MGIEasy

FS DNA Library Prep Set FAQ

Cat. No. 1000006987, 1000006988, 1000017572

Kit Version: V2.1

FAQ Version: A1

Chapter 1: Overview

1, How many specifications does the MGIEasy FS DNA Library Prep Set V2.1 have? What kits are included?

A: Currently we have 3 different models in total, see details as follows:

Product	Component kit	Configuration
MGIEasy FS DNA Library Prep Set (Cat. No. 1000006987)	MGIEasy FS DNA Library Prep Kit (Part. No. 1000005254)	16 RXN
	MGIEasy DNA Adapters-16 (Tube) Kit (Part. No.1000005284)	16 x 10 μL
	MGIEasy DNA Clean Beads (Part. No. 1000005278)	8 mL
	MGIEasy Circularization Module (Part. No. 1000005260)	16 RXN
MGIEasy FS DNA Library Prep Set (Cat. No. 1000006988)	MGIEasy FS DNA Library Prep Kit (Part. No. 1000005256)	96 RXN
	MGIEasy DNA Adapters-96 (Plate) Kit (Part. No.1000005282)	96 x 10 μL
	MGIEasy DNA Clean Beads (Part. No. 1000005279)	50 mL
	MGIEasy Circularization Module (Part. No. 1000005260)	16 RXN
MGIEasy FS DNA Library Prep Set (Cat. No.1000017572)	MGIEasy FS DNA Library Prep Kit (Part. No. 1000005256)	96 RXN
	MGIEasy DNA Adapters-96 (Plate) Kit (Part. No.1000005282)	96 x 10 μL
	MGIEasy DNA Clean Beads (Part. No. 1000005279)	50 mL
	MGIEasy Circularization Module (Part. No.1000017573)	96 RXN

2, What kind of sample can be used with the MGIEasy FS DNA Library Prep Set V2.1?

A: This MGIEasy FS DNA Library Prep Set V2.1 is applicable for gDNA sample from human sample (saliva, blood, tissue) and common animals, plants, fungus and Meta.

3, Do I have to prepare any special equipment for library preparation?

A: Thermocycler, Pipets, Magnetic rack DynaMag is needed. Rack with high magnetism is recommended like ALPAQUA, Part#A000400 for the better recovery.

4, What's the requirement of the sample quality?

A: Integrity and Purity of the input DNA are needed for the MGIEasy FS DNA Library Prep Set V2.1. It is strongly recommended to use high quality genomic DNA (gDNA) samples (A260/A280=1.8-2.0, A260/A230>2.0, no degradation or degraded slightly) for fragmentation. For the gDNA with low quality, it is recommended to do fragmentation test using part of gDNA to adjust the optimal fragment time, but risk of failure remains.

5, If the sample's quality fails to meet the requirement, can it be used for library preparation?

A: Attempt for library construction can be considered. We recommend tune the fragmentation parameters and PCR cycles accordingly. Increase QC points in the first library preparation (e.g. size QC after fragmentation, concentration QC after size selection, concentration QC after adapters ligation and purifications).

6, Is there any recommendation for input amount in the MGIEasy FS DNA Library Prep Set V2.1?

A: The recommended input amounts for different sample types are showed as below. The input amount could be increased if the quality of sample is poor.

Sample type	Input Range	Recommended Input amount	Recommended Concentration
Complex genome	50-400 ng	200 ng	≥15 ng/μL
Simple genome	5-400 ng	100 ng	≥7.2 ng/μL
Microbiome	5-400 ng	100 ng	≥7.2 ng/μL
Meta	5-400 ng	100 ng	≥7.2 ng/μL
Pathogenic samples	5-100 ng	100 ng	≥7.2 ng/μL

7, How to identify High / low complexity genome?

A: Generally, a genome less than 20 MB is a simple genome, such as microbial genome, Meta genome, virus genome, etc. A genome more than 20 MB is a complex genome, such as human genome, polyploidy plant genome, etc.

8, Can the library be constructed with a higher input amount than that recommended in user manual?

A: For the MGIEasy FS DNA Library Prep Set V2.1, the maximum input amount for end repair is 100ng. On

the basis of 25% selection efficiency by calculation, 400ng gDNA can be allowed for fragment selection process (two-step magnetic beads purification after fragmentation). If the input amount is increased, there may be a risk of incomplete enzymatic reactions in each step.

9, 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.

10, What's the exact temperature when it refers to "heated lid: On"?

A: The heated lid should be set 5-10℃ higher than reaction temperature when using the Thermocycler with adjustable lid. For example, if the reaction condition is 37℃, the heated lid should be set to 42-47℃. 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℃.

11, What extra reagents, kits or materials should I prepare for the MGIEasy FS DNA Library Prep Set V2.1?

