



Size selection and PCR clean-up reagent Sera-Mag Select

Sera-Mag™ Select PCR clean-up and size selection reagent is based on well-known solid phase reversible immobilization technology, for selective binding of DNA fragments for applications such as next generation sequencing (NGS) and PCR clean-up. It combines the convenience of magnetic bead technology, using the exceptional binding characteristics of Sera-Mag Carboxyl Speedbeads with an optimized binding solution in a ready-to-use formulation.

Sera-Mag Select provides a flexible solution for DNA clean-up, recovery and size selection in one ready-to-use solution. By adjusting the amount of reagent that is mixed with a fixed volume of sample, the DNA size range isolated from the process can be adjusted to suit the user's requirements, from total recovery to narrow range size selection. As a general rule, the smaller the DNA fragment to be bound, the larger the volume of the reagent that should be added (relative to the initial sample volume). DNA of 100 bp size or greater can be reliably recovered using Sera-Mag Select, allowing effective removal of primers, nucleotides and adapters. Different modes of use are described in detailed in the [User Guide](#).

PCR clean-up

Sera-Mag Select can be used to clean up PCR products post-cycling, removing unused oligonucleotides, PCR buffer components and enzymes. By eluting into a smaller buffer volume than that of the input sample, the concentration of the eluted DNA can be increased.

A PCR product (1352 bp) was generated from lambda DNA template using 28 mer forward and reverse primers. This crude product was spiked with additional primers to a final concentration of 20 μ M to stress the purification. Clean-up was carried out by adding 80 μ l of Sera-Mag Select to 100 μ l of PCR product (0.8x ratio). After washing and drying, the 1352 bp PCR product was eluted into varying volumes of 1x TE buffer and analyzed by gel electrophoresis (Fig 1).



Using 0.8x ratio, we see effective removal of all the spiked-in primers from the PCR product, even when eluted in a small volume and loaded on the gel. By choosing the correct ratio, this left-sided purification mode is ideally suited to removing unused primers, nucleotides and adapters.

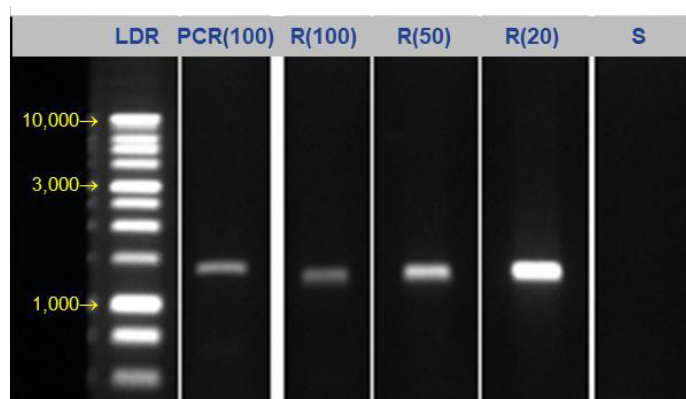


Fig 1. Spiked PCRs (100 μ l) were mixed with 80 μ l of Sera-Mag Select reagent and processed as described in the protocol: Lane PCR(100) shows PCR with excess primer; Lanes R(x) show elution at 100 μ l, 50 μ l and 20 μ l volumes.

DNA recovery

Recovery can be affected by the ratios of reagent used. We recommend optimizing ratios prior to implementation into new workflows for optimal results. When effective ratios have been established, there should be no need to change from run to run. Sera-Mag Select gives reproducible results each time.

To show recovery performance, a commercial 250 bp DNA fragment was bound using Sera-Mag Select and following elution, recovery was calculated as a percent of input.

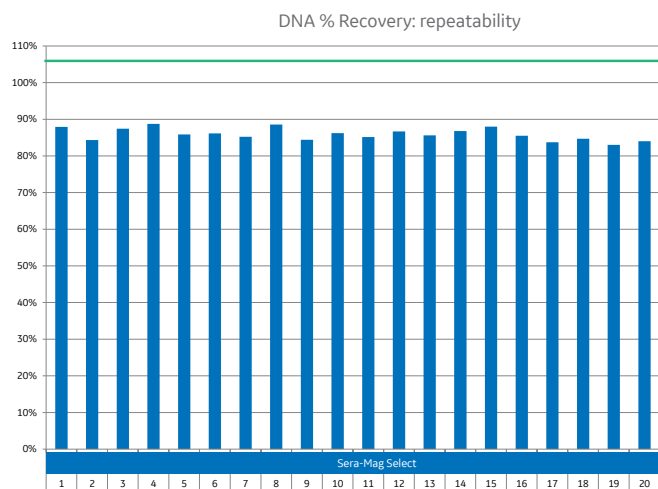


Fig 2. 20 replicates of a 250bp DNA fragment were bound and recovered using Sera-Mag Select reagent. Resulting samples were analyzed by Qubit dsDNA HS Assay.

