

Highly Efficient Micro RNA Enrichment Procedure using Solid Phase Reverse Immobilization Magnetic Bead Technology

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Introduction

Over the past few years, the scientific community has placed great importance on characterizing small RNA molecules, in particular the microRNAs (miRNA). miRNAs are ribonucleic acids that range from 18 nucleotides to 40 nucleotides, and the majority of the miRNA is composed of approximately 22 nucleotides. Most of the commercially available miRNA extraction protocols co-purify the miRNA and total RNA. Therefore, the extracted samples still contain ribosomal RNA and messenger RNA with only a low percentage of miRNA. In order to measure miRNA more precisely and achieve better quality miRNA sequencing data, it is desirable to remove the larger unwanted ribosomal and other species of RNAs.

This technical note describes the use of Beckman Coulter's SPRIselect reagent kit for miRNA enrichment. The Solid Phase Reverse Immobilization (SPRI) magnetic bead method is a nucleic acid purification procedure that does not require centrifugation or filtration steps. This simple, rapid, automation friendly and produces high quality samples.

Enrichment of miRNA from the total RNA can be achieved in three binding steps.

Step 1: larger ribosomal RNAs are preferentially immobilized onto the SPRI beads, leaving smaller fragments in solution. Supernatant is then aspirated and transferred for the second binding.

Step 2: the supernatant is treated with the binding condition that preferentially immobilizes the intermediate RNA fragment sizes onto the SPRI beads, leaving the smallest RNA fragments in solution. Supernatant is then aspirated and transferred for the third binding.

Step 3: the supernatant is treated with the binding condition that preferentially binds the smallest fragments containing miRNAs and small RNAs.

The micro-particles with bound miRNA are thoroughly washed with molecular biology-grade ethanol. In the elution step, the purified miRNA is easily recovered from the micro-particles under aqueous conditions, which provides maximum flexibility for downstream applications. The results showed that SPRIselect successfully enriched miRNA from total RNA samples and gave a higher percentage enrichment of miRNA when compared to a column method. In addition, the SPRIselect procedure provided flexibility to purify small, intermediate and larger RNA fragments.

Materials and Methods

For the single stranded DNA oligonucleotide experiments: 50 μ L of sample containing 1 μ g of 22 and 53 base oligonucleotides were incubated with different ratios (volume: volume) of SPRIselect (Beckman Coulter B23318) to 100% isopropanol (American Bioanalytical, AB07015) at room temperature for 5 minutes and then separated on an Agencourt SPRIPlate 96 – Ring Super Magnet Plate (Beckman Coulter Life Sciences, A32782) for 5 minutes. The nucleic acid bound beads were then washed with 300 μ L of 85% ethanol (prepared from 100% ethanol, American Bioanalytical, AB00138). The beads were air dried for 5 minutes and eluted with 50 μ L of nuclease free water. 1 μ L of eluted sample was loaded on an E-Gel EX 4% agarose electrophoresis (Life Technologies, G4010-04).

For the miRNA enrichment using SPRIselect: 50 μ L of sample containing 1 μ g tissue Total RNA (Ambion, #AM7950) was mixed thoroughly with 90 μ L of SPRIselect and incubated at room temperature for 5 minutes and then separated on the magnet plate for 5 minutes. During this binding step, ribosomal RNA and mRNA was bound to the beads and the ribosomal RNA/mRNA depleted supernatant (about 140 μ L) was transferred into a new well.



The supernatant was incubated with 70 μL of the SPRIselect plus 90 μL of 100% isopropanol for 5 minutes and then separated on the magnet plate for 5 minutes. During this binding step, the beads preferentially bind the RNA fragments from 90 to 200 nucleotides. The supernatant containing miRNA and small RNA (300 μL) was transferred to a new well and incubated with 90 μL of SPRIselect plus 270 μL of 100% isopropanol for 5 minutes and then separated on the magnetic plate for 15 minutes. This step preferentially binds the miRNA and small RNAs from 18 to 65 nucleotides. After washing twice with 300 μL of freshly made 85% ethanol the beads were air dried for 5 minutes and eluted with either 20 μL or 50 μL of nuclease free water. 1 μL of the purified miRNA was analyzed and the percent miRNA enrichment was measured on a chip from the Agilent 2100 Small RNA Kit (Agilent Technologies, 5067-1548).

For single stranded DNA oligonucleotide and miRNA enrichment using MinElute Column: the experiments were performed according to the modified RNeasy MinElute Clean Up Kit for miRNA enrichment (Qiagen 74204). 100 μL of sample containing 1 μg RNA was mixed thoroughly with 350 μL RLT +250 μL 100% ethanol. The sample mixture was applied to the first column and centrifuged for 15 sec at 10,000 rpm. 50 μL of RLT and 500 μL 100% ethanol were then added into the collected flow through. The flow through was mixed well with RLT/ethanol and was transferred to the second column. After centrifugation, the column was washed with RB buffer and then ethanol, the purified miRNA was eluted with either 14 μL , 20 μL or 50 μL of nuclease free water.

