



棉花中高效的CRISPR/Mb2Cas12a 基因组编辑工具

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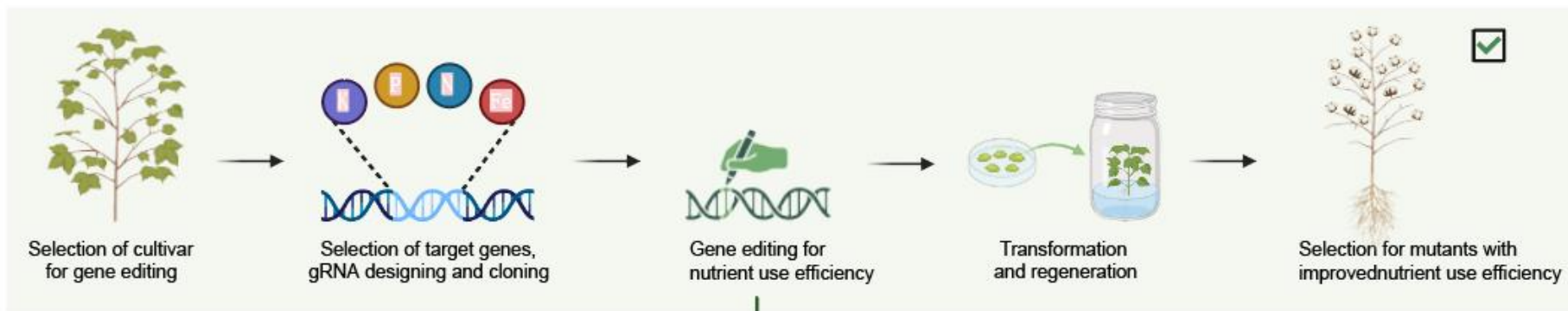


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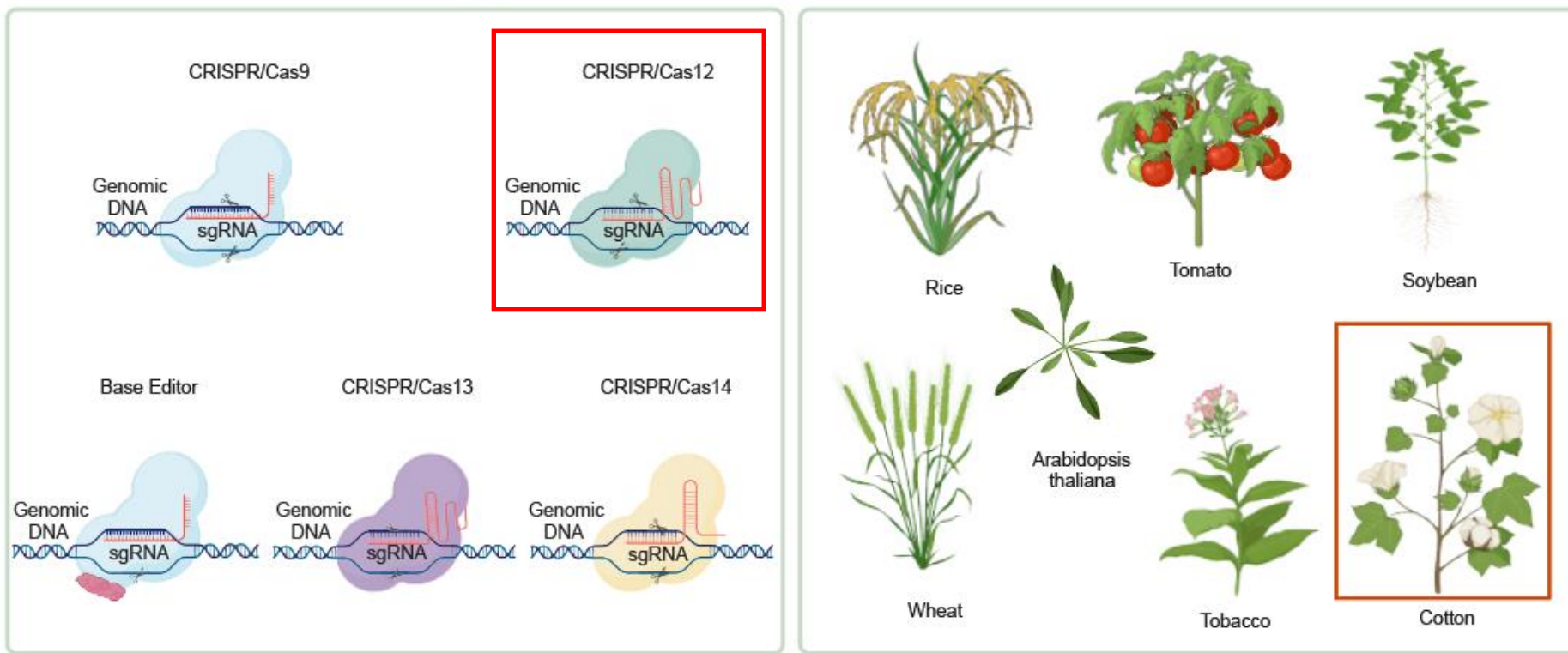


