FS PCR-Free DNA Library Prep Set FAQ

Cat. No. 1000013454 (16 RXN), 1000013455 (96 RXN)

Kit Version: V1.2 (16 RXN)， V1.3 (96 RXN)

FAQ Version: A1

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1, Does the MGIEasy FS PCR-Free DNA Library Prep Set have any requirement for the sample concentration and quality?

A: Integrity and Purity of the input DNA are needed for the MGIEasy FS PCR-Free DNA Library Prep Set. It is strongly recommended to use high quality genomic DNA (gDNA) samples (A260/A280=1.8-2.0, A260/A230≥1.7, no degradation or degraded slightly) for fragmentation. If the integrity or purity does not meet the requirement, attempt for library construction can be considered, but risk of low library yields or failure remains. For the input DNA that purity do not meet the requirements, we recommend do the purification with 2x beads before library construction. The accuracy of DNA input concentration should be quantified and guaranteed. Ensure no more than 1000ng of highest input for manual library construction and 900ng for automatic platform.

2, Do I have to prepare any special equipment for library construction?

A: Rack with high magnetism is recommended like ALPAQUA, Part#A000400 for the better recovery.

3, For the MGIEasy FS PCR-Free DNA Library Prep Set, is there any special requirement on DNA storage buffer?

A: Since the FS Enzyme Mix II is sensitive to the pH and content of DNA storage buffer, we recommend using 1x TE buffer (PH 8.0) or H_20 for dissolution of DNA. If other buffers (10mM Tris (PH 6.8-8.0), AE Buffer (PH8.5), 0.1XTE (PH 8.0)) are present, please follow the scheme in 3.2 of the manual for pilot test first. If other special buffers are present, please do a test first. We recommend the use of pH7.0-8.5 DNA storage buffer for testing. On the basis of base parameter--the fragmentation time of 11 min at pH 8.0, the lower pH, the shorter shearing time. The higher pH, the longer. If the test result is not good, please re-purified the gDNA and elute in 1x TE buffer (PH 8.0).

4, What are the factors that affect Fragmentation Enzyme?

A: pH of DNA storage buffer will affect the fragmentation effect of the Fragmentation Enzyme. Do the test on the basis of fragmentation condition that recommended (pH8.0) in user manual. In addition, protein, phenolic and other contaminations in DNA samples may affect the fragmentation effect as well. Bead purification or trizol extraction is recommended if too many impurities.

5, How to determine the fragment size is suitable?

A: Followed by addition of 20 μL En-TE after fragmentation reaction, we recommend extract 5 μL reaction product and then add 10 μL En-Beads for purification. The resulting sample is analyzed by Agilent2100. The fragment selection can be conducted (two-step magnetic beads purification) following

the suggested condition if main fragment sizes fall in the range of 300-800 bp (see user manual for peak shape). The peak insert size is around 380 bp after sequencing and data analysis. Otherwise, the peak of insert size should be around 320 bp under one-step magnetic beads purification process.

6, What are the DNA concentration and requirements after size selection?

A: If the library is prepared with 1000 ng gDNAs, the concentration of size-selected DNA is generally greater than 3 ng/μL and the total amount is more than 120 ng. If the concentration is in the range of 1.5-3 ng/μL and total amount is more than 60ng, library preparation can be attempted, but with the risk of low library yields or failure. The total amount of purified DNA fragments before end repair needs to be strictly controlled in the 80-200ng range.

7, What is the quantity of gDNA input when using the MGISP-960 for library preparation?

A: There are two scripts for the MGIEasy FS PCR-Free DNA Library Prep Set by using MGISP-960: JB-A09-102 script for fragment selection process (two-step magnetic beads purification process) and JB-A09-104 script for purification process (one-step magnetic beads purification process). To ensure high success rate, 900 ng/48μL of gDNA input quantity is recommended for JB-A09-102 script and 320 ng/48 μL for JB-A09-104 script.

8, How to do quality control when using the MGISP-960 for library preparation?

A: There are two scripts for the MGIEasy FS PCR-Free DNA Library Prep Set by using MGISP-960: JB-A09-102 script for fragment selection process (two-step magnetic beads purification process) and JB-A09-104 script for purification process (one-step magnetic beads purification process) after fragmentation. Please follow the corresponding manuals of each script to do quality control. Quality control requires manual operation.

