Sample prep

Specific capture of nucleic acid on Dynabeads™ magnetic beads for target enrichment from liquid biopsies

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Abstract

Dynabeads™ monodisperse superparamagnetic beads can be covalently coupled to oligonucleotides for the specific capture of nucleic acid targets through hybridization. The coupled oligonucleotide density is tunable and stable, enabling the adaptation to any assay and application. As a proof-of-concept, oligonucleotide-coupled Dynabeads™ magnetic beads have been successfully used for the specific capture of nucleic acid from liquid biopsies as a part of a new sample prep workflow with the following attributes: ultra-rapid, automatable, versatile, highly sensitive, alcohol-free and scalable.

Introduction

Molecular diagnostics is a fast-growing market that uses nucleic acids (NA) for diagnostics, prevention and therapeutics. Biotech companies developing such molecular diagnostics demand powerful and reliable tools for the isolation of specific NA targets. Dynabeads™ monodisperse superparamagnetic beads provide a robust, customizable, and automation-friendly tool for specific capture of NA that enable the isolation and manipulation of any desired NA target.

Dynabeads™ MyOne™ Carboxylic Acid beads are covalently coupled to oligonucleotides for the isolation of specific NA targets through specific hybridization. The binding capacity is tunable, ranging from 50 to 2000 pmol of NA target per mg beads, to enable adaptation to any assay and application.

The resulting DNA-bead conjugates successfully capture down to 10 copies of viral NA targets, from a virus-containing biological sample ranging from 50 µL up to 5000 µL. The optimized sample prep workflow enables efficient lysis of the virus, binding, and release of the NA target in less than 10 minutes. The elution of the NA target from the beads is not required, since the beads are compatible with various downstream applications, including qPCR and isothermal amplification.

Together, our findings demonstrate that bead-based specific capture of nucleic acid is a powerful method for targeted sequence enrichment required in various molecular diagnostics approaches.

Materials and methods

Reagents and Samples

- Dynabeads™ MyOne™ Carboxylic Acid, SKU 65011/65012 for research use only or SKU 35401 for OEM and industrial use only, from Thermo Fisher Scientific.
- Custom DNA oligonucleotides from IDT DNA Technologies. The sequences of the oligonucleotides immobilized on Dynabeads™ magnetic beads are:
  - Oligo$(dN)_{20}$: 5' ATACTTTTGCGGGAGAAGCC 3'
  - Oligo$(dX)_{20}$: 5' AATACGCAAACCGCCTCTCC 3'
- Virus model: M13 KO7 helper phage, Thermo Fisher Scientific.
- Plasma sample: Pooled human plasma, Apheresis derived K2 EDTA, Innovative Research, Inc.

Test Methods

- DNA coupling to Dynabeads™ magnetic beads

The coupling procedure is proprietary.

- Hybridization assay

The bead coupled oligo density is assessed by measuring the amount of complementary oligonucleotides recovered in the eluates after a bind-wash-elute procedure with a sample matrix containing a large excess of the complementary oligonucleotide. The amount of complementary oligonucleotide is measured by absorbance at 260 nm. The detailed procedure can be shared upon request.

- Specific capture assay

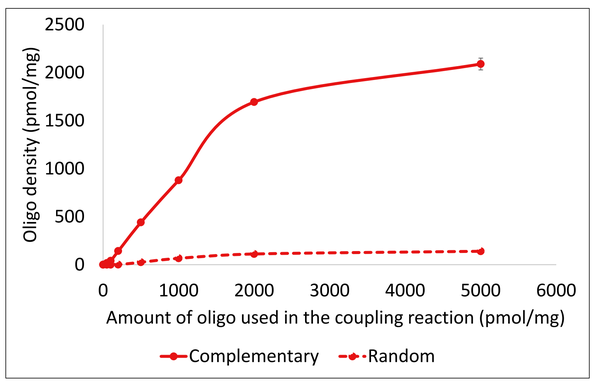
The performance of the oligo-bead conjugate is measured by its capacity to recover a nucleic acid target from a plasma sample spiked with a known quantity of viral particles beforehand. The assay consists in the lysis of the viral particles, binding of the extracted nucleic acid target on Dynabeads™ magnetic beads, washing of the beads, elution of the nucleic acid target (optional) and nucleic acid detection by quantitative PCR (qPCR) or loop-mediated isothermal amplification (LAMP). The assay was performed both manually or automated on KingFisher™ Flex and Apex instruments. The detailed procedure can be shared upon request.

