Application Note

Genomics

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Three-day Turnaround Time for Routine Oncology Testing Using the MagnisDx NGS Prep System

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Abstract

The MagnisDx NGS Prep system has been designed for the walkaway automation of complex next-generation sequencing (NGS) target enrichment-based assays with minimal hands-on time. During this study, Glasgow Precision Oncology Laboratory (GPOL) evaluated the MagnisDx system for use in oncology testing with a specific focus on rapid turnaround time (TAT). We show that the MagnisDx system enables a three-day TAT for routine oncology testing. Its walkaway automation capabilities and batching flexibility allow for rapid target enrichment NGS workflows. The MagnisDx instrument is a perfect system to use alongside existing high-throughput workflows in the lab.

Introduction

NGS technology is a key tool used by researchers to understand the complex genomic events which drive disease in the context of oncology. There is also growing evidence that routine comprehensive genomic profiling of tumors could enable guided therapies for many cancer patients either by accessing clinically approved therapies or via enrollment into ongoing clinical trials[1]. GPOL has focused on developing a suite of target-enrichment-based cancer assays that enable broad genomic profiling in the majority of adult cancers[2]. In order to realize the full potential of comprehensive genomic profiling in routine oncology testing, there is a need to improve the TAT from sample receipt to patient report. Additionally, any rapid turnaround test still needs to be compatible with samples from formalin-fixed paraffin-embedded (FFPE) tissues and require as little starting material as possible.

Within GPOL, we have validated a hybrid-capture workflow based on SureSelect technology which consists of DNA extraction, NGS library prep, and target enrichment, followed by sequencing with the NextSeq, HiSeq, or NovaSeq instruments (Illumina, Inc.). The sequencing data is then passed through an automated sequencing analysis pipeline to call and interpret variants and generate a report. Throughout 2019, we achieved an average TAT of 10 days (with the fastest TAT being 5 days) using our standard workflows. However, the wet lab processes have historically been a bottleneck within the lab. Despite the use of automated liquid handlers such as the Agilent Bravo system, it can still take two (SureSelect XT Low Input) or three (SureSelect XT) days to get an enriched library ready for sequencing.

Here we present our evaluation of the MagnisDx NGS Prep system, an instrument that provides walkaway automation of the full SureSelect target enrichment workflow. Using the MagnisDx system, we were able to reduce the library prep and enrichment process from two days to just nine hours, as well as reducing hands on-time from 2.5 hours to just 10 to 15 minutes. When combined with the rest of our workflow, we were able to achieve a 72-hour TAT from sample to results (Figure 1).

Materials and Methods

Validation of the MagnisDx system as the new NGS workflow for oncology

The MagnisDx system was validated by GPOL over a series of six runs from clinically derived FFPE samples. These samples were extracted from a mixture of relatively recent fresh FFPE tissue as well as older archived samples.

gDNA isolation

Whole blood samples were prepared and gDNA isolated using Chemagen blood extraction kits (PerkinElmer, Inc.) automated on a Chemagic STAR liquid handler (Hamilton Bio) following the manufacturer's protocol. Alternatively, lower volumes of blood had gDNA isolated using the QIAsymphony DNA Mini Kit (QIAGEN) using the automated QIAsymphony liquid handler following the manufacturer's protocol.

FFPE gDNA was isolated using Maxwell FFPE DNA extraction kits (Promega Corporation) on a Maxwell 16 liquid handler following the manufacturer's protocol. Samples with low tumor content were targeted by macrodissection. Isolated targeted material was then extracted using the same Maxwell FFPE extraction protocol.

Isolated DNA was analyzed on a Nanodrop spectrophotometer (Thermo Fisher Scientific) with the 260/280 and 260/230 ratios used to assess extraction purity. All material was quantified using a Qubit fluorometer (Thermo Fisher Scientific) with the Qubit dsDNA BR Assay Kit prior to normalization for downstream applications.

Shearing of gDNA

Shearing of gDNA from both FFPE and whole blood was performed using an LE220-plus Focused-ultrasonicator (Covaris). The protocol is optimized to produce a range of DNA fragment sizes with a peak at about 150bp using Covaris 8 microTUBE strips. 50-µL aliquots of gDNA are used for sonication of each sample. 50 ng (1 ng/µL) is prepared for processing on the MagnisDx SureSelect XT HS protocol, while 200 ng (4 ng/µL) is prepared for processing on the Bravo SureSelect XT protocol. The LE220-plus was used according to the manufacturer's guidelines with the following conditions for both fresh and FFPE gDNA:

- Peak Incident Power: 450W
- Duty Factor: 15%
- Cycles per burst: 200
- Treatment time: 350 seconds.

