RNA cleanup and size selection using sparQ PureMag Beads

Keywords: sparQ PureMag Beads, cleanup, size selection, RNA, purification, RNase

ABSTRACT

Nucleic acid purification is a necessary step in almost all molecular biology applications. In addition to DNA and cDNA, RNA cleanup is required in a growing number of applications, such as RNA-seq, purification of probes and *in vitro* transcription experiments. The reversible nucleic acid binding properties of magnetic beads has been optimized in sparQ PureMag Beads and is widely validated for DNA purification. In this application note, we demonstrate that sparQ PureMag Beads are also a highly efficient and reproducible method for RNA cleanup and size selection, further validating their flexibility and performance.

INTRODUCTION

RNA purification is a key step in a wide variety of laboratory and clinical analysis. To provide relevant and reliable results, pure and intact molecules of RNA must be separated from a variety of mixtures. sparQ PureMag Beads use the reversible nucleic acid binding properties of magnetic beads for fast, efficient and flexible nucleic acid purification for multiple downstream applications. In particular, sparQ PureMag Beads can be used for the purification of RNA and cDNA from enzymatic reactions such as reverse transcription, *in vitro* transcription or during next generation sequencing (NGS) RNA-seq workflows. An important consideration in such workflows is that reagents are free from RNases that could degrade the RNA sample. In this application note, we assessed the efficiency of ssRNA cleanup with sparQ PureMag Beads, using a low range ladder as the template to assess the capture of different RNA fragment lengths. We then tested the stability of RNA in the sparQ PureMag bead solution, comparing results to a certified RNase-free bead-based purification product (RNAClean™ XP, Beckman Coulter).

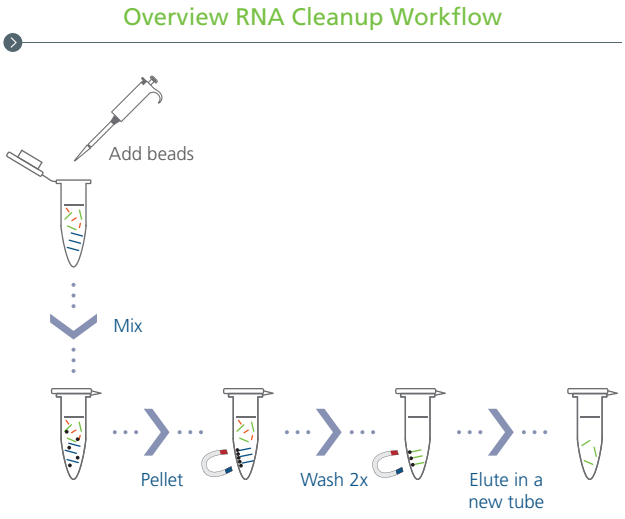

Figure 1 **Overview RNA cleanup workflow.** (1) Beads are added to the RNA sample and mixed. (2) The beads are pelleted and the supernatant discarded. (3) The pellet is held on the magnet and washed twice. (4) The RNA is eluted in RNase-free water.

MATERIALS AND METHODS

RNA cleanup

Low Range ssRNA Ladder (NEB, #N0364S) was diluted 1:50 and mixed with sparQ PureMag Beads (Quantabio) or RNAClean XP (Beckman Coulter). The mixture was incubated at room temperature for 5 minutes then the beads pelleted on a magnet. The supernatant was discarded and the bead-bound RNA washed twice with 200 µl 80% ethanol. The beads were air-dried then the RNA eluted in 20 µl RNase-free water.

Residual RNase testing (RNA stability)

Low Range ssRNA Ladder was diluted 1:50 and mixed with 1X sparQ PureMag Beads or RNAClean XP. The mixtures were incubated for 0 or 4 hours (h) at room temperature followed by RNA cleanup.

Saliva was used as a positive control and RNase-free water was used as a negative control, in place of beads. Following incubation at room temperature for 4 h, 1X sparQ PureMag Beads were added to control samples and RNA cleanup carried out.

