Predictable and automation-friendly isolation of genomic DNA using Dynabeads® SILANE

Ellen Kleveland*, Darren Ellis, and Marie Bosnes
Dynal® Bead Based Separations, Invitrogen Group
Ullernschausséen 52, NO-0379 Oslo, Norway

* To whom correspondence should be addressed: Ellen Kleveland, Product Manager, Nucleic Acid IVD & Biotech OEM, ellen.kleveland@invitrogen.com

Introduction

Technologies for nucleic acid separation have evolved. The use of automated systems allows for more robust, systematic, and efficient capture of nucleic acids, reducing labor time and using less reagents.

Invitrogen has developed a new product for automation-friendly and highly predictable isolation of pure and intact genomic DNA (gDNA). The Dynabeads® SILANE genomic DNA kit offers enhanced performance beyond the capabilities offered by alternative magnetic separation systems. The protocol is rapid, reliable, and well-suited for automated molecular assays. The product has been developed to meet the high requirements for solid-phase sample preparation for diagnostic and biotech OEM customers.

Dynabeads® magnetic separation technology

By pioneering biomagnetic separation technology in the 1980s, Dynal revolutionized separation methodologies. Magnetic particles from other suppliers often have a random size range distribution, surface area, and binding capacity (Figures 1A and 1B). This variability can compromise the reproducibility of your assay results.

Dynabeads® are manufactured under validated and highly controlled, reproducible production processes. The resulting magnetic beads have a uniform size and have defined silica-like surface characteristics (Figures 1C and 1D) that will ensure optimal reproducibility, capacity, and performance in your automated assays.

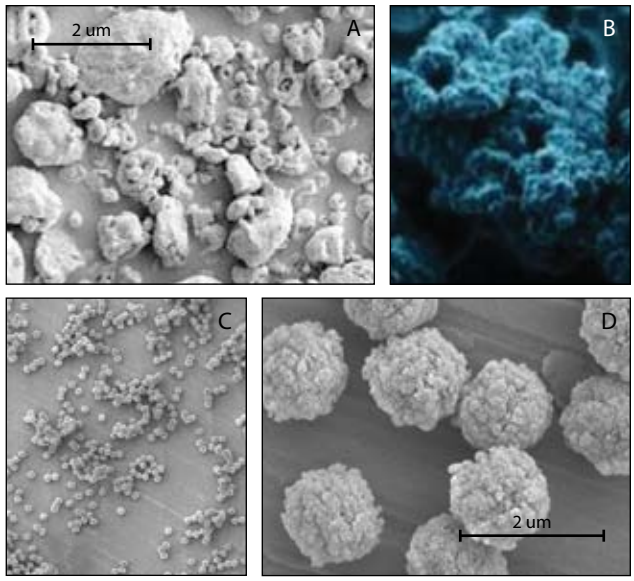


Figure 1—No compromises on reproducibility. The random size of magnetic particles from other suppliers (A, B) can compromise the reproducibility of your assay results. Dynabeads® MyOne™ SILANE (C, D) are monosized, 1-µm magnetic beads with a large surface area, providing highly reproducible results.

Materials and methods

Product description: The Dynabeads® SILANE genomic DNA kit (Cat. no. 370-12D) contains Dynabeads® MyOne™ SILANE (5 ml, 40 mg/ml), binding/washing/elution buffers, and a protocol optimized for isolation of gDNA from human whole blood. One isolation using 50 µl (2 mg) of beads will typically isolate 10 µg gDNA from a 350 µl blood sample. The kit contains reagents sufficient for 96 isolations.

Dynabeads® MyOne™ SILANE are uniform, monosized magnetic beads, 1 µm in diameter. They are composed of highly cross-linked polystyrene with evenly distributed magnetic material. Their optimized silica-like surface chemistry and high specific surface area provide efficient kinetics and excellent binding capacity. The beads have increased magnetic strength compared to the main Dynabeads® portfolio. This ensures rapid magnetic mobility and efficient isolation of viscous gDNA. They also feature a low sedimentation rate and favorable reaction kinetics, making them particularly well-suited for automated liquid handling.

Protocol description: The Dynabeads® SILANE genomic DNA kit provides an excellent tool for automated gDNA isolation, following a simple and scalable protocol (Figure 2). A buffer is first added to the sample for lysis, followed by incubation with Dynabeads® MyOne™ SILANE. When placed in a magnetic field, the beads with bound gDNA are pulled to the side of the tube and unbound material removed by aspiration. Magnetic separation facilitates simple washing and elution of the isolated gDNA. Dynabeads® protocols are flexible, scalable, and easily adapted to automated liquid handling platforms.

Dynabeads® MyOne™ SILANE are chemically stable and can be used with a variety of buffer systems. The best isolation results are achieved with the buffers supplied in the kit (results not shown). Another set of buffers has been developed for highly sensitive isolation of viral DNA/RNA, and are part of the Dynabeads® SILANE viral NA kit (Cat. no. 370-11D). Please contact Invitrogen if you would like to discuss specific application and system developments, validation, or a potential OEM arrangement.

