Sample prep

Bead-based target enrichment generates high-quality library preparation for enhanced next-generation sequencing readouts

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Abstract

Purpose: Enhanced next-generation sequencing (NGS) readouts with bead-based target enrichment for generation of high quality NGS libraries

Methods: Exome sequencing (contract research organization) and binding capacity for oligonucleotides (in-house)

Results: Equal or better performance in NGS with Dynabeads™ Streptavidin for Target Enrichment compared to internal control and 4 alternative streptavidin bead suppliers.

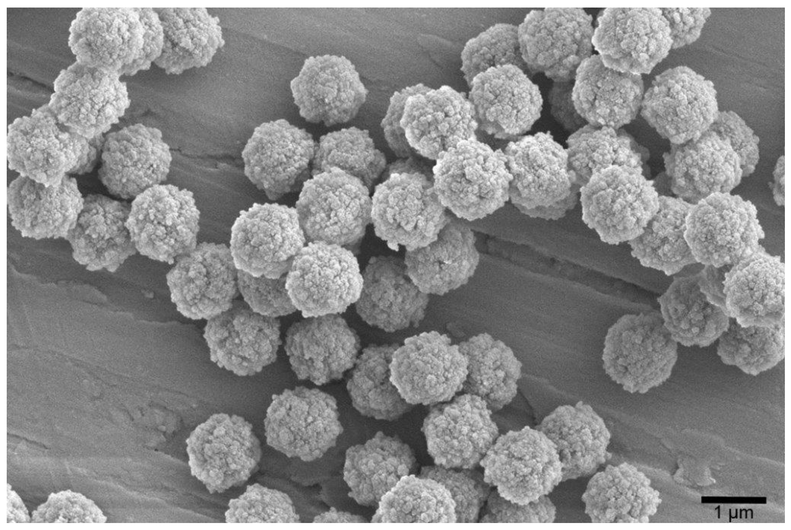

Figure 1. Monosized superparamagnetic Dynabeads™ magnetic beads.

Introduction

Today, landmarks are made in liquid biopsy based diagnostic tests. Highly invasive procedures to procure the necessary biopsies for molecular analyses for applications such as tumor detection, cancer prognosis and monitoring can be replaced by simpler, safer minimally invasive liquid biopsies. To understand a disease state, genomic material obtained from liquid biopsies is used for downstream NGS analysis.

To enhance sensitivity while lowering cost, an efficient and robust capture of targeted sequences is vital. Targeted NGS allows researchers to use genomic data retained from liquid biopsies to study plausible treatments for cancers and genetic diseases and rapid identification of rare genetic variations. Targeted NGS facilitates for better insight of the disease-driving molecular alterations by targeting regions of interest. Here, Dynabeads Streptavidin for Target Enrichment has been specifically designed for targeted sequences enrichment for NGS library workflows.

Figure 2. An example workflow of library preparation and target enrichment used prior to downstream NGS analysis.

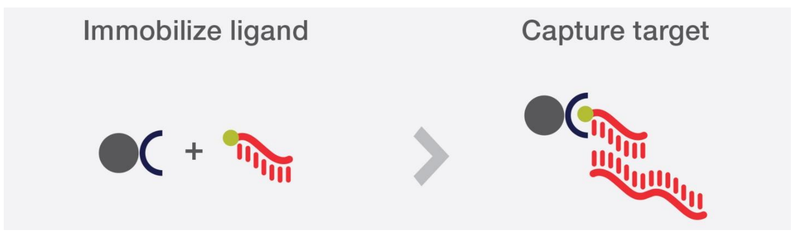

Figure 3. The hybridization to the targeted sequences can be performed prior to or after the bead immobilization to the biotinylated capture probe. The figure demonstrates the direct capture method. Dynabeads magnetic beads binds to the biotinylated DNA probe in the immobilization step. Subsequently, the beads with the immobilized ligand is added to the sample for hybridizing to the targeted regions of interest.

