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基因编辑
操作手册
Mb2Cas12a

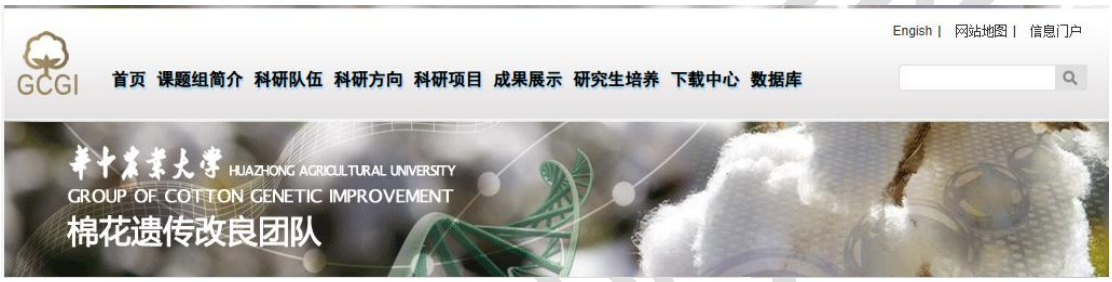
华中农业大学作物遗传改良全国重点实验室
湖北洪山实验室
棉花遗传改良团队

二0二五年六月·武汉

基因编辑平台
GENOME EDITING PLATFORM

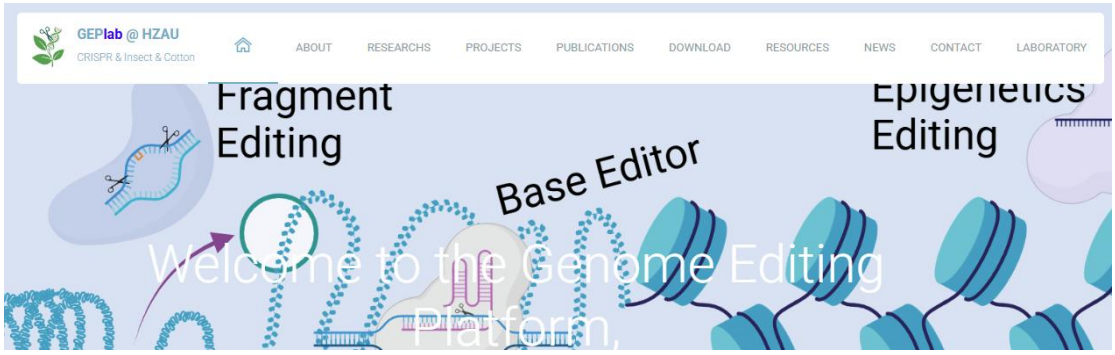
目录

棉花遗传改良团队：https://cotton.hzau.edu.cn/



湖北洪山实验室：https://hbhs.hzau.edu.cn/

基因编辑平台：http://jinlab.hzau.edu.cn/GenomeEditingPlatform/



Mb2Cas12a protocol（中文版）

CRISPR/Mb2Cas12a 基因编辑载体构建（Kan）方法：

（以 CRISPR/Mb2Cas12a-**tRNA 为例**）：

1. 骨架载体 Mb2 利用 BsaI 核酸酶（NEB）进行酶切，电泳检测酶切效果，凝胶回收试剂盒进行纯化、测浓度

表 1 酶切体系

	100μl 体系
Mb2	10μg
10X cut smart Buffer	10μl
BSA1	4μl
ddH₂O	up to 100μl

2. 单靶标载体构建：tRNA-crRNA scaffold-gRNA-tRNA-TTTTTTT

- 方法 1：选择基因合成的方式：南京金斯瑞
- 方法 2：实验室构建方法：

以 Gh360（kan）为骨架载体，扩增 tRNA-crRNA scaffold 片段

2.1 第一次 PCR 扩增：扩增靶标序列

1041 F: AAGCATCAGATGGGCAAACAAAGCACCAGTGGTCTAG

1041R:

CGAAAATAGCCATCGCCCCATGAATCTACCATAGTAGAAATTATGCACC

(黄色：靶标反向互补序列；绿色：crRNA scaffold；红色：tRNA)

黄色部分根据自己设计的靶标设计相应的引物

ddH₂O	**16.1**	
Buffer	**2**	
dNTP	**0.3**	
S primer	**1041F**	**0.2**
AS primer	**1041R**	**0.2**
Taq	**0.2**	
模板	**1**	

PCR 条件：

预变性：95℃ 5min
变性：95℃ 30S
退火：55℃ 30S
延伸：72℃ 20S
循环：3C
变性：95℃ 30S
退火：60℃ 30S
延伸：72℃ 20S
循环：27C
最后延伸 72℃ 5min
电泳检测

第二次 PCR 扩增：添加重组接头序列

以第一次 PCR 产物为模板

1042 F: AAGCATCAGATGGGCAAACAAA

1042 R: CACTGGTGCTTTGTTCGAAAATAGCCATCGCCCCATGA

(红色：tRNA；黄色：靶标反向互补序列)

ddH_2O	**16.1**	
Buffer	**2**	
dNTP	**0.3**	
S primer	**1042F**	**0.2**
AS primer	**1042R**	**0.2**
Taq	**0.2**	
第一次 PCR 产物	**1**	