Results

Highly predictable and consistent yields of pure and intact gDNA can be isolated from both fresh and frozen blood samples, and with a wide range of anti-coagulants. The purity of the isolated gDNA is excellent (Figures 3, 5 and 6) and the yield is directly proportional to the amount of gDNA (i.e., number of white blood cells (WBC)) present in the sample (Figure 4A). As magnetic separation is gentle with minimal shearing, the isolated gDNA is of high integrity (Figure 4B). Dynabeads® secure a high level of reproducibility and optimal performance in automated liquid handling (Figures 4A, 5, and 6).

The results shown demonstrate how Dynabeads® MyOne™ SILANE will improve gDNA sample preparation. The data presented are from model systems testing bead functionality, and do not reflect the results of an optimized automated molecular assay.

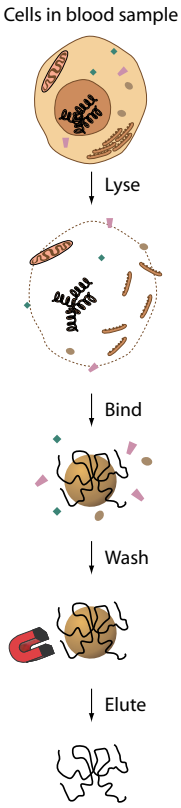
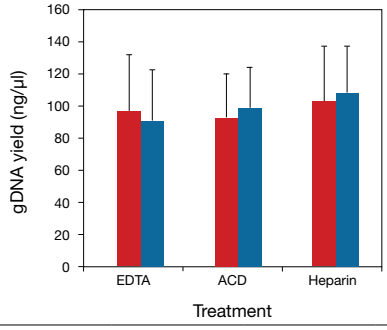


Figure 2—Quick and automated magnetic separation protocol. The illustration shows the simple steps (lyse, bind, wash, and elute) of the protocol for isolation of gDNA from human blood using the Dynabeads® SILANE genomic DNA kit. For a complete protocol, refer to the package insert.



Anti-coagulant	EDTA	ACD	Heparin
A_{260}/A_{280} (fresh)	1.78	1.76	1.74
A_{260}/A_{280} (frozen)	1.79	1.78	1.79
A_{260}/A_{230} (fresh)	1.35	1.29	1.26
A_{260}/A_{230} (frozen)	1.21	1.34	1.30

Figure 3—High purity and yields of gDNA from both fresh and frozen blood with a variety of anti-coagulants. The gDNA was isolated from 6 different blood samples containing EDTA, 6 samples with ACD, and 6 samples with heparin anti-coagulant (350 µl). Both fresh (red) and frozen (blue) parallels of each sample were analyzed. The purified gDNA was eluted in a final volume of 100 µl and the concentration (ng/µl) and purity (A_{260}/A_{280} and A_{260}/A_{230}) were measured spectrophotometrically. The values are comparable for all, irrespective of the anti-coagulant added. Error bars reflect the biological variation between blood samples (i.e. WBC).

Technical tips for automated gDNA isolation from blood

Preparations

- Choice of anti-coagulant: If possible, use ACD or heparin, as EDTA pellets tend to be fluffier and require longer elution times.
- All reagents should be at room temperature prior to use.
- Required gDNA yield: The automated procedure should be set up to use the minimum blood volume for your downstream application. Lower blood volumes will improve the handling performance of the system (pellets easier to handle and resuspend). If the blood input volume is decreased, the buffer volumes used should still remain the same.

Lyse and bind

- The gDNA adsorbed to the beads is not subject to normal shear forces and can be vortexed/pipetted safely.
- The first magnetic separation step is the most challenging, as the lysed blood preparation is both viscous and opaque. Allow extra time (2–3 min) for the beads to collect on the magnet.
- The gDNA and beads form a gelatinous pellet which will easily dissociate from the tube walls. Pipette slowly to remove supernatant. Use wide-bore tips to prevent blockage of pellets.

Wash

- The first magnetic separation will remove most of the viscous, unwanted blood components, permitting reduced time on the magnet and increased pipetting speeds.
- The gelatinous DNA-bead complex is quite robust and can be handled without risk of damaging or shearing the DNA. This pellet needs to be thoroughly washed throughout the procedure, but it is not necessary to break the pellet up or to resuspend the pellet to form a totally homogenous suspension.
- Carefully remove the alcohol-containing wash buffer and allow all residues to evaporate before elution.

Elute

- Elution from the bead pellet is easier if the DNA-bead complex is first allowed to 'soften' in elution buffer for a few minutes. Then, pipette slowly back-and-forth for several more minutes before removing the beads by magnetic separation.

