Case study

Sera-Mag Select in a targeted resequencing approach

Background

Dr. rer. nat. Stefanie Stepanow, an expert in next generation sequencing (NGS) in a molecular diagnostic laboratory in Cologne, is responsible for an NGS core facility and the establishment and validation of new assays into the routine. She tested Sera-Mag™ Select (Cytiva) in a targeted resequencing approach in August, 2018.

Sera-Mag Select is a ready-to-use reagent based on Cytiva's Sera-Mag Carboxyl SpeedBead and the well-known solid phase reversible immobilization technology. It provides optimal binding characteristics for both polymerase chain reaction (PCR) clean-up and DNA fragment size selection for NGS library preparation.

Material and methods

DNA

Genomic DNA from four reference samples purchased from Coriell Institute for Medical Research were used:

- NA12878 (HG001)
- NA24385 (HG002)
- NA24149 (HG003)
- NA24143 (HG004)

Published high confidence data sets are available for these samples, which can be used for the assessment of accuracy, sensitivity and specificity of a next-generation targeted sequencing approach.

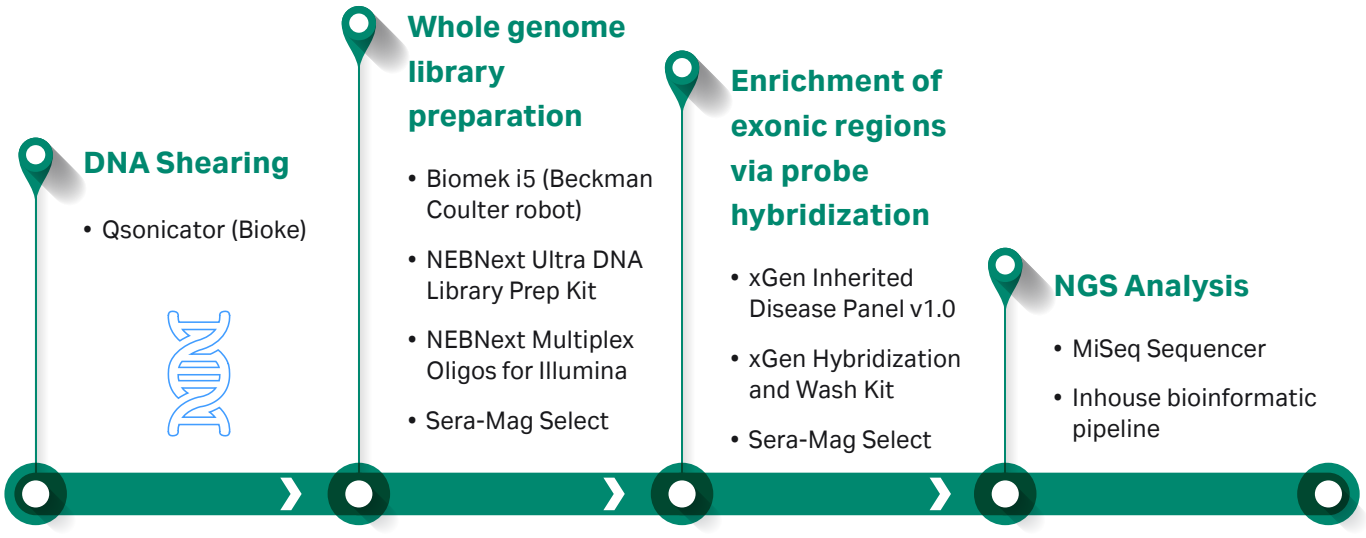

Fig 1. Standard NGS workflow showing the products used in the targeted sequencing approach.

Reagents for library preparation

The libraries were constructed by using NEBNext™ Ultra (New England Biolabs Inc.) library preparation kit for the Illumina™ platform. Then the xGen™ Inherited Disease Panel (Integrated DNA Technologies), which enables deeper sequencing of genomic regions containing genes and SNPs associated with inherited diseases, was utilized to explore the presence of inherited diseases. (Protocol can be found here: http://sfvideo.blob.core.windows.net/sitefinity/docs/default-source/protocol/xgen-hybridization-capture-of-dna-libraries.pdf?sfvrsn=ab880a07_10.)

Kit	Component	Vendor
NEBNext Ultra DNA Library Prep Kit for Illumina	E7370	NEB
NEBNext Multiplex Oligos for Illumina (Dual Index Primer Set 1)	E7600S	NEB
xGen Inherited Disease Panel v1.0 (16×)	1016352	IDT
xGen Hybridization and Wash Kit (16×)	1080577	IDT

Sera-Mag Select magnetic particles (Cytiva) were used for size selection and PCR clean-up during all of the steps, following the standard protocols for these products.