Materials and methods

Sample Preparation

- All lab work related to exome sequencing was executed by a third-party vendor
- Genome in a bottle - genomic DNA was isolated before the initiation of library preparation
- Enrichment of NGS libraries using Dynabeads magnetic beads and 4 alternative streptavidin bead vendors

Test Methods

- In-house hybridization assay to determine the binding capacity for oligonucleotides
- Targeted Exome sequencing
- NGS library quality control with Invitrogen Qubit™ Fluorometer and capillary electrophoresis with Advanced Analytical Technologies Fragment Analyzer™

Data Analysis

- Raw data from exome sequencing was processed with fastp [1]

Results

Binding capacity for oligonucleotides

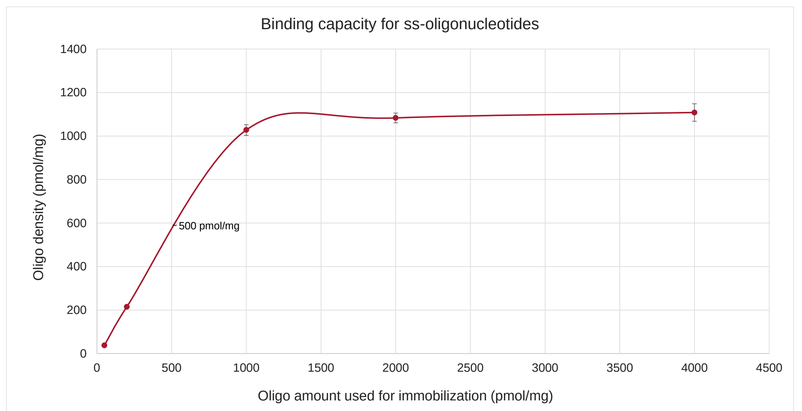

Figure 4. Titration study of the binding capacity of single-stranded (ss) oligonucleotides immobilized to the Dynabeads magnetic beads. The X-axis shows the added amounts of ss-oligonucleotides used in the immobilization and the Y-axis represents the oligo density (bound). The recommended oligo density (pmol/mg beads) is in the middle of the linear response at 500 pmol/mg bead. There is approximately 100 % binding efficiency up to 1000 pm/mg.

Assessing the quality and the concentration of final libraries to ensure good reads in downstream NGS

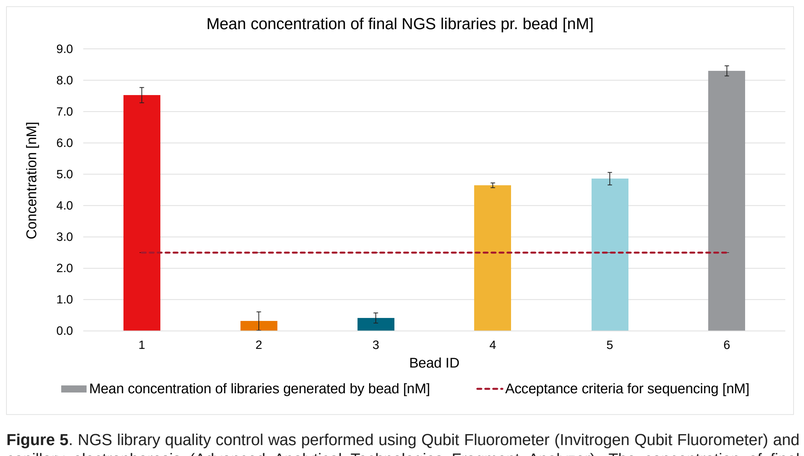

Figure 5. NGS library quality control was performed using Qubit Fluorometer (Invitrogen Qubit Fluorometer) and capillary electrophoresis (Advanced Analytical Technologies Fragment Analyzer). The concentration of final libraries [nM] is shown on the Y-axis and the different beads tested is shown on the X-axis. The error bar is the standard deviation (n=3). Dynabeads magnetic beads (1), alternative streptavidin magnetic bead vendors (2-5), internal control (6). The highest recovery is achieved with Dynabeads Streptavidin for Target Enrichment.