A: There are no additional reagents or kits you need to buy from MGI since most of the reagents (including library preparation reagents, circularization reagents, adapters and clean beads) are included in the MGIEasy FS DNA Library Prep Set V2.1. For a list of other normal reagents and materials that are not packaged in the set and need to be prepared in advance, please check the section "Equipment and Materials required but not provided" in the User Manual.

12, For the MGIEasy FS DNA Library Prep Set V2.1, what kinds of DNA storage buffers can be compatible with it?

A: The compatibility of Fragmentation Enzymes in the MGIEasy FS DNA Library Prep Set V2.1 has been greatly improved. Compatible DNA storage buffers include water, EB, 0.1×TE, Buffer AE, TE and other common storage buffers. It is suggested that the sample be dissolved in water, EB or low TE (0.1×TE). For other buffers, we suggest exploring the optimal fragmentation time before use.

13, Can I fragment the samples into insert size exceed 250-350bp range?

A: Fragment with insert size±50bp can be used for normal preparation. 400bp can be accepted for PE100 sequencing, but size less than 250bp is not recommended for PE150 because of the overlap.

14, Can I use the WGS library constructed using the MGIEasy FS DNA Library Prep Set V2.1 to do SE

sequencing?

A: We recommend PE sequencing for the sake of good performance of data analysis in complex genome species sequencing. SE sequencing can be done as well but it does not guarantee to get the same data performance as that can get with PE sequencing under the same depth.

15, Can I skip the size-selection step?

A: The size selection step can be replaced with one-step purification in the MGIEasy FS DNA Library Prep Set V2.1. Ranged size may influence the sequencing quality.

16, Can I use amplicon to do the library preparation using the MGIEasy FS DNA Library Prep Set V2.1?

A: Yes. It is recommended to explore the optimal fragmentation condition first. After fragmentation, the main band should be located in the target range.

17, Can I use the beads from other vendors?

A: You can use AMPURE XP to replace MGI Clean Beads. It is recommended to do tests before the replacement can be used if beads are from other vendors.

18, Which sequencers can the MGIEasy FS DNA Library Prep Set V2.1 be adapted to?

A: SE50/PE100/PE150 on MGISEQ-2000, and PE100 on BGISEQ-500/DNBSEQ-T7 are the recommended sequencing strategies. More sequencing strategies and sequencers are currently being tested.

19, What is the difference between the MGIEasy FS DNA Library Prep Set V2.1 and the MGIEasy Universal DNA Library Prep Set V2.0?

A: The MGIEasy FS DNA Library Prep Set V2.1 has additional Fragmentation Enzyme and buffer and it is compatible with the laboratory that do not have DNA shearing machine and/or automatic library construction system.

Chapter 2 Experimental Questions

20, How many fragmentation reactions can the MGIEasy FS DNA Library Prep Set V2.1 supply?

A: Based on the standard SOP in the user manual, the amount of the Frag Buffer II and Frag Enzyme II of the MGIEasy FS DNA Prep Kit (16RXN, Part No. 1000005254) can supply 16 manual fragmentation reactions and about 1-2 extra manual fragmentation reactions. And that of the MGIEasy FS DNA Prep Kit (96RXN, Part No. 1000005256) is enough to prepare 96 manual fragmentation reactions and about 20-24 extra manual fragmentation reactions. When using MGISP-960 for library preparation, that of the

kit (96RXN, Part No. 1000005256) can supply 96 automated fragmentation reactions per run and about 1-2 extra manual fragmentation reactions. We recommend do tests using these extra reagents reasonably before formal experiment.

21, What should be done if the concentration of fragmented samples is low and the volume exceeds the requirement of the fragmentation system?

A: ① it is recommended to make libraries with low input; ② Increase the volume of the fragmentation mixture based on the final volume of the sample. Keep the elute volume as 42 μL after size selection or purification and transfer 40 μL superment for ER step. It should be noticed that the maximum reaction of the kit is limited.

22, What are the factors that affect Fragmentation Enzyme?

A: For the upgraded Fragmentation Enzyme II, the tolerance of EDTA in the DNA storage buffer increases, and the fragment sizes show no significant difference when DNA sample dissolving in TE or Tris solution of the same pH. But the DNA fragmentation is relatively sensitive to pH of the DNA storage buffer. It needs to fine-tune the shearing time after DNA normalization if the DNA storage buffer does not belong to common storage buffer (e.g. water, EB, 0.1× TE, Buffer AE, TE), or the pH of the DNA storage buffer is out of the range between 6.8 and 8.5. The fine-tuning method is suggested to extend/shorten the fragmentation time by 2 min for each 200 bp oversize/undersize of the main band.

In addition, protein, phenolic and other contaminations in DNA samples may affect the fragmentation effect as well. Beads purification or trizol extraction is recommended if too many impurities.

23, Can I skip the quantification step before ER step?

A: If the DNA yield is more than 100ng, the quantification step should be done and choose no more than 100ng to do next ER step. Otherwise, the quantification can be skipped.

24, What is the concentration of the MGIEasy DNA Adapters?

A: 10 μM.

