Small RNA Sequencing FAQ

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1. What is small RNA sequencing?

   Small RNAs (sRNA) from Illumina HiSeqTM 2000 deep sequencing cover almost every kind of RNA, including miRNA, siRNA, piRNA, rRNA, tRNA, snRNA, snoRNA, repeat associated sRNA, and degraded tags of exon or intron. By comparing our sequences with those in databases and identifying the overlap of genome location between our data and the databases, sRNAs can be annotated into different categories. Those which can not be annotated are used to predict novel miRNA using our in-house software called Mireap.

2. What is the experimental process?

   Small RNA are special kind of molecules in organisms that induces gene silencing and play an important role in the regulation of cell growth, gene transcription, and translation. The small RNA digitalization analysis based on Illumina HiSeqTM 2000 high-throughput sequencing uses SBS-sequencing by synthesis, which can decrease the loss of nucleotides caused by the secondary structure. It is also advantageous due to the small quantity of sample required, high through-put, high accuracy, and simplicity of the automated platform. This analysis can obtain millions of small RNA sequence tags in one shot, comprehensively identify small RNA of certain species in specific conditions, predict novel miRNA, and construct the small RNA differential expression profile between samples, allowing it to be used as a powerful tool for small RNA function research. The experimental process of small RNA sequencing is shown in Figure 1.

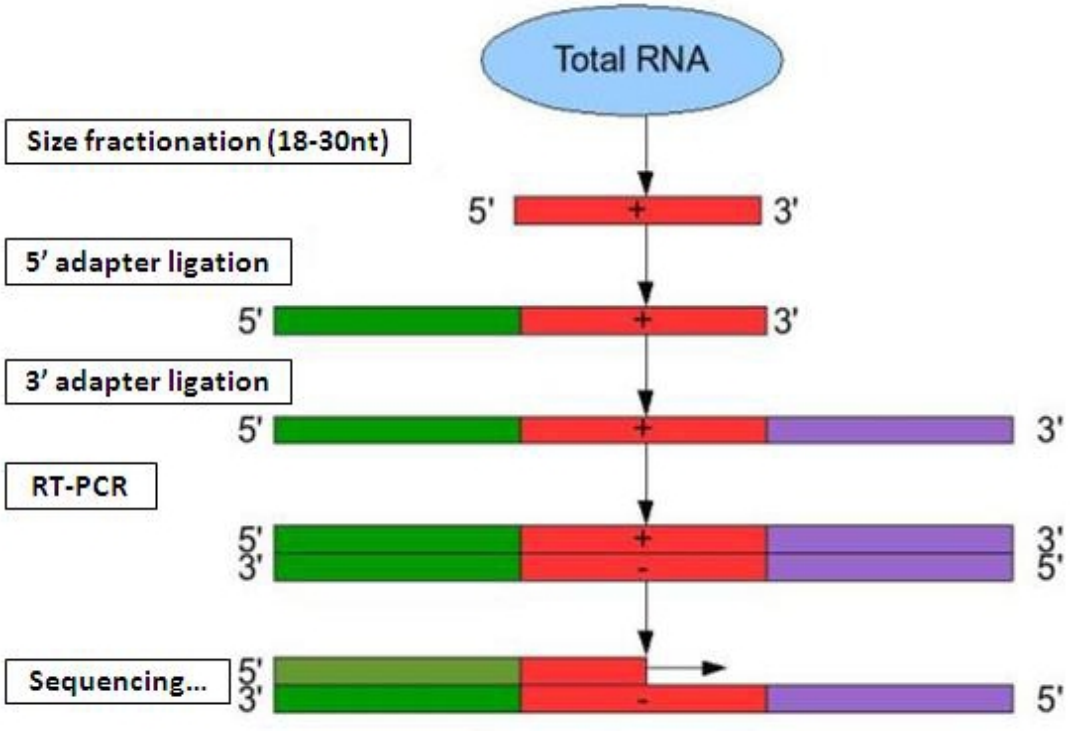

Figure 1 Experimental process for small RNA sequencing

3. What data analysis processes are applied?

   The data for 49nt sequence tags from Illumina HiSeqTM 2000 sequencing are initially cleaned, which includes removing low quality tags and several kinds of contaminants. Length distribution of clean tags is then summarized. Standard bioinformatics analysis annotate the clean tags into different categories and use those that cannot be annotated to any category to predict the novel miRNA and base edit of potential known miRNA.

4. Are there any special requirements for total RNA isolated from tissues?

   Note the following regarding isolation:

   - Avoid isolating total RNA using the Qiagen column kit.
   - Avoid using lithium chloride (LiCl) for RNA preparation.

5. What are the requirements for total RNA isolated from serum samples?

   For RNA isolated from serum samples, provide more than 5 ml of serum.

6. What type of samples can be used for small RNA sequencing?

   Samples that can be used are total RNAs, small RNA fragments, DNAs reverse transcribed from small RNAs, tissues, serum, and suspension cell lines etc.

7. What are the requirements for RNA samples?

   Requirements for RNA samples include?

   - Sample condition: integrated total RNA samples. Avoid protein contamination during RNA isolation. We recommend that you provide a sample amount that is enough for two-time use.

   - Sample quantity:

     - general requirements: total RNA must be ≥ 10 μg;

     - for plasma/serum or if used for co-IP: total RNA must be ≥ 100 ng (≥ 5ml for serum sample);

     - for < 200 nt small RNA isolated from mirVana™ miRNA Isolation Kit : total RNA must be ≥1 μg.

   - Sample concentration:

     - general requirements: concentration must be ≥ 400 ng/μl;

     - for plasma/serum or if used for co-IP: concentration must be ≥ 5 ng/μl;

     - for < 200 nt small RNA isolated from mirVana™miRNA Isolation Kit supplied: concentration must be ≥ 20 ng/μl.

   - Sample purity:

     - OD260/280 = 1.8 to 2.2;

     - for animal samples, RNA 28S:18S ≥ 1.5, RIN ≥ 8.0;

     - for plant samples, no requirements for RNA 28S:18S and RIN value.

8. Can we use our library for sequencing?

   You can use your library. If the adapters you use are same as what BGI uses, the library can be used in Illumina sequencing directly; otherwise, you are required to provide primers. Agilent 2100 Bioanalyzer is used to test the segment size. The accurate quantification is determined by Q-PCR.

9. When and where can I find a complete final report? Does it include an interpretation of the results?

   Our final report is provided in a Web html format, which provides the complete report and detailed description and interpretation.

10. When are the data returned to me?

    Normally, it requires 30 working days to complete an entire small RNA sequencing analysis, from sample test to bioinformatics analysis. We will provide you with a complete report when the analysis is done.