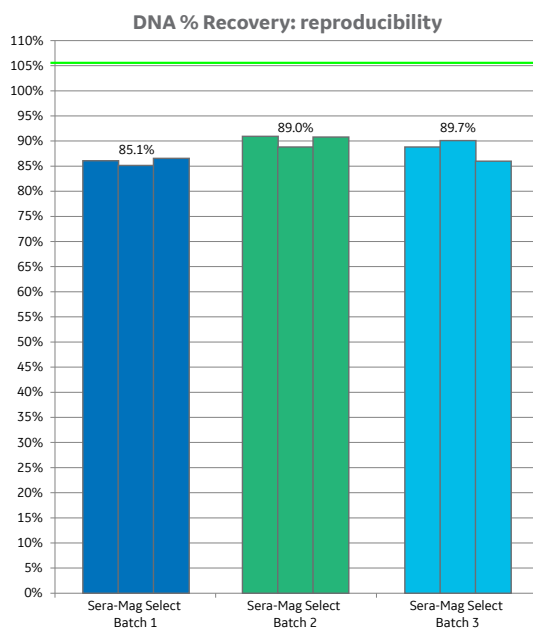


Fig 3. Three different batches of Sera-Mag select were each used to bind and recover three replicates of a 250 bp DNA fragment. Resulting samples were analyzed by Qubit dsDNA HS Assay.

Multiple aliquots (50 μ l) of 250 bp DNA fragment were individually purified with Sera-Mag Select. DNA was bound using 1.5x ratio (75 μ l reagent) and processed otherwise according to the standard recovery protocol. Recovered fragments were analyzed by Qubit (ThermoFisher Scientific) (Fig 2).

The level of recovery across the multiple replicates using Sera-Mag Select is consistent and shows excellent repeatability for recovery of a 250 bp DNA fragment.

To demonstrate batch to batch consistency we tested three different batches of Sera-Mag Select for recovery efficiency, again using a 250 bp DNA fragment to determine reproducibility between batches.

Three replicates (50 μ l) of 250bp DNA fragment were individually purified with each of three different batches of Sera-Mag Select. DNA was bound using 1.5x ratio (75 μ l reagent) and processed otherwise according to the standard recovery protocol. Recovered fragments were analyzed by Qubit (Fig 3).

From discreet testing of three different batches of Sera-Mag Select, it can be seen that DNA recovery is reproducible between batches, providing reliable performance time after time.

Dual-sided size selection

In addition to effective PCR clean-up, good size selection is critical in many library prep workflows for next generation sequencing (NGS). The ability to successfully select a narrow range of sizes from a fragmented DNA sample improves sequencing efficiency, with better quality data as a result. By choosing the correct ratios of reagent to sample, the size selection can be optimized around a given target size, by removal of higher molecular weight DNA in a first round, followed by removal of lower molecular weight DNA in the second round.

Human genomic DNA was fragmented using NEBNext™ dsDNA Fragmentase™ (New England Biolabs) and 100 ng of fragmented DNA was subject to dual-sided size selection with each of three different reagent ratios (Table 1). Recovered DNA was eluted in 50 μ l TE and 1 μ l was run on Agilent™ Bioanalyzer™ 2100 using a High Sensitivity DNA Chip (Fig 4).

Table 1. Left- and right-sided ratios of Sera-Mag Select used to prepare high (HI), medium (MED) and low (LO) DNA fragment sizes.

Size selection reagent applied levels			
Size range	Round 1 (left-sided)	Round 2 (right-sided)	Final ratio of Sera-Mag Select
HI	0.40x	0.10x	0.50x
MED	0.50x	0.15x	0.65x
LO	0.65x	0.35x	1.00x

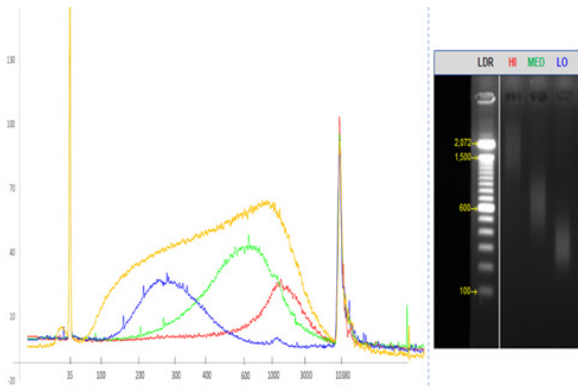


Fig 4. Three different size selections, high (**HI**), medium (**MED**) and low (**LO**) isolated from fragmented genomic DNA using three different ratios of Sera-Mag Select. Input sample is included in (**yellow**). Analysis was by Qubit (left) and gel (right).

