Data Sheet

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Library Preparation Performance on the Magnis NGS Prep System with Reagent and Protocol Updates

Introduction

The Agilent Magnis NGS Prep system is a fully walkaway automation platform for preparing sequencing-ready NGS libraries from genomic DNA. This instrument offers ease-of-use, batching flexibility, and reproducibility to help improve laboratory operational efficiency, shorten the turnaround time of NGS testing, and make NGS results more reliable for both clinical and research labs. To provide greater convenience and less storage space, the library preparation reagent, target capture probes, and sample index oligos are pre-aliquoted. These reagents are ready to load on the instrument after brief vortexing.

Agilent recently upgraded its manufacturing processes to help meet the growing demand for Magnis reagent kits while maintaining stringent quality standards for the SureSelect library preparation target enrichment chemistry. The Agilent Magnis SureSelect XT HS Rev B kit (Rev B), produced by the upgraded manufacturing process, supports the same Agilent SureSelect XT HS chemistry. However, several modifications have been made to the original Agilent Magnis SureSelect XT HS kit to enable performance advantages.

Modifications for the Rev B Kit

The Rev B kit differs from the original Rev A kit in several ways:

1) The reagent plate of Rev B is made from a plate containing removable vials. Each vial is covered with a primary seal, then the plate top is covered with a secondary seal. The reagent fill volume of each vial on the reagent plate has been optimized for output library performance and production efficiency.
2) The capture probe plate of Rev B is preloaded into one single vial of the 8-vial probe strip in each run.[1] This format helps to ensure well-to-well pipetting consistency of small volumes during the hybridization step.
3) The resolution of barcode images on individual component strips in the Rev B kit has been improved to minimize barcode scanning errors.

Performance of Rev B Kits and Protocols

The run protocol for the Rev B kit was modified from the original Rev A protocol (SureSelectXT HS-Illumina_ v1.0.1) to accommodate the new reagent format.[2] However, reaction conditions for the library prep and target enrichment stages, including the 62.5 °C hybridization temperature profile, remain unchanged. Due to these similarities, the performance of output libraries generated by the Rev B kit and protocol are comparable to libraries prepared with the Rev A kit and protocol.

This was illustrated with the Agilent SureSelect Cancer All-In-One (AIO) Lung assay, a targeted NGS panel that detects SNVs, indels, CNVs, and translocations in 20 lung cancer-relevant genes with a 226 kb design size. Libraries prepared on the Magnis NGS Prep system system with Rev A and Rev B protocols and kits using 50 ng of input DNA for the SureSelect AIO lung assay were compared (Figure 1). The key sequencing metrics for duplicate rate, on-target rate, uniformity, and coverage (>20X) exhibit high similarity between the Rev A and Rev B kit. Other important sequencing metrics, such as AT and GC dropout rate, are similar between the Rev A and Rev B kit.

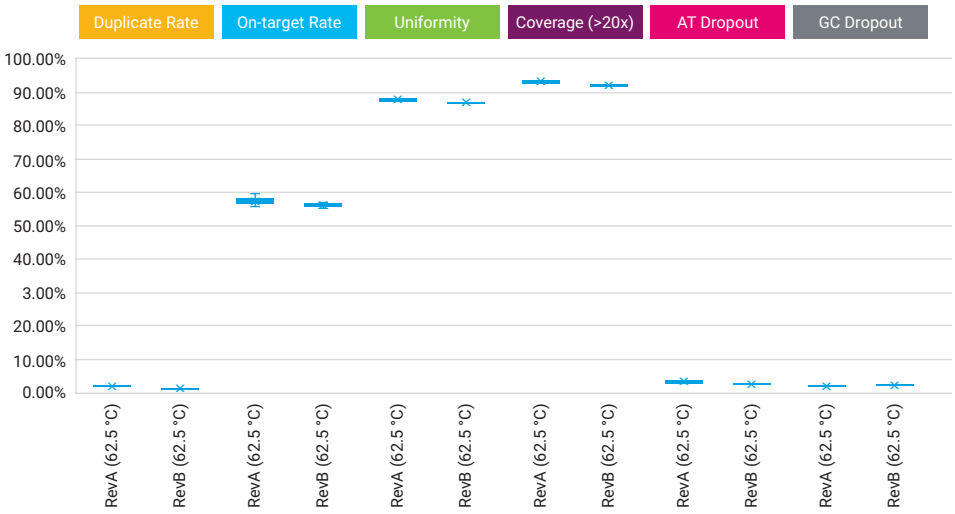

Figure 1. Comparable performance between Rev A and Rev B kits and protocols for key sequencing metrics. Each sample was prepared with 50 ng of mechanically sheared HapMap NA 18507 DNA using the SureSelect Cancer AIO Lung assay. All samples were run with a hybridization temperature of 62.5 °C. Libraries were sequenced on an Illumina MiSeq instrument with 2x100 bp reads. Both kits and their associated run protocols exhibited high similarity across all tested sequencing metrics.

