Performance of oncoReveal™ MLH1 & MGMT Methylation Panel from Pillar Bioscience

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Introduction

Methylation of DNA occurs on cytosine residues, especially on CpG dinucleotides enriched in small regions of DNA (<500 bp) known as CpG islands. They are clustered around the regulatory region of MLH1 and MGMT genes and can affect the transcriptional regulation of these genes. Methylation of CpG islands by DNA methylase has been shown to be associated with gene inactivation and plays an important role in the development of cancer. Despite progress in studies correlating DNA methylation with cancer, the adoption of methylation tests for solid tumors in clinical trials and patient testing remains challenging due to assay complexity and lack of test standardization. In this study we evaluated performance of NGS sequencing method using oncoRevealTM MLH1 & MGMT Methylation Panel from Pillar Bioscience

Materials and Methods

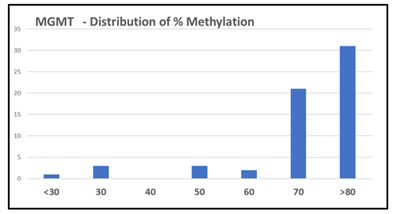
Materials:

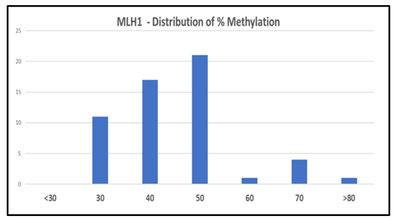
DNA from 61 MGMT and 57 MLH1 FFPE clinical samples with known methylation status were used to evaluate clinical accuracy. DNA extraction was performed using MaxWell® from Promega, followed by exposure of DNA to bisulfite treatment using Epitech Fast DNA Bisulfite Kit (Qiagen, #59824). ACL results were confirmed by a secondary orthogonal methylation method such as (MassArray or ddPCR).

Method:

The oncoReveal™ Essential MPN Panel (Pillar Biosciences) utilizes proprietary SLIMamp™ (stem-loop inhibition mediated amplification) technology, allowing amplification of regions of interest in a single-tube, multiplex reaction. The ACL MLH1 gene promoter assay covers 13 CpG islets; the MGMT gene assay covers 7 CpG islets which are present in exon 1 pDMR2 region. NGS libraries were prepared manually and sequenced on the Illumina MiSeq™ platform using Nano kit v2 (300 cycles). Sample results and reports were generated using PiVAT® (Pillar Biosciences' Variant Analysis Toolkit).

Limit of detection



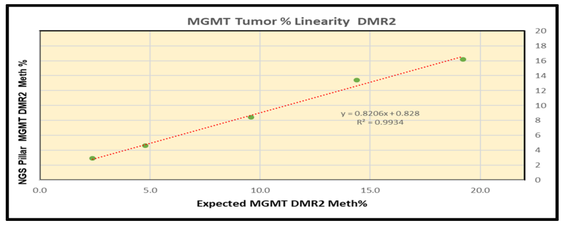


MLH1 LoD DNA conc				
Sample Number	DNA conc	% Meth	Cov Prm1.deg	Cov PMR1
IS23-1376-0.5-F	0.5 ng/uL	63	890	5158
IS23-1376-1.0-F	1.0 ng/uL	65	4164	12105
IS23-1376-1.5-F	1.5 ng/uL	65	4793	13631
n/t				
MLH1 LoD DNA conc				
SW48 96/97	0.25ng/uL-A	96	8244	14577
SW48 96/97	0.25ng/uL-B	97	9738	15437
SW48 96/97	0.5ng/uL-A	97	8031	16990
SW48 96/97	0.5ng/uL-B	97	8291	18821
SW48 96/97	1.0 ng/uL-A	97	4590	18758
SW48 96/97	1.0 ng/uL-B	97	4764	18315
SW48 96/97	2.0 ng/uL-A	97	11534	21065
SW48 96/97	2.0 ng/uL-B	97	8850	16232
SW48 96/97	5.0 ng/uL-A	97	7955	19965
SW48 96/97	5.0 ng/uL-B	97	8882	21158
SW48 96/97	10 ng/uL-A	97	8380	20091
SW48 96/97	10 ng/uL-B	97	7317	18863
SW48 96/97	20 ng/uL -A	97	6453	18782
SW48 96/97	21 ng/uL -B	97	6491	19262
	Ave	97	7628	19025
	STND	0.1	1821.1	1397.4
	%CV	0.14	23.87	7.34

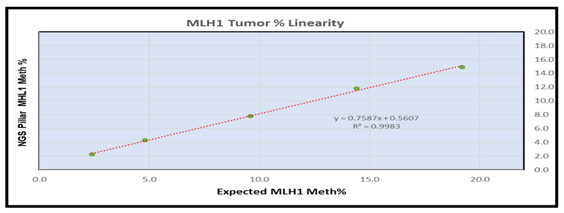
MGMT LoD DNA conc				
Sample Number	DNA conc	% Meth	Cov DMR2	Cov DMR1
NS21-6846-0.5-F	0.5 ng/uL	70	3508	1899
NS21-6846-1-F	1.0 ng/uL	71	4680	2895
NS21-6846-1.5-F	1.5 ng/uL	70	4540	3261
NS21-6846-2-F	**2.0 ng/uL**	**71**	**4828**	**3163**
MGMT LoD DNA conc				
SW48 96/97	0.25ng/uL-A	97	5787	3932
SW48 96/97	0.25ng/uL-B	97	5684	4652
SW48 96/97	0.5ng/uL-A	97	6215	4244
SW48 96/97	0.5ng/uL-B	97	5618	3505
SW48 96/97	1.0 ng/uL-A	97	7455	2821
SW48 96/97	1.0 ng/uL-B	96	6980	2825
SW48 96/97	2.0 ng/uL-A	97	6744	5643
SW48 96/97	2.0 ng/uL-B	97	5305	5215
SW48 96/97	5.0 ng/uL-A	97	6608	4980
SW48 96/97	5.0 ng/uL-B	97	6764	5427
SW48 96/97	10 ng/uL-A	97	7335	5937
SW48 96/97	10 ng/uL-B	97	6189	5600
SW48 96/97	20 ng/uL -A	97	6456	5437
SW48 96/97	21 ng/uL -B	97	6407	5146
	Ave	97	6506	4732
	STDEV	0.3	603.9	1061.2
	%CV	0.28	9.28	22.43

