

Abstract

Introduction: SLIMamp allows multiplex-PCR of tiled amplicons in a single tube, which enables targeting of large exons for NGS analysis with a streamlined process. This technology was previously demonstrated to be a sensitive and robust NGS library prep method for Illumina instruments. To benefit the largest number of patients and researchers, SLIMamp library prep was made compatible with multiple sequencing platforms that are common worldwide including the MGISEQ.

Materials and Methods: Five ONCO/Reveal assays for MGI were evaluated (multi-cancer, multi-cancer with CNV, multi-cancer cfDNA, lung fusions, and lung and colon cancer) on the MGISEQ-2000. Libraries were prepared and sequenced by two laboratories (Zhengu and MGI). 14 CRC samples were tested and compared on the MGISEQ-2000 and NextSeq CN500 platforms. Automated library preparation by the MGISP-100 was also evaluated.

Results: Across the five assays, mapping and on-target rates and coverage uniformity at 0.2x of the mean demonstrated high performance (generally >95%). Variant calls from 14 CRC samples for the ONCO/Reveal Multi-Cancer Panel sequenced on the MGISEQ-2000 and NextSeq CN500 demonstrated an R² value of 97%. Variants were concordantly called from 2% to 65% VAF. The MGISP-100 reduced hands-on-time to 30 minutes.

Conclusions: All ONCO/Reveal assays demonstrated high performance when sequenced on MGISEQ-2000 and Illumina platforms. Automated library preparation utilizing the MGISP-100 reduced hands-on time while providing sensitive and robust performance. Concordance was demonstrated across platforms, specifically the NextSeq CN500 and MGISEQ-2000. ONCO/Reveal assays are sensitive and robust solutions across multiple sequencing platforms that can be used by researchers across the globe.

Methods and Assay Design

Panel Name	Mapping Rate	On Target Rate	Coverage Uniformity >0.2x Mean Coverage
ONCO/Reveal Multi-Cancer Panel	99.2% ± 0.1%	99.3% ± 0.3%	92.2% ± 1.3%
ONCO/Reveal Multi-Cancer with CNV Panel	97.1% ± 0.2%	96.3% ± 0.3%	95.1% ± 2.4%
ONCO/Reveal PillarHS Multi-Cancer Panel (O/R Multi-Cancer cfDNA Panel)	97.6% ± 0.1%	93.6% ± 0.5%	94.9%
ONCO/Reveal Lung and Colon Cancer Panel	99.5% ± 0.1%	97.5% ± 0.4%	93.2% ± 0.8%

Figure 1: All ONCO/Reveal panels demonstrated high mapping, on target, and coverage uniformity when sequenced on the MGISEQ-2000.

O/R Multi-Cancer with CNV Panel (Automated Library Prep)

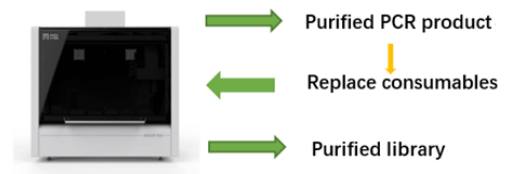


Figure 2: The MGISP-100 reduced hands on time to <30 minutes with an automated run time of 4.5 hours. Performance of the ONCO/Reveal Multi-Cancer with CNV Panel was similar compared to manual library prep.

Sample Type	DNA Input (ng)	Library Concentration (ng/ul)
HD701	20.8 (n=2)	4.83 ± 0.15
HD200	24.7 (n=6)	6.40 ± 1.29

Results and Conclusions

ONCO/Reveal Multi-Cancer Panel

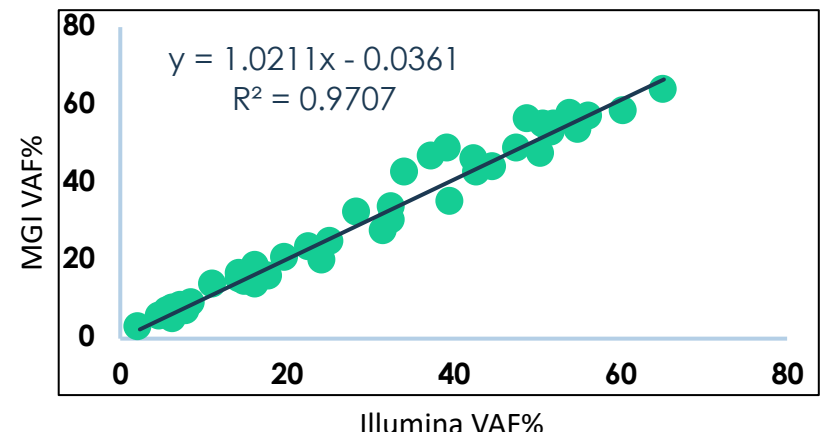


Figure 3: 14 CRC samples tested on the Illumina NextSeq CN500 and MGISEQ-2000 demonstrated high concordance. Sequencers: NextSeqCN500, PE150; MGISEQ-2000, PE100q

ONCO/Reveal Lung Fusion NA Panel

Fusion Pattern	COSMIC ID	HD796 FFPE (Junction reads per million)	HD796 FFPE	HD796 FFPE	Negative FFPE	Negative FFPE
EML4(13)-ALK(20)	COSF463	+ (6515)	+ (7289)	+ (3587)	-	-
CCDC6(1)-RET(12)	COSF1272	+ (7670)	+ (4803)	+ (6845)	-	-
SLC34A2(4)-ROS1(32)	COSF1197	+ (6493)	+ (11129)	+ (11650)	-	-

Figure 4: The ONCO/Reveal Lung Fusion RNA Panel was evaluated using a commercially available reference standard (Horizon Discovery HD796) and FFPE samples without fusions. The assay detected all fusions within its design without any false positives.

ONCO/Reveal Panel Performance Summary:

- Pillar Biosciences ONCO/Reveal panels are robust, sensitive, and easy-to-use
- Demonstrate concordance (R² =97%) between Illumina and MGI sequencers
- Achieve High mapping, on target, and coverage uniformity >0.2x mean coverage
- Comparable library prep results when automated with hands on time <30 minutes
- A global solution for clinical oncology researchers