BECKMAN COULTER
Life Sciences

Impact of Clean-Up Kits on DNA Sequencing Quality and Efficiency

The applications of DNA sequencing in biological research are growing. A significant driver of this growth is Next-Generation Sequencing (NGS), a modern DNA sequencing technology instrumental in achieving complete DNA sequences or genomes of humans, many animals, plants, and microbial species.

NGS can offer rapidity, reliability, and cost-effectiveness. However, these benefits also depend on the quality of the DNA being sequenced. Poor quality or inefficiency in the early stages of the sequencing process can mean additional costs of errors, rework, or waste later. When DNA sequencing drives targeted drug development, testing, and manufacturing, or the tracking and containment of dangerous viruses and epidemics, outcomes must not be put at risk through ill-advised shortcuts upstream.

Clean-up kits are a pivotal part of the NGS process. They have an immediate impact on efficiency and on quality, as well as key impacts downstream. Clearly, it is not worth jeopardizing entire DNA sequencing operations and more, simply to save pennies on the price per sample of cheaper but lower performance, poorer quality, and less efficient kits.

It is therefore critical to examine a DNA sequencing clean-up kit before selection and compare its overall performance with others, either when starting NGS operations or when considering changing to a different clean-up kit solution. While clean-up kit cost is always a factor, switching to cheaper or other products is not necessarily more advantageous and focusing exclusively on price can have highly undesirable consequences.

Factor 1 – Yield

The first and perhaps most obvious factor is percent yield, the final DNA concentration divided by the starting DNA concentration, multiplied by 100%. For optimal quality and efficiency, the percent yield should be between 80% to 95%. Lower yields may compromise downstream data quality. Not all clean-up kits can achieve consistently high yield (see Figure 1).

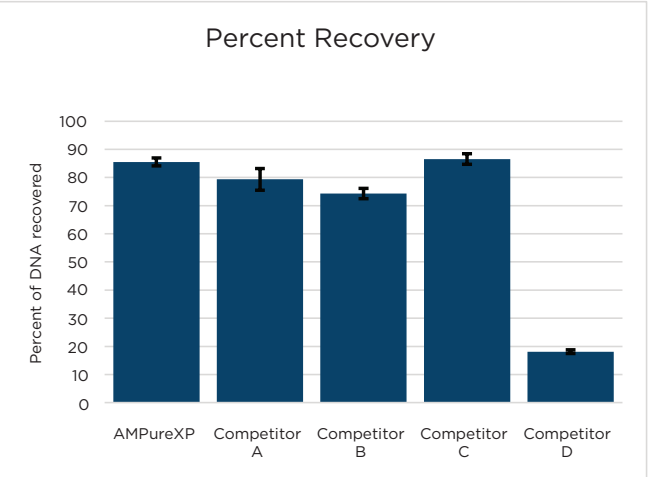


Figure 1

Factor 2 – Purity

Good quality DNA should have an A260/A280 ratio of at least 1.8. A lower rating indicates contaminating proteins. Similarly, an A260/A230 ratio of less than 2.0 indicates other contaminates.

Factor 3 – Time

Time is often the most valuable resource. Shorter clean-up times are better. A difference between 30 and 45 minutes per clean-up step (see figure 2) is multiplied each time an additional clean-up step is needed when running library construction kits. For example, 3 clean-up steps mean a difference between 1.5 hours and 2.25 hours. This difference is multiplied again as overall NGS activity increases.

