03 How to increase sequencing quality on MGI platform?

The sequencing quality is closely related to library quality. It depends on whether the library concentration meets quality control standards and is sufficient for sequencing, library size distribution, base complexity and the selection of barcodes also need attention.

(1)Control of library size distribution

Since the BGI platform library needs additional cyclization and DNB preparation steps before sequencing, too long or too short fragments size distribution will lead to significant differences in cyclization efficiency. The ideal library peak is smooth and concentrated with an obvious single main peak. The ideal library size distribution should not contain adapter dimers, small fragment contamination, or large fragment residues. The different sub-library within the same pooling should have the same library size distribution, and the size difference of the main peak should not be more than 100bp.

(2)Use balanced library

Generally, two or four photos will be taken for each cycle in NGS（Next-generation sequencing) and the library base complexity has a great impact on sequencing data. For libraries with low base complexity (like methylated libraries, small RNA libraries and amplicon libraries), a certain proportion of base-balanced libraries can be added for mixed sequencing to help balance the fluorescence signals generated from each sequencing cycle, thus improving the output and quality of sequencing.

04 How to improve the uniformity of sequencing data?

(1)Quantification of library concentration

Before library pooling, it is recommended to use Qubit to quantify the sub-library. During pooling, the input of each sub-library needs to be consistent.

(2)Nucleotides should be in balance in each barcode position

A group of the balanced barcode should have four bases (A/T/C/G) in each position and the proportion of each base is not less than 12.5%. So that it can guarantee the sequencing output and quality. If the barcode combination is not good, the sequencing quality of indexes will decrease and some or all of the index bases will be recognized incorrectly. Then a part of the data will become undetermined data that can not be attributed to any sample, resulting in wastage.

Vazyme has developed a barcode selection tool for the MGI platform. Users only need to input the number of sub-libraries and the catalogue number used for library preparation, then you can get the best barcode combination recommended for the current catalogue number.

Vazyme

MGI platform

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Product List

Library Preparation Kits

Product Type	Product Name	Cat.No	Product Features
Transposase Library	TruePrep® DNA Library Prep Kit for MGI	TDM501–503	Finish library construction quickly in 2h.
Transposase Library	TruePrep® Flexible DNA Library Prep Kit for MGI	TDM504	Compatible with100 pg–500 ng input DNA ,quick library construction in 1.3h
Transposase Library Adapter	TruePrep® Index Kit for MGI	TDM101–TDM104	Each set contains 24 types of indexes. Four sets offer 96 types in total.
Normal DNA Library (Physical Fragmentation)	VAHTS® Universal DNA Library Prep Kit for MGI	NDM607	High library conversion rate, High uniformity
Normal DNA Library (include damage repair)	VAHTS® Universal Pro DNA Library Prep Kit for MGI	NDM608	Combine base damage, cutting, nicks
Normal DNA Library (Including Enzymatic Fragmentation)	VAHTS® Universal Plus DNA Library Prep Kit for MGI V2	NDM627	Decreased false positives detected by SNP and InDel
Normal Library Adapter (Single index)	VAHTS® DNA Adapters set 8 for MGI	NM108	Single indexed, 96 types
Normal PCR–free Library Adapter (Single index)	VAHTS® PCR–Free DNA Adapters for MGI	NM10901–NM10904	Single indexed, 96 types
Normal Library Adapter (Dual UDI UMI)	VAHTS® DNA Dual UMI UDB Adapters for MGI	NM35101–NM35108	Up to 192 types of index
RNA Seq Library Preparation	VAHTS® Universal V8 RNA–seq Library Prep Kit for MGI	NRM605	Efficient library construction for low initial quantity and quality samples
RNA Adapter	VAHTS® RNA Adapters set 8 for MGI	NM208	Single indexed, 96 types

Modules for Library Preparation

Product Type	Product Name	Cat.No
Amplification Module for Transposes Library	TruePrep® Amplify Enzyme	TD601
Amplification Module	VAHTS® HiFi Universal Amplification Mix 2 for MGI(SI)	NM618
Single-end Barcode Circularization	VAHTS® Circularization Kit for MGI	NM201
Single/Pair-End Barcode Circularization	VAHTS® Universal Circularization Kit For MGI	NM202
MGI Library Conversion	VAHTS® Library Conversion Kit For MGI	NM401

Compatible for MGI Automated Library Preparation

MGISP–100　　MGISP–960

Automated scripts for MGISP-960 available

FAQ

01 How to sequence my Illumina Library on MGI platform?

Universal library conversion reagents can be used for both normal libraries and transposase libraries. After selecting Vazyme #NM401 or MGI APPA universal library conversion reagents to obtain single-chain cycled products, DNB can be prepared and sequenced by machine. Corresponding sequencing reagents should be selected according to the library type or sequencing primers should be changed.

Library Type	Normal Library	Transposase Library
Conversion Reagents	Vazyme #NM401 or MGI APP-A universal library conversion reagents	
Sequencing Reagents	Plan A：Use APP-A sequencing total kit Plan B：Use APP-A sequencing primer kit or synthesis related primers to replace all primers in normal sequencing kit	Use APP-B sequencing primer kit or synthesis related primers to replace all primers in normal sequencing kit

02 How to sequence my MGI Library on MGI platform?

For a normal BGI platform library, the cyclization reagent is selected according to the single barcode connector or double barcode connector, as shown in the figure below. After the single-chain cycled products were obtained, DNB was prepared and sequenced by machine. Corresponding sequencing reagents should be selected or sequencing primers should be changed according to the library type.
For the BGI transposase platform library, only the single barcode sequencing strategy exists currently. After obtaining the single-chain cyclization product with the single-ended cycling reagent, DNB will be prepared and sequenced on the machine. It should be noted that additional sequencing primers for the transposase library should be selected.

Library Type	Single Barcode Normal Library	Double Barcode Normal Library	Single Barcode Transposase Library
Conversion Kit	Vazyme #NM201	Vazyme #NM202	Vazyme #NM201
Sequencing Kit	Normal Sequencing Kit	Normal Sequencing Kit, need add corresponding CPAS barcode primer (buy or synthesis)	TM high-throughput sequencing primer kit need to be purchased or synthesize related primers,to replace all primers in normal sequencing reagent set.