Sera-Mag Select

SIZE SELECTION AND PCR CLEAN-UP REAGENT

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 polymerase chain reaction (PCR) PCR clean-up (Fig 1). 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 detail 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 µM to stress the purification. Clean-up was carried out by adding 80 µL of Sera-Mag Select to 100 µL 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 2).

Fig 1. Sera-Mag Select PCR clean-up and size selection reagent is based on reversible immobilization technology for selective binding of DNA fragments.

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.

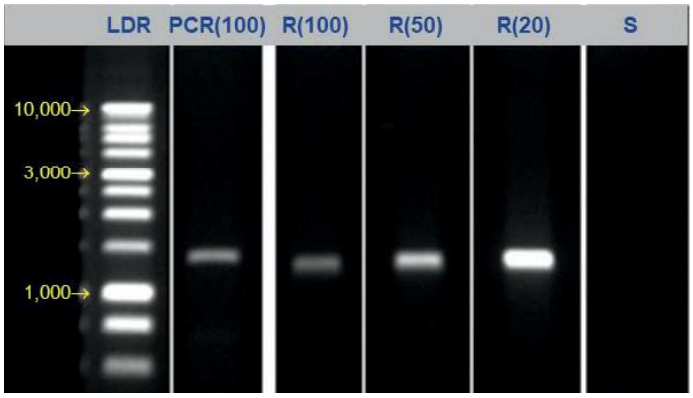

Fig 2. Spiked PCRs (100 µL) were mixed with 80 µL of Sera-Mag Select reagent and processed as described in the protocol: Lane PCR(100) shows PCR with excess primer; Lanes R(x) show elution at 100 µL, 50 µL and 20 µL volumes.

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.

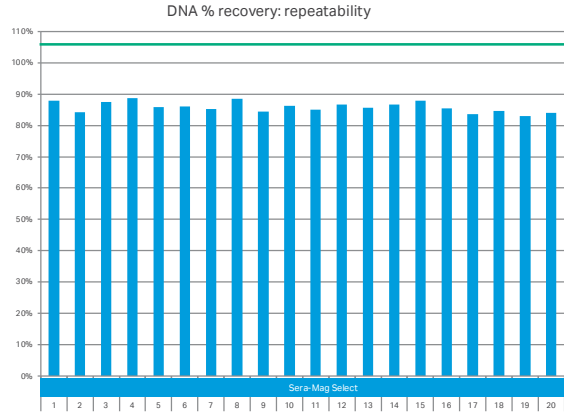

Fig 3. 20 replicates of a 250 bp DNA fragment were bound and recovered using Sera-Mag Select reagent. Resulting samples were analyzed by Qubit dsDNA HS Assay.

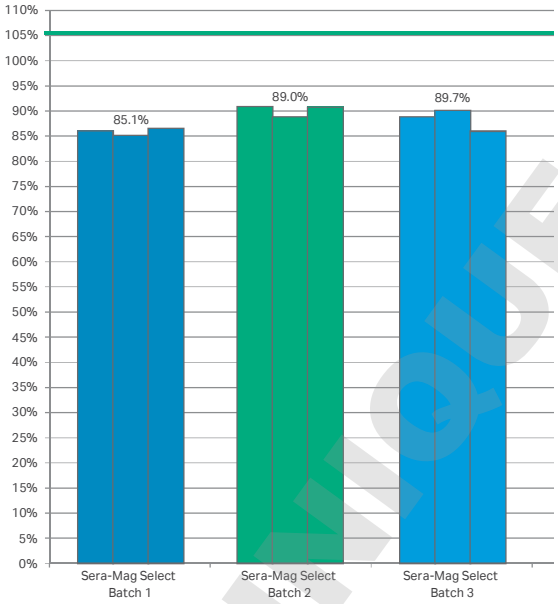

Fig 4. Three different batches of Sera-Mag select were each used to bind and recover three replicates of a 250 bp DNA fragment. Resulting samples were analyzed by Qubit dsDNA HS Assay.

Multiple aliquots (50 µL) of 250 bp DNA fragment were individually purified with Sera-Mag Select. DNA was bound using 1.5x ratio (75 µL reagent) and processed otherwise according to the standard recovery protocol. Recovered fragments were analyzed by Qubit™ (Thermo Fisher Scientific) (Fig 3).

