Transcriptome Sequencing (mRNA-seq) FAQ

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1. What are the advantages of transcriptome or mRNA-seq compared to genotyping chips?

   The digital signals produced by mRNA-seq are more accurate and reliable than using genotyping chips. Not only can mRNA-seq detect known genes and their expression profile, it is also able to detect RNA of low quantity, novel mRNA, alternative splicing, and gene fusion. It can also determine the exact intron/exon boundary.

2. What are the advantages of mRNA-seq compared to digital gene expression (DGE)?

   DGE is appropriate for quantitative analysis. However, mRNA-seq is capable of both qualitative and quantitative analysis. mRNA-seq can also detect alternative splicing and optimizing gene structure.

3. What is the turnaround time for mRNA-seq services?

   If the sample passes quality control, sequencing and data analysis can typically be performed within 50 workdays. A project report is delivered at the end of the 50 day project. If the sample size is larger than usual or library construction failed, the turnaround time can be longer.

4. What are the sample requirements for mRNA-seq? What is the minimum amount required?

   For mammalian samples (e.g. human and mice), provide sample concentration ≥80 ng/μl and total RNA quantity ≥5 μg. For other animals, provide sample concentration ≥200 ng/μl and total RNA sample quantity ≥10μg.

   Generally, RNA sample purity OD260/280 = 1.8~2.2.The non-degraded RNA sample should be purified and treated with DNase to remove protein and DNA. If there is extra RNA, provide twice the amount for backup to ensure that the schedule for sequencing is maintained.

5. What types of samples (tissue samples or suspended cell lines) can be used for RNA-Seq, total RNA, and mRNA?

   All types are acceptable.

6. What are the sample requirements for tissue and blood samples?

   General requirements include:

   - Samples should be packaged appropriately to ensure the freshness and avoid freezing and thawing.
   - The sample should be divided into appropriate size, e.g. 100mg for individual packing and shipping, to prevent adverse effect in sample extraction.

Specific requirements are as follows:

- Human and animal tissues

  Tissue sample should be rinsed with RNase-free 0.9% physiological saline to remove blood and contaminants. Other tissues that are not for experimental use such as connective tissue and fatty tissue should also be removed. Samples should be cut into small pieces of about 50mg (the smaller the size, the better the preservation). The sample should then be placed into 1.5 ml or 2 ml RNase-free EP tubes. RNAlater or other types of reagents that can prevent RNA degradation should be added. Reagents from ABI are strongly recommended, and be sure to follow the procedures stated in the operation manual. The sample should be frozen using liquid nitrogen and sealed with sealing film, then placed the EP tube into 50ml centrifuge tube or sealed mini plastic bag, and shipped with dry ice. Ensure that amount of dry ice is sufficient to reach BGI. If no dry ice and liquid nitrogen are available, please ship the sample at room temperature using ice bags.

- Blood samples

  Separate white cells separated in advance, then put sample into RNAlater or TRIZOL. After freezing using liquid nitrogen, ship with dry ice.We do not accept whole blood sample.

7. What RNA quality checks are included at BGI?

   BGI uses the Agilent 2100 Bioanalyzer for quality checks, and the data that are provided includes concentration, volume, total quantity, 28S/18S, and RIN value.

8. What is the standard in determining the qualified RNA sample?

   We determine if there are multiple, miscellaneous peaks in the testing report diagrams. We also see if the total quantity meets the requirements, such as 28S/18S and RIN value. Because of the differences in species, there are different standards for quality. We will assess according to the different species.

   For human and mice samples, critical indications for consideration are:

   - total quantity $\leq 5\mu g$
   - 28S/18S value < 1
   - RIN value < 7

   RIN=RNA integrated number, which is the number of RNA molecules. It directly reflects the quality of RNA. The higher the number of RIN, the better the RNA quality is. This model of assessing RNA quality of mammals (mainly human and

mice) has been developed by Agilent and is based on much human and mouse RNA quality data.

9. What is the workflow for transcriptome sequencing?

   The workflow is as follows:

   1. QA RNA samples
   2. Construct library
   3. Test library
   4. Perform clustering
   5. Prepare platform
   6. Perform sequencing
   7. Perform bioinformatics analysis.

10. What is the sequencing strategy used in mRNA sequencing?

    BGI uses the 91PE or 101PE sequencing approach used by the Illumina HiSeqTM2000.

11. What bioinformatics analysis results are generated with mRNA resequencing?

    The following analysis results are generated:

    - Sequencing evaluation (alignment statistics, randomness, assessment, and distribution of reads in reference genome)
    - Gene expression annotation
    - Differentially expressed genes (two or more samples)
    - Optimization of gene structures (eukaryote *only*)
    - Identification of alternative spliced transcripts (eukaryote *only*)
    - Novel transcripts prediction
    - SNP analysis

12. How is data provided and how is confidentiality of the data ensured?

    For data sets less than 4GB we transfer via the Web. For data sets between 4GB and 40GB we deliver via CD-ROM. For datasets greater than 40GB we transfer via hard disk.

    For data downloaded via the Web, your project manager will inform you via e-mail of the download address, username, and password. The data is accessed via an ftp server. No data will be disclosed to other parties unless we have a signed letter of confirmation with a designated contact name.

13. How are the unused and unqualified samples handled?

    BGI stores the samples during the project and for a period of one month after the project completion without any charge. Please contact the project manager within 15 days after the testing report issued if the samples need to be returned. Expired product will be destroyed 15 days after testing report is issued.

    BGI stores the samples 30 days after the summary report is sent. Please contact the project manager in a timely manner if the samples need to be returned. Expired samples are usually destroyed 30 days after the summary report is submitted.