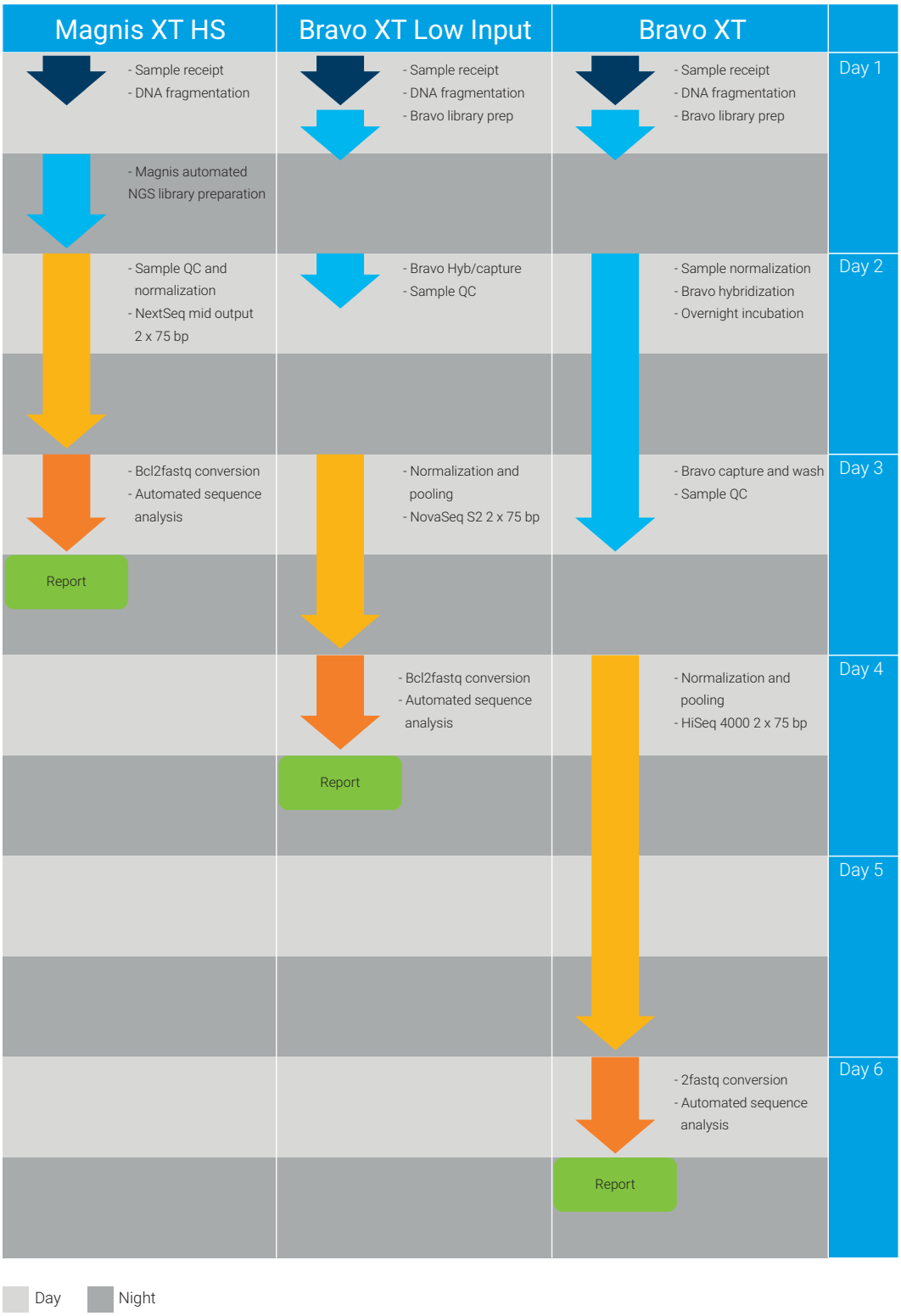

Figure 1. Comparison of workflow timings of the Glasgow cancer assays when using both MagnisDx- and Bravo-based workflows. The MagnisDx system enables the rapid TNT of comprehensive cancer genomic insights within 72 hours. The stated sequencing time assumes the Bravo projects were running only 16 samples captured on a 25 Mb panel. The recent inclusion of the NovaSeq platform into the low-input workflow increases capacity up to 48 samples and reduces the required time to four days (though FASTQ generation and analysis may take longer). As noted previously, the XT Low Input and XT HS workflows use the same chemistry and hybridization time, so the observed difference in TAT between protocols is due solely to the MagnisDx system's workflow.