简介



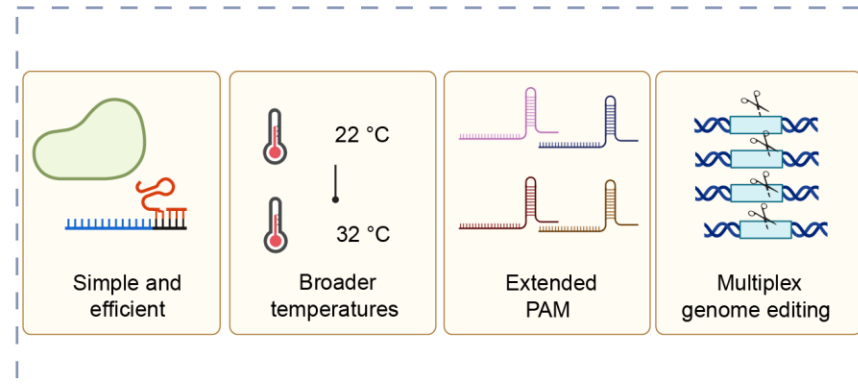
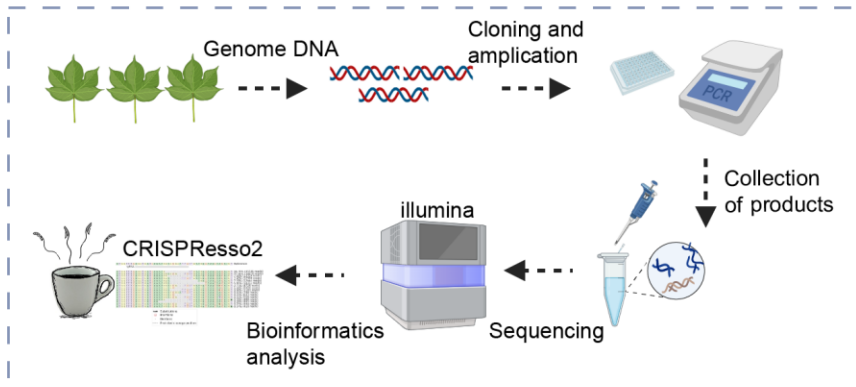
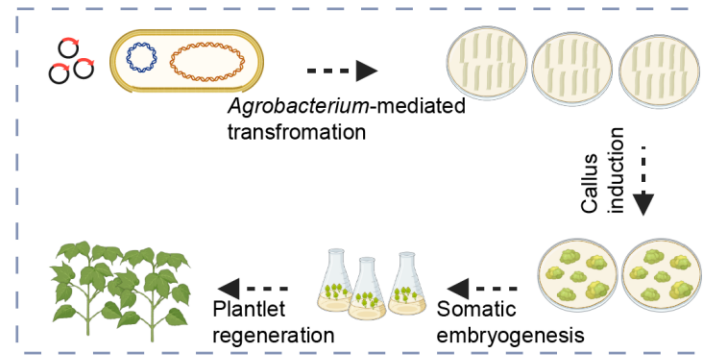
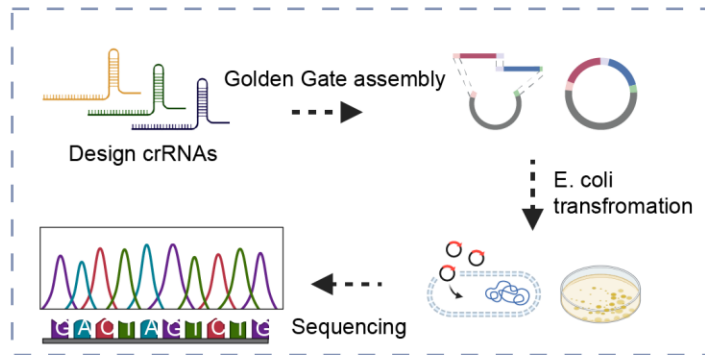
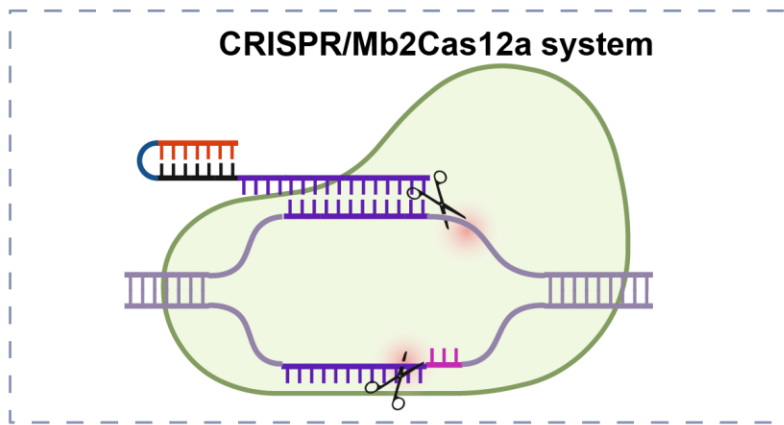
Genome Editing Tools