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 µL) 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 µL reagent) and processed otherwise according to the standard recovery protocol. Recovered fragments were analyzed by Qubit (Fig 4).

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 / NEB) 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 µL TE and 1 µL was run on Bioanalyzer™ (Agilent™) 2100 using a High Sensitivity DNA Chip (Fig 5).

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 (right-sided)**	**Round 2 (left-sided)**
HI	0.40x	0.50x
MED	0.50x	0.65x
LO	0.65x	1.00x

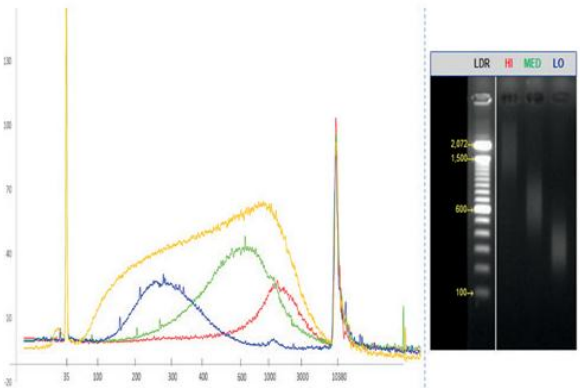

Fig 5. Three different size selections, high (**HI**), medium (**MED**) and low (**LO**) isolated from fragmented genomic DNA using three different ratios of Sera-Mag Select. Input sample is included in (**yellow**). Analysis was by Bioanalyzer and gel electrophoresis (left and right respectively).

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

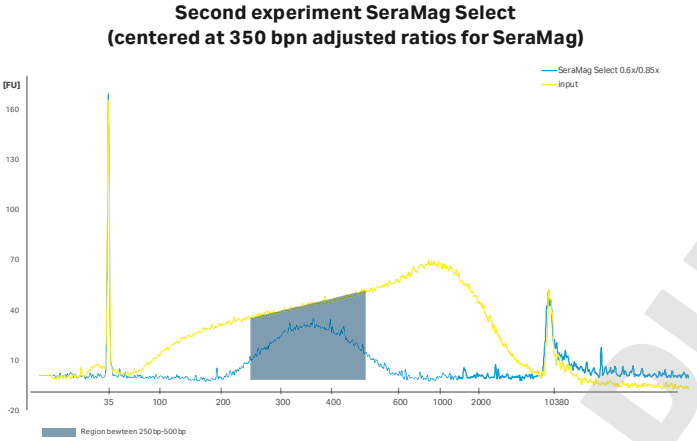

Fig 6. Dual size selection of DNA fragments with Sera-Mag Select (**blue**) centered on 350 bp isolated from fragmented genomic DNA and analyzed with Bioanalyzer 2100 using High Sensitivity DNA Chip (Agilent).

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 (NEB) 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 Bioanalyzer 2100 using High Sensitivity DNA Chip (Figure 6).

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 (right-sided)**	**Round 2 (left-sided)**
Sera-Mag Select	0.60x	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.

Batch to batch consistency

When implementing a new solution into any NGS workflow, reproducibility and reliability are vital considerations to deliver consistency to every operation, every time. To remove these concerns, production and quality control of Sera-Mag Select has been designed to ensure process variation is kept to a minimum. To demonstrate batch to batch reproducibility in dual size selection, four different batches of Sera-Mag Select were used to perform size selection on fragmented gDNA. The results were compared (Fig 7).

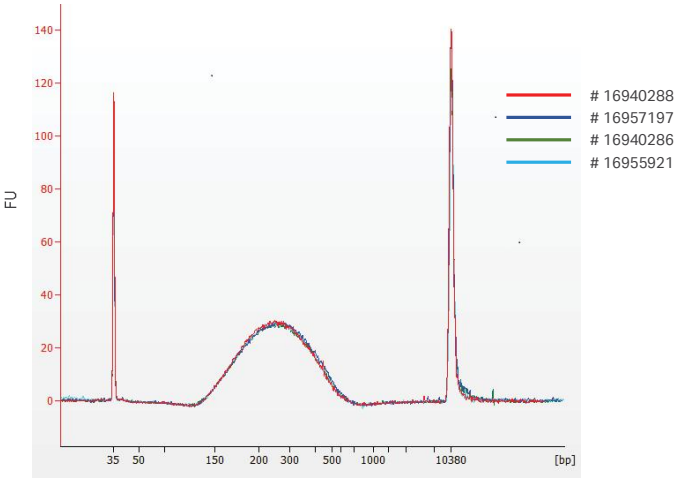

Fig 7. Dual size selection of fragmented DNA; Sera-Mag Select™ batch to batch comparison.

