ThermoFisher SCIENTIFIC

Next-generation sequencing

Bead-based target enrichment generates high-quality libraries for enhanced NGS readouts

Keywords

Dynabeads Streptavidin for Target Enrichment, NGS, sequencing, Automation, KingFisher Apex, KingFisher Flex, sample preparation, liquid biopsy, genomics, library prep

In this technical note, we show:

- Successful target enrichment using Dynabeads magnetic beads before downstream next-generation sequencing (NGS)
- Excellent sequence coverage enabling variant calling with high confidence while helping reduce costs with targeted sequencing
- A simple and efficient method for successful target enrichment, seamlessly integrated with sequencing products

Today landmark discoveries are made using liquid biopsy–based diagnostic tests. For molecular applications such as tumor detection and cancer prognosis and monitoring, highly invasive procedures to procure necessary biopsies can now be replaced by simpler, safer, and 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, efficient and robust capture of target 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 to rapidly identify rare genetic variations. Targeted NGS helps facilitate better insight into disease-driving molecular alterations by targeting genomic regions of interest.

Invitrogen™ Dynabeads™ Streptavidin for Target Enrichment beads are superparamagnetic (Figure 1) and designed for optimal enrichment of target sequences, which can lead to high-quality libraries for robust and reproducible NGS analysis (Figures 2 and 3). These uniform 1.0 µm beads, which have a streptavidin monolayer covalently coupled to their hydrophobic surface, are ideally poised for automation. The bead-based enrichment process can be automated using any of the Thermo Scientific™ KingFisher™ instruments or the Thermo Scientific™ KingFisher™ Presto Purification System in combination with liquid handlers. Scalable, automatable target enrichment from circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), genomic DNA (gDNA), and cell-free DNA (cfDNA) can be obtained using Dynabeads Streptavidin for Target Enrichment beads for seamless integration with downstream NGS.

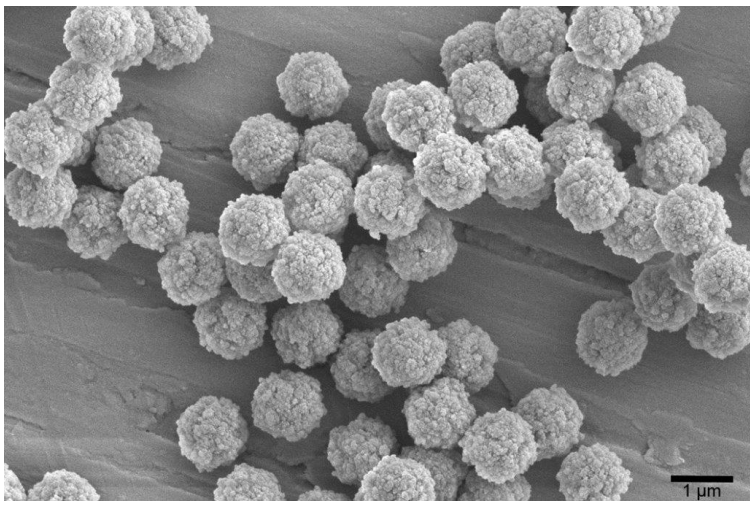

Figure 1. Monosized superparamagnetic 1 µm Invitrogen™ Dynabeads™ magnetic beads.

invitrogen

Here we present Dynabeads Streptavidin for Target Enrichment beads that enable efficient and robust capture of target sequences with high accuracy that yields trustworthy NGS sequencing data.