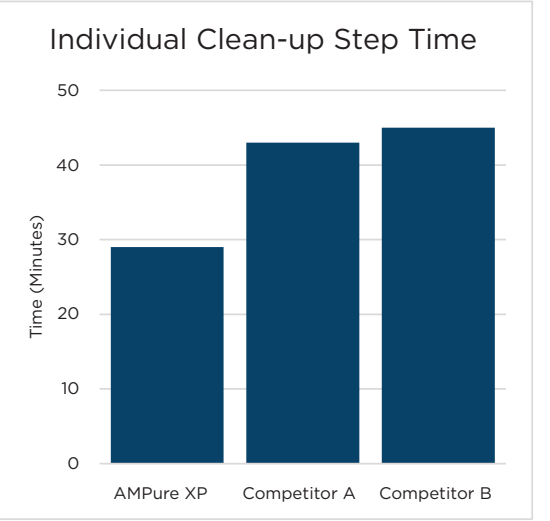
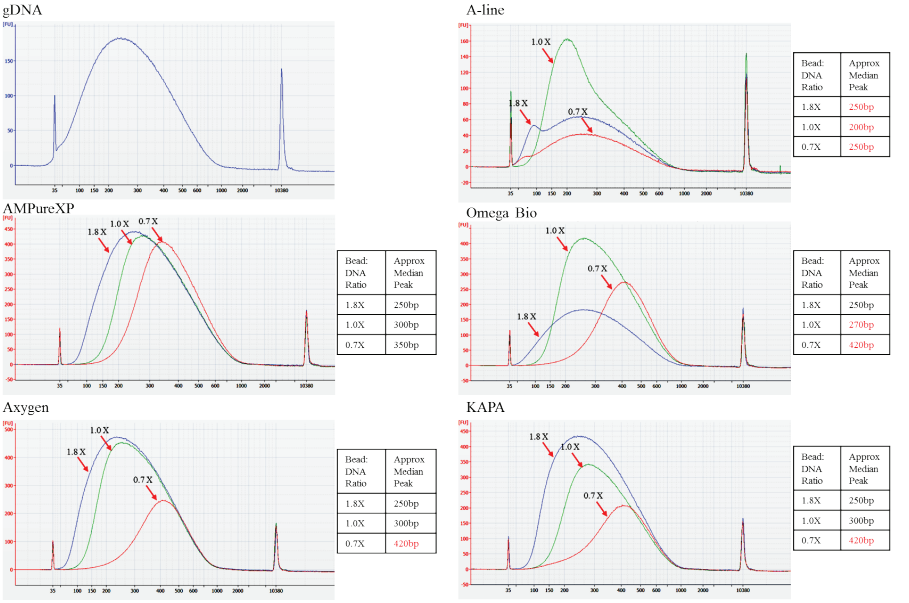


Figure 2

Factor 4 – Predictable Performance

Uniformity of DNA fragment size affects both quality and efficiency of DNA sequencing. Initial fragment size must be correctly targeted to allow for additional base pair (bp) length as adapters are added to fragments for sequencing. Ideally, fragment size concentrations will remain high and predictable as different binding ratios are used (see graph for AMPure XP in Figure 3). This is an important factor if a user is considering switching from one clean-up kit to another. Traces below show the relative differences in peak height and recovery (Figure 3).



Factor 5 – Risks of Switching

The example above of binding ratios giving different DNA fragment size concentrations for different clean-up kits is one risk of switching to another clean-up kit. Others are:

- The extra time and effort needed to evaluate a new clean-up kit
- The risk of having to redo sequencing later if the new product does not perform as well as the original product
- A library kit supplier withdrawing its support for resolving issues if used “off-label” with a clean-up product that was not recommended by that library kit supplier.

Conclusion

Cheap DNA sequencing clean-up kits may perform poorly and jeopardize the overall results of NGS. Risks include wasted time, suboptimal efficiency, and incomplete discovery.

By comparison, AMPure XP targets what really matters to businesses and organizations working with DNA sequencing: consistency and reliability of performance lot-to-lot, quality for manufacturing, and on-time delivery.

AMPure XP is recommended by 215 library construction kit manufacturers. The suppliers of these kits recommend AMPure XP often as the sole clean-up option. AMPure XP has also been cited in over 21,000 articles on Google Scholar.

Using AMPure XP as your clean-up kit, you can keep your sequencing projects on track and continue to provide the best results possible to your customers, patients and journals.

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