FAQ

MGIEasy FS DNA Library Prep Set V2.0

MGIEasy 酶切 DNA 文库制备试剂盒 V2.0

Version: A0

版本号：A0

第一章 概述篇

1.1 Which sample can be used in MGIEasy FS DNA Prep Set?

MGIEasy 酶切 DNA 文库制备试剂盒是否所有样本类型都可以?

A：MGIEasy FS DNA Prep Set is suitable for human (saliva, blood, tissue), plant, animal, fungus, Meta gDNA.

答：MGIEasy 酶切 DNA 文库制备试剂盒可进行常规人（来源于唾液，血液，新鲜和冻存的组织），植物，动物，真菌和宏基因组的 gDNA 文库构建。

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

客户建库的时候，需要准备什么特殊的仪器设备吗?

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

答:客户只需要准备 PCR 仪、移液枪和磁力架，推荐使用 0.2 mL PCR 管适用的磁性较强的磁力架（如：ALPAQUA, Part#A000400），可以达到最大的回收效率。

1.3 What's the requirement of the sample quality?

MGIEasy 酶切 DNA 文库制备试剂盒对于样品浓度和样本质量有什么要求?

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

答：MGIEasy 酶切 DNA 文库制备试剂盒要求对投入打断的 DNA 的完整度及纯度要相对较好，DNA 完整或轻微降解，A260/A280 = 1.8~2.0, A260/A230>2.0，对于降解或者纯度不达标的 gDNA，可以先取少部分样本进行打断测试，摸索最优打断时间后，再进行建库，但仍有失败的风险。

1.4 Is there any recommendation for different input in MGIEasy FS DNA Library Prep Set?

MGIEasy 酶切 DNA 文库制备试剂盒对于不同样本起始量有什么推荐?

A: The recommendation of different input shows as below. The input could be increased if the quality is pool.

Sample type	Input Range	Recommended Input	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

答：不同样本起始量推荐如下，若样本质量偏低可适当提高投入量：

样本类型	起始量范围	推荐起始量	推荐浓度
复杂基因组	50-400 ng	200 ng	≥15 ng/μL
简单基因组	5-400 ng	100 ng	≥7.2 ng/μL
微生物基因组	5-400 ng	100 ng	≥7.2 ng/μL
Meta 样本	5-400 ng	100 ng	≥7.2 ng/μL
病原样本	5-100 ng	100 ng	≥7.2 ng/μL

1.5 How to identify Complex/Simple genome?

如何定义复杂/简单基因组?

A: Simple genome means genome size less than 20 Mb. For example: Microbiome/Meta genome/Virus. Complex genome means genome size more than 20 Mb. For example: human, polyploidy plant.

答：低复杂度的样本（简单基因组）：基因组小于等于 20 Mb 左右，例如微生物基因组、宏基因组、病毒等；高复杂度的样本（复杂基因组）：基因组大于 20 Mb，例如：人、复杂多倍体植物。

1.6 Is there any stopping point during library Preparation? How long can the product be saved?

是否有建库操作安全终止节点? 每个安全终止节点产物可保存多久?

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.

答：具体终止点详见说明书。在每步纯化之后，纯化的 DNA 在-20℃可至少保存半年，最终的单链环文库可至少保存 3 个月。

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

说明书中 PCR 仪的热盖推荐"ON"，具体调节多少℃呢?

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

We recommend the heated lid remain unclosed when the condition below 25℃ when using the Thermocycler without adjustable lid. 105℃ could be set under the other conditions.

答：若可调节热盖温度的 PCR 仪，请选择比最高反应温度高 5-10℃的热盖温度，如最高反应温度为 37℃，可调整热盖温度为 42℃-47℃之间。根据反应温度热盖温度能保证最好的数据表现。

若是不可调节热盖温度的 PCR 仪，我们推荐在 25℃及以下反应温度时，不盖或不扣紧 PCR 仪的热盖，其他温度条件可使用 105℃热盖。

1.8 Can I use fragment with insert size exceed 250-350bp range?

说明书中推荐 PE100 主带 250bp，PE150 主带 350bp，若片段不在这个主带是否可以建库?

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.

答：说明书中推荐的主带是推荐的磁珠片段筛选后产生的主带，由于打断主带及个人操作有差异，片段大小 ± 50bp 均可正常建库上机测序。另外较大的片段，例如 400bp 插入片段也可以测 PE100，但是 250bp 不建议测 PE150，会有较多的 overlap。

1.9 Can the SOP used for PE200?

是否可以实现 PE200 建库?

