Performance Validation of Magnis BR: a full-automatic capture-based library preparation platform for next-generation sequencing (NGS)

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Abstract

Background

NGS with capture-based multigene panel has been widely used in cancer genomic profiling and therapy selection due to its capability of detecting biomarkers for both targeted- and immuno- therapies. However, the library preparation process involving hybrid capture requires highly skilled experimental operators and is time consuming, which limits its execution within hospital laboratory. To solve the issue, we have developed the first full-automatic capture-based library preparation system-Magnis BR. The system can not only complete the library preparation by fully walkaway automation but also shorten the process from 24-36 hrs to 9 hrs. Herein, we prepared DNA libraries on the Magnis BR with a 520-gene assay (OncoScreen IO Tissue assay) and a 168-gene assay (OncoCompass Target ctDNA assay) to further validate its performance.

Methods

All samples were subjected to library preparation on Magnis BR and sequenced on NextSeq 500. Reference Materials with different types and different abundances of variants were prepared for each assay. Each standard underwent 3 batches of library preparation with replication in each batch to verify sensitivity and precision. NA12878 and GM24385 served as the negative standard for evaluating specificity. Three MSI-High positive cancer cell-lines with gradient dilution were also prepared to evaluate the LOD of MSI detection for ctDNA assay. To verify accuracy, parallel manual and automatic library preparations were performed with 16 clinical FFPE samples and 34 plasma samples.

Results

In the analytical validation of OncoScreen IO Tissue assay, the sensitivities of detecting SNV, INDEL and fusion all reached 100% under the AF of 2%. The sensitivity for CNV in a CN of 4 was 100%, and MSI was detected with 100% sensitivity under a tumor cell fraction of 10%. The repeatability and reproducibility both achieved 100%. In the 16 FFPE samples, the concordance rate between Magnis BR and manual prep for total 717 variations was 98.7%, and 100% for MSI-H.

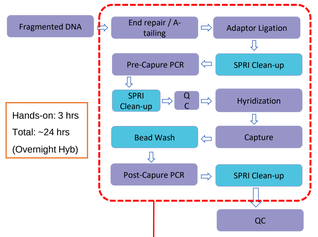
In the analytical validation of OncoCompass Target ctDNA assay, the sensitivity of detecting SNV achieved 96% under the VAF of 0.2%, and the sensitivity of detecting other type of variants achieved 100% under the VAF of 0.2%, CN of 2.5 and tumor cell fraction of 1%. The repeatability and reproducibility of all variant types achieved 100%. The specificity achieved 100% with no false positive variant detected. The sensitivity of MSI detection achieved 100% under the tumor cell fraction of 0.25% at median sequencing depth of 15000X. In the 34 plasma samples, Magnis BR showed higher coverage of mutation hotspots compared with manual process. The concordance rate for total 120 variations was 97.5% and 100% for MSS.

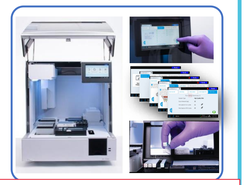
Conclusions:

The performance validation shows that the library preparation approach using the fully automated Magnis BR system and 2 assays from Burning Rock have excellent sensitivity, precision, specificity and accuracy, which meets the requirement of clinical applications. Adoption of Magnis BR system can routinely achieve a 3-day turnaround time from a submitted biological sample to a final report. Eight samples per run makes it more suitable for daily use in hospital laboratories.

Introduction

- **Magnis BR** is a **fully automated capture-based** NGS library preparation system co-developed by Agilent Technologies and Burning Rock. Currently it is distributed within China.
- It can prepare 8 libraries per run **within 9 hrs** and up to 16 libraries per day by overnight runs.
- **Capture-based** library preparation can detect almost all types of mutation, such as SNV, InDel, Fusion, CNV, MSI, LGR, HRD, TMB and LOH.
- **The OncoScreen IO Tissue assay** designed by Burning Rock is a 520-gene panel , which is intended to detect microsatellite instability (MSI), tumor mutation burden (TMB) and mutations in FFPE samples of pan-cancer patients with paired WBC samples.
- **The OncoCompass Target ctDNA assay** designed by Burning Rock is a 168-gene panel , which is intended to detect microsatellite instability (MSI) and mutations in plasma samples of pan-cancer patients.





