Bisulfite Sequencing FAQ

2011-04-28 (Version 1)

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1. Is Bisulfite-Seq adequate for methylation research without a known reference genome?

   Bislufite-Seq strongly relies on reference genome, and the quality of the reference genome directly influences the result of the bioinformatics analysis. Therefore, Bislufite-Seq is very appropriate for methylome research in which the reference genome is known.

2. What is the definition of a methylation pattern?

   A methylation pattern refers to the species-specific methylated features in a genome. In bioinformatics analysis, the methylation pattern analysis refers to the proportion of mCs in CG, CHG, and CHH that are located in a specific gene region or in a genome. Also it can refer to the proportion of different levels of methylated cytosine.

3. How much data is generated through bisulfite sequencing?

   Bisulfite sequencing calculates the rate of methylation of every cytosine base. As such a sufficient amount of data is needed for an accurate analysis. The recommended minimum amount is 40X the genome size. We do recommend generating more data than this amount. Note that 40X cannot be used to obtain 120G. For species that have a small genome size such as microorganisms, we recommend 2G of data.

4. What quality control tests are used in bisulfite sequencing?

   We use Qubit® from Invitrogen to measure the sample concentration and amount. Electrophoresis analysis is used to check DNA sample size.

5. Why do some other service providers use NanoDrop instead of Qubit?

   NanoDrop testing is based on detecting light absorbance and is affected by protein, RNA, and salt ions. Assuming no contamination, result should be the same for Qubit and NanoDrop. However, contamination due to organic ions and protein can be detected. Qubit testing is based of detecting the light absorbance of fluorescence light that is emitted resulting from the stimulation of a laser after hybridization of fluorescence dye and double-strand DNA. Qubit therefore detects indirectly the quantity of DNA and is not affected by other contaminants. In most situations, the resulting of concentration detected in the test is lower in Qubit than in NanoDrop.

6. What is the minimum sample size for constructing a library for bisulfite sequencing?

   An initial sample of 100ng can be used to build one library. 7G of raw data can be obtained from each library. According to a preliminary study, data alignment is satisfactory using 100ng in regular libraries.

7. How is the cytosine methylation defined in bisulfite sequencing?

   Cytosine is defined as methylated when the reads that support methylation are equal to or greater than an expected value.

8. Can methylation frequency be calculated in bisulfite sequencing?

   Yes, the frequency can be calculated. However, it is not included in standard bioinformatics analysis. Methylation frequency indicates how many bases (ATCG) that are apart are methylated cytosine. The results of a related study using animal models (Blue mustard and human) are available in Nature.