A:there is no PE200 sequencing data yet, Library for PE200 can be done using less fragmentation time and less size-selection beads. For example, the fragmentation condition could be set to "32℃ for 3minutes", and the size selection condition could be set to "0.55+0.15"

答：暂无 PE200 数据。理论上，减少打断时间，减少双选的磁珠用量可以实现 PE200 建库：如 32℃ 3min 打断，0.55+0.15 双选后，进行建库。

1.10 Can I use the WGS library to do the SE sequencing?

文库是否可以进行 SE 测序?

A：PE sequencing is recommended for complex genome species sequencing. SE sequencing can also be done with good sequencing quality but MGI doesn't commit the alignment performance.

答：MGI 建库试剂盒推荐 PE 测序是为了保证大基因组（如人）样品 WGS/捕获分析数据表现。文库本身可以进行 SE 测序，但是不代表同深度下能达到 PE 测序一样的数据表现。

1.11 Can I skip the size-selection step?

MGIEasy 酶切 DNA 文库制备试剂盒 V2.0 能不能不双选建库?

A：The size selection step can be changed to one-turn purification in the FS library prep set. Ranged size may influence the sequencing quality.

答：可以实现打断后纯化后（不双选）建库。需要注意文库弥散度会影响测序质量。

1.12 Can I use the amplicon product to make libraries?

是否支持扩增子产物打断建库?

A: Make the library by fragmenting the size as we recommended when dealing with the amplicon product.

答：可实现扩增子建库，建议摸索打断条件，打断后 DNA 主带在目的区域即可。

1.13 Can I use the adapter from the other vendor?

可否使用其他品牌接头替代试剂盒中含有的接头?

A：Adapter with a "T" base extrude is all available. MGI doesn't commit any performance of the adaptor from the other vendor.

答：可以。只要是 T 突出接头，最终序列可用于 MGI 平台测序的接头，理论上都可以替代试剂盒中的接头，但是需要测试，性能、污染率等 MGI 不能保证。

1.14 What is the difference between MGIEasy "FS" and MGIEasy "Universal"?

MGIEasy 酶切 DNA 文库制备试剂盒 V2.0 和 MGIEasy 通用 DNA 文库制备试剂盒 V1.0 的差别是什么?

A: the "FS" one includes Fra Enzyme to do the fragmentation for libraries without fragmentation machine and automatic platform.

答：MGIEasy 酶切 DNA 文库制备试剂盒 V2.0 比 MGIEasy 通用 DNA 文库制备试剂盒 V1.0 多 DNA 打断酶及配套打断缓冲液的试剂，适用于无打断仪的实验室和/或基因组 DNA 起始的自动化建库。

1.15 Can reagents that shared the same name but from different set (such as Universal/FS) be used as substitutions?

不同试剂盒的试剂（如通用/酶切的同名未修试剂）是否可以混用?

A：Reagents with the same item number could be used freely.

答：Item 号一样就可以代替使用。

第二章 实验篇

2.1 How many frag-reaction can MGIEasy FS DNA Library Prep Set supply?

MGIEasy 酶切 DNA 文库制备试剂盒中 Frag Buffer 和 Frag Enzyme 可支持几个打断反应?

A：we recommend costumers to do several test before formal experiment. MGIEasy FS DNA Prep Set（Lot：1000005254）have 18 reaction for real.(2 for extra experiment) , MGIEasy FS DNA Prep Set（Lot：1000005256）have 120 reaction for real.(24 for extra experiment), If the set is used in MGI SP-960, the test should be no more than 2 samples.

答：我们鼓励客户在进行大批量酶切建库前，先进行几个打断测试，以找到最合适的打断条件，得到最优的数据。MGIEasy 酶切 DNA 文库制备试剂盒（货号：1000005254）实际含有 18 个反应的打断试剂，有 2 个多余试剂可进行测试。MGIEasy 酶切 DNA 文库制备试剂盒（货号：1000005256）实际含有 120 个反应的打断试剂，有 24 个多余试剂可进行测试，但如果客户将使用本 96RXN 试剂盒在 MGISP-960 上进行建库，建议只测试 1-2 个样本即可。

2.2 What will influence the fragmentation result?

影响酶切打断的因素有什么?

A：① Under 32℃, the more fragmentation time being cost, the smaller the size is. ② more EDTA decreases the fragmentase activity. ③ High concentration of divalent cation improves the fragmentase activity. ④ the size increased slightly when more gDNA was input into fragmentation. ⑤ Wrong storage condition will significantly decrease the activity.⑥ Vortex is necessary for fragmentation Mix preparing, or the fragmentation will be effected seriously.

