

Long non-coding (lnc) RNA Sequencing FAQ

1. What is long non-coding RNA? What is the role of long non-coding RNA?

Although 5% to 10% of the mammalian genome is stably transcribed, only about 1% of this sequence encodes proteins. The remaining 4% to 9% consists of transcribed non-coding RNA. Non-coding RNAs are functional RNA molecules that are not translated into proteins. Long non-coding RNAs are longer than 200 nucleotides in length. They play important roles by affecting the expression of neighboring genes, regulating protein activity and position, producing small RNA molecules, and helping to regulate other RNAs.

2. How does lncRNA-Seq compare with the microarray method?

Novel lncRNA prediction and specific lncRNA enrichment are added benefits of lncRNA-Seq compared to the microarray method.

3. What is the purpose of investigating non-coding RNAs?

The main applications of research focused on non-coding RNAs are screening for disease-specific markers and functional genomics.

4. Can we perform low-input total RNA library construction with lncRNA-Seq?

lncRNA-Seq is not yet suitable for construction of low-input total RNA libraries.

5. What is BGI's experience with long non-coding RNA?

BGI has successfully accomplished more than one hundred projects involving long non-coding RNA sequencing. Most of our experience is with human samples. We lack experience with other species. We currently offer lncRNA-Seq for human, rat, and mouse. lncRNA-Seq for other species will require evaluation in advance.

6. What is the strategy for constructing lncRNA-Seq libraries?

We use the Ribo zero kit to remove ribosomal RNA (rRNA) and apply a strand-specific method to construct libraries.

7. What are the risks involved in a lncRNA-Seq contract?

The efficiency with which rRNA is removed is unstable, potentially leading to a high level of residual rRNA. The method for removing rRNA is more suitable for species with good reference genomes. Coding and non-coding sequences can be distinguished through follow-up alignment.

8. Which software products are used for the bioinformatics analyses?

We use Tophat and Cufflinksto to build the transcript, Infernal for family classification, CPC to predict the transcript-encoding capability, Blast for comparisons, and SOAP to filter rRNA that is not removed in the experiment and for express quantitative comparisons of lncRNAs.

9. Why do we remove only rRNA during the library construction?

Similar to mRNA, some lncRNAs have polyA tails. Exclusive removal of rRNA helps retain lncRNAs that contain polyA tails. The universal approach for removing mRNA uses OligodT beads. However, this method removes lncRNAs that contain polyA tails. If the customer requires removal of both rRNA and mRNA, at least 20 µg of total RNA input will be required.