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

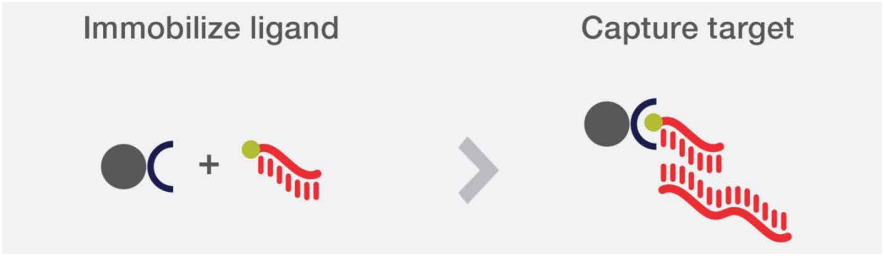

Figure 3. Target enrichment by the direct capture method. Hybridization to the targeted sequences can be performed before or after bead immobilization to the biotinylated capture probe. Using the direct capture method, Dynabeads Streptavidin for Target Enrichment beads bind to the biotinylated DNA probe in the immobilization step. Subsequently, the beads with the immobilized ligand are added to the sample for hybridizing to the targeted regions of interest.

The binding capacity for oligonucleotides

We performed a titration study of the binding capacity of Dynabeads Streptavidin for Target Enrichment beads for different input amounts of single-stranded (ss) oligonucleotides (20 nt) (Figure 4). The recommended oligo density (pmol/mg of beads) is in the middle of the linear response at 500 pmol/mg of beads. There is a 100% binding efficiency up to 1,000 pmol/mg of beads (where the beads are saturated). For oligonucleotides, the maximum binding capacity is inversely correlated to the molecule size (number of bases). This reduction in binding capacity for larger DNA fragments is most likely due to steric hindrance.

The binding capacity for dsDNA

We performed a titration study of the binding capacity of Dynabeads Streptavidin for Target Enrichment beads for different input amounts of 288 bp double-stranded (ds) DNA (Figure 5). The high binding capacity and excellent reaction kinetics of the beads enable efficient immobilizations. The recommended amount of dsDNA is 20 µg when using 1 mg of beads. There is a 100% binding efficiency for up to 20 µg of 288 bp dsDNA per mg of beads. For higher amounts of dsDNA, the binding efficiency decreases.

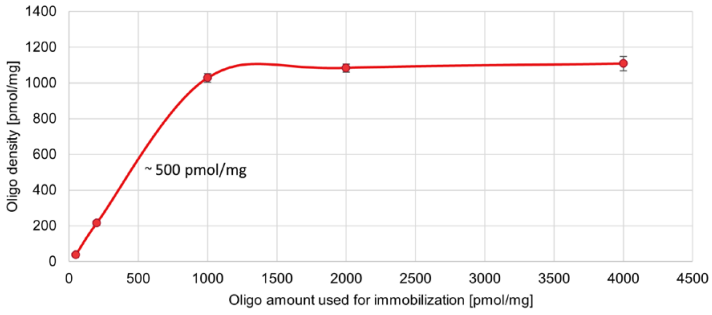

Figure 4. The binding capacity of ss oligonucleotides immobilized to the Dynabeads Streptavidin for Target Enrichment beads. Error bars are standard deviation. The x-axis shows the amounts of ss oligonucleotides used for immobilization, and the y-axis represents the density of bound oligonucleotides.

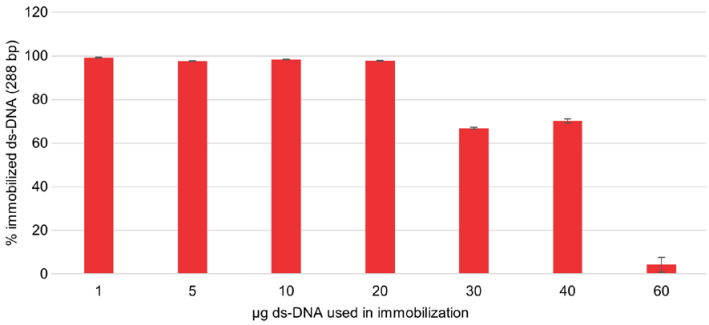

Figure 5. The binding capacity of dsDNA (288 bp) immobilized to the Dynabeads Streptavidin for Target Enrichment beads. The error bars are standard deviation. The x-axis shows the amount of dsDNA used in the immobilization reaction, and the y-axis represents the percentage of immobilized dsDNA.

