MGIEasy Fast PCR-FREE FS DNA Library Prep Set V2.0 FAQ

Cat. No.: 940-000886-00（16 RXN, V2.0）
940-000884-00（96 RXN, V2.0）
940-000882-00（384 RXN, V2.0）

FAQ Version: 1.0

For Research Use Only.
MGI Tech Co., Ltd. All rights reserved

1. Does the MGIEasy Fast PCR-FREE 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, attempt for library preparation can be tried, but risk of low library yields or failure remains. For the input DNA that purity do not meet the requirements, we recommend to preform the purification with 1.8x~2x beads before library preparation. 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. For the MGIEasy Fast PCR-FREE FS DNA Library Prep Set V2.0, is there any special requirement on DNA storage buffer?

A: The Fast FS Enzyme II is sensitive to the pH. If the sample are solved in AE Buffer (pH8.5)/EB Buffer (pH8.0)/ 1x TE buffer (pH 8.0) or H_20, please according to the user manual to prepare library. If not, please do the fragmentation time titration. On the basis of base parameter--the fragmentation time of 8.5 min at pH 8.0. The incubation time can be titrated from 7.5 to 15 min. 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. For amplificon products, the fragmentation time should be tested because of the amplificon length, sequence and GC content. On the basis of base parameter--the fragmentation time of 8.5 min at pH 8.0.

5. How to determine the fragment size is suitable?

A: For the first fragmentation test, it is recommended that you take 10 μL of product from step 7 in section 3.2.2 for purification with 0.8 x magnetic beads and elute in 8 μL of En-TE. Take 1 μL of elute product for Agilent 2100 High Sensitivity test and ensure that the smear size is 200 bp - 3000 bp with the peak size between 600 bp - 850 bp and the region of long insert size(1000bp-3000np) should be less than 8%.

6. How to get longer or shorter insert size?

A: By increasing or decreasing the fragmentation time to get different insert size. The longer fragmentation time, the smaller insert size peak of fragment. When you adjust the length of insert size, you also should adjust the input for DNB preparation. The final input for DNB preparation can be measured by is its DNB concentration (10~15ng/μL).

For longer insert size: please shorten the fragmentation time and adjust En-beads amount. The longer insert size, the lower ESR result of sequence.

For shorter insert size: please increase the fragmentation time and En-beads amount. But it will have the risk of adapter dimer which will affect the sequencing results of ESR and Q30. For the En-beads amount, please do the titration first and then optimize it based the library and sequencing results. Considering to adapter dimer, the insert size can not be less than 120 bp.

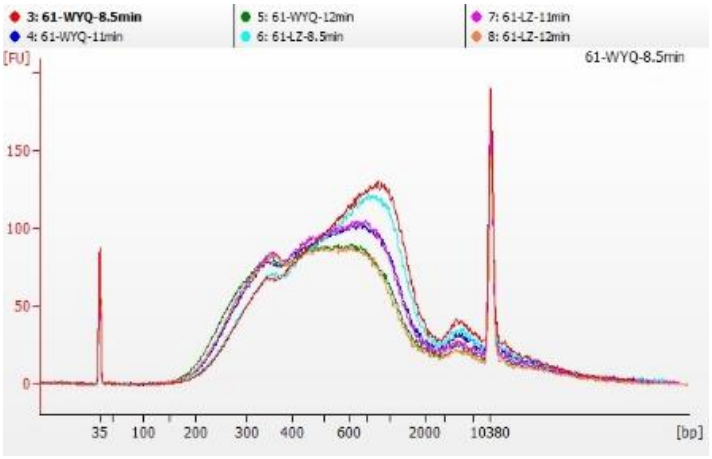


Figure 1 Agilent 2100 Bioanalyzer results of 0.8 x beads purification fragmentation product from different person and incubation time (8.5/11/12min)

7. What is the minimum gDNA input when library prepared by double-sided size selection and combined with DNBSEQ Onestep DNB Make Reagent Kit.

A: 500 ng is recommended, 300 ng also can be tried.

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. For adapter ligation step, should we pay attention to the sequence of adding the ligation mixture and adapter?

A: Yes. Different adding sequence will result in adapter or insert dimer which will affect the ssCir or DNB results. Please add the adapter first and then add the ligation mixture.

12. Why En-TE should be added after adapter ligation? What is the effect, if En-TE is not added?

A: It can reduce the adapter dimer contamination. If you forget to add En-TE, it has smaller effect on the pooled samples that will do the second purification. But it will have bad effect on the samples that will not pooled with other samples. Thus, if the ligation product is enough (more than 1.5 ng/μL), please perform a purification refer to H-940-000883-00-01 MGIEasy Fast_PCR-FREE FS Library Prep Set User Manual 5.1.4 Cleanup2 of adapter-ligated ligation product.

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 or/and too low to be sequenced.