Result: (Input sample reference trace in **orange**).

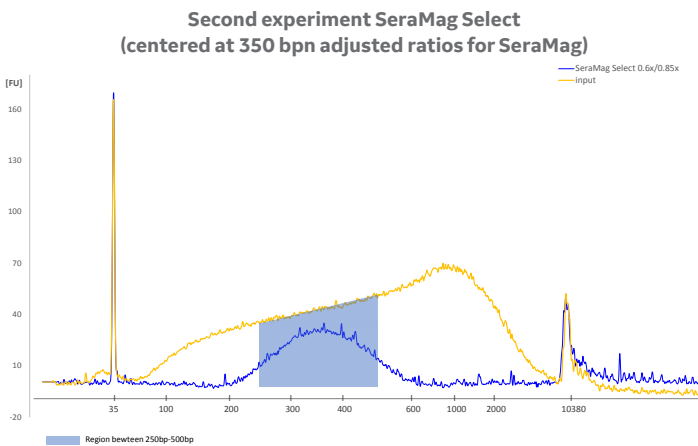


Fig 5. Dual size selection of DNA fragments with Sera-Mag Select (**blue**) centered on 350 bp isolated from fragmented genomic DNA and analyzed with Agilent Bioanalyzer 2100 using a High Sensitivity DNA Chip.

By controlling the reagent volume in relation to sample volume, it is clear that size selection can be optimized to meet the needs of the user. Increasing the amount of Sera-Mag Select reagent that is added in the first round, followed by a higher final ratio generated by the second round lowers the average size recovered.

To further investigate performance, fragmented human genomic DNA input samples were subject to processing to isolate target fragment sizes centered around 350 bp following the protocols supplied with the products.

Human genomic DNA was fragmented with NEBNext™ dsDNA Fragmentase™ (New England Biolabs) and 100 ng of fragmented DNA (50 µl) was subject to dual-sided size selection with given ratios of reagent volume to input sample volume (Table 2). Recovered DNA was run on Agilent Bioanalyzer 2100 using a High Sensitivity DNA Chip (Figure 5).

Table 2. Relative ratios of size selection reagents used to isolate 350 bp range from fragmented human genomic DNA.

Size selection reagent applied levels			
Size selection chemistry	Round 1 (left-sided)	Round 2 (right-sided)	Final ratio of Sera-Mag Select
Sera-Mag Select	0.60x	0.25x	0.85x

In summary, Sera-Mag Select provides a single solution for both PCR clean-up and DNA size selection, with the convenience and proven performance of Sera-Mag Carboxyl Speedbeads magnetic particles. We have demonstrated the effective removal of oligonucleotides from PCR product and have shown DNA recovery and size selection performance in line with industry requirements.



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Ordering information

Product	Code	USD	Euro	GBP	Yen	RMD
Sera-Mag Select 5 ml	29343045	\$217	204 euro	£185	27,700 Yen	1,488 CNY
Sera-Mag Select 60 ml	29343052	\$519	487 euro	£441	66,300 Yen	3,559 CNY
Sera-Mag Select 450 ml	29343057	\$2,950	2,768 euro	£2,502	376,600 Yen	20,300 CNY

Related products

Product	Description	Product code
illustra™ GenomiPhi™ V2 DNA Amplification Kit	25 reactions	25660030
illustra GenomiPhi V2 DNA Amplification Kit	100 reactions	25660031
illustra GenomiPhi V2 DNA Amplification Kit	500 reactions	25660032
illustra Ready-To-Go™ GenomiPhi V3 DNA Amplification Kit	24 reactions	29013586
illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit	96 reactions	29013587
illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit	480 reactions	29013588
illustra Single Cell GenomiPhi DNA Amplification Kit	25 reactions	29108107
illustra Single Cell GenomiPhi DNA Amplification Kit	100 reactions	29108039

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GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden

GE Healthcare Europe, GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752 USA

GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073 Japan

For local office contact information, visit www.gelifesciences.com/contact