The Agilent ClearSeq Comprehensive Cancer panel, a targeted NGS panel 800 kb in size that covers 151 key cancer-associated genes, was also analyzed. Libraries were generated on the Magnis with Rev A and Rev B protocols and kits using 10 ng of input DNA were evaluated (Figure 2). The key sequencing metrics for duplicate rate, on target rate, uniformity, and coverage (>20X) are comparable between the Rev A and Rev B kit. AT and GC dropout rate exhibit high similarity between the Rev A and Rev B kit.

The results from these two targeted panels indicate that the user would likely obtain comparable performance when switching from the Rev A to Rev B kit with a 62.5 °C hybridization protocol on custom SureSelect designs. The level of concordance, however, will vary depending on the design profile of capture probes.

In addition to the Rev B protocol supporting a 62.5 °C hybridization temperature, another protocol was created to support a 65 °C hybridization condition.[2] For many Agilent SureSelect XT HS-boosted custom panel designs, particularly large panels, output library sequencing metrics are enhanced using the 65 °C hybridization temperature protocol.

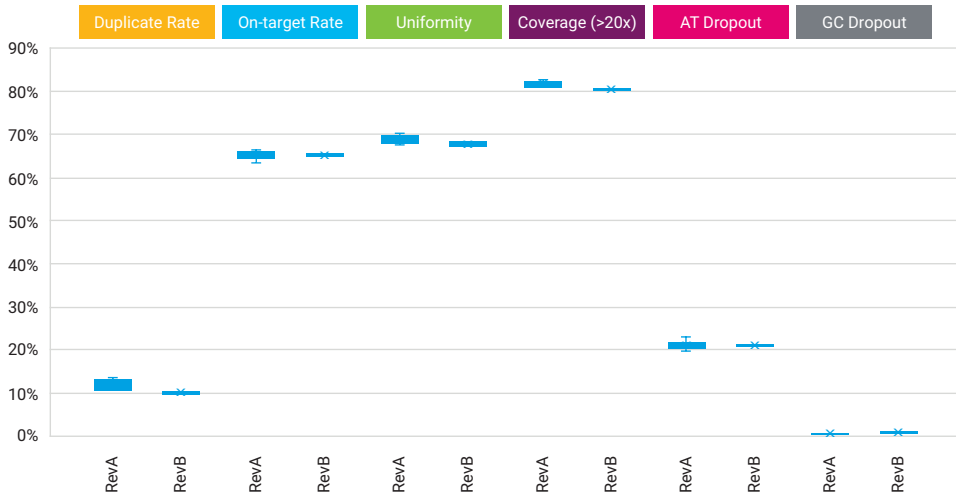

Figure 2. Comparable performance between Rev A and Rev B kits and protocols for key sequencing metrics. Each sample was prepared with 10 ng of mechanically sheared HapMap NA 18507 DNA using the ClearSeq Comprehensive Cancer panel. Libraries were sequenced on an Illumina MiSeq instrument with 2x100 bp reads. All samples were run with a hybridization temperature of 62.5 °C. Both kits and their associated run protocols exhibited high similarity across all tested sequencing metrics.

This optimization was illustrated in a case using the Agilent SureSelect Human Exome V7 kit, a widely adopted targeted sequencing panel that is used in both clinical testing and in research applications. The Exome V7 panel is an XT HS-boosted design and is available as a catalog panel for the Magnis NGS Prep system system (p/n G9771C and G9771D). The Magnis Human Exome V7 Rev B kit was used to generate libraries with hybridization temperatures of either 62.5 or 65 °C, then these libraries were sequenced (Figure 3). Libraries prepared with 10 ng of DNA using the 65 °C protocol exhibited a nearly identical duplicate rate compared to those from the 62.5 °C protocol. However, on-target rate, uniformity of sequencing, and coverage rate were all noticeably higher with the 65 °C protocol than those of the 62.5 °C protocol. Libraries also exhibited an increased AT dropout rate and a decreased GC dropout rate using the 65 °C protocol. Repeating these experiments with 200 ng of input DNA showed similar results (data not shown). Therefore we highly recommend using the 65 °C protocol with the Magnis Exome V7 Rev B kit.