Kit	Component	Vendor
Sera-Mag Select 60 mL	29343052	Cytiva

Sequencing

A MiSeq™ Reagent v3 600 cycle kit (Illumina) was used to allow for paired end sequencing and read lengths up to 2 × 300 bp.

Kit	Component	Vendor
MiSeq Reagent Kit v3, 600 cycles	MS-102-3003	Illumina

Results

The performance of Sera-Mag Select was evaluated based on the quality control (QC) results of the whole genome and the enriched libraries and by analyzing the sequencing data of the prepared libraries.

DNA shearing

The gDNA was sheared using Qsonica Sonicator (Bioke), following an in-house protocol. The target DNA fragment size after shearing was 150-200 bp: the BioAnalyzer™ traces show that all of the samples have a peak centered around the desired size range (Fig 2).

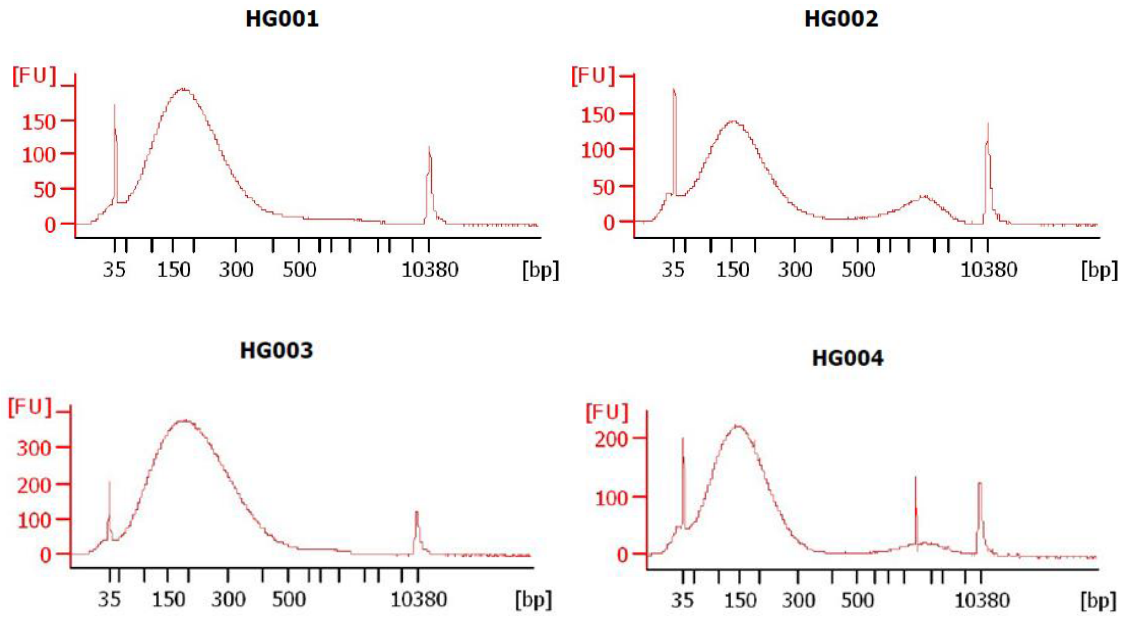

Fig 2. BioAnalyzer DNA HS Assay showing peaks centred around the desired size range.

Whole genome library QC

Following library preparation, the xGen Inherited Disease Panel v1.0 (IDT) was used to select specific targets of interest, and Sera-Mag Select (Cytiva) was used for PCR clean-up post capture PCR enrichment. The final library QC results are shown below, with the peak centered at around 300 bp indicating that the adapters have successfully ligated to the library fragments. (Fig 3).

Table 1. Final library QC results.

Probe	Index 7xx	Index 5xx	Conc. in ng/µL	Volume in Seq-Pool in µL	Seq Run #
HG001	702	508	33.8	14.8	425
HG002	703	508	17.8	23.0	
HG003	704	508	56.7	8.8	
HG004	705	508	48.8	10.2	