DNA coupling to Dynabeads™ magnetic beads

Broad DNA binding capacity

Dynabeads™ MyOne™ Carboxylic Acid beads are covalently coupled to various amounts of a 20-mer oligonucleotide, and the oligo density at the bead surface is measured by hybridization assay. The oligo density ranges from 50 to 2000 pmol/mg beads. The density is directly proportional to the amount of oligonucleotide used in the coupling reaction up to 2000 pmol/mg. Beyond 2000 pmol/mg, the beads are saturated and do not couple more oligonucleotide with larger amounts than 2000 pmol/mg used in the coupling reaction.

Figure 1. Oligonucleotide density measured after DNA coupling to Dynabeads™ MyOne™ Carboxylic Acid magnetic beads.



Reproducible DNA coupling process

The intraday (repeatability) and interday (intermediate precision) variability of the DNA coupling process are assessed for beads coupled to Oligo$(dN)_{20}$ at either low density (200 pmol/mg) or high density (2000 pmol/mg). The relative standard deviations (RSD) of the repeatability and of the intermediate precision are below 5% and 13%, respectively, demonstrating that the DNA coupling to Dynabeads™ magnetic beads is highly reproducible.

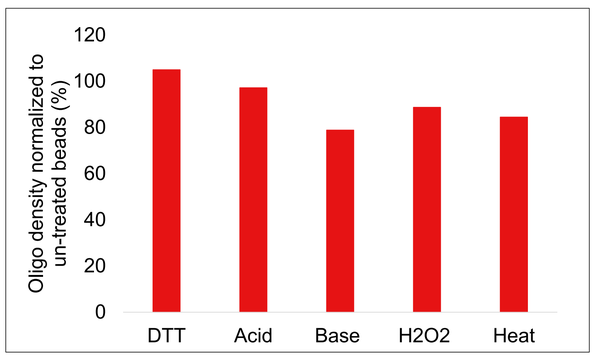
Table 1. Relative standard deviation (%) of the repeatability and intermediate precision of the DNA coupling process.

	Low density coupling	High density coupling
Repeatability	2.6%	4.3%
Intermediate precision	12.7%	9.8%

Stable over a variety of stresses

The oligonucleotide-coupled Dynabeads™ conjugates are subjected to harsh chemical and thermal stresses: 0.1M DTT for 10 min at 95ºC (DTT), 0.5N HCl for 30 min (Acid), 0.5N NaOH for 30 min (Base), 1% H_2O_2 for 30 min (H2O2) and heating at 95ºC for 1 h (Heat). The binding capacity is then measured by hybridization assay. The DTT and acid treatments have no deleterious effect on the oligo-coupled Dynabeads™ conjugates, while the base, oxidative and thermal stresses have only minor impact on the binding capacity of the beads, indicating that the bead conjugates generated with our coupling process are resistant and stable over a variety of stresses.

Figure 2. The binding capacity on oligonucleotide-coupled beads subjected to various chemical and thermal stresses is measured and normalized to the un-treated beads.



Robust nucleic acid capture workflow

Fast capture workflow

The oligonucleotide-coupled Dynabeads™ magnetic beads are used for the specific capture of a nucleic acid target from a biological sample (Figure 3). After spiking a known quantity (1.4x10^5 pfu) of the M13 phage in plasma (400 µL final volume), the sample is lyzed and the released nucleic acid target captured on oligo-coupled bead conjugates through hybridization. The nucleic acid target is then eluted from the beads and quantified by qPCR. While the recovery rate is around 25% when the lysis/binding step is only 2 minutes long at room temperature, it reaches 70% when the lysis/binding time is increased to 10 min and occurs at 55ºC, demonstrating that the assay conditions are adjustable according to the capture efficiency requirements (Figure 4).

