MGIEasy Fast FS DNA Library Prep Set V2.0 FAQ

Cat. No.: 940-001193-00 (16 RXN, V2.0)
940-001194-00 (96 RXN, V2.0)
940-001196-00 (192 RXN, V2.0)

FAQ Version: 1.0

For Research Use Only.

MGI Tech Co., Ltd. All rights reserved

1. Does the MGIEasy Fast FS Library Prep Set V2.0 have any requirement for the sample concentration and quality?

A: It is strongly recommended to use high quality genomic DNA (gDNA) samples ($1.8 \leq OD_{260}/OD_{280} \leq 2.0$, $OD_{260}/OD_{230} \geq 1.7$, no degradation or degraded slightly) for fragmentation. If the integrity or purity does not meet the requirement, It is possible to proceed library construction with risk of low library yields. For the input DNA that purity do not meet the requirements, clean up the DNA with 1.8x~2x beads prior to library prep. If there are RNA contamination, please perform a RNase A digestion before library preparation. The accuracy of DNA input concentration should be quantified and guaranteed. Qubit® dsDNA HS Assay Kit is recommended for sample quantification.

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. What are the factors that affect Fragmentation Enzyme?

A: FS Enzyme II is sensitive to pH and components of DNA storage buffer. Genomic DNA (gDNA) dissolved in EB/TE/H2O can be processed according to the User Manual. For DNA dissolved in other buffers, please adjust the fragmentation conditions as recommended in the User Manual. If there are impurities such as proteins, RNA, phenolic compounds, etc., remaining in the DNA sample, it may affect the efficiency of fragmentation. It is recommended to use bead purification, RNase digestion, or phenol-chloroform re-extraction for purification. When the input DNA are long amplicons of DNA, fragmentation time should be tested due to differences in amplicon length and GC content.

4. How to determine the size of the fragmented DNA is appropriate?

A: Samples with ≥100 ng of gDNA: take 5 μL of the sample after the fragmentation reaction, purify with 1x En-Beads, resuspend it in 5 μL of TE, and then use the Agilent Bioanalyzer with High Sensitivity DNA Kit to analyze the size of the fragments. The size should range from 100 to 2000 bp, with a peak between 400 and 700 bp; this method can also be applied to analyze the size of fragments after PCR purification. Samples with <100 ng of gDNA: it is recommended to analyze the fragment distribution after PCR purification. The PCR products from this library construction process range from 300 to 2000 bp, with bead single size selection for peaks of 500 to 750 bp, and bead double size selection for peaks of 450 to 550 bp.

5. Is it possible to use sheared DNA as input material with MGIEasy Fast FS Library Prep Set V2.0?

A: For sheared DNA inputs, we recommend using the MGIEasy UDB Universal Library Prep Set (Item No.: 1000022803/1000022804/1000022805), or MGIEasy Universal DNA Library Prep Set (Item No.: 1000006985/1000006986/1000017571)

6. what is the suggestion when my input DNA does not meet the sample quality requirements?

A: It is possible to proceed library construction by risk. You can Clean up the DNA prior to library prep, adjust the fragmentation parameters, extend the ligation reaction time, and increase the number of PCR

cycles. During the first library construction process, add quality control points, such as taking out 5 μL of the sample after the fragmentation, purifying with 1x En-Beads and then performing fragment size analysis and DNA quantification. Additional quantification should be done after the adapter ligation purification as well.

7. What is the minimum gDNA input when library prepared by double-sided size selection.

A: 500 ng is recommended. Increase the number of PCR cycles if you minimize the gDNA input to 200ng.

8. Can we vortex Fast FS Enzyme II?

A: No. Do not vortex Fast FS Enzyme II. If you vortex it, please ensure that you only vortex it within three times. If it is vortex more than three times, you should do more verification before you use it for real test.

9. How long the Adapter ligation mixture can be stored after it is prepared?

A: the adapter ligation mixture must be stored on ice and used in 30min.

10. What we should note when using adapters?

A: 1) Only adapter kits listed in user manual can be used.

2) The adapter kit should be thoroughly mixed and centrifuged before use.

3) The adapter needs to be diluted according to the initial amount of library preparation. For the dilution ratio, refer to Table 23 Relationship between UDB PF Adapter dilution ratio and gDNA input.

4) It is recommended to using TE for adapter dilution and freshly preparing adapter dilution.

11. Can I pre-mix adaptor with Ligation Mix?

A: Do not add adaptor to ligation master mix. This can cause increased adaptor-dimer formation. For best results, add adaptor to sample, mix, and then add Ligation Mix, mix again.

12. primers dimer remaining after PCR

A: Primers dimer is about 130 bp an visible on Bioanalyzer. Perform another 0.75 x En-beads cleanup to remove the dimer.

13. 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.

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 first opened for use. The DNA library yield will not significantly change if the Ligation Enhancer undergoes 1 or 2 freeze-thaw cycles, but it will dramatically decrease if Ligation Enhancer undergoes more than 7 freeze-thaw cycles. Stop using the reagent if you see a precipitate in Ligation Enhancer.

15. Is there any other brand of purification beads in library preparation?

A: No. Customers should do the demo test by themselves. If they want to try other brand purification beads, the results of library yield, the peak and distribution of insert size should be same with control test.

16. What is the effect of adding 80% ethonal to wash in fragmentation step?

A: Except the lower yield, there is no effect. You can continue to perform the library preparation.

17. 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 transportthe circularized ssDNA libraries on dry ice.

18. Is it necessary to dilute the UDB adapter when the input of gDNA is 1~25 ng?

A: There is no obvious effect on library yield, but the adapter contamination will increase and data quality may be compromised. Perform another 0.7 x En-beads cleanup to remove the dimer.

19. What should I do if it is found that the PCR product concentration is particularly high (>100ng/ul)? Can I proceed to make libraries?

A: ① Re-measure the concentration; ② Analyze the fragments by Agilent 2100, etc. If the fragment sizes meet the expectation, library preparation can be resumed.

20. Which step can we pool libraries

A: It is recommended to do the pooling step after the step of "QC of PCR product", "QC of PCR product" or "DNB preparation".

21. What are the differences and scenarios between DNBSEQ One-Step DNB Preparation Kit V2.0 (OS-DB) and DNBSEQ One-Step DNB Preparation Kit?

A: For the difference, 1) V2.0 has optimized components based on V1.0, which is more friendly to high GC coverage. 2) the reaction condition is different.

Based on this, the recommended application scenarios are below:

1) Selectively use the one-step DNB preparation kit version recommended by the sequencing platform or existing applications.

2) For new applications or WGS sequencing, DNBSEQ One-Step DNB Preparation Kit V2.0 (OS-DB) is preferred.

22. Whether the input of library for DNB preparation should be half, if you use half volume for DNB preparation?

A: Yes.