SureSelect target enrichment bait library design

The custom-designed Cancer PLUS panel is a 4 Mb design that includes 353 genes and a 1 Mb-resolution copy number backbone. Details on this panel and other associated designs are available from the Agilent SureSelect Community Designs for Oncology Research catalog[2].

SureSelect XT HS library preparation using the MagnisDx system

The recommended protocol was followed while using the SureSelect XT HS kit on the MagnisDx system. During instrument setup the presets for 50 ng of FFPE-derived gDNA were selected. Out of consideration for the panel design (>3 Mbp) and DNA input parameters, one modification was made to the standard protocol. The number of pre-capture PCR cycles was reduced to 13 while keeping the number of post-capture PCR cycles at nine.

SureSelect XT library preparation using the Bravo liquid handler

The protocol for the Bravo instrument uses the manufacturer's recommendations for FFPE-derived genomic material with several optimizations. 200 ng of FFPE- or whole blood-derived fragmented gDNA was used for each sample. Undiluted adapters were used in the ligation reaction setup. 750 ng of an adapter-ligated DNA library was used for each hybridization reaction. The maximum volume (30 µL) of captured DNA library was added to the final enrichment PCR. PCR cycles were optimized from the manufacturer's recommendations for panels >1.5 Mbp and mid-quality FFPE. 11 pre-capture and 10 post-capture PCR cycles were used.

SureSelect XT low input library preparation using the Bravo liquid handler

The protocol for the SSXT low input workflow on the Bravo follows the manufacturer recommended method for the gDNA input and bait type used. 50ng of intact gDNA from FFPE extracted tissue or fresh frozen whole blood was used for each sample. 12 cycles of pre-capture PCR are used for all samples to ensure compatibility with FFPE extracted material. 9 cycles of post-capture PCR are used as recommended for capture panel designs over 5Mb in size.

Sequencing

Final prepared libraries were checked using the High Sensitivity D1000 ScreenTape assay (p/n 5067-5585) and quantified with the Quant-It PicoGreen dsDNA Assay kit prior to pooling. MagnisDx-produced libraries were run on a NextSeq v2.5 Mid-output flow cell with 75 bp paired-end reads. Bravo-produced libraries using this assay were clustered in pools of up to 16 on the cBot2 and run on the HiSeq 4000 with paired end 75 bp reads. Bravo-produced libraries using this assay can also be sequenced on NovaSeq S2 flow cells in pools of up to 24 samples with paired-end 75 bp reads.

Data analysis

Sequencing data was processed using HOLMES v1.2, a proprietary pipeline developed at GPOL. The estimated TAT from sequence completion to report being issued was approximately 12 hours. This estimate covers samples captured using the Cancer PLUS panel design on the MagnisDx system and sequenced using NextSeq Mid-output flow cells.

Results

Workflow comparison

We used the two methods established in our lab to compare the workflow features of the MagnisDx system (Table 1). The SureSelect XT protocol used at GPOL has historically been used with the Agilent Bravo liquid handler. More recently, we have validated the SureSelect XT Low Input workflow on the Bravo liquid handler to enable the use of lower quantities of starting material. In comparison to the Bravo protocols, the MagnisDx system produces target-enriched, sequencing-ready libraries in roughly half the time. It has similar input requirements as the SureSelect XT Low Input protocol and can also handle a wide variety of sample types (including FFPE). The MagnisDx system has a throughput of eight samples per run, in comparison to the Bravo protocols which can process up to 96 at one time, meaning that high-priority samples do not have to wait until enough samples are available to perform a run. In addition, the MagnisDx system offers full walkaway automation, allowing runs to be set up at the end of a working day with the instrument running overnight. This can free up the lab staff to attend to higher throughput runs on the Bravo liquid handler.

Table 1. Key features of the workflows and default settings which have been established by GPOL for patient testing. SureSelect XT is a legacy workflow which has now been replaced by the SureSelect XT Low Input workflow on the Bravo system. The XT Low Input and XT HS workflows use the same chemistry and hybridization time, so the observed difference in TAT is solely due to the MagnisDx system's workflow.

