Single-cell RNA Sequencing FAQ

1. How many reads are recommended for single-cell RNA-Seq?

We recommend at least 30 M clean reads for RNA-Seq analysis; however, some cell types, except mammalian embryonic cells, do not have saturation curves.

2. Why are 30 M clean reads recommended for single-cell RNA-Seq analysis, whereas only 5 M reads are recommended for traditional RNA-Seq analysis?

Because of the bias during multiple cycles of PCR amplification, we need more reads to detect low-abundance genes and to obtain saturation.

3. What is the detection efficiency for low-abundance transcripts in single-cell RNA-Seq analysis?

Because of the bias produced during amplification, some low-abundance transcripts will inevitably be omitted, similar to traditional RNA-Seq analysis. According to our experience, more than 90% of the genes that have 80% of transcripts with RPKM>1 will be detected with fewer than 50 M SE50 reads. This indicates that we can detect a certain number of low-abundance transcripts in addition to high-abundance transcripts.

4. Can customers prepare the lysis buffer on their own?

Only BGI's buffer is compatible with the subsequent amplification system, so the cell lysis buffers prepared by customers are not acceptable.

5. Does single-cell RNA-Seq evaluate the amplification efficiency of housekeeping genes for quality control?

Expression levels of housekeeping genes change during various cell states, especially during early embryonic development. Therefore, it is difficult to select a specific value for quality control. Consequently, there is no quality control for housekeeping genes. At present, we evaluate the amplification efficiency by first-round concentration detection and second-round electrophoresis.

6. Can bias be prevented with random primers instead of an oligod(T) primer?

Amplification with random primers requires mRNA isolation, which cannot be achieved with a single cell. Therefore, single-cell RNA-Seq amplification is based on the polyA tail structure.

7. Do customers need to include a positive and negative control?

We will set up negative and positive controls with normal tissues or standard samples if the cell state is unknown. In the event that amplification is not successful, we will troubleshoot the problem by performing amplification reactions for controls and samples simultaneously.

8. Is single-cell RNA-Seq available for other cell types?

The successful demo case for single-cell RNA-Seq is the mammalian embryonic cell. We would like to perform additional experiments if the customer agrees to accept the risk due to the lack of experience with other cell types. Single-cell transcriptome sequencing is not available, because the maximum length we have amplified is about 3 kb, which is less than the length of the whole transcriptome.