25, Ligation 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, or the yield will be influenced.

26, What is the concentration of PCR Primer Mix?

A: 20 μM.

27, What should I do if it is found that the PCR product concentration is particularly high (>100ng/ul)? Is that normal? 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.

28, What if circularized ssDNA library is too low to be detected while the concentration of PCR product is normal?

A: Most likely there is something wrong in the circularization step. We suggest adding a positive control (PCR products that were previously circularized successfully) to do the circularization again.

29, Can the circularization step be proceeded if only 100ng PCR product is obtained? Is there any influence on sequencing data?

A: 100 ng PCR products can be circularized and the circularization efficiency will not be affected. However, there are many factors affecting the sequencing data performance. If the PCR yield is obviously abnormal with the empirical value under the condition of the same input amount and library construction SOP, it might result from abnormalities in library preparation. It is recommended to find out the reason and reconstruct libraries.

30, What if the yield of circularized ssDNA is very high (>4 ng/uL)?

A: Circularization efficiency is generally less than 30%. In this case, re-measure the concentration and reset the standard curve first. If the re-measure result is still too high, the possible reasons are as follows: ①PCR product is not denatured; ② incomplete digestion. Re-circularization is recommended.

31, What if the DNB concentration is lower than the requirement?

A: It is recommended to increase the input to make DNB. Sequencing can be attempted if the DNB concentration ⩾6 ng/μL, but with the risk of failure.

32, Which step can we pool libraries?

A: It is recommended to do the pooling step after the step of "Quality Control of PCR Product" or "Quality Control of Enzymatic Digestion Product" in the User Manual. It is not recommended to do library pooling after the step of "Adapter-Ligated DNA Cleanup" in the User Manual, which can avoid a small number of residual adapters to participate in the next reaction resulting in contamination between samples.

33, 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.

34, Why barcode split rate is low?

A: Generally, the success rate of splitting should be greater than 98%. The base imbalance of barcode will affect the split rate. It is recommended to do the pooling according to the recommended barcode combinations.

35, Is the MGIEasy FS DNA library Prep set V2.1 compatible for automatic platform?

A: The MGIEasy FS DNA Library Prep Set V2.1 is compatible for MGISP-100 and MGISP-960.

The MGIEasy FS DNA Library Prep Set (16RXN (with 16RXN circularization), Cat. No. 1000006987) is compatible for preparing 16 single circle libraries each time on MGISP-100.

The MGIEasy FS DNA Library Prep Set (96RXN (with 96RXN circularization), Cat. No. 1000017572) is compatible for preparing 96 single circle libraries each time on MGISP-960.

The MGIEasy FS DNA Library Prep Set (96RXN (with 16RXN circularization), Cat. No.100006988) is compatible for preparing 96 PCR products using MGISO-960 and make 16 single circle by manual or using MGISP-100.

Product	Configuration#	Part Number	Product Version	Chinese User Manual Version ##	Engish User Manual Version ##	NO. of RXNs by manual	NO. of RXNs per run × N runs by MGISP-100	NO. of RXNs per run × N runs by MGISP-960
MGIEasy FS DNA Library Prep Set	16RXN (with 16RXN circularizations)	1000006987	V2.1	B4	B3	16RXN	16RXN × 1 run	/
	96RXN (with 16RXN circularization)	1000006988	V2.1	B4	B3	96RXN **	/	96RXN × 1 run **
	96RXN (with 96RXN circularization)	1000017572	V2.1	B4	B3	96RXN	/	96RXN × 1 run

* Configuration = NO. of RXNs by manual

\# For more information of user manuals, please visit the MGI website (https://en.mgitech.cn/) or obtained the user manuals from your MGI account manager or technical support engineers.

** The set contains 96 RXN library kit + 96 RXN adapters + 50 mL clean beads + 16 RXN circularization module. Up to 96 samples libraries can be constructed by properly pooling according to the number of circularization module and the number of samples before circularization during libraries construction. Consult your local MGI technical support engineers for more advice on the usage of the product.

36, What are the advantages of the MGI Easy FS PCR-Free DNA Library Prep Set V2.1?

- High-quality, low bias fragmentase
- Simple operation, automation friendly
- Good uniformity
- No obvious GC bias
- More accurate InDel calling

37, For the MGIEasy FS DNA Library Prep Set V2.1, what sequencing applications can be recommended?

A: The MGIEasy FS DNA Library Prep Set V2.1 can be applied in human whole genome sequencing, exome sequencing, animal and plant genome sequencing, pathogen sequencing, metagenome sequencing,

et. It's suitable for anyone who is willing to or is using WGS sequencing on DNBSEQ platform as a tool for scientific research. As the MGI Easy FS DNA Library Prep Set V2.1 is an upgraded version of ordinary WGS and has a better data performance, especially suitable for those who have the capability for large sample input amounts and are looking for a simple procedure that results in better variant calling performance and better WGS sequencing data.