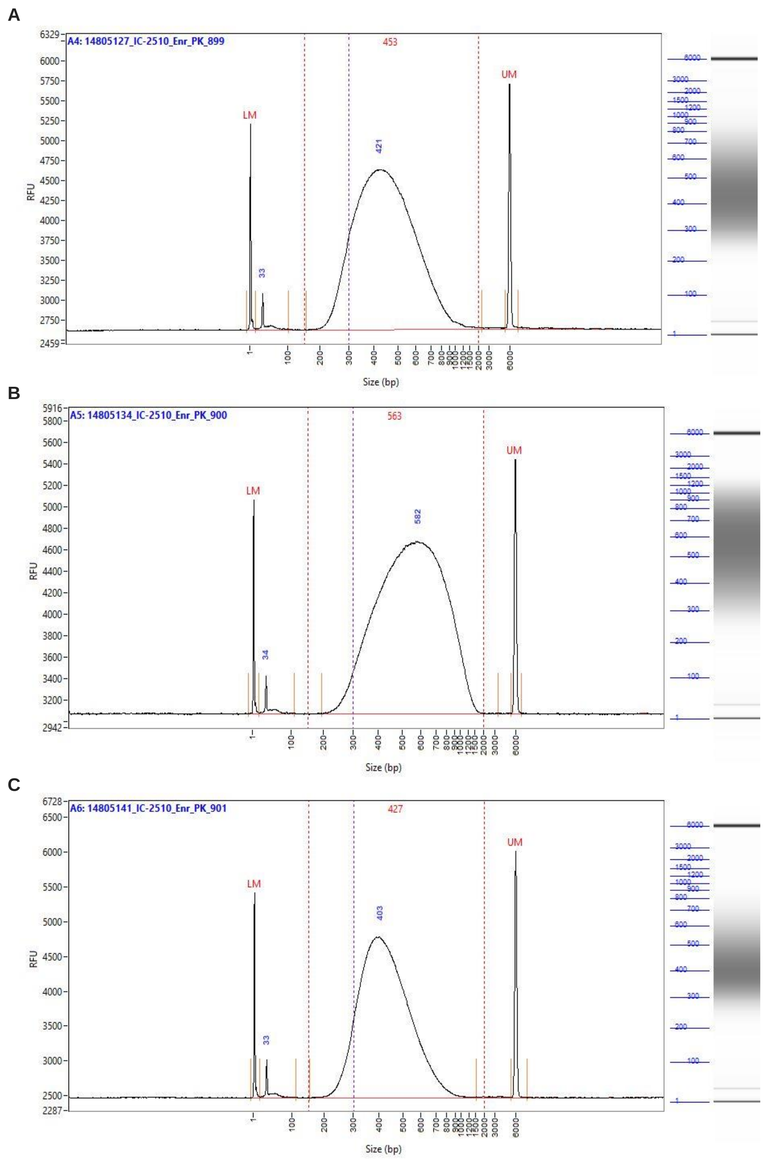

Figure 6 A, B and C. Fragment Analyzer assessing the final libraries as part of quality control prior to downstream sequencing. These three NGS libraries generated by Dynabeads magnetic beads show the high reproducibility of the beads.

Highly accurate results with deep coverage in an exome sequencing workflow

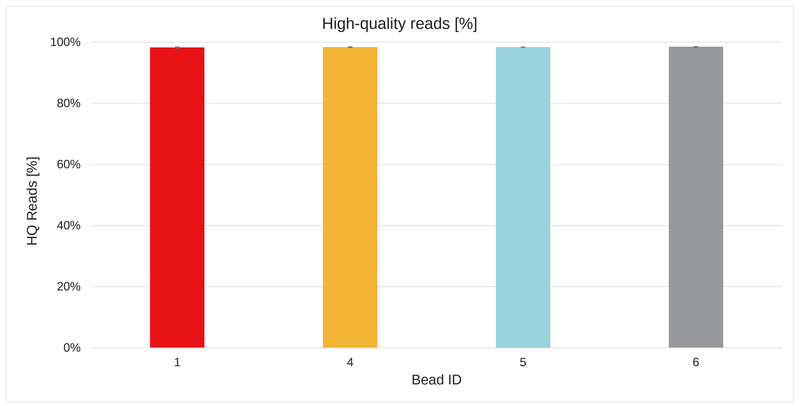

Figure 7. The percentage (%) of high-quality reads is on the Y-axis, while the beads are on the X-axis. The error bar is the standard deviation (n=3). Dynabeads magnetic beads (1), alternative streptavidin magnetic bead vendors (4 and 5), internal control (6). High-quality NGS libraries obtained with Dynabeads Streptavidin for Target Enrichment comparable to internal control bead.