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 1 or 2 freeze-thaw cycles, but it will dramatically decrease if Ligation Enhancer undergoes more than 7 freeze-thaw cycles. If precipitation of particulate matter occurs, stop using the reagent.

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 or adapter ligation purification step?

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

17. What is the effect of forget to add 80% ethonal wash in adapter ligation purification step?

A: The purity of library is worse than normal library preparation. It may affect the ESR, Q30 and the adapter contamination. If you find it when you finish adapter ligation 3.5 Cleanup of adapter-ligated product, you can perform a purification refer to H-940-000883-00-01 MGIEasy Fast_PCR-FREE FS Library Prep Set User Manual 5.1.4 Cleanup2 of adapter-ligated ligation product.

18. 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.

19. What the size distribution of ligation product by agarose QC?

1) The agarose concentration is 2%. The amount of each sample is 20ng.

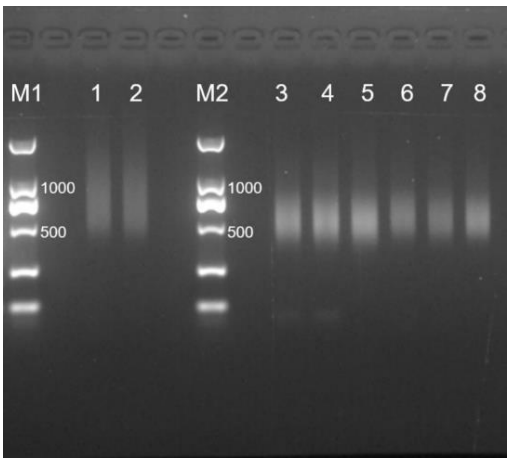


Figure 2 Agarose gel results of adapter-ligated libraries

M1, M2: D2000 ladder

1，2: 20ng ligation product prepared by single-sided selection (gDNA input 200ng)

3~4: 20ng ligation product prepared by double-sided selection (gDNA input 900ng)

5~8: 20ng ligation product prepared by double-sided selection (gDNA input 500ng)

20. What is the effect if you forget to dilute UDB PF adapter in library preparation (gDNA 25~500 ng)?

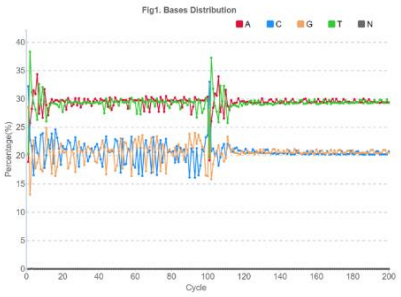
A: There is no obvious effect on library yield, but the adapter contamination will increase, and it maybe

affect sequencing quality, for example, the base distribution. If so, you can perform a purification refer to H-940-000883-00-01 MGIEasy Fast_PCR-FREE FS Library Prep Set User Manual 5.1.4 Cleanup2 of adapter-ligated ligation product.

21. Why base distribution of sequencing report is abnormal? How to ensure the adapter contamination of adapter-ligated libraries?

A: 1) There are adapter contamination in adapter-ligated library. If so, you can perform a purification refer to H-940-000883-00-01 MGIEasy Fast_PCR-FREE FS Library Prep Set User Manual 5.1.4 Cleanup2 of adapter-ligated ligation product.

Abnormal base distribution



Normal base distribution

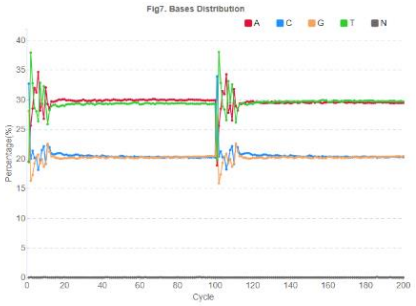
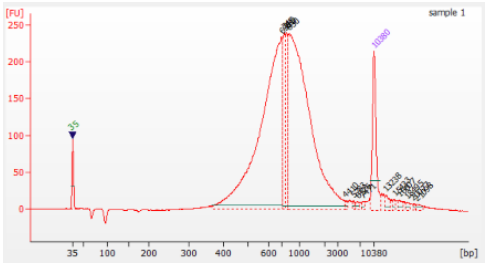


Figure 3 Base distribution of DNBSEQ-T7 PE100

2) Take 5 ng adapter-ligated libraries for Agilent 2100 High Sensitivity test. If there are small peak near 100bp, it indicates there are adapter contamination. Note: because of adapter configuration, the 2100 results only can reflect the adapter contamination, the peak size is not the real size of libraries.

Adapter-ligated libraries with adapter contamination



Adapter-ligated libraries(normal)

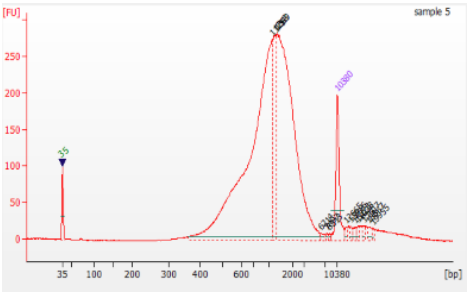


Figure 4 Agilent 2100 Bioanalyzer results of adapter-ligated libraries

22. What is the effect for sequencing results, when fragmentation product purified by single-sided size selection or double-sided size selection?

