Comments to the Authors,

This manuscript provided a comprehensive design to evaluate the influence of purification method, miRNA-seq method (Hiseq or Miseq), RNA quality or degradation and sequencing coverage to miRNA-seq. And the authors found RNA degradation, Hiseq or Miseq would not bring dramatically effect to miRNA-seq while purification method in library preparation would bring significantly change to miRNA-seq. The study was performed rigorously and the findings are interesting. However, the manuscript needs more careful editing since the design is very complicated which would make the reader confused without perfect and smooth manuscript. In general, I'd recommend publication if the authors can address the following concerns and prepare a more concise draft.

**Major Compulsory Revisions**

1, In the background section, The function of the miRNA, LincRNA, rRNA, piRNA and T-UCRs, however, please provide some explicit evidences that they could be taken as biomarkers, or else, please shorten these comprehensive description. In addition, DNA methylation also has been considered to be a great biomarker for some complex disease. It should be mentioned in the background.

2, In the background section, large content were used to introduce miRNA, piRNA and so on. However, these information is non-informative for the manuscript. However, the most important thing of Table 1 is lack of enough description. Please change the styles.

3, Please make sure about the GEO accession ID for the dataset is correct in the line 5 of page 11.

4, All the commas in the Tables should be replaced with points throughout Tabel 2,4,5 and 6

5, In the section of “Bioinformatic output measures for small RNA sequencing quality control”, the sentence of “was consistent with published results” and “was externally validated with \*\*” was confusing. Please make this sentence clearer. What’s your conclusion for this section?

6, The authors should give some interpretation to the reason why PPS give so many raw reads than other methods in the table 2.

7, It would be perfect for authors to make a comprehensive comparison between different RNA purification method with a table in the supplementary.

**Minor Essential Revisions**

8, Please change all the points in “has.miR.485.5p” as “has-miR-485-5p”

9, How RIN was measured should be introduced in the background section. In addition, please define it when you use it at the first time.

10, Please provide the rank correlation of shared miRNAs in the Table 6. I assume the correlation of the rank between the identified miRNAs in Hiseq2500 and Miseq would be very strong.

11, Please provide a short eventual and explicit recommendation or highlight for the readers in the summary section as the main discovery in the study such as RIN would not affect the miRNA-seq and so on.

12, Please provide the detailed numbers of the miRNAs in Figure 6, except the proportions.

13, Please provide corresponding heatmap plot based on the data of Figure 4 and Figure 7, respectively, as the supplementary or in the main body.

14, Which factors in the RNA-seq would affect “Surviving Reads” as mentioned in Table 2,4,5,6?

15, Table 3, specific values would be prefer than relative description.

16, Figure 1 should provide more information. For example, the purification method can be labelled in Figuare 1B and so on.

17, it seems there is no any difference between AMPure and control group with the data of Table 2. Why?

18, In the table 1, what does it mean “we removed reads with a quality scores<30”? Any reads which have any base score <30 will be filter out?