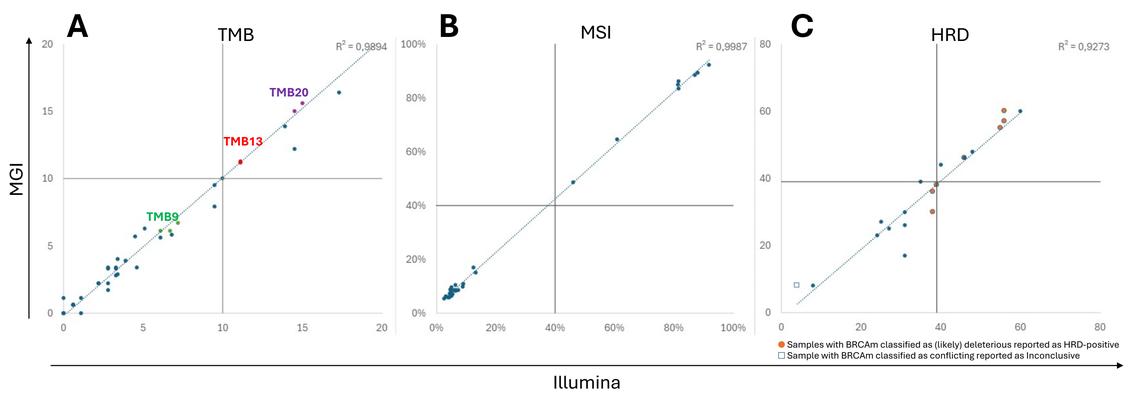


Figure 4: Correlation of genomic signatue (TMB, MSI and HRD) generated via OncoDEEP panel and sequenced on MGI versus Illumina showing a high concordance of the two platforms.

Conclusion: The **OncoDEEP®** is a **cost-effective, end-to-end solution**, based on Twist Bioscience technology and Illumina sequencing, offering a full characterization of the tumor. **MGI** platform was shown to be an **alternative to Illumina** by generating **high quality data**. **All variants and fusion detected, as well as the calculation of genomic signatures were comparable to those using an Illumina platform**. This allows the reduction of sequencing costs, **increasing the cost-effectiveness of the OncoDEEP® solution**, and therefore making CGP more accessible to patients.