Results and Discussion

The SPRIselect enrichment procedure showed higher binding efficiency for the 22 base oligonucleotides than the MinElute column.

A mixture of 22 and 53 base oligonucleotides were used to determine the optimal binding conditions that can separate them. Figure 1 shows that the 53 base oligonucleotides were successfully removed from the 22 base when the optimal SPRIselect binding conditions were used (lane 5). The optimal binding conditions were then selected for the miRNA enrichment.

To compare the binding efficiency for the 22 base oligonucleotide using the column method, the same mixture of the 22 and 53 base oligonucleotides was applied to the RNeasy MinElute Clean up kit. Figure 2 shows that the MinElute column preferentially bound the 53 base oligonucleotides and was less effective for binding the 22 base oligonucleotides (lane 4-6) whereas the SPRIselect beads bound the 22 base oligonucleotides more effectively (lane 3).

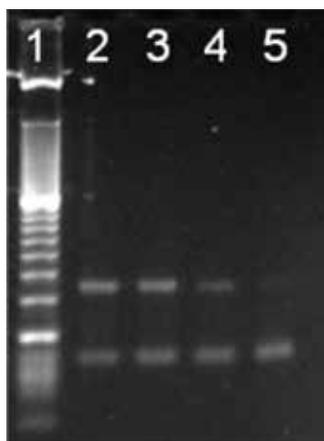


Figure 1. Different binding conditions for the 22 base oligonucleotides. 1 μL of sample was loaded into each lane and analyzed on an E-Gel EX 4% agarose electrophoresis. Lane 1 was the 10 bp DNA size marker ladder (Life Technologies, 10821-015), lanes 2-5 were samples recovered from different SPRIselect binding conditions. Lane 5 showed the highest binding efficiency for the 22 base oligonucleotides. Upper bands = 53 base oligonucleotides; lower bands = 22 base oligonucleotides.



Figure 2. The SPRIselect enrichment method showed better binding efficiency for the 22 base oligonucleotides than the MinElute column. Lane 1: 1 μL 10 bp DNA size marker ladder; lane 2: 1 μL of unpurified control DNA containing 25 ng of the 22 and 53 base oligonucleotides; lane 3: 1 μL of sample recovered from SPRIselect as lane 5 in Figure 1; lanes 4-6: different amount of the MinElute eluted samples were loaded, lane 4 (1 uL), lane 5 (2 uL) and lane 6 (1 uL of 1:4 dilution). Upper bands = 53 base oligonucleotides; lower bands = 22 base oligonucleotides.

SPRIselect was more efficient at enriching miRNA than the MinElute columns.

To determine whether the SPRIselect has better miRNA enrichment efficiency compared to the MinElute column method, a total of twenty human RNA samples were tested. Eight samples were purified using the SPRIselect and the other twelve samples were purified using the MinElute column. One μL of eluted miRNA was analyzed on the Agilent Small RNA Chips to determine miRNA enrichment. The results showed that the average miRNA enrichment using the SPRIselect was at 61.63% and the average miRNA enrichment using the MinElute column was at 51.33%. T test results showed that the p value was at 0.00016 suggesting that the difference is statistically significant (Table 1).

Table 1: Average miRNA % enriched from from SPRIselect and MinElute column

Method	Average miRNA% +/-Standard Deviations	p value
SPRIselect	61.63 +/- 4.3	0.00016
MinElute	51.33 +/- 5.3	

Figure 3 shows the miRNA profiling, same amount of miRNA samples were analyzed on the Small RNA Chip. The SPRIselect successfully excluded fragments greater than 100 nucleotides (Figure 3a.), however, the recovered miRNA samples from the MinElute columns still contained RNA fragment sizes between 100-150 nucleotides (Figure 3b).

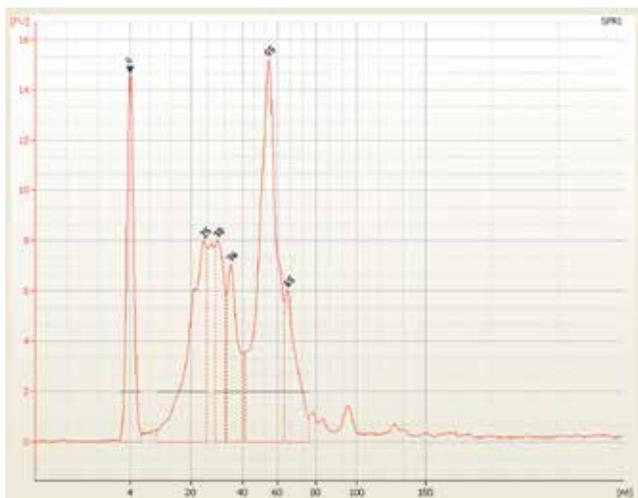


Figure 3a. 1 μL of SPRIselect miRNA samples were analyzed on the Small RNA chips.

