FAQ

MGIEasy FS PCR-Free DNA Library Prep Set

Version：A0

1. Does the MGIEasy FS PCR-Free DNA Library Prep Set have any specific sample requirement? Is this set applicable to all sample types?

A: This library prep set can be used for samples from common animals, plants, fungus, bacteria etc. including human (blood, saliva, or fresh tissue samples), mice, rice, E. coli and microbes. Stable performance across all such sample types is expected. In addition, purified WGA DNA is also compatible. However, as different WGA methods may have different results，it is recommended take a sufficient validation test before widely used.

2. What special equipment do I need for MGIEasy FS PCR-Free DNA Library construction?

A: A magnetic separation rack compatible with 0.2 mL PCR tubes is recommended in order to reach the maximum product recovery efficiency. （recommend ALPAQUA, Part#A000400）

3. What's the DNA input requirement?

A: High quality DNA is recommended. The DNA should be intact or slightly degraded, A260/A280 = 1.8~2.0, A260/A230>2.0. If the integrity or purity does not meet the requirement, there will be some risk of low library yields or library failure.

4. What are the DNA concentration and fragment size distribution requirements after size selection?

A: Since ideal insert size for sequencing is 475±50 bp, therefore, after size selection, size distribution of fragmented product is recommended to range from 275±50 bp to 675±50 bp. Smaller size may lead to low variant calling sensitivity, and larger size may lead to low sequencing quality. In addition, a concentrated size distribution increases sequencing quality, while broader size distribution may lead to low sequencing quality and low data output.The concentration of size selected DNA is recommended to be more than 3 ng/μL. If the concentration is 1.5-3 ng/μL, library preparation can be attempted, but with the risk of failure. Otherwise, do not proceed.

5. 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 the Elute Enhancer is not used to elute purified

DNA, the library yield may be lower than expected or/and too low to be sequenced.

6. Are there any stopping points during library construction? How long can the products be kept after each stopping point?

A: The stopping points are after each purification step. The purified DNA product can be stored -20℃ for 6 months, the purified circularized ssDNA library can be stored -20℃ for 3 months.

7. In the user manual, every PCR program has a specific temperature setting for the thermocycler lid. What will happen 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℃.

8. Can I replace PF Adapters with Adapters from MGIEasy DNA Adapters Kit?

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

9. The Ligation Enhancer needs to be stored at room temperature and away from light. Will storing it at -20℃ make any difference?

A: This reagent should be stored at room temperature and avoid sunlight. The FS PCR-Free DNA library yield will not significantly change if the Ligation Enhancer undergoes 1 or 2 freeze-thaw cycles, but will dramatically decrease if the Ligation Enhancer undergoes more than 2 freeze-thaw cycles. If precipitation of particulate matter occurs, stop using the reagent.

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

A: After thawing, the Ad-Lig Buffer should be vortexed thoroughly on a vortex mixer before using. After adding DNA sample, the ligation reaction mixture should also be thoroughly vortexed until the color is a homogenous light yellow.

11. Why should I add 20 μL of En-TE after adapter ligation? What will happen if I forget this step?

A: The En-TE will decrease the remained adapters after purification. If you forget to add it, the adapter contamination rate will increase, leading to a decrease of data utilization. If you do forget to add the En-TE buffer, and the ligation yield is high enough, (the concentration of purified ligation products ≥ 1.5 ng/μL), we recommend that you re-purify the ligation product with 1x En-Beads (50 μL En-Beads).

12. Can I skip the ligation product quantification step?

A: It is not suggested to skip this step except for the situation that expected sequencing results can be consistently repeated using the same recipe and similar samples.

13. 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.

14. Can I store the circularized ssDNA library before sequencing? If can, what is the recommended storage condition and what is the recommended shelf life? Is the library transportable before sequencing?

A: Of course, the circularized ssDNA libraries can be kept at -20℃ for up to 3 months. You can transport the libraries on dry ice.

15. What is the difference between PCR-Free DNA Library Prep Set and FS PCR-Free DNA Library Prep Set?

A: FS PCR-Free DNA Library Prep Set has the Shearing Enzyme and buffer and it is compatible with automatic sample

preparation systems.

16. What are the factors that affect the Shearing Enzyme?

A: The EDTA component in DNA lysis buffer will affect the fragmentation effect of the Shearing Enzyme. Please make sure that the final concentration of EDTA in 50 μL Fragmentation Mixture is 0.7 mM. In addition, protein, phenolic and other contaminations in DNA samples may affect the fragmentation effect of the Shearing Enzyme.

17. How to make sure the final concentration of EDTA in 50 μL Fragmentation Mixture is 0.7 mM?

A: Make sure the EDTA concentration in the gDNA elute buffer by using 35mM EDTA solution and following the formula below

The final concentration of EDTA: 0.7 mM=(a*35+35*b)/50

Note: "a" indicates the EDTA concentration in the gDNA elute buffer, "b" indicates the volumn of 35 mM EDTA you need to added.

Examples are given in the following table:

EDTA concentration in the gDNA elute buffer, mM	gDNA volumn after normalization, μL	Volumn of 35 mM EDTA added, μL	Volumn of Fragmentation Mixture, μL	The final concentration of EDTA, mM
a	35	b	50	0.7
1	35	0	50	0.7
0.75	35	0.25	50	0.7
0.5	35	0.5	50	0.7
0.25	35	0.75	50	0.7
0	35	1	50	0.7
Note: Make sure to use the same buffer for gDNA normalization as the gDNA elute buffer.				

18. Which step can we pool libraries?

A: It is recommended to do the pooling step after the step of "3.9 Exo Digestion" in the User Manual. It is not recommended to do library pooling after the step of "3.6 Cleanup of Adapter-ligated DNA" in the User Manual, which

can avoid a small amount of residual adapters to participate in the next reaction resulting in contamination between samples. If the pooling step is performed after the step of "3.6 Cleanup of Adapter-ligated DNA" in the User Manual, 0.8x En-Beads should be used for re-purification after the pooling.