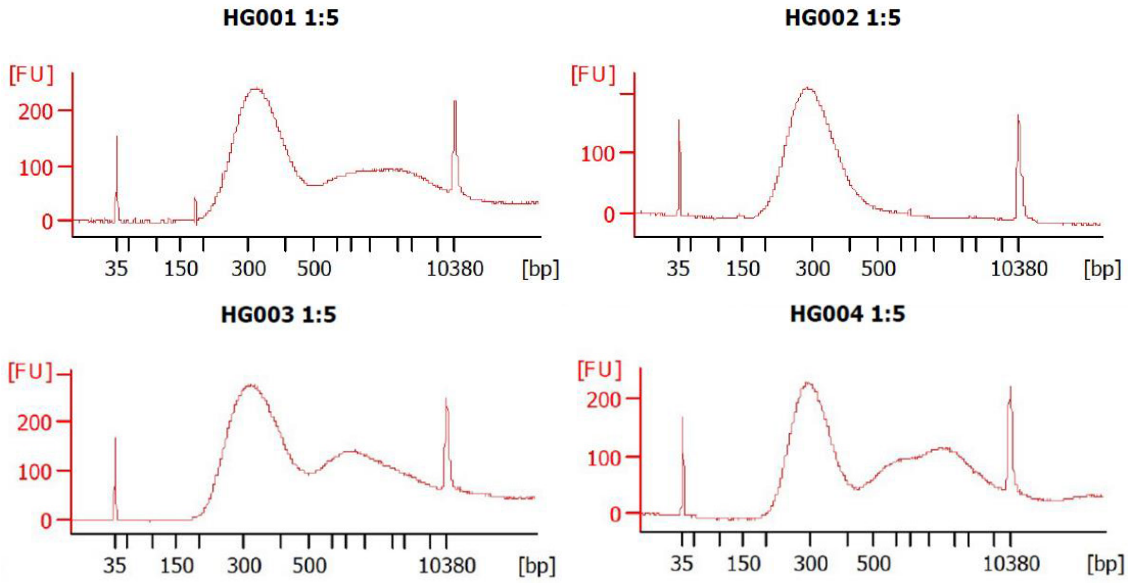

Fig 3. BioAnalyzer DNA HS Assay with peaks centred around 300 bp.

Enriched NGS libraries

The enriched whole genome libraries were pooled, and the results of the final pooled library are shown in Fig 4 and Table 2.

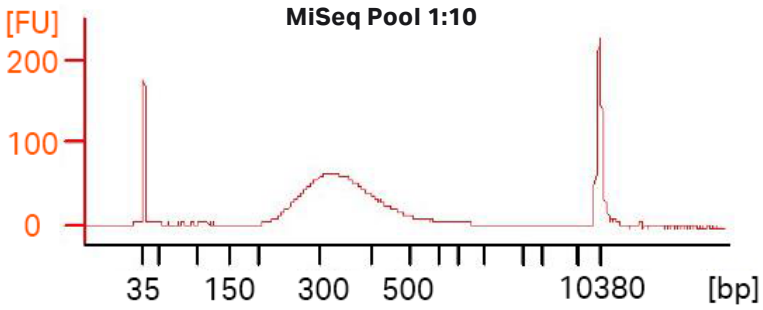

Fig 4. BioAnalyzer DNA HS Assay showing the results of the final pooled library.

Table 2. Results of final pooled whole genome libraries.

Sample	Conc. In ng/µL	Mean fragment length in bp	Molarity in nM
Library Pool	6.17	364	25.68

Sequencing run QC results

The pooled samples were diluted to 4 nM and then denatured and further diluted to 8 pM following the Illumina protocol for the MiSeq system (#15039740 v03). The sequencing was performed on the MiSeq instrument to generate 2×150 bp paired-end reads using the v3 Reagent Kit. The following run metrics were observed:

Table 3. Metrics for Sera-Mag Select (Cytiva) on MiSeq run #425.

Density (K/mm²)	Cluster PF (%)	Phas/ Prephas Read 1 (%)	Phas/ Prephas Read 4 (%)	Reads (M)	Reads PF (M)	% >= Q30	Error rate PhiX total (%)
1177	91.59	0.137 / 0.121	0.117 / 0.071	28.23	25.85	93.92	0.60

NGS data QC

The resulting NGS data for the samples was analyzed via an inhouse bioinformatics pipeline.

Table 4. Sequencing results for Sera-Mag Select (Cytiva).

Sample	Total reads	% Q20	% Q30	% mapped reads	% on target reads	Mean coverage	% 30x covered
HG001	14 745 128	99.66	94.17	99.84	89.33	122.2	96.90
HG002	8 031 000	99.69	94.77	99.85	89.79	59.7	82.86
HG003	13 989 730	99.67	89.98	99.86	89.70	122.3	94.80
HG004	11 453 576	99.67	94.01	99.85	89.98	95.5	93.05

Table 5. Sera-Mag Select (Cytiva): averaged data.

Sample	Total reads	% Q20	% Q30	% mapped reads	% on target reads	Mean coverage	% 30x covered
Combined	12 054 859	99.67	93.23	99.85	89.70	99.93	91.90

Conclusions

The sequencing results show that the average percentage of bases with a Q30 score is 93.23%, indicating the high quality of the data. Also, an average of 91.90% of the bases were covered 30×, which allows for confidence that the regions of interest will be covered adequately for study.

This study demonstrates that using Sera-Mag Select in the above workflows for both the preparation and enrichment of NGS libraries yields high quality sequencing data with a high percentage of Q30 reads achieved.

It also shows that Sera-Mag Select works effectively for library preparation completed on an automated platform.

For further information on Sera-Mag Select and to request a free sample, please visit: **cytiva.com/sera-mag**.

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CY14829-09Dec20-CS