Plants





图文摘要





结果：实现高效的靶向突变

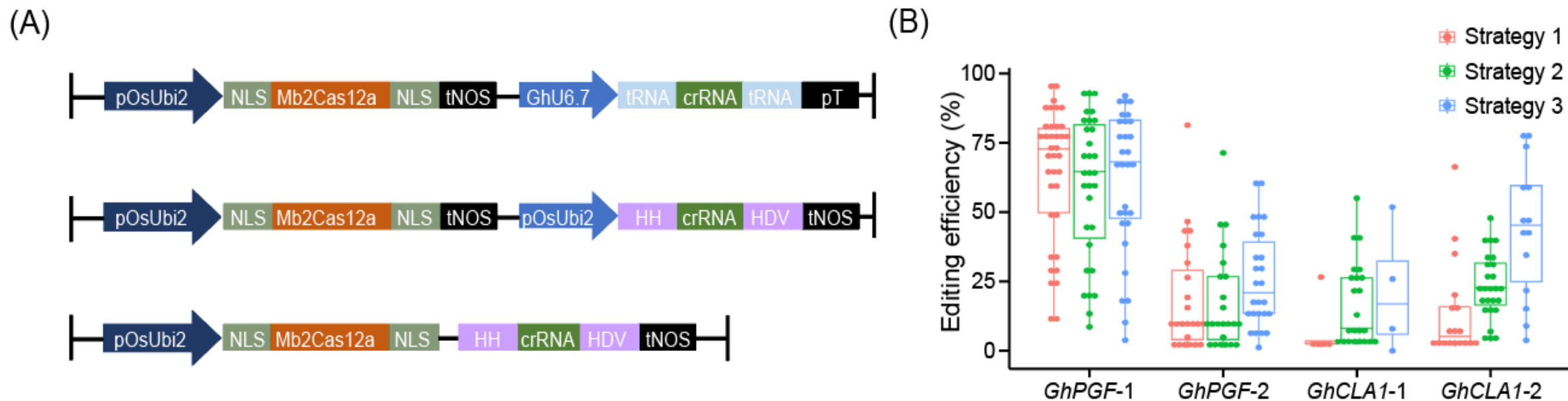
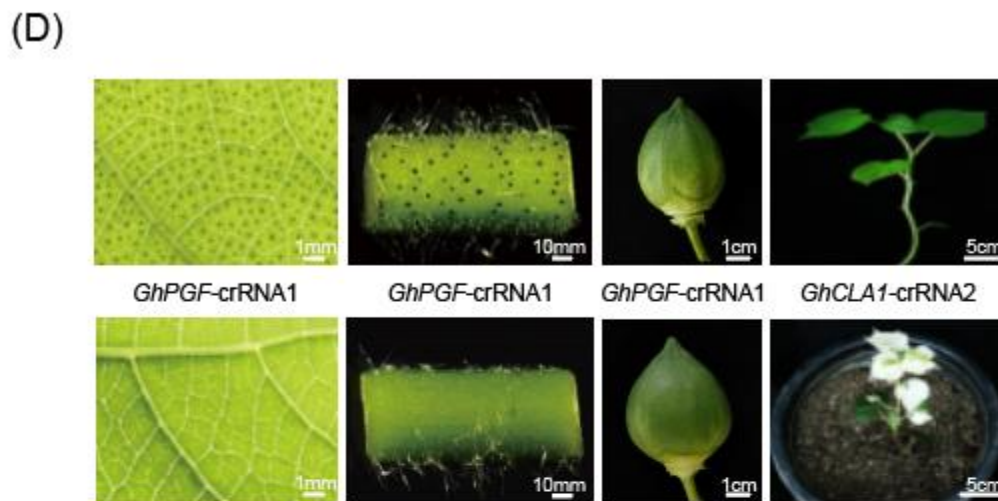
