华大科技
BGI·tech

RNA-Seq (Quantification) FAQ

1. **What methods are available to study gene expression profiling?**
   Northern blot, real-time PCR, SAGE based on RNA sequencing, cDNA chip based on hybridization, and gene expression clustering are used to study gene expression profile.

2. **Compared to microarrays, what are the advantages of RNA-Seq (Quantification)?**
   Microarrays employs the hybridization of probe and sample mRNA to acquire mRNA sequence information. However, new transcripts cannot be identified. In addition, the background optical signal of hybridization is very high. mRNA sequence can be obtained directly by RNA-Seq (Quantification). New transcripts can be identified and a wide range of transcripts copies can be detected. RNA-Seq (Quantification) is more sensitive than microarrays. Whole genome expression can be obtained based on RNA-Seq (Quantification).

3. **Are eukaryotes and prokaryote appropriate for RNA-Seq?**
   All eukaryote, prokaryote as well as bacterium can be analyzed using RNA-Seq (Quantification).

4. **Are biological repeats needed for RNA-Seq (Quantification)? If yes, how many repeats are needed?**
   At least two biological repeats are necessary and more than three repeats are better. Hansen's study in July 2011 showed that genes express differently between biological repeats, and this is not related to the detection technology or to the bioinformatics pipeline. Editors can refuse to publish a manuscript if no biological repeats are used.

5. **Which kind of reference can be utilized in RNA-Seq (Quantification)?**
   Genome sequence references are preferable for RNA-Seq (Quantification) analysis. If a genome sequence is unavailable, a unigene sequence, an mRNA sequence, or a CDS sequence can be used as the reference.

6. **What criteria are used for gene expression difference analysis and identification of the differentially expressed genes?**
   For RNA-Seq (Quantification), an FDR≤0.001 and the absolute value of Log2Ratio

(treatment/control)≥1 are used as the threshold to judge the significance of gene expression differences. More stringent criteria with a smaller FDR and greater fold-change value can be used to identify differentially expressed genes.

7. Are there any limitations for low-input (200ng) RNA-Seq (Quantification)?

Low input (200ng) RNA-Seq (Quantification) is an update of RNA-Seq (Quantification) for trace sample quantification, and there is no species limitation.

8. For customer's total RNA samples, will BGI perform Dnase treatment before library construction?

We recommend that you perform DNase treatment before sample delivery, but we will do it again before library construction.

9. Which primer will be used in reverse transcription of RNA-Seq (Quantification) library construction, random primers or oligo (dT)?

Random primers are used in library construction.