Human genomic DNA was fragmented using NEBNext dsDNA Fragmentase and 100ng of fragmented DNA (100 µL in TE) was subjected to dual side size selection centred around 300bp using four different manufacturing batches of Sera-Mag Select. Briefly, the beads were mixed with the sample at 0.575x sample volume by vortexing and incubated for 10 mins at room temperature. The beads were allowed to settle down on the magnet and the supernatant was retained and subjected to the second round of size selection using a total ratio of 0.9x. Following 10 mins incubation at room temperature, the beads were allowed to settle down on the magnet and the supernatant was discarded. The beads were washed twice with 85% ethanol and dried for 5 mins at room temperature. The DNA was eluted using 50 µL of TE buffer following 10 mins incubation at room temperature and 1 µL was run on Bioanalyzer using High Sensitivity DNA Chip.

In summary, for each of the four batches of Sera-Mag Select tested, size distribution of DNA fragments from each size selection are shown to overlap, demonstrating a very high level of consistency from batch to batch.

Benchmarking

Sera-Mag Select was tested with several alternative products in extensive side-by-side studies to clearly demonstrate performance in clean-up and size selection (Fig 8–11).

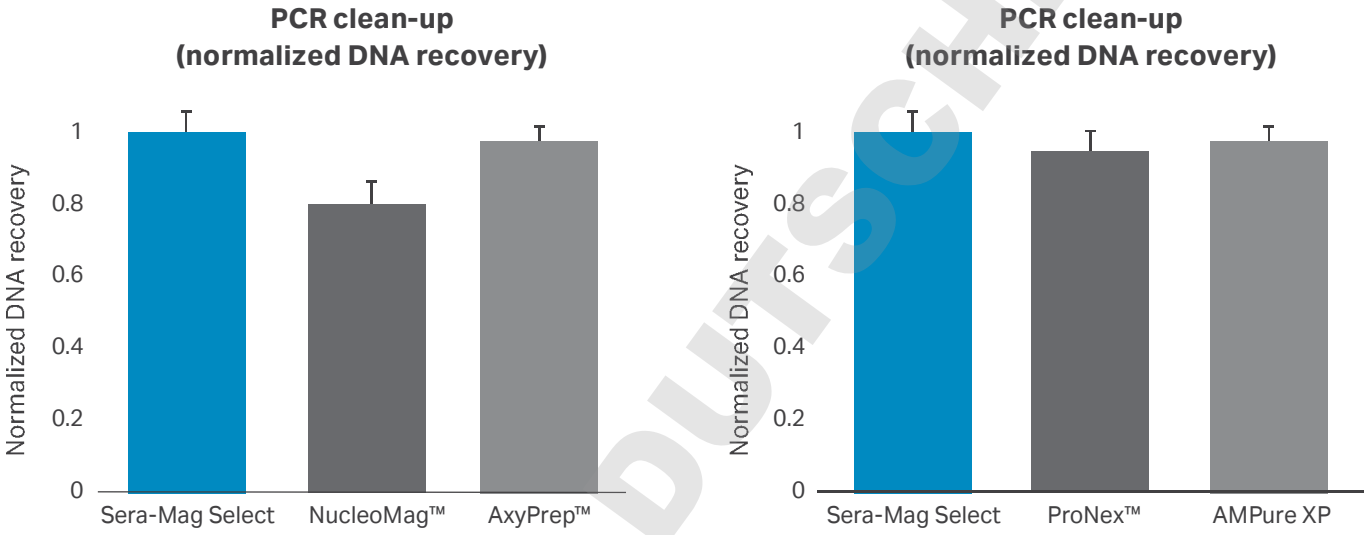

Fig 8. Total DNA recovery in PCR clean-up.