A: The single-sided size selection library has a wider insert size distribution than the double-sided size selection library, resulting in lower sequencing quality (for example, the ESR, total reads will be lower). Take T7 PE100 sequencing as an example: the data output of double-sided size selection library is basically above 5000M, and the Q30 of Read2 is normally raised. The data output of single-selection

library will fluctuate between 4500M and 5100M according to the difference of pooling libraries (such as different library construction input volume, different sample types when performing pooling test).

Unfilter Q30 of double-sided size selection libraries Unfilter Q30 of single-sided size selection libraries

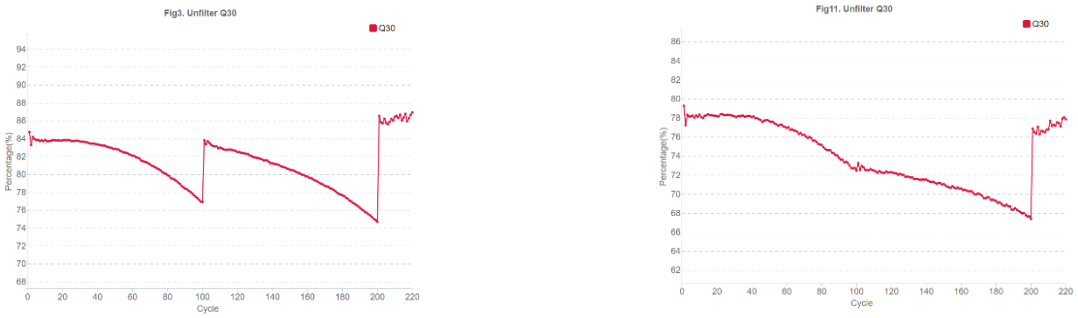


Figure 5 Unfilter Q30 of DNBSEQ-T7 (Slide is E1 series) with libraries prepared by different size selection methods

23. How to increase the pooling uniformity?

A: 384 barcodes are selected which can be used and pooled the ligation product without quantification. Here we show 384 barcodes to show the barcode splint results. The debarcode CV can be 20.53% as shown in the below figure. The libraries are pooled at ligation product by same volume. And the libraries are prepared from 65 persons (88 libraries) , blood gDNA from 56 persons and NA12878 (18 libraries).

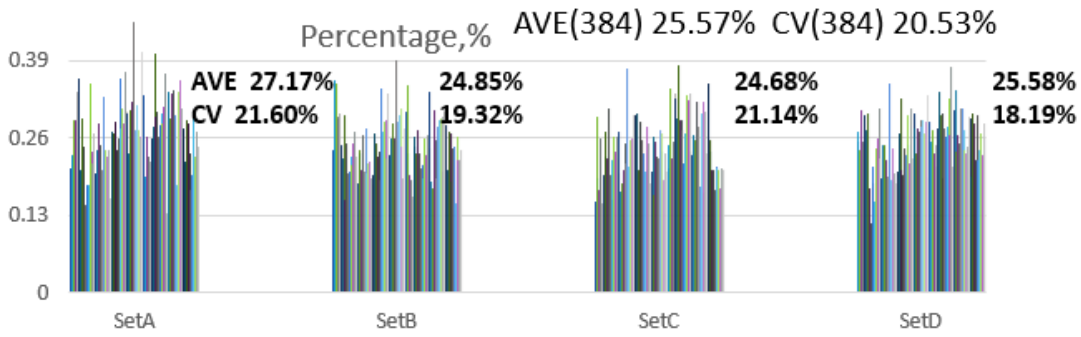


Figure 6 Debarocde rate of each barcode and debarcode CV

But the debacode CV will vary according to the sample's quality, quantification bias. So, we have some pooling strategies to improve the pooling uniformity.

1) Pool samples that has the same sample type, library preparation input and the sample library preparation protocol.

2) Combined with MGIEasy Dual Barcode Circularization Module (Cat. No.: 1000020570), pool samples by the same mass after the step of 4.1.4 "QC of digestion product" in the user manual which will be better than pooling at ligation product strategy.

3) Combined with DNBSEQ Onestep DNB Make Reagent Kit V2.0 (OS-DB, Cat. No.: 940-000036-00) or DNBSEQ Onestep DNB Make Reagent Kit (OS-DB, Cat. No.: 1000026466), pool samples by the same

DNB mass after DNB making which will be better than pooling at ligation product strategy.

4) The uniformity will be worse, if you pool the samples that are different sample type or sample input. But if you have to pool them, you can refer to pooling strategy 2) or 3) or pool them at ligation product by same mass to improve the uniformity.

24. 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.

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

A: Yes.