RNA analysis

The quality of eluted RNA was visualized using RNA High Sensitivity Screen Tape on a 4200 TapeStation system (Agilent Technologies). RNA concentrations were measured using a Nanodrop™ 8000 spectrophotometer (Thermo Fisher Scientific).

RESULTS

RNA cleanup efficiency

High recovery of RNA during bead cleanup can be critical for workflows with limited input material. Two input concentrations of ssRNA ladder were purified using 1.8X sparQ PureMag Beads or RNAClean XP and the output concentration measured (Figure 2). Average percentage recovery was similar from the two bead types at both input RNA concentrations tested. For example with 500 ng input RNA, average recovery was 81% for purification with sparQ PureMag Beads and 77% with RNAClean XP. Therefore, sparQ PureMag Beads provide a highly efficient RNA cleanup method.

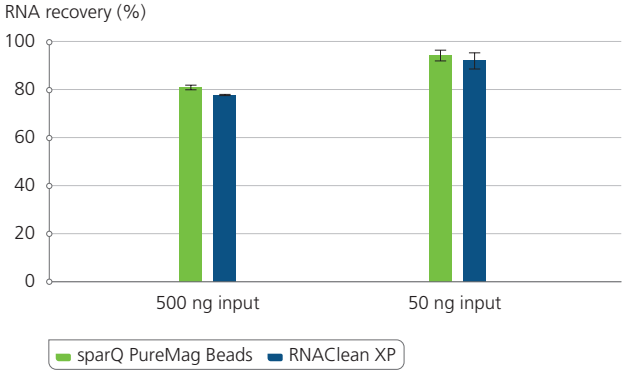

Figure 2 **RNA recovery efficiency.** 500 ng or 50 ng ssRNA ladder was purified using 1.8X sparQ PureMag Beads or RNAClean XP. RNA was quantified by Nanodrop. Bars show mean average ± s.d. of two experiments.

Size selection of RNA by sparQ PureMag Beads

During the bead-binding step, the concentration of PEG and NaCl in the solution determines the size of nucleic acid molecules that bind to the beads. Therefore, sparQ PureMag Beads could be used as a highly flexible method of fragment size-selection by altering the ratio of beads in the mixture.

50 µl aliquots of diluted ssRNA ladder were purified with 1X, 0.8X or 0.6X sparQ PureMag Beads and the eluted RNA compared to ssRNA ladder (Figure 3). TapeStation analysis revealed effective RNA size selection by alteration of bead to sample ratio. RNA cleanup with 1X or 0.8X beads removed short fragments less than 80 nucleotides (nt) and retained longer fragments. Cleanup with 0.6X beads resulted in more stringent size selection with removal of fragments less than 150 nt in length, however, this did result in an overall reduction in yield. Most importantly, all cleanup ratios provided high-quality RNA, with no visible sample degradation demonstrating that sparQ PureMag Beads enable effective size selection of RNA. Optimization with specific input RNA conditions or sample matrixes is recommended.