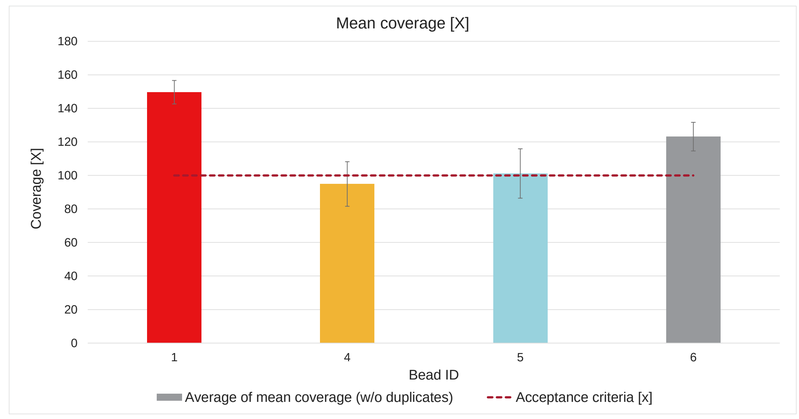

Figure 8. The coverage [X] is on the Y-axis, while the beads are on the X-axis. The error bar is the standard deviation (n=3). Dynabeads magnetic beads (1), alternative streptavidin magnetic bead vendors (4 and 5), internal control (6).

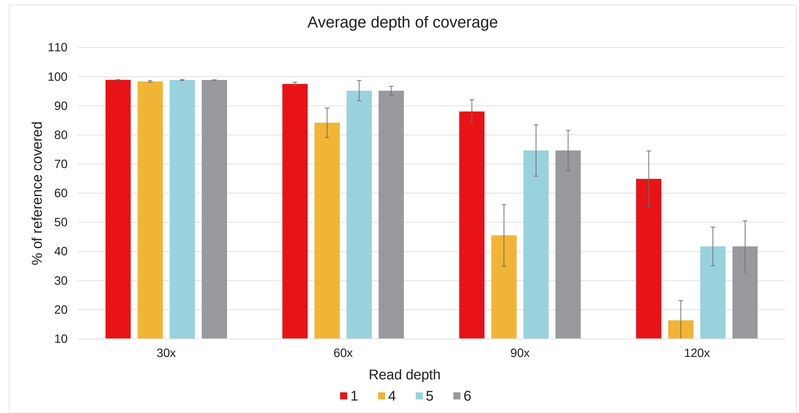

Figure 9. The percentage (%) of the reference sequence covered is on the Y-axis, while the coverage/read depth is on the X-axis. The error bar is the standard deviation (n=3). Dynabeads magnetic beads (1), alternative streptavidin magnetic bead vendors (4 and 5), internal control (6). Libraries captured and enriched by the Dynabeads Streptavidin for Target Enrichment gave the deepest coverage, which is particularly important for clinical applications and identification of rare variants.

Conclusion

Efficient and robust enrichment of target sequences for the preparation of NGS libraries is vital to secure accurate and high-quality sequencing data. Here, we have shown that Dynabeads Streptavidin for Target Enrichment yields:

- **Highly reproducible target capture**
- **Efficient and robust capture of targeted sequences**
- **High-quality reads from the generated NGS libraries**
- **Excellent coverage enables variant detection with high confidence**
- **More prominent advantage the deeper the sequencing**

References

1. Shifu Chen, Yanqing Zhou, Yaru Chen, and Jia Gu. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, 17 (September 2018), i884–i890. DOI:https://doi.org/10.1093/bioinformatics/bty560

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