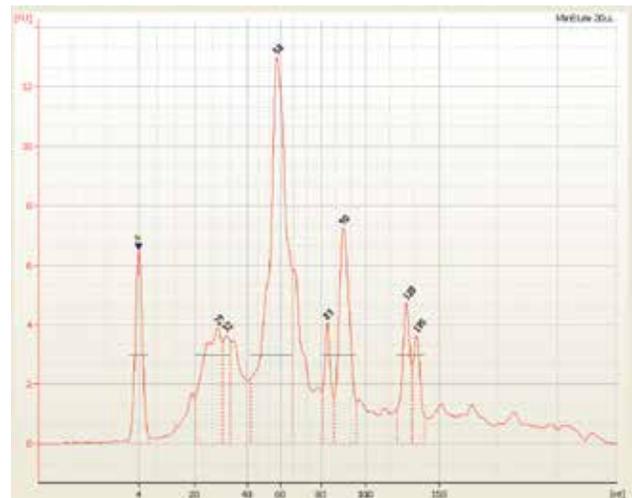


Figure 3b. 1 μL of MinEluted miRNA samples were analyzed on the Small RNA chips.

SPRIselect demonstrated flexibility in recovery of different RNA fragment sizes.

The SPRIselect miRNA enrichment procedure provided flexibility when recovering different fractions of the RNA fragments from human tissue RNA. There were three binding steps in the miRNA enrichment where no RNA was discarded. During the three binding steps, the supernatant can be saved and RNA can be recovered out of them. The first binding condition preferentially binds the larger fragments and excludes the ribosomal RNA and mRNA from the sample. Therefore these RNA species can be recovered from the beads used in the first binding step (not shown). Combining the first supernatant with additional binding conditions preferentially attracts the fragments between 90 to 200 nucleotides. Therefore, these fragments can be recovered from the beads used in the second binding step (Figure 4a). Combining the second supernatant with the third binding condition preferentially binds miRNA and small RNA (18-60 nucleotides) and therefore, miRNA can be recovered from beads used in the third binding step (Figure 4b). Skipping the second binding enables recovery of fragment sizes between 18 to 200 nucleotides by removing only the ribosomal and larger mRNA species (Figure 4c).

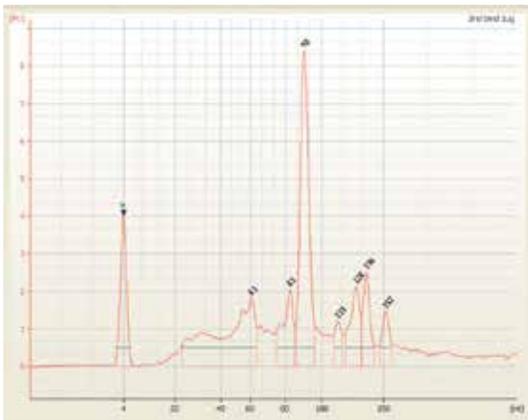


Figure 4a. The second binding step enriched the fragment sizes primarily from 90-200 nucleotides.

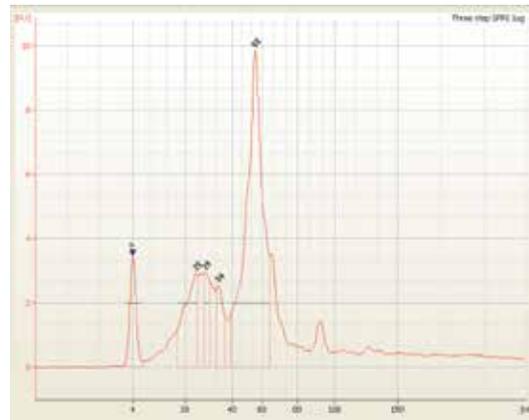


Figure 4b. The third binding step enriched the fragment sizes primarily from 18-60 nucleotides.

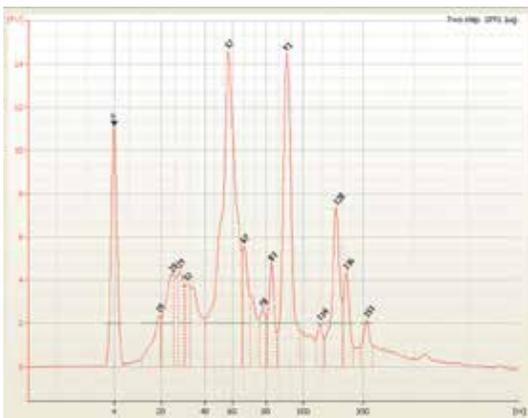


Figure 4c. Skipping the second binding step enriched the fragment sizes primarily from 18-200 nucleotides.

Conclusions

The data from this study shows that SPRIselect provides better miRNA enrichment when compared to the column method. First, it provides flexibility to separate different fragment sizes. Second, it shows higher miRNA binding efficiency. Third, it enables users to keep all fractions of RNA species during miRNA enrichment.