Results Comparison

MGMT LoD Tumor % conc						
Sample Number	Exp Tum %	% Meth	Cov DMR2	% Meth	Cov DMR1	Reporducibility
SW48 96/97 Full	2.4	2.9	4469	1.6	7094	2/2.
SW48 96/97 Half		3.0	5161	1.5	8567	2/2.
SW48 96/97 Full	4.8	4.6	4414	2.5	7963	2/2.
SW48 96/97 Half		5.0	5007	1.7	8309	2/2.
SW48 96/97 Full	9.6	8.4	3856	4.7	6474	2/2.
SW48 96/97 Half		9.3	4552	4.5	6223	2/2.
SW48 96/97 Full	14.4	13.4	3949	5.9	3949	2/2.
SW48 96/97 Half		13.6	3773	5.9	5690	2/2.
SW48 96/97 Full	19.2	16.2	3760	7.5	5836	2/2.
SW48 96/97 Half		16.8	4932	7.7	7055	2/2.



MLH1 LoD Tumor % conc					
Sample Number	Exp Tum %	% Meth	Cov Prm1.deg	Cov PMR1	Reporducibility
SW48 96/97 Full	2.4	2.3	4627	14364	2/2.
SW48 96/97 Half		2.3	6437	15999	2/2.
SW48 96/97 Full	4.8	4.3	4663	12693	2/2.
SW48 96/97 Half		3.5	5756	14377	2/2.
SW48 96/97 Full	9.6	7.8	4660	15072	2/2.
SW48 96/97 Half		8.2	5874	16527	2/2.
SW48 96/97 Full	14.4	11.8	4230	12430	2/2.
SW48 96/97 Half		11.9	5274	11925	2/2.
SW48 96/97 Full	19.2	14.9	4320	13208	2/2.
SW48 96/97 Half		15.4	6618	16414	2/2.



Analytical specificity and sensitivity				
ACL VALIDATION		CAP, Zymo, Cell Lines		
		+	-	Total
NGS MLH1	+	24		24
Illumina	-		32	32
			Total	56
%				
100.0	PPA - Positive percent agreement (sensitivity)			
100.0	NPA - Negative percent agreement (specificity)			
100.0	Positive Predictive Value (PPV)			
100.0	Negative Predicative Value (NPV)			
100.0	Accuracy			

Analytical specificity and sensitivity				
ACL VALIDATION		CAP, Zymo, Cell Lines		
		+	-	Total
NGS MGMT	+	34		34
Illumina	-		33	33
			Total	67
%				
100.0	PPA - Positive percent agreement (sensitivity)			
100.0	NPA - Negative percent agreement (specificity)			
100.0	Positive Predictive Value (PPV)			
100.0	Negative Predicative Value (NPV)			
100.0	Accuracy			

ACL VALIDATION		ARUP MLH1		
		+	-	Total
ACL LDT MLH1	+	32		32
NGS	-		25	25
			Total	57
%				
100.0	PPA - Positive percent agreement (sensitivity)			
100.0	NPA - Negative percent agreement (specificity)			
100.0	Positive Predictive Value (PPV)			
100.0	Negative Predicative Value (NPV)			
100.0	Accuracy			

ACL VALIDATION		ARUP/UPMC		DMR2
		+	-	Total
ACL LDT MGMT	+	37	0	37
MGMT	-	3	21	24
			Total	61
%				
92.5	PPA - Positive percent agreement (sensitivity)			
100.0	NPA - Negative percent agreement (specificity)			
100.0	Positive Predictive Value (PPV)			
87.5	Negative Predicative Value (NPV)			
95.1	Accuracy			

Results

For MGMT methylation, 61 FFPE brain-biopsy samples were tested; 58/61 samples (95.1%) correlated. Three low-positive samples were called unmethylated. For MLH1 methylation, 57 FFPE samples (different tumor types) were tested; 57/57 samples (100%) correlated. Both assays showed linear response to tumor dilution with R2=0.998 (MLH1) and R2=0.994 (MGMT DMR2). Analytical linear detection range of this assay is 3%-100% methylation. Clinical sensitivity is 6% methylation for MGMT and 10% methylation for MLH1. Analytical sensitivity was 100% (68/68). Analytic specificity was 100% (63/63).

Workflow Comparison

The average laboratory TAT was shortened by 7-10 days, and the cost was sufficiently reduced, with the oncoRevealTM MLH1 & MGMT Methylation Panel compared to the previous send out processes.

Conclusions

This study demonstrates that oncoReveal™ MLH1 and MGMT Methylation Panel performed very well against comparator methods. The performance characteristics and workflow benefits are suitable for clinical testing.

References

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