Hands-on: 5 min
Total: 9 hrs
(Fast Hyb)

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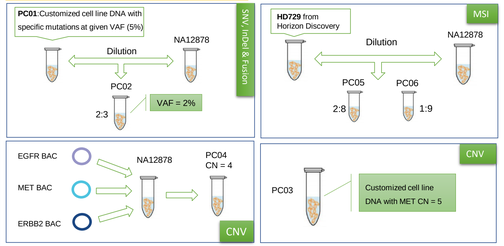
OncoScreen IO Tissue Assay Analytical Validation

Reference Materials Design



Standard Name	Mutation Type	Gene	Variant	VAF or CN
PC01/ PC02	SNV	EGFR	p.T790M	5%/ 2%
		EGFR	p.L858R	
		KRAS	p.G12V	
		BRAF	p.V600E	
	InDel	EGFR	p.E746_A750del	
	Fusion	ALK	EML4-ALK	
PC03	CNV	MET	Amplification	5
PC04	CNV	EGFR	Amplification	3
		ERBB2		
		MET		

Standard Name	Mutation Type	Tumor cell Fraction
PC05	MSI-High	20%
PC06	MSI-High	10%
NC(NA12878)	NA	NA

- Each standard underwent 3 batches of library preparation with replication in each batch to verify sensitivity and precision.
- Data analysis was conducted with each sample down sampled to 1000X at median sequencing depth.

Study of Precision, Sensitivity and Specificity

Precision

Standard	Variant Type	Expected VAF or CN	Concordance	Avg.VAF or CN（95%CI）
PC01	SNV	5%	100%	4.92% (3.45%,6.39%)
	INDEL		100%	4.7% (2.92%,6.49%)
	FUSION		100%	4.15% (2.15%,6.15%)
PC03	CNV	5	100%	5.43 (5.32,5.55)

Standard	Variant Type	Expected outcome	Concordance	Avg.msi. Percentage (95%CI)
PC05	MSI	MSI-H	100%	97.87% (95.57%,100.17%)

Sensitivity

Standard	Variant Type	Expected VAF or CN	Concordance	Avg.VAF or CN（95%CI）
PC02	SNV	2%	100%	2.44% (1.50%,3.39%)
	INDEL		100%	3.43% (2.38%,4.48%)
	FUSION		100%	2.08% (1.02%,3.14%)
PC04	CNV	4	100%	4.78 (4.63,4.94)

Standard	Variant Type	Expected outcome	Concordance	Avg.msi. Percentage (95%CI)
PC06	MSI	MSI-High	100%	77.24% (73.54%,80.95%)

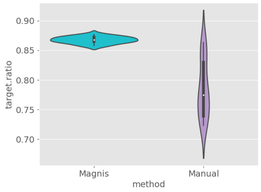
Specificity

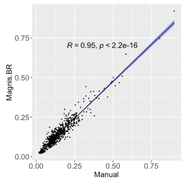
Sample	Variant Type	Expected outcome	Concordance
NC	NA	NA	100%

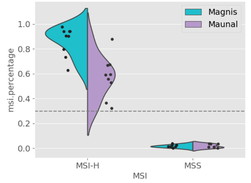
- The sensitivity of detecting all variant types achieved 100% under the VAF of 2%, CN of 4 and tumor cell fraction of 10%.
- The repeatability and reproducibility also achieved 100% under the VAF of 5%, CN of 5 and tumor cell fraction of 20%.
- The specificity achieved 100% with no false positive variant detected.