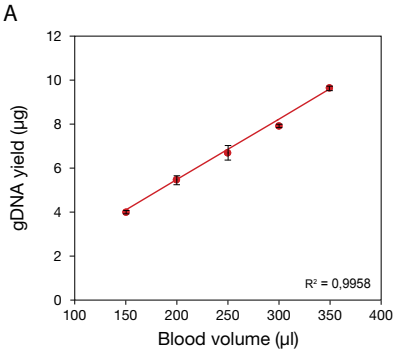


Figure 4—Linear yields of high-integrity gDNA relative to WBC counts. A. gDNA was isolated from varying amounts of human whole blood (150–350 µl) containing a known number of WBC. **B.** The isolated gDNA is of high integrity. 10 µl (1/10th) of the gDNA isolated from different starting volumes of blood (100–350 µl), is loaded onto the gel.

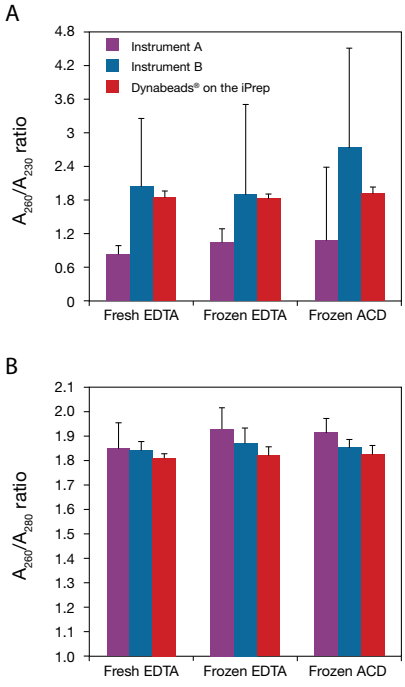


Figure 6—Better purity and reproducibility with Dynabeads®. gDNA was isolated from fresh and frozen blood samples containing different anti-coagulants (EDTA or ACD), following the respective suppliers' automated protocols. The Dynabeads® isolation protocol was run on the Invitrogen iPrep™ instrument. **A.** The A_{260}/A_{230} purity with Dynabeads® (red) is consistently above 1.2, and shows a higher level of reproducibility compared to the other systems tested (blue and purple). **B.** Comparable results are achieved for A_{260}/A_{280} purity.

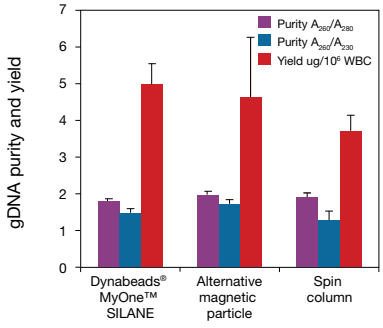


Figure 5—Benchmarking yield and purity. gDNA was isolated from whole blood following respective suppliers' protocols. Dynabeads® and the alternative magnetic particles provide comparable high yields (µg/10^6 WBC). A higher level of reproducibility for both yield and purity is achieved with Dynabeads®.

Summary

Consistent and highly reproducible

Dynabeads® MyOne™ SILANE hold reputable Dynal® high standards with respect to intra- and inter-lot reproducibility. This ensures:

- Absolute consistency that reduces assay variability
- Linear range of gDNA yield relative to WBC count
- Highly predictable binding per mg of beads
- High integrity and purity of isolated gDNA
- Highly consistent range and capacity
- Successful isolation from fresh or frozen blood with different anticoagulants
- Quicker and more cost-effective processing, yet performs to the same level as spin columns
- Automation-friendly protocols (slow sedimentation rate and high magnetic mobility)

Ordering information

Product	Quantity*	Cat. no.
Dynabeads® SILANE genomic DNA Contains Dynabeads® MyOne™ SILANE and specific buffers optimized for predictable isolation of gDNA from human whole blood.	96 isolations	370-12D
Related products		
Dynabeads® MyOne™ SILANE Manufactured under validated production processes. Can be supplied in bulk quantities.	5 ml (40 mg/ml)	370-02D
Dynabeads® SILANE viral NA Contains Dynabeads® MyOne™ SILANE and specific buffers optimized for sensitive isolation of viral DNA/RNA from human serum/plasma samples.	96 isolations	370-11D
iPrep™ PureLink™ gDNA Blood Kit For purification of gDNA from human blood using the iPrep™ Purification Instrument.	52 isolations	IS-10005

*Alternative product formats can be made available.

Dynabeads® for specific capture of nucleic acids

A comprehensive range of Dynabeads® for specific capture of nucleic acids is also available, across different bead sizes and surface functionalities. Some Dynabeads® are precoated with streptavidin, allowing for capture of biotinylated molecules in a wide variety of protocols. Other Dynabeads® have a specific surface chemistry for coupling of nucleic acids (e.g., hybridization probes/primers) and/or other ligands.

Custom development

Our strong assay development and immobilization competencies enable us to respond to our customers' needs, and work with them for validation and customization on an OEM level.

If you would like to discuss a potential collaboration or OEM agreement, please contact us at ivd@invitrogen.com.