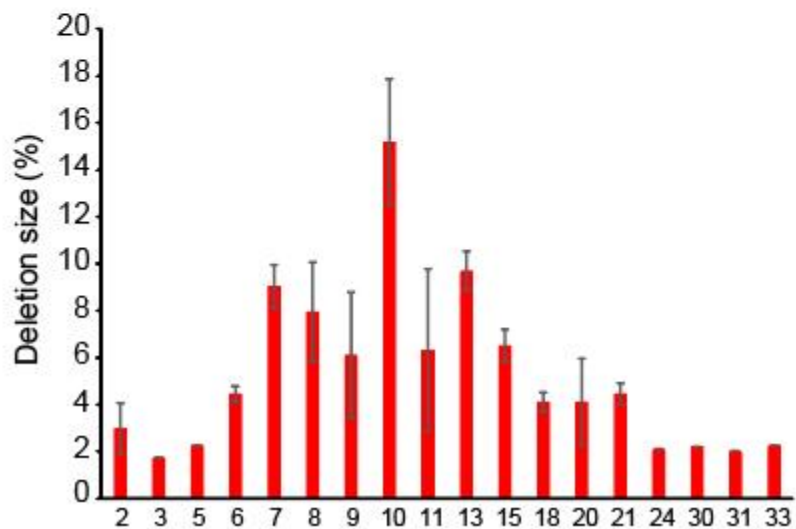
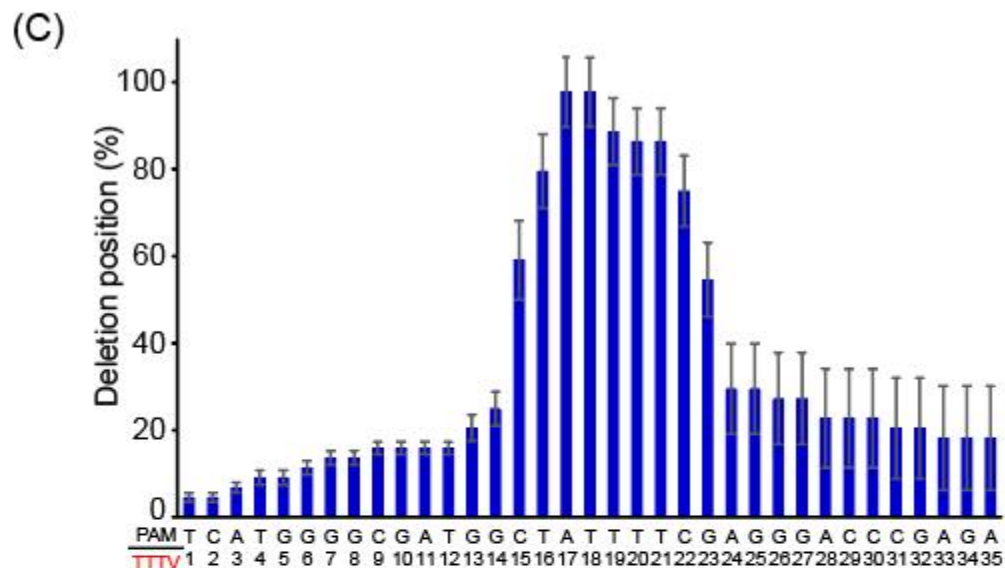