Study of Accuracy with clinical FFPE samples

Patient (n=16)	Age	Gender	Cancer Type
PT1	NA	NA	Colorectal
PT2	54	M	Colorectal
PT3	61	M	Prostate
PT4	64	M	Other
PT5	64	F	Colorectal
PT6	71	M	Gastric
PT7	84	F	Colorectal
PT8	NA	NA	Cervical
PT9	53	F	Cervical
PT10	61	F	Lung
PT11	36	F	Gastric
PT12	57	M	Lung
PT13	57	F	Lung
PT14	NA	NA	Other
PT15	43	F	Breast
PT16	55	M	Colorectal



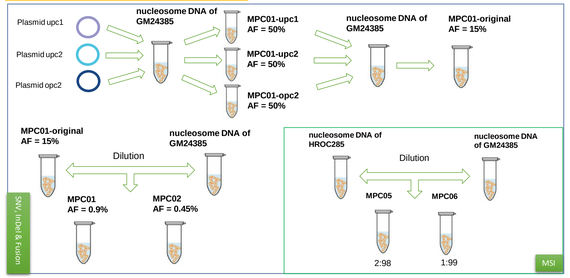




- Parallel manual and automatic library preparations were performed with 16 clinical FFPE samples (input of 200 ng) paired with WBC samples (input of 50 ng).
- Magnis BR improved the capture on target ratio by an average of 8% (86% vs 78%).
- The concordance rate for total 717 variations was 98.7%, and 100% for MSI-H (Jaccard index).
- The proportion of MSI sites identified by Magnis BR was significantly increased versus that by the manual process (86% vs 57%) in MSI-H samples.

OncoCompass Target ctDNA Assay Analytical Validation

Reference Materials Design



Standard Name	Mutation Type	Gene	Variant	AF
MPC01/ MPC02	SNV	EGFR	p.L858R	0.40%/ 0.20%
		EGFR	p.T790M	
		KRAS	p.G12V	
		BRAF	p.V600E	
		MET	c.3028+1G>T	
		NRAS	p.Q61K	
		KRAS	p.G12C	
		PIK3CA	A:p.H1047R	
	InDel	EGFR	p.A767_V769dup	
		EGFR	p.E746_A750del	
		ERBB2	p.Y772_A775dup	
		BRCA1	p.Y1853fs	
		BRCA2	p.L1908fs	
	Fusion	ALK	EML4-ALK	
		RET	CCDC6-RET	
		ROS1	CD74-ROS1	
		NTRK1	TPM3-NTRK1	

Standard Name	Mutation Type	Gene	Variant	CN
MPC03/ MPC04	CNV	EGFR	Amplification	3.0/ 2.5
		ERBB2		
		MET		
		FGFR1		

Standard Name	Mutation Type	Tumor cell Fraction
MPC05/ MPC06	MSI-High	2%/1%

Cell-line	Mutation Type	Tumor cell Fraction
SW48	MSI-High	2%,1%,0.5%,0.25%
LOVO		2%,1%,0.5%,0.25%
RKO		2%,1%,0.5%,0.25%

- MPC03/04 (CN = 3.0/2.5) were prepared in the same way as MPC01/02 with corresponding BAC plasmids.
- Each standard underwent 3 batches of library preparation with replication in each batch to verify sensitivity and precision.
- Data analysis was conducted with each sample down sampled to 15000X at median sequencing depth.
- The standards for MSI-LOD test were prepared in the same way as MPC05 with different cell-lines and gradient dilution by GM24385.

Study of Precision, Sensitivity and Specificity

Precision

Standard	Variant Type	Expected VAF or CN	Concordance	Avg.VAF or CN（95%CI）
MPC01	SNV	0.40%	100%	0.52% (0.06%,0.97%)
	INDEL		100%	0.37% (0.18%,0.56%)
	FUSION		100%	0.62% (0.14%,1.11%)
MPC03	CNV	3	100%	3.4 (3.21,3.59)

Standard	Variant Type	Expected outcome	Concordance	Avg.*msi. Score (95%CI)
MPC05	MSI-High	MSI-High	100%	0.76 (0.76, 0.76)

Sensitivity

Standard	Variant Type	Expected VAF or CN	Concordance	Avg.VAF or CN（95%CI）
MPC02	SNV	0.20%	96%	0.29% (0.05%,0.53%)
	INDEL		100%	0.23% (0.08%,0.37%)
	FUSION		100%	0.42% (0.13%,0.70%)
MPC04	CNV	2.5	100%	2.8 (2.41,3.19)

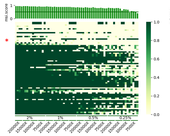
Standard	Variant Type	Expected outcome	Concordance	Avg.*msi. Score (95%CI)
MPC06	MSI-High	MSI-High	100%	0.77 (0.70,0.83)