	SureSelect XT HS (MagnisDx)	SureSelect Low Input (Bravo)	SureSelect XT (Bravo)
Input quantity	50 ng	50 ng	200 ng
Fragmentation	Sonication	Enzymatic	Sonication
Indexing strategy	Single index with molecular barcodes	Dual index	Single index
Total run time	9 hours	2 day	3 to 4 days
Walk-away processing	Yes	No	No
Number of samples per run	up to 8	up to 96	up to 96

Yield

Since the majority of cancer samples are derived from FFPE material, it was important to us to evaluate how the MagnisDx system handles the range of samples that we receive. Figure 2 shows the pre- and post-capture yields that were achieved over the six validation runs. We observed good consistency in both pre- and post-capture yields when using an input of 50 ng (Figure 2). The mean yield was 2387.8 ng (SD: 793.2 ng) and 16.4 nM (SD: 5.4 nM) for pre- and post-capture, respectively. We also tested varying input levels of 25, 50, and 100 ng using the same pre- and post- capture PCR strategy and observed comparable yields, indicating robust performance (data not shown).

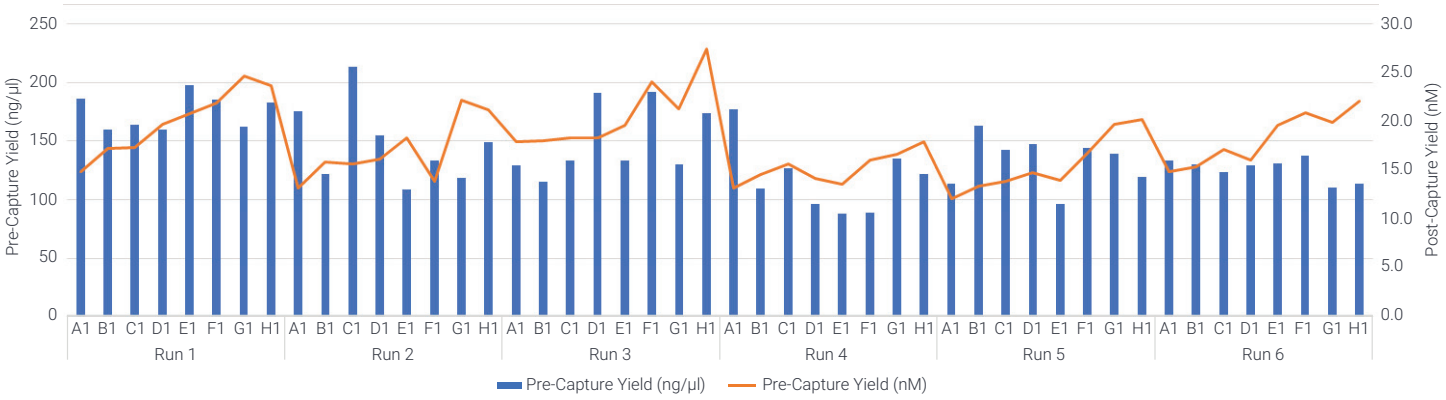

Figure 2. Comparison of pre and post capture yield obtained during six validation runs on the MagnisDx system. 50 ng of mechanically sheared FFPE DNA was used as input. PCR cycle numbers for pre- and post-capture amplification were 13 and 9 cycles, respectively. Pre-capture yield for each sample is shown in the blue bars, while post-capture yield is shown as an orange line. Overall, there is good consistency between samples across the runs.

Sequencing performance

We assessed sequencing performance of the validation samples through a series of NextSeq Mid-output runs using 75 bp paired-end sequencing. Each sample received approximately 2 Gb of sequencing for the 3.8 Mb panel. Table 2 shows a subset of samples across four sequencing runs. In general, key metrics such as coding depth, and on-target rate remained stable across a wide variety of samples from blood-derived intact gDNA as well as tumor-derived FFPE gDNA. We observed the expected increase in duplication rate for the more degraded input material. In addition, we also tested a range of DNA inputs including 25, 50, and 100 ng while maintaining the same amplification strategy. Here we observed comparable sequencing metrics indicating a robust assay performance (data not shown).

Table 2. Sequencing metrics for eight samples prepared on the MagnisDx system using 50 ng of input DNA and sequenced across four independent NextSeq mid-output runs (75 bp paired-end reads). Average read depth, coding depth, and on-target metrics are relatively stable across all samples indicating robust performance. On target percentage is calculated following deduplictaion.