PCR reaction mix (50 µL) containing 250bp DNA fragment was subjected to DNA clean-up. Briefly, Sera-Mag Select was mixed with the sample at 1.5x sample volume by vortexing and incubated for 10 mins at room temperature. The beads were allowed to settle down on the magnet and the supernatant was discarded. The beads were washed twice with the wash buffer (70% ethanol, 20mM Tris pH 8.5, 50mM NaCl) and dried for 10 mins at 40°C. The DNA was eluted using 50 µL of TE buffer following 15 mins incubation at 40°C.

For NucleoMag (Macherey-Nagel), AxyPrep (Dutscher), ProNex (Promega Corporation) and AMPure XP (Beckman Coulter), PCR clean-up was performed performed following manufacturers' protocol. DNA was quantified using Qubit dsDNA HS Reagent. Graphs represent data obtained in three independent experiments normalised to Sera-Mag Select, error bars represent standard deviation (Fig 8). Statistical analysis was performed using one-way Anova with Dunnett's post-hoc test.

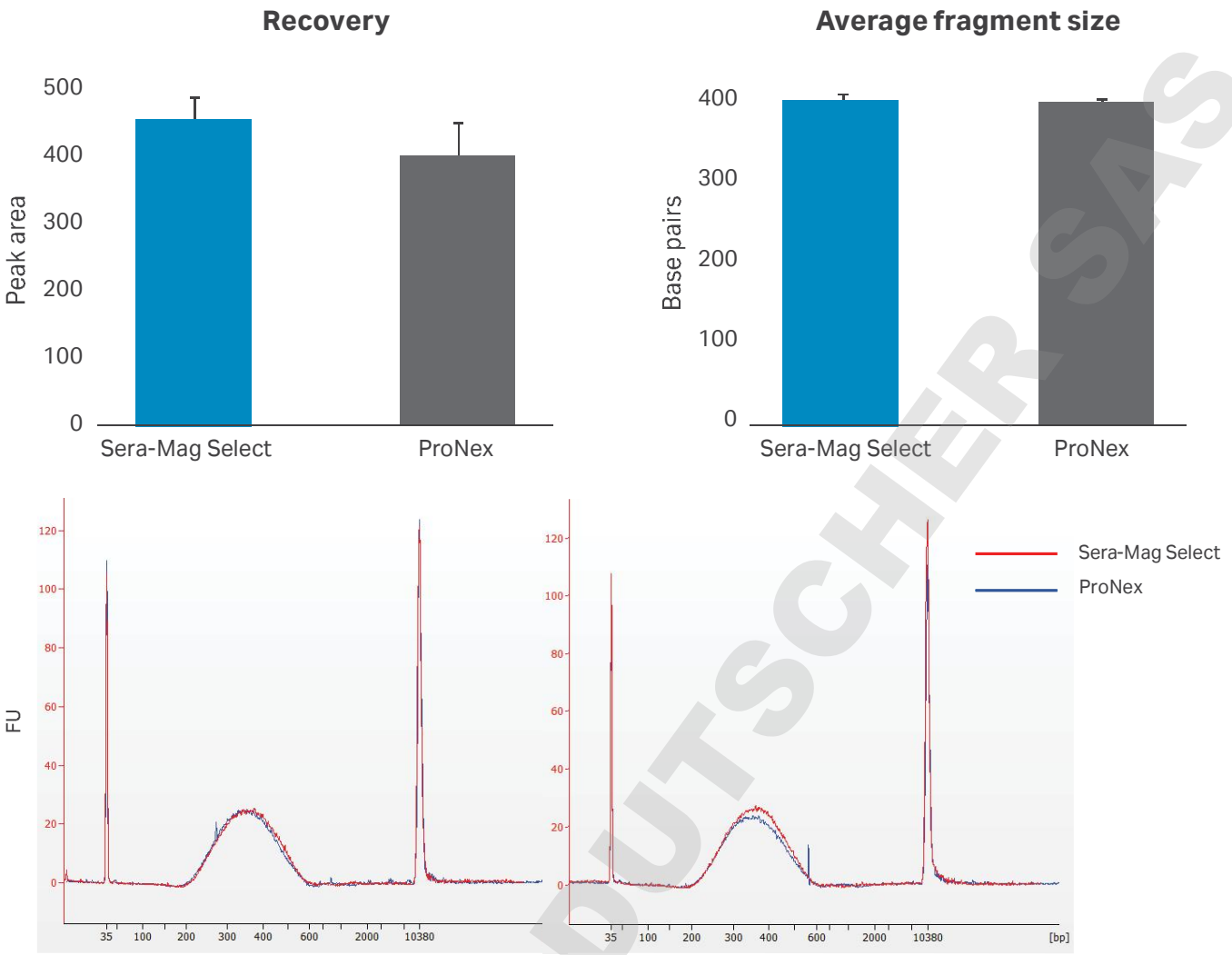

Fig 9. Dual size selection of fragmented DNA; Sera-Mag Select versus ProNex.