Specificity

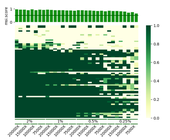
Sample	Variant Type	Expected outcome	Concordance
MNC(GM24385)	NA	NA	100%

- The sensitivity of detecting SNV achieved 96% under the VAF of 0.2%, and the sensitivity of detecting other type of variants achieved 100% under the VAF of 0.2%, CN of 2.5 and tumor cell fraction of 1%.
- The repeatability and reproducibility of all variant types achieved 100% under the VAF of 0.4%, CN of 3 and tumor cell fraction of 2%.
- The specificity achieved 100% with no false positive variant detected.

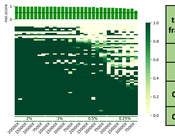
Study of MSI-LOD



msi.score of SW48 dilution samples



msi.score of LOVO dilution samples



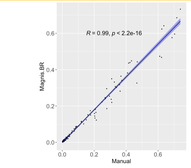
msi.score of RKO dilution samples

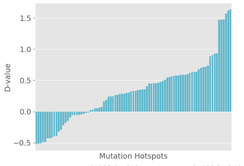
tumor fraction	MSI-H sensitivity			
	20000X	15000X	10000X	7500X
2%	100%	100%	100%	100%
1%	100%	100%	100%	100%
0.50%	100%	100%	100%	100%
0.25%	100%	100%	90.6%	93.8%

- SW48 dilution samples underwent 2 batches of library prep with replication in each batch, and the rest samples only underwent 1 batch with replication.
- All samples were down sampled to 20000X, 15000X, 10000X and 7500X at median sequencing depths to evaluate MSI LOD.
- The sensitivity of MSI detection achieved 100% under the tumor cell fraction of 0.25% at median sequencing depth of 15000X.

Study of Accuracy with clinical Plasma samples

Patient (n=34)	Age	Gender	Cancer Type
PP1	68	M	Lung
PP2	73	F	Lung
PP3	66	M	Lung
PP4	35	M	Lung
PP5	66	F	Lung
PP6	56	F	Lung
PP7	48	F	Lung
PP8	NA	NA	Lung
PP9	47	F	Lung
PP10	55	M	Lung
PP11	57	M	Lung
PP12	47	M	Lung
PP13	67	M	Lung
PP14	49	M	Lung
PP15	56	M	Lung
PP16	67	F	Lung
PP17	52	F	Lung
PP18	56	F	Lung
PP19	64	F	Lung
PP20	65	F	Lung
PP21	71	F	Lung
PP22	64	F	Lung
PP23	76	F	Lung
PP24	71	F	Lung
PP25	48	F	Lung
PP26	68	F	Lung
PP27	67	F	Lung
PP28	82	M	Lung
PP29	70	F	Lung
PP30	58	F	Lung
PP31	52	F	Lung
PP32	65	M	Lung
PP33	71	F	Lung
PP34	54	F	Lung





$$D-value = Median_{Magnis\ BR}\left(\frac{POS_depth}{median_depth}\right) - Median_{Manual}\left(\frac{POS_depth}{median_depth}\right)$$

- Parallel manual and automatic library preparations were performed with 34 clinical plasma samples with DNA input at 80ng.
- The concordance rate for total 120 variations was 97.5% (Jaccard index).
- Magnis BR improved coverage of most of the mutation hotspots, compared with manual process.

Conclusion

- The performance validation shows that the library preparation approach using the fully automated Magnis BR system and 2 assays from Burning Rock have excellent sensitivity, precision, specificity and accuracy, which meets the requirement of clinical applications.
- Magnis BR system has improved the capture on target ratio with the OncoScreen IO Tissue assay and boosted coverage of mutation hotspots with the OncoCompass Target ctDNA assay.
- Adoption of Magnis BR system can routinely achieve a 3-day turnaround time from a submitted biological sample to a final report. Eight samples per run makes it more suitable for daily use in hospital laboratories.

Reference

*Detection of Microsatellite Instability from Circulating Tumor DNA by Targeted Deep Sequencing.

Z Cai et al. *J Mol Diagn* 2020, 22(7), 860~870