答：影响酶切打断的主要因素是 32℃打断时间及体系中 EDTA 含量，时间越长，打断越剧烈。EDTA 含量越高，打断越困难。建议样品溶于水、EB 或 low TE（0.1×TE）中，确保打断体系 EDTA 终浓度为 0-0.1mM。此外，DNA 样本中若有蛋白质、酚类等杂质残留，可能会影响酶切打断效果。

2.3 Can I skip the quantification step before ER step?

打断纯化/磁珠片段筛选后必须做定量吗?

A: If the DNA yield is considered to be 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.

答：必要时才做，当你的打断纯化/磁珠片段筛选后的产量有可能超过 100ng 时，需要定量后，取 100ng 进行随后的末端修复步骤。若预估的纯化后产量不足 100ng，可不做定量，直接做末端修复。理论上单选的回收率为 50%-100%，双选的回收率为 10%-25%。

2.4 What is the concentration of the MGIEasy DNA Adapters?

MGIEasy DNA Adapters 的浓度是多少?

A:10 μM

答：10 μM

2.5 How to make sure the buffer mix is homogeneous as the ligation buffer is sticky?

Ligation Buffer 很粘稠，如何保证 Buffer 是均匀的?

A: Thaw the Ligation Buffer at room temperature and vortex 6 times (6 s each) , which ensuring homogeneous. If the mix remain sticky after sample added, 6 times (6 s each) vertexing should be done again, or the yield will be influenced.
答：Ligation Buffer 从冰箱取出室温溶解后，请务必在涡旋仪上混匀 6 次以上，每次 6 秒以上，保证液体充分震荡混匀。加入样本后，反应液仍然较粘稠，请务必在涡旋仪上混匀 6 次以上，每次 6 秒以上，保证液体充分震荡混匀。此步骤是否混匀关系到后续产量。

2.6 Why is 20 ul TE Buffer added after ligation step? What will happen when forgot?

连接完成之后为什么要添加 20 uL TE Buffer，如果忘记加了会怎样?

A: 20 ul TE Buffer can mitigate the adaptor residue while the efficiency remains good. If this step is missed: ①during step 3.5.2, 20 ul TE could be added into the beads. ② If the purification step is finished, 1X beads purification once more is recommended.
答：20 ul TE Buffer 的添加可以在保证纯化效率的前提下，减少磁珠纯化时的小片段残余，从而减少体系中接头的残余。如果忘记添加这一步的 TE Buffer：①已加入 50μL 纯化磁珠，未丢弃上清：可再次加入 20μLTE，混匀后重新结合 5 min；②已纯化结束，推荐补足 50uL 体系，使用 50 uL DNA Clean Beads 将连接产物进行二次纯化以保证测序质量。

2.7 What is the concentration of the PCR Primer Mix

PCR Primer Mix 的浓度是多少?

A: 20 uM

答：20 μM

2.8 What happened if I got no yield of single circle DNA when the PCR Product is normal?

PCR 有浓度，单链环没有浓度，怎么办?

A: Most likely there is something wrong in the circularization step. It is recommended to add a positive control combine

with the product to do the circularization again.

答：环化过程出错，建议增加阳性参照（之前环化成功的 PCR 产物），重新环化。

2.9 Can I use the DNB to do sequencing if the circularization efficiency is very low?

单链环效率非常低，但是够 make DNB，能不能上机?

A: We don't recommend doing that.

答：不建议上机。

2.10 Can I pool single circle with various insert size?

不同片段的单链环文库可以 Pooling 在一起上机吗?

A: if the size range >100nt, the sequencing quality may be influenced. We don't recommend to pool libraries like that.

答：片段差距太大的文库（＞100nt）可能会影响测序质量和拆分，不建议 pooling 在一起。

2.11 Is MGIEasy FS DNA library Prep set compatible for automatic machine?

MGIEasy 酶切 DNA 文库制备试剂套装是否可以兼容自动化?

A: MGIEasy FS DNA Library Prep Set is compatible for MGISP-100, MSP-960 and most kind of automatic machine (need additional test to confirm).Set 1000017572 is compatible for preparing 96 single circle libraries each time on MGISP-960. Set 100006988 is compatible for preparing 96 PCR product using MGISO-960 and make 16 single circle using MGISP-100.

答：可以兼容 MGISP-100、MGISP-960 及市面上大部分自动化仪器（需测试）。其中套装货号：1000017572 可在 MGISP-960 上实现 96 个文库构建（打断-环化），无需增加购买环化模块。套装货号：1000006988 适合构建 96 个 PCR 产物，使用手动或者 MGISP-100 pooling 后再进行环化（16RXN）。

MGI WeChat

■ 基本信息

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