Human genomic DNA was fragmented using NEBNext dsDNA Fragmentase and 100ng of fragmented DNA (100 µL in TE) was subjected to dual side size selection centred around 350/400bp. Briefly, Sera-Mag Select was mixed with the sample at 0.585x sample volume by vortexing and incubated for 10 mins at room temperature. The beads were allowed to settle down on the magnet and the supernatant was retained and subjected to the second round of size selection using total ratio of 0.85x. Following 10 mins incubation at room temperature, the beads were allowed to settle down on the magnet and the supernatant was discarded. The beads were washed twice with the wash buffer (70% ethanol, 20mM Tris pH 8.5, 50mM NaCl) and dried for 10 mins at 40°C.

The DNA was eluted using 50 µL of TE buffer following 15 mins incubation at 40°C. The ratio of 1.1x/1.45x was used for ProNex as recommended by manufacturer's protocol. DNA recovery was calculated using Bioanalyzer smear analysis in the region between 200-800bp (peak area and average fragment size). Graphs represent data obtained in four independent experiments, error bars represent SEM. Statistical analysis was performed using paired 2-tailed Student's t-test (upper panel). Lower panel shows representative Bioanalyzer traces of size selected DNA using Sera-Mag Select and ProNex (in red and blue respectively) (Fig 9).