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

For NGS data generation, a third-party vendor was used. Dynabeads Streptavidin for Target Enrichment beads successfully captured and enriched the target libraries to be used in downstream sequencing. The concentrations of the final libraries (nM) for Dynabeads Streptavidin for Target Enrichment beads, internal control beads, and streptavidin magnetic beads from four alternative suppliers are shown in Figure 6. All samples were prepared in triplicate. The quality control of the target libraries was performed using an Invitrogen™ Qubit™ Fluorometer and capillary electrophoresis (Advanced Analytical Technologies Fragment Analyzer™ system). To avoid both over- and under-loading and prevent a negative impact on data quality and sequencing yield, the final NGS libraries generated were quantified for optimum loading and cluster generation. Accurate quantifications of the concentrations of the final libraries were achieved by combining the size distribution determined by capillary electrophoresis and the concentration of the NGS libraries (Figure 7). For this particular downstream exome sequencing assay, the acceptance criterion for the concentration of final libraries was 2.5 nM. For comparison, commercially available streptavidin magnetic beads from four different suppliers, used at the same bead concentration and volume, were also tested. The two sequencing libraries that did not pass the acceptance criterion were generated with beads from other suppliers (IDs 2 and 3) (Figure 6). However, it should be noted that a direct comparison is challenging because of differences in bead properties between suppliers, such as bead surface chemistry.

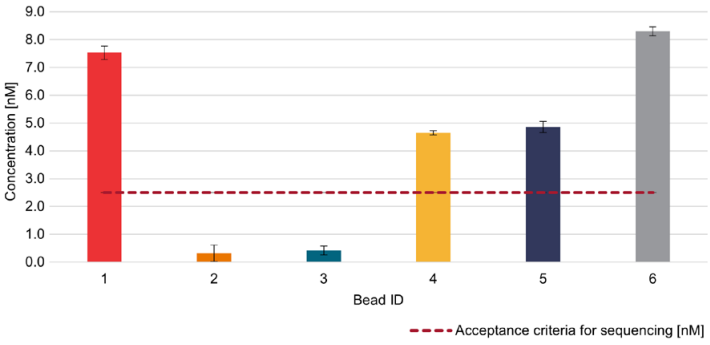

Figure 6. Mean concentration of final NGS libraries. The concentration of final libraries is shown on the y-axis, and the different beads are shown on the x-axis. The error bars are standard deviation (n = 3). The different beads are Dynabeads Streptavidin for Target Enrichment beads (1), streptavidin magnetic beads from other suppliers (2–5), and internal control beads (6).