PCR 条件：

预变性：95℃ 5min
变性：95℃ 30S
退火：60℃ 30S
延伸：72℃ 25S
循环：28C
最后延伸 72℃ 5min
电泳检测

In-fusion 连接：最终目标序列连接在 **Mb2 载体的** BsaI 处。

目的片段	100ng（2ul）

线性化表达载体	100ng（1ul）
Exnase	0.5μl
CE Buffer	1μl
ddH2O	Up to 5 ul

37℃水浴 30min，冰上放置 5min，可-20℃保存备用。

热激转化，挑单克隆送测序，正确的质粒保菌。

3. 双（多）靶标：tRNA-crRNA scaffold-gRNA-tRNA-crRNA scaffold-gRNA- tRNA-TTTTTTT 序列。

在单靶标的基础上进行重叠延伸 PCR 以此类推。

tRNA:

>AACAAAGCACCAGTGGTCTAGTGGTAgaatagtaccctgccacggtacagacccgggTTCGATTCCCGGCTGGTGCA

crRNA scaffold:

>TAATTTCTACTATGGTAGAT

Target:23bp

附件：

1. Mb2Cas12a 骨架载体（Mb2）
2. Gh360 模板载体
3. Mb2Cas12a 靶标设计
4. 可供参考的最终重组载体（Mb2Cas12a-Bsa1 构建后参考序列）

Mb2Cas12a protocol（英文版）

CRISPR/Mb2Cas12a Gene Editing Vector Construction (Kan) Method

(Taking CRISPR/Mb2Cas12a-tRNA as an example):

Digestion of Skeleton Vector Mb2

The skeleton vector Mb2 was digested with BsaI nuclease (NEB). The digestion effect was detected by electrophoresis, and the product was purified using a gel recovery kit followed by concentration measurement.

Table 1. Digestion System

	100μl 体系
Mb2	10μg
10X cut smart Buffer	10μl
BSA1	4μl
ddH₂O	up to 100μl

2. Single Target Vector Construction: tRNA-crRNA scaffold-gRNA-tRNA-TTTTTTT

- Method 1: Gene synthesis by Nanjing 金斯瑞 (Genscript).
- Method 2: Laboratory construction using Gh360 (kan) as the skeleton vector to amplify the tRNA-crRNA scaffold fragment.

2.1 First PCR Amplification: Amplify Target Sequence

Primers:

1041 F: AAGCATCAGATGGGCAAACAAAGCACCAGTGGTCTAG

1041R:

CGAAAATAGCCATCGCCCCATGAATCTACCATAGTAGAAATTATGCACC

(Yellow: Target reverse complementary sequence; Green: crRNA scaffold; Red: tRNA)

The yellow part should be designed according to the user's target sequence.

PCR System:

ddH₂O	**16.1**
Buffer	**2**
dNTP	**0.3**
S primer	**1041F 0.2**
AS primer	**1041R 0.2**
Taq	**0.2**
模板	**1**

PCR Conditions:

Pre-denaturation: 95℃, 5 min

Denaturation: 95℃, 30 s

Annealing: 55℃, 30 s

Extension: 72℃, 20 s

Cycles: 3 cycles

Denaturation: 95℃, 30 s

Annealing: 60℃, 30 s

Extension: 72℃, 20 s

Cycles: 27 cycles

Final extension: 72℃, 5 min

Electrophoresis detection required.

2.2 Second PCR Amplification: Add Recombinant Adaptor Sequence

Using the first PCR product as a template.

Primers:

1042 F: AAGCATCAGATGGGCAAACAAA

1042 R: CACTGGTGCTTTGTTCGAAAATAGCCATCGCCCCATGA

(Red: tRNA; Yellow: Target reverse complementary sequence)

PCR System:

ddH_2O	**16.1**
Buffer	**2**
dNTP	**0.3**
S primer	**1042F 0.2**
AS primer	**1042R 0.2**
Taq	**0.2**
第一次 PCR 产物	**1**

PCR Conditions:

Pre-denaturation: 95℃, 5 min

Denaturation: 95℃, 30 s

Annealing: 60℃, 30 s

Extension: 72℃, 25 s

Cycles: 28 cycles

Final extension: 72℃, 5 min

Electrophoresis detection required.

In-fusion Ligation

Ligate the final target sequence into the BsaI site of the Mb2 vector.

Ligation System:

Component	Volume (μl)
Target Fragment	100ng（2ul）
Linearized Expression Vector	100ng（1ul）
Exnase	0.5μl
CE Buffer	1μl
ddH2O	Up to 5 ul

Incubate at 37℃ for 30 min, place on ice for 5 min, and store at -20℃ for later use.

Heat shock transformation, pick single colonies for sequencing, and preserve the correct plasmids in bacterial cultures.

3. Dual (Multiple) Targets: tRNA-crRNA scaffold-gRNA-tRNA-crRNA scaffold-gRNA-tRNA-TTTTTTT

Based on the single-target vector, perform overlap extension PCR sequentially.

Sequences:

tRNA:

AACAAAGCACCAGTGGTCTAGTGGTAgaatagtaccctgccacggtacagacccgggTTCGATTCCCGGCTGGTGCA

crRNA scaffold:

TAATTTCTACTATGGTAGAT

Target: 23 bp

Attachments:

1. Mb2Cas12a skeleton vector (Mb2)
2. Gh360 template vector
3. Mb2Cas12a target design
4. Reference sequence for the final recombinant vector (Mb2Cas12a-Bsa1 after construction)