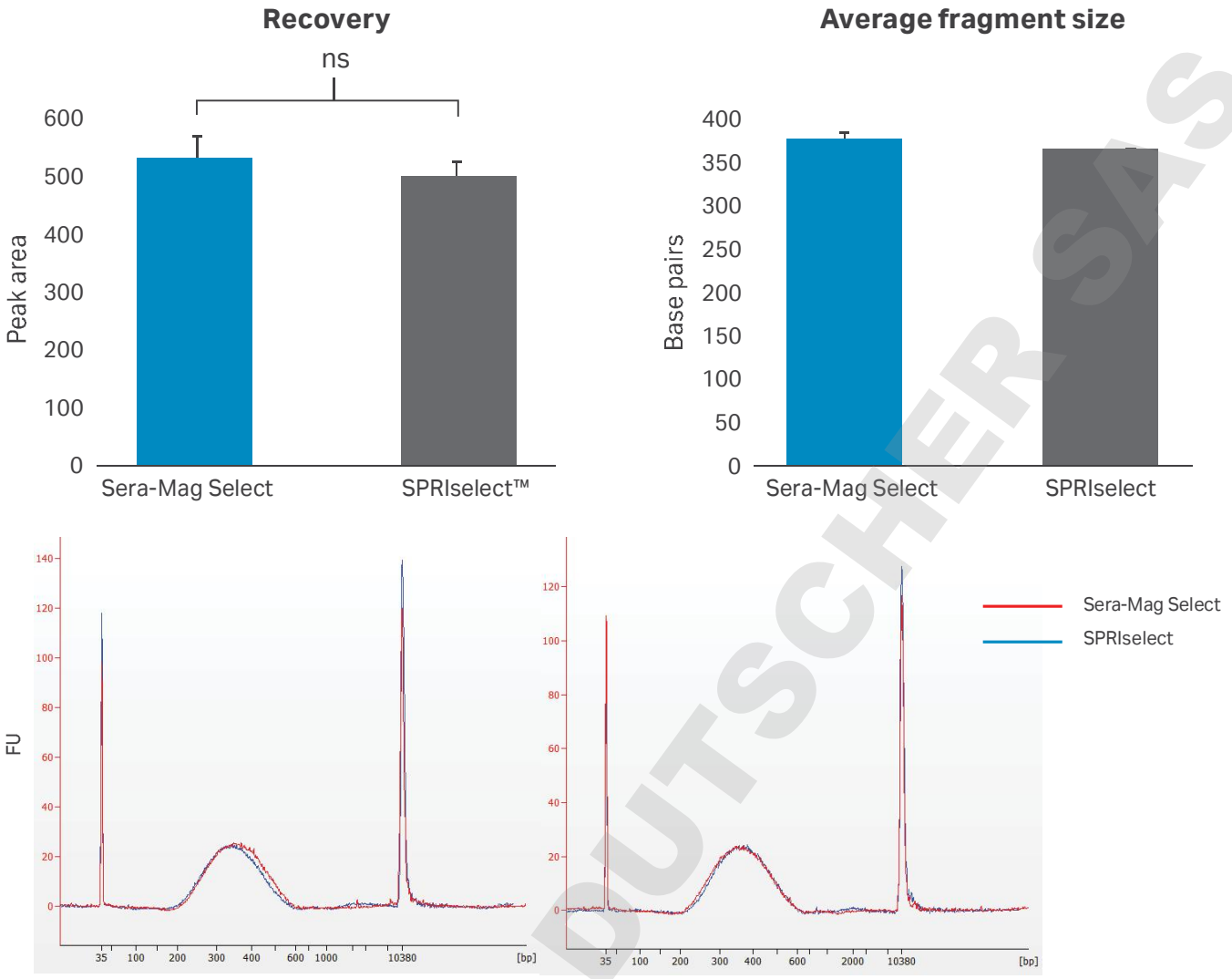

Fig 10. Dual size selection of fragmented DNA; Sera-Mag Select versus SPRIselect.

Human genomic DNA was fragmented using NEBNext dsDNA Fragmentase and 100ng of fragmented DNA (100 µL in TE) was subjected to dual side size selection centred around 350/400bp. Briefly, Sera-Mag Select was mixed with the sample at 0.6x sample volume by vortexing and incubated for 10 mins at room temperature. The beads were allowed to settle down on the magnet and the supernatant was retained and subjected to the second round of size selection using total ratio of 0.825x. Following 10 mins incubation at room temperature, the beads were allowed to settle down on the magnet and the supernatant was discarded. The beads were washed twice with 85% ethanol and dried for 5 mins at room temperature. The DNA was eluted using 50 µL of TE buffer following 10 mins incubation at room temperature. The ratio of 0.6x/81x was used for SPRIselect (Beckman Coulter) as recommended by manufacturer's protocol. DNA recovery was calculated using Bioanalyzer smear analysis in the region between 200–800bp (peak area and average fragment size). Graphs represent data obtained in five independent experiments, error bars represent SEM. Statistical analysis was performed using paired 2-tailed Student's t-test (upper panel). The lower panel shows representative Bioanalyzer traces of size selected DNA using Sera-Mag Select and SPRIselect (in red and blue respectively). (Fig 10).