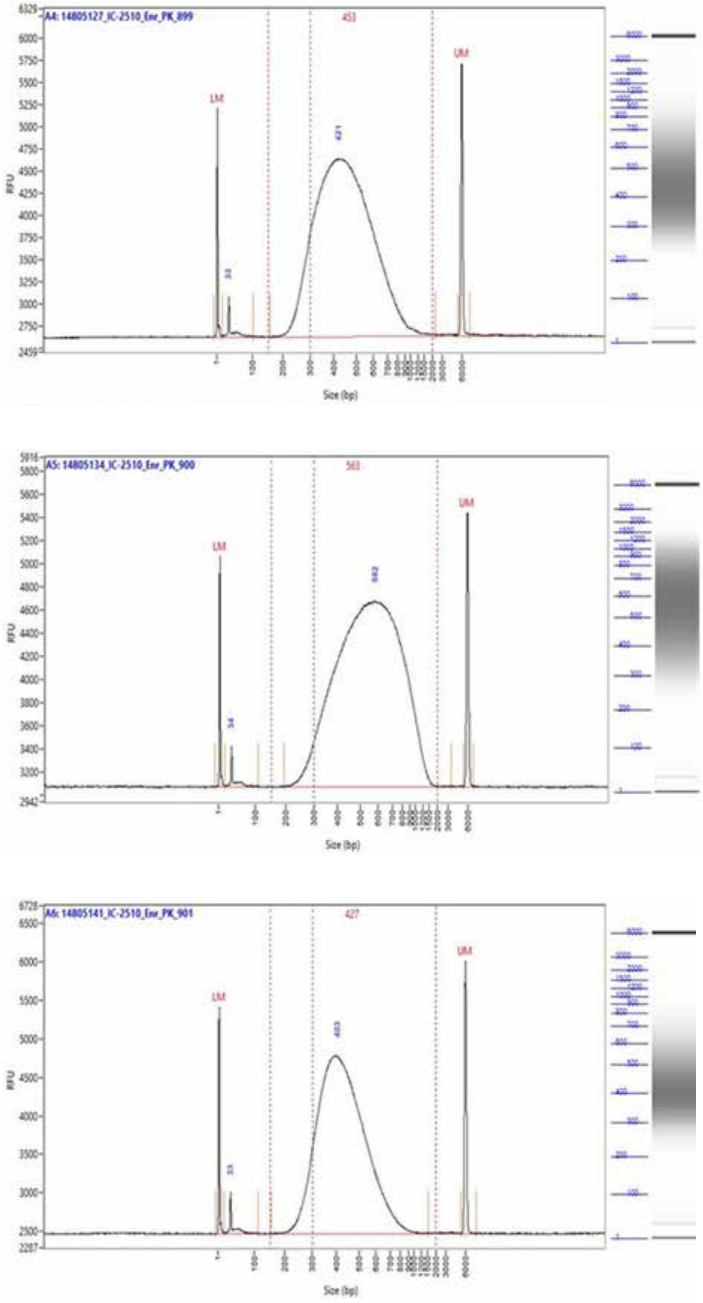

Figure 7. Quality control of the final libraries prior to NGS. The graphs for the three NGS libraries generated using the Dynabeads Streptavidin for Target Enrichment beads show high reproducibility.

Highly accurate results with deep coverage in an exome sequencing workflow

Dynabeads Streptavidin for Target Enrichment beads efficiently captured the NGS libraries and ensured high-quality reads comparable to the internal control beads (Figure 8). The sequencing coverage for Dynabeads Streptavidin for Target Enrichment beads, internal control beads, and streptavidin magnetic beads from the two alternative suppliers that passed the NGS library quality control criteria are shown in Figure 9. In clinical applications, deep coverage is essential for rare-variant calling. Libraries captured and enriched by the Dynabeads Streptavidin for Target Enrichment beads gave the deepest coverage after duplicates removal. A greater depth of coverage generally increases confidence in results and enables rare-variant calling with high accuracy.

The percentage of the reference sequence covered versus the read depth for Dynabeads Streptavidin for Target Enrichment beads, two alternative suppliers' beads, and the internal control beads is shown in Figure 10. For the Dynabeads Streptavidin for Target Enrichment beads at 60x coverage, 97.53% of the reference is covered with a minimum of 60 reads. This performance attribute is particularly important for clinical applications and identification of rare variants where deep coverage (e.g., >60x) is essential. Within cancer research, the sequencing depth typically starts at 80x; at 90x depth, Streptavidin for Target Enrichment beads shows an even more prominent benefit compared to other suppliers' beads. Dynabeads Streptavidin for Target Enrichment beads performed significantly better compared to the streptavidin magnetic beads from alternative suppliers and the internal control beads. The deeper the sequencing, the bigger the discrepancies were between Dynabeads Streptavidin for Target Enrichment beads and other suppliers' products.

Performance highlights

- Efficient and robust capture of high-quality target sequences was achieved using Dynabeads Streptavidin for Target Enrichment beads.
- Excellent coverage enables high confidence in variant detection.
- The beads are suitable for high-throughput and walk-away solutions with automation as demonstrated in this study, where the NGS data were generated using a fully automated platform.