Figure 3. The specific capture workflow consists in the lysis of the viral particles, binding of the extracted nucleic acid targets to Dynabeads™ magnetic beads, washing and elution. The total workflow time is less than 10 minutes.

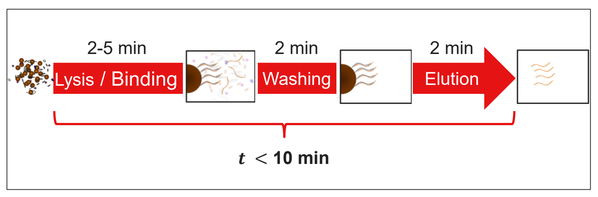
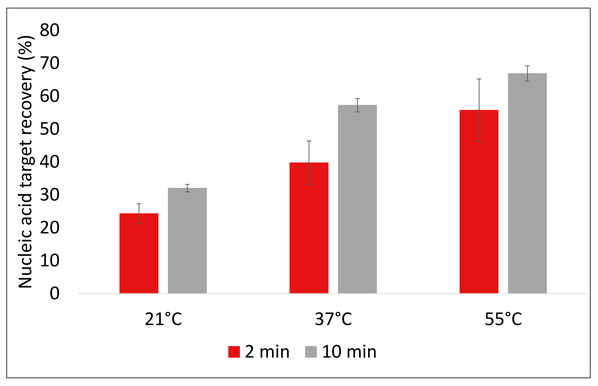


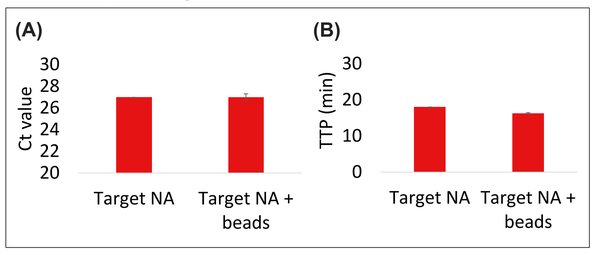
Figure 4. The recovery of the nucleic acid target by the oligonucleotide-coupled beads is assessed at different temperatures and times during the lysis/binding step.



Versatile capture workflow

The specific capture workflow is tested in combination with two downstream applications, in the presence or absence of beads in readout. The qPCR readout is not affected by the presence beads, with similar Ct values in the absence and presence of beads. In the same way, the LAMP readout leads to similar time to product with or without beads in the amplification reaction, demonstrating that oligonucleotide-coupled Dynabeads™ magnetic beads are fully compatible with these downstream applications.

Figure 5. The specific capture workflow is compatible with qPCR (A) and LAMP (B), in the presence or absence of beads. TTP, time to product.



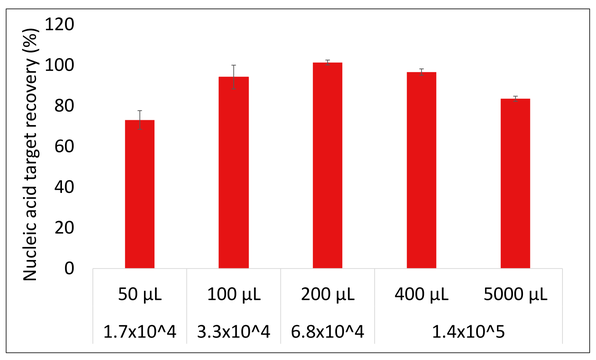
Highly sensitive sequence enrichment

Broad sample volume range

The scalability of the specific capture workflow is assessed by decreasing the reaction volume while maintaining the NA target:reaction volume ratio constant. The recovery rate stands above 70% between 50 and 400 µL, indicating that the specific capture workflow is scalable and compatible with small volumes-based setups (i.e. point of care testing).

The efficacy of the specific capture workflow is assessed by spiking 1.4x10^5 copies of nucleic acid target in 400 or 5000 µL reaction volumes and measuring the recovery rate. More than 80% of the nucleic acid target is still captured in a 5000 µL reaction volume, showing that the efficacy of the capture workflow is very high.