For JB-A09-102 script, after fragmentation and the pop-up operation prompt appears on the screen, take out the corresponding deep hole plate according to the manual, place the deep-well plate with magnetic beads on a 96-well magnetic rack, take 1 μL supernatant for quantification and 1 μL supernatant for Agilent 2100 size analysis.

For JB-A09-104 script, after the completion of stage 1 (purification), please follow the manual to do quality control.

9, When can the intermediate products of quality control be discarded?

A: The intermediate products of quality control can be discarded when the library construction and sequencing successfully or there is no obvious need for repeated quality inspection.

10, Why is Elute Enhancer necessary during DNA elution? What is the consequence if it's not used?

A: Elute Enhancer can reduce DNA loss during the purification process. If Elute Enhancer is not used during purification, the library yield may be lower than expected or/and too low to be sequenced.

11, Are there any stopping points during library preparation? How long can the products be stored after each stopping point? Is the circularized ssDNA library transportable before sequencing?

A: The stopping points are after each cleanup step. The purified DNA product can be stored at -20℃ for up to 6 months. The circularized ssDNA libraries can be stored for up to 3 months. You can transport the circularized ssDNA libraries on dry ice.

12, In the user manual, every PCR program has a specific temperature setting for the thermocycler lid. What if the thermocycler lid is not programmable?

A: If the thermocycler lid is not programmable and can only be kept to 105℃, it is recommended to keep lid open or loosen when reaction temperature is below 25℃ and close the lid when the reaction temperature is above 25℃.

13, Can I replace PF Adapters with Adapters from the MGIEasy DNA Adapters Kit?

A: No, those two adapters have different designs. Adapter ligation using adapters from the MGIEasy DNA Adapters Kit will lead to failure of library construction.

14, The Ligation Enhancer needs to be stored at room temperature and away from light. Will store or transport it at -20℃ make any difference?

A: For the Ligation Enhancer, be transported at -20℃ makes no difference to its function. It should be stored at room temperature and avoid sunlight after being received or first opened for use. The DNA library yield will not significantly change if the Ligation Enhancer undergoes 2 or 3 freeze-thaw cycles, but will dramatically decrease if Ligation Enhancer undergoes more than 7 freeze-thaw cycles. If precipitation of particulate matter occurs, stop using the reagent.

15, Ad-Lig Buffer seems very viscous, what should I do to ensure the buffer is homogenous before using?

A: Thaw the Ligation Buffer at room temperature and vortex 6 times (6 s each) to ensure homogeneous. If the mix remain viscous after sample added, 6 times (6 s each) vertexing should be done again to ensure that the ligation reaction mixture be thoroughly vortexed until the color is a homogenous light yellow, or the yield will be influenced.

16, The suggested input of circularized ssDNA for sequencing is 75 fmol. What will happen if the circularized ssDNA yield does not meet the requirement of minimum input?

A: If you use less than 75 fmol of circularized FS PCR-Free ssDNA for DNB making, the DNB concentration

may be too low to be sequenced or the data output will be reduced.

17, What is the difference between the MGIEasy PCR-Free DNA Library Prep Set and the MGIEasy FS PCR-Free DNA Library Prep Set?

A: The MGIEasy FS PCR-Free DNA Library Prep Set has additional Fragmentation Enzyme and buffer and it is compatible with the laboratory that do not have DNA shearing machine and/or automatic process with genomic DNA as input.

18, Which step can we pool libraries?

A: It is recommended to do the pooling step after the step of "Quality Control of Digestion Product" in the User Manual. It is not recommended to do library pooling after the step of "Cleanup of Adapter-ligated DNA" in the User Manual, which can avoid a small number of residual adapters to participate in the next reaction resulting in contamination between samples. If the pooling step is performed after the step of "Cleanup of Adapter-ligated DNA" in the User Manual, 0.8x En-Beads should be used for re-purification after pooling.

19, Will it make any difference for sequencing quality after version update?

A: After version update, it is normal if the unfilter Q30 of the first 1-2 bp drops and then rises again. This is caused by the fact that the sequencing signal was still in the lifting stage during the first two bp sequencing, and this has no effect on the following data analysis.