图1. (A) CRISPR/Mb2Cas12a双元载体T-DNA区域示意图 (B) 使用不同载体编辑*GhPGF*和*GhCLA1*靶标位点的编辑效率



结果



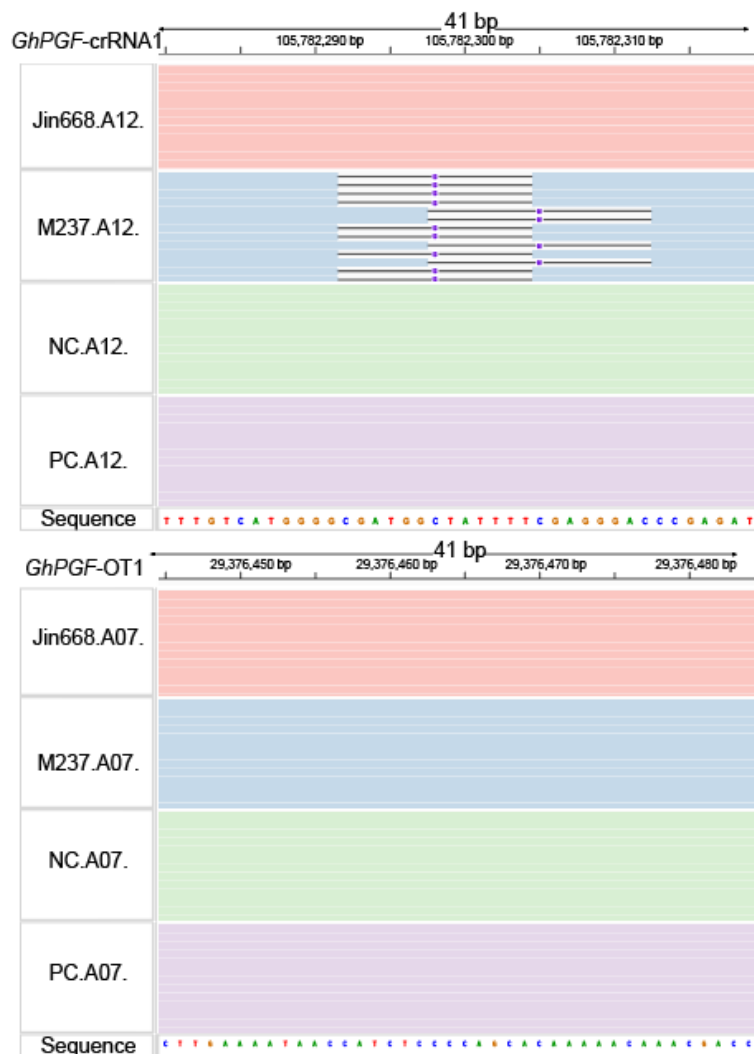
- 编辑窗口主要在PAM位点下游13到26 nt之间；
- 缺失大小主要集中在7到18 bp之间；
- 叶片和茎中棉酚腺体的密度明显下降；
- *GhCLA1*-crRNA2靶标编辑的植株叶片表现出部分漂白。

图1. (C) Mb2Cas12a诱导的*GhPGF*-crRNA1靶标位点DNA缺失位置和大小频率 (D) *GhPGF*和*GhCLA1*靶标突变的T0棉花植株表型