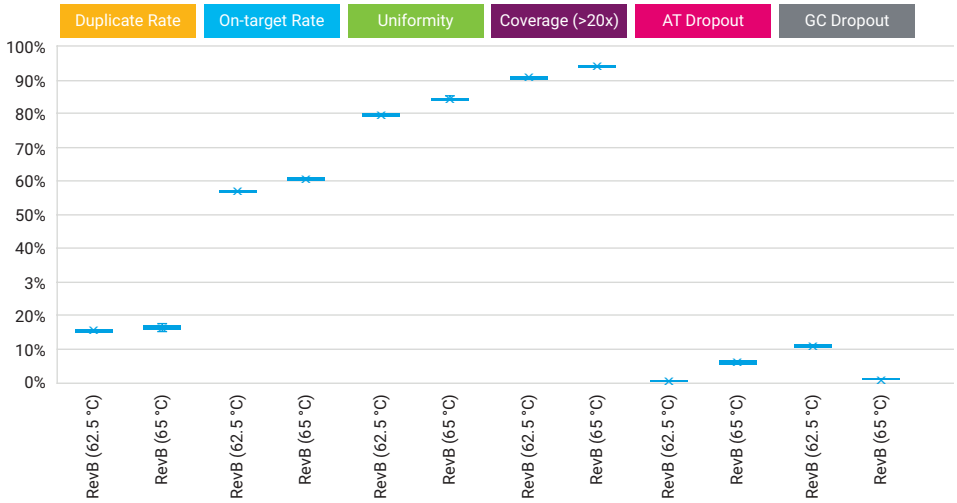

Figure 3. Greater performance with a 65 °C hybridization temperature using the Exome V7 NGS panel. Each sample was prepared with 10 ng of mechanically sheared HapMap NA 18507 DNA using the SureSelect Human Exome V7 kit. Libraries were sequenced on an Illumina HiSeq 4000 instrument with 2x100 bp reads. The duplicate was comparable between the two conditions; however, on-target rate, uniformity, and coverage were all noticeably higher in libraries prepared with the 65 °C protocol. Additionally, the AT dropout rate increased while GC dropout decreased in the 65 °C protocol compared to the 62.5 °C protocol.

The performance of the Magnis Rev B kit on the Cancer AIO Lung assay was also evaluated using both 62.5 and 65 °C protocols (Figure 4). Unlike the Exome V7 panel data, libraries generated with the Cancer AIO Lung assay demonstrated high similarity in duplicate rate, uniformity, coverage, AT and GC dropout rate between the two protocols. However, the on-target rate was observed to be higher using the 65 °C protocol compared to the 62.5 °C protocol. The findings between Exome V7 and AIO lung panel underscore that the effect of hybridization temperature on output library performance is dependent on probe profile. The results are consistent with other empirical data that the performance of large panels is more likely to benefit from a 65 °C hybridization temperature profile than that of small panels.

The performance of the Magnis SureSelect XT HS Rev B kit with its protocols is illustrated by several Agilent catalog panels. For custom designed panels, probe profile of each panel design is unique, concordance level between Rev A/Rev B kit and associated protocols varies with the targeted genomic region and design profile.

In conclusion, the Magnis SureSelect XT HS Rev B kit and its protocols provides comparable or superior performance to Rev A kit. For customers switching from Rev A to Rev B kits, we ar confident that performance will be maintained along with the improved robustness.

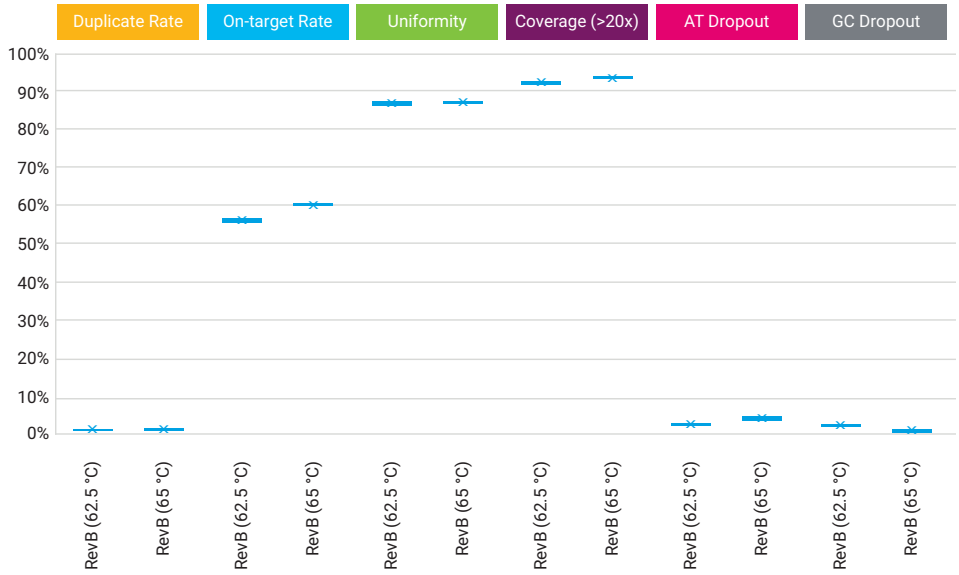

Figure 4. Similar performance across two hybridization temperatures using a Cancer AIO Lung panel. Each sample was prepared with 50 ng of mechanically sheared HapMap NA 18507 DNA using the SureSelect Cancer AIO Lung assay. Libraries were sequenced on an Illumina MiSeq instrument with 2x100 bp reads. The performance of libraries generated at 62.5 °C was similar to libraries generated at 65 °C hybridization temperatures for duplicate rates, uniformity, and coverage, except libraries generated at 65 °C exhibited noticeably higher on-target rates.

References

1. Refer to Magnis reagent kits with probe pre-loaded on the probe plate. Magnis No-Probe reagent kit, which doesn't contain probe, requires user self-filling each of the 8 wells in the probe strip.
2. The protocol name supporting the 62.5 °C hybridization temperature profile is LT-SSEL XTHS-RevB-ILM_v1.1.1. For more information, see Magnis Protocol Download Page.

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