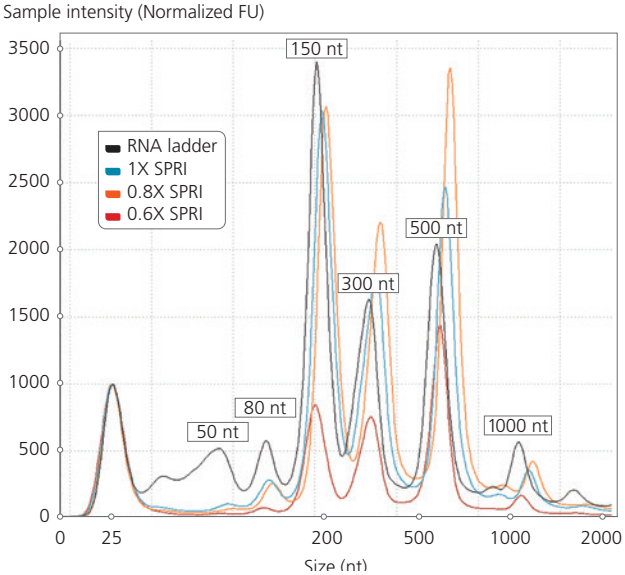

Figure 3 **Size selection of RNA with sparQ PureMag Beads.** ssRNA ladder was purified with 1X (blue), 0.8X (orange) or 0.6X (red). The eluted RNA, and a control sample of RNA ladder (black), was diluted 10-fold and analyzed on a TapeStation RNA High Sensitivity Screen Tape. An overlay of electropherograms for each sample is shown with labelled peaks.

RNA stability testing

Control of RNase contamination is important to maintain the integrity of RNA for many applications. The use of RNase-free reagents is central to these operations. To test for the potential presence of RNase in sparQ PureMag Beads, RNA was purified with 1X sparQ PureMag Beads or RNAClean XP either directly, per standard instructions, or after 4 h incubation at room temperature. The quality of the eluted RNA was assessed by gel electrophoresis on a TapeStation RNA High Sensitivity Screen Tape. RNA purified by either sparQ PureMag Beads or RNAClean XP was comparable. For both products, after 4 h incubation at room temperature, the bands of the ssRNA ladder were clearly visualized with no detectable smearing which would indicate RNA degradation These results demonstrate the absence of detectable RNase in sparQ PureMag Beads.

The negative control of ssRNA incubated with RNase-free water also produced clearly visible bands, confirming that the ladder itself did not contain RNase. In contrast, bands were not clearly visualized for the positive control of ssRNA incubated with saliva, indicating the presence of RNase and RNA degradation in this positive control.

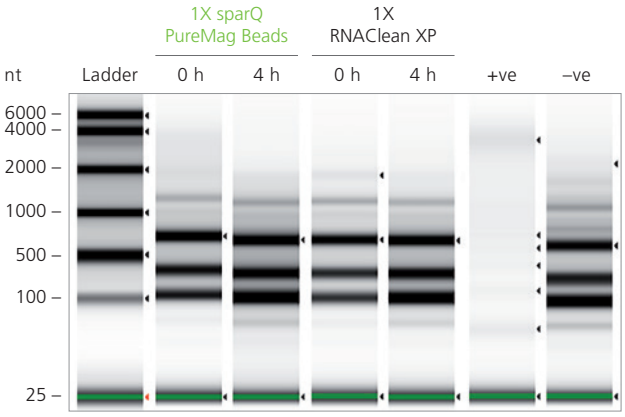

Figure 4 **RNA stability with sparQ PureMag Beads compared to RNAClean XP.** ssRNA ladder was mixed with 1X sparQ PureMag Beads or RNAClean XP and either cleanup carried out immediately (0 h) or the mixture incubated at room temperature for 4 h before cleanup (4 h). ssRNA was incubated for 4 h with saliva as a positive control (+ve) or RNase-free water as a negative control (-ve). Combined digital gel images for TapeStation High Sensitivity tape analyses are shown.

DISCUSSION

In this application note, we have demonstrated RNA purification with high recovery using sparQ PureMag Beads with up to 89% recovery with 1.8X bead cleanup. Additionally, by varying the bead to sample ratio, sparQ PureMag Beads can be used to select RNA fragments of a specific size ranges. Although, at very low bead to sample ratios, there is a risk of reduced yield.

We also conducted a test of RNA stability in the bead buffer solution. Preservation of RNA integrity following extended incubation at room temperature demonstrated that sparQ PureMag Beads were RNase-free. We compared these results to purification with RNAClean XP and found results to be equivalent to sparQ PureMag Beads.

In conclusion, sparQ PureMag Beads provide a fast, flexible and cost-effective alternative for RNA purification. sparQ PureMag Beads can easily be incorporated into a range of manual or automated RNA workflows to provide superior RNA recovery and quality for downstream applications.

MK-AN-0004 REV 03 RNA cleanup 0121