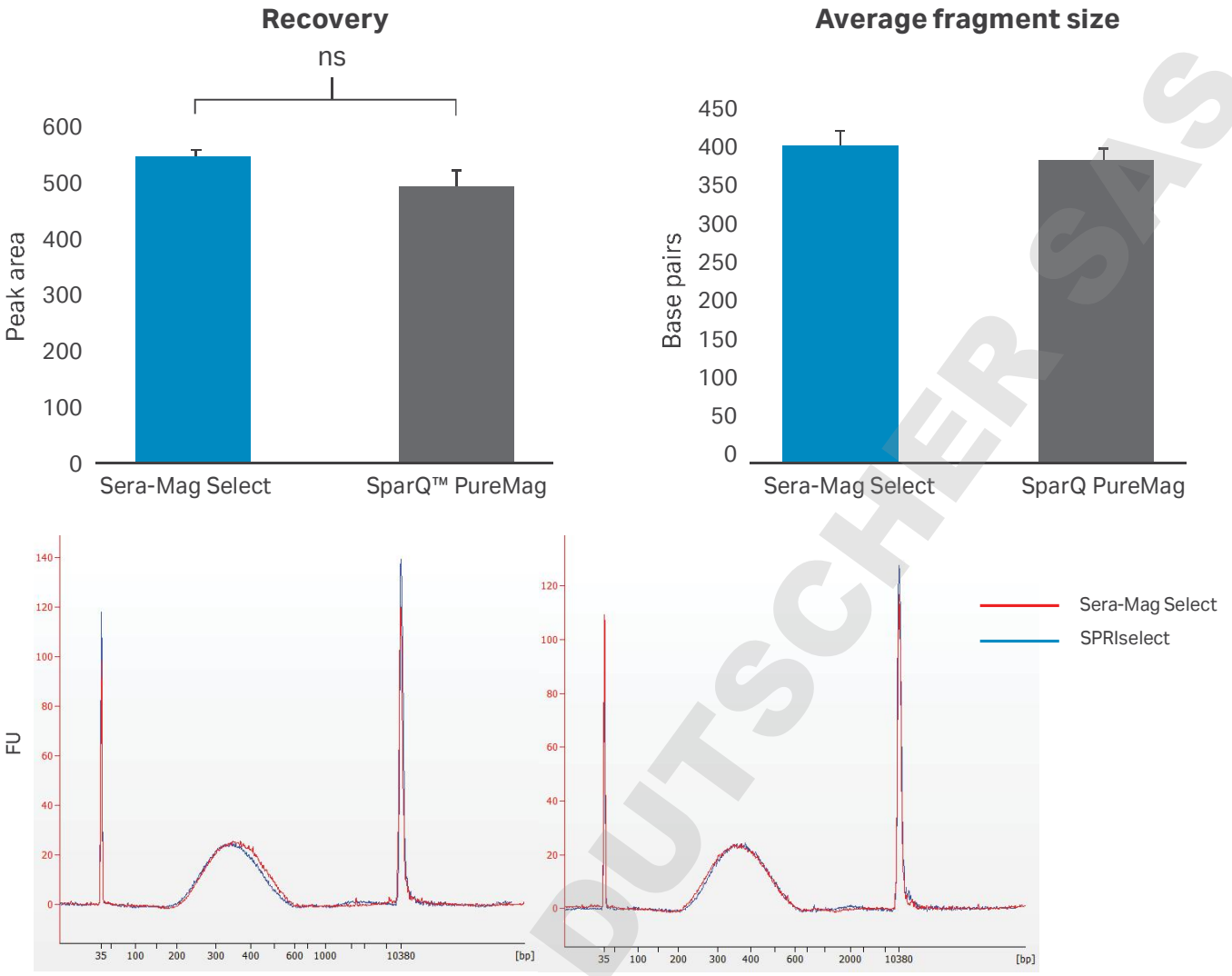

Fig 11. Dual size selection of fragmented DNA; Sera-Mag Select versus SparQ PureMag beads.

Human genomic DNA was fragmented using NEBNext dsDNA Fragmentase and 100ng of fragmented DNA (100 µL in TE) was subjected to dual side size selection centred around 350/400bp. Briefly, Sera-Mag Select was mixed with the sample at 0.57x sample volume by vortexing and incubated for 10 mins at room temperature. The beads were allowed to settle down on the magnet and the supernatant was retained and subjected to the second round of size selection using total ratio of 0.78x. Following 10 mins incubation at room temperature, the beads were allowed to settle down on the magnet and the supernatant was discarded. The beads were washed twice with 85% ethanol and dried for 5 mins at room temperature. The DNA was eluted using 50 µL of TE buffer following 10 mins incubation at room temperature. The ratio of 0.6x/8x was used for SparQ PureMag beads (Quantabio) as recommended by the manufacturer's protocol. DNA recovery was calculated using Bioanalyzer smear analysis in the region between 200–800bp (peak area and average fragment size). Graphs represent data obtained in seven independent experiments, error bars represent SEM. Statistical analysis was performed using paired 2-tailed Student's t-test (upper panel). Lower panel shows representative Bioanalyzer traces of size selected DNA using Sera-Mag Select and SparQ PureMag beads (in red and blue respectively) (Fig 11).

This data is based on a minimum three independent experiments / replicate trials including at least two replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol/recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during September–December 2018 and February-April 2019 (total DNA recovery and size selection respectively) and is held at this location.

Ordering information

Product	Pack size	Product code
Sera-Mag Select	5 mL	29343045
Sera-Mag Select	60 mL	29343052
Sera-Mag Select	450 mL	29343057

Related products

Product	Pack size	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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