Sample Name	Sample Type	DNA Input [ng]	Avg. Insert Size [bp]	Avg. Coding Depth	Duplicates [%]	On Target [%]	NextSeq Run
Sample 1	Whole Blood	50	173	606	19	60	Run 1
Sample 2	Tumor FFPE	50	180	510	21	57	Run 1
Sample 3	Whole Blood	50	185	482	21	58	Run 2
Sample 4	Tumor FFPE	50	162	444	26	55	Run 2
Sample 5	Whole Blood	50	181	525	15	62	Run 3
Sample 6	Tumor FFPE	50	161	414	38	49	Run 3
Sample 7	Tumor FFPE	50	143	484	36	55	Run 4
Sample 8	Tumor FFPE	50	174	571	28	54	Run 4

Parallel processing on the MagnisDx and Bravo systems

To compare the performance of the MagnisDx system to the laboratory's current method, two samples were processed in parallel on the Bravo liquid handler (using the SureSelect XT workflow) and on the MagnisDx system (using the SureSelect XT HS workflow). Samples were then sequenced on a HiSeq 4000 or NextSeq 550 instrument, respectively. The recommended input of 200 ng was used for the SureSelect XT workflow on the Bravo instrument, while a lower input of 50 ng was used on the MagnisDx system. We observed comparable performance across both platforms in terms of on-target rate and sequencing depth, in addition to a reduction in duplicate percentage for samples processed with the MagnisDx system in spite of the lower input used on this platform (Table 3). It is unclear with this data whether the reduction in duplicates is related to the MagnisDx system, the SureSelect XT HS chemistry, or the sequencing platform.

Table 3. Sequencing metrics following parallel processing of two samples on the MagnisDx and Bravo instruments. Sequencing was performed on the NextSeq and HiSeq 4000 instruments for MagnisDx and Bravo samples, respectively.

Platform	Sample Name	Sample Input [ng]	Avg. Coding Depth	Duplicates [%]	On Target [%]
MagnisDx	Sample 1	50	596	21	59
MagnisDx	Sample 2	50	667	13	62
Bravo	Sample 1	200	596	36	64
Bravo	Sample 2	200	700	31	62

Feedback from GPOL on our experiences with the MagnisDx system

GPOL facility operates several liquid handling platforms to automate library preparation for NGS workflows. Library preparations, quality control (QC) setup, normalizations, and pooling are all carried out by liquid handlers using both customized and vendor supplied methods. The integration of the MagnisDx instrument and its associated workflow into GPOL has been relatively easy. Our typical process of testing, validating, and training while introducing new workflows was streamlined by its ease of use.

Our MagnisDx system has been used to process samples overnight in most cases which gives us the flexibility to fit the new workflow around our existing commitments. User hands-on time involves one to two hours for upfront sample preparation (mechanical shearing and quantification) followed by 10 minutes of hands-on time to start the MagnisDx system. Further reductions in sample preparation can be achieved when using enzymatic fragmentation of samples; however this was not attempted as part of our evaluation. The resulting libraries are target-enriched and then ready for sequencing

A typical run on the MagnisDx instrument will process eight FFPE-derived samples over approximately nine hours. Although longer than a typical working day, within a 24-hour period multiple runs are obviously possible allowing for larger batches to be processed. GPOL has successfully trialed multiple runs on the MagnisDx system in the same day. While this required a change to work patterns, it allowed up to 16 samples to be batched for sequencing while still maintaining the rapid turnaround requirement.

Conclusion

If sample testing is provided on an as-needed basis, traditional sample batching and high-throughput processing in NGS workflows introduce delays. The envisaged application for the MagnisDx NGS Prep system within GPOL was to facilitate the rapid return of biologically relevant data that could be used to inform patient treatment or suitability for a clinical trial.

The workflow presented above for the MagnisDx system is suitable for up to eight samples captured using a custom 5 Mb panel design and sequenced using the Illumina NextSeq platform. A patient-specific report is available within 72 hours, representing a significant improvement in time savings over our current high-throughput workflow using the Agilent Bravo platform. The addition of the MagnisDx platform thus complements GPOL's current range of capabilities: the Bravo system provides large-scale, cost-effective target enrichment, while the MagnisDx system allows focus on rapid TNT and small-batch processing when needed.

For our new rapid turnaround workflow, some equivalency to existing processes at GPOL can be drawn by comparing typical NGS metrics (Table 2 and 3). Libraries produced on the MagnisDx system used significantly less starting gDNA and were captured on a smaller panel design, but still compared favorably to those prepared with the Bravo SureSelect XT workflow.

References

1. Beer, P. A.; Cooke, S. L.; Chang, D. K.; Biankin, A.V. Reasons to be testing: the dawn of complex molecular profiling in routine oncology practice. *Annals of Oncology*. **2019**, 30(11), 1691–1694
2. SureSelect Community Design Glasgow Cancer Panels p/n 5995-1477EN https://www.agilent.com/cs/library/flyers/public/flyer-genomics-glasgow-panel-5994-1477en-agilent.pdf

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For In Vitro Diagnostic Use
PR7000-2678

Agilent has not verified or validated the SureSelect Community Design Glasgow Cancer Panels.
MagnisDx NGS Prep system is a CE-IVD instrument.

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