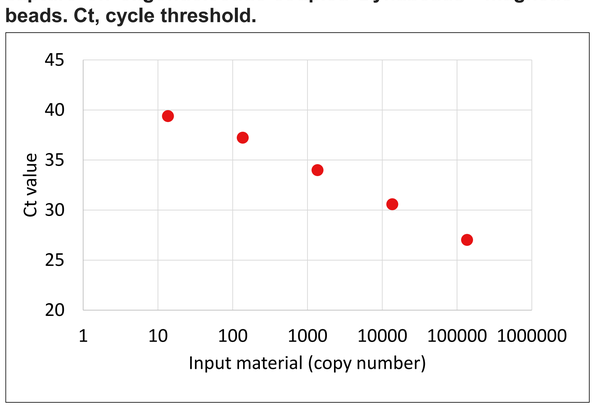
Figure 6. The specific capture workflow is scalable down to 50 µL and up to 5000 µL, while maintaining a high efficacy.



High sensitivity

In order to assess the sensitivity of the specific capture workflow, decreasing quantities of nucleic acid target, down to 14 copies, are spiked in a 400 µL-sample volume and the recovery rate is measured by qPCR. The semi-log regression line plot of Ct value versus log of input material shows the nearly 100% capture efficiency at all tested amounts of input material. Most importantly, the lowest quantity of nucleic acid target – 14 copies – is successfully captured and detected by qPCR with a Ct around 39, demonstrating the very high sensitivity of the specific capture workflow.

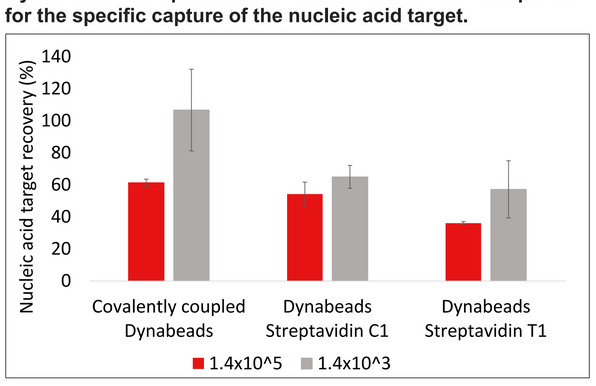
Figure 7. The amount of nucleic acid target spiked in the plasma sample is plotted against the Ct value after specific capture on oligonucleotide-coupled Dynabeads™ magnetic beads. Ct, cycle threshold.



Higher nucleic acid recovery than Dynabeads™ Streptavidin beads

The performance of the covalently coupled Dynabeads™ magnetic beads is compared to Dynabeads™ Streptavidin C1 and T1 beads. After spiking either 1.4x10^5 or 1.4x10^3 copies of the nucleic acid target in plasma, the amount of nucleic acid target after specific capture is quantified by qPCR. The covalently oligonucleotide-coupled Dynabeads™ magnetic beads recover 60% and 100% of the nucleic acid target when 1.4x10^5 and 1.4x10^3 copies of M13 are spiked, respectively. The recovery rates obtained with Dynabeads™ Streptavidin C1 and T1 beads are lower than for the covalently coupled Dynabeads™ magnetic beads for both amounts of input material.

Figure 8. Covalently coupled Dynabeads™ magnetic beads, Dynabeads™ Streptavidin C1 and T1 beads are compared for the specific capture of the nucleic acid target.



Conclusions

- Dynabeads™ magnetic beads are effectively functionalized with a broad density of oligonucleotides.
- The DNA coupling process is highly reproducible.
- The resulting DNA-bead conjugates are stable over a variety of stresses.
- Dynabeads™ magnetic beads functionalized with oligonucleotides successfully capture specific nucleic acid targets by hybridization from liquid biopsies.
- The capture workflow adapts to various downstream applications, is fast and highly sensitive.
- The capture workflow is alcohol-free, automatable and scalable.

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