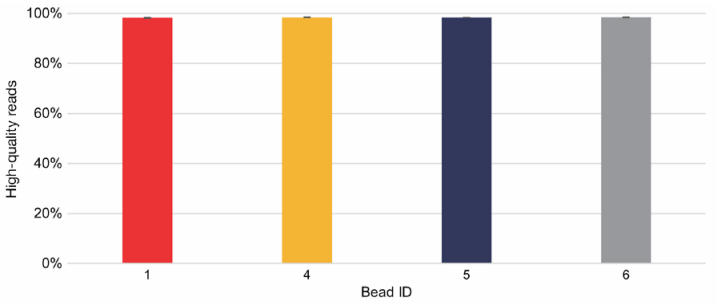

Figure 8. High-quality reads obtained from target sequences captured using Dynabeads Streptavidin for Target Enrichment beads. The percentage of high-quality reads is on the y-axis, while the bead are on the x-axis. The error bars are standard deviation (n = 3). The different beads are Dynabeads Streptavidin for Target Enrichment beads (1), streptavidin magnetic beads from other suppliers (4 and 5), and internal control beads (6).

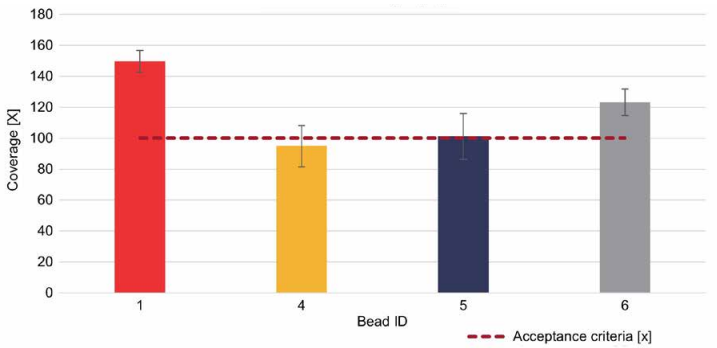

Figure 9. High sequence coverage of target sequences captured using Dynabeads Streptavidin for Target Enrichment beads. The coverage is on the y-axis, while the beads are on the x-axis. The error bars are standard deviation (n = 3). The different beads are Dynabeads Streptavidin for Target Enrichment beads (1), streptavidin magnetic beads from other suppliers (4 and 5), and internal control beads (6).

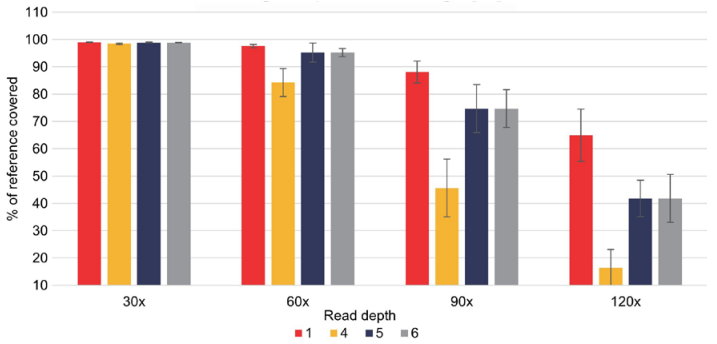

Figure 10. High read depth coverage of target sequences captured using Dynabeads Streptavidin for Target Enrichment beads. The percentage of the reference sequence covered is on the y-axis, while the coverage/read depth is on the x-axis. The error bars are standard deviation (n = 3). The different beads are Dynabeads Streptavidin for Target Enrichment beads (1), streptavidin magnetic beads from other suppliers (4 and 5), and internal control beads (6).

Conclusions

We present Dynabeads Streptavidin for Target Enrichment beads, a solution for NGS sample preparation to facilitate efficient and robust capture of target sequences, which is vital to secure accurate and high-quality sequencing data. The fast-binding kinetics, yield of high-quality reads, and deeper coverage than that of other suppliers' products position the Dynabeads Streptavidin for Target Enrichment beads as superior for the identification of essential genomic insights. The high-performing Dynabeads Streptavidin for Target Enrichment beads (for research use only) facilitate the need for accurate results to meet the increased demand for genomics-based personalized medicine.

Ordering information

Product	Quantity	Cat. No.
Dynabeads Streptavidin for Target Enrichment	2 mL	65605D
	10 mL	65606D
	50 mL	65607D
DynaMag-2 Magnet	1 each	12321D

Please contact us at **oemdynal@lifetech.com** if you want to discuss the commercialization of the beads in your assay or service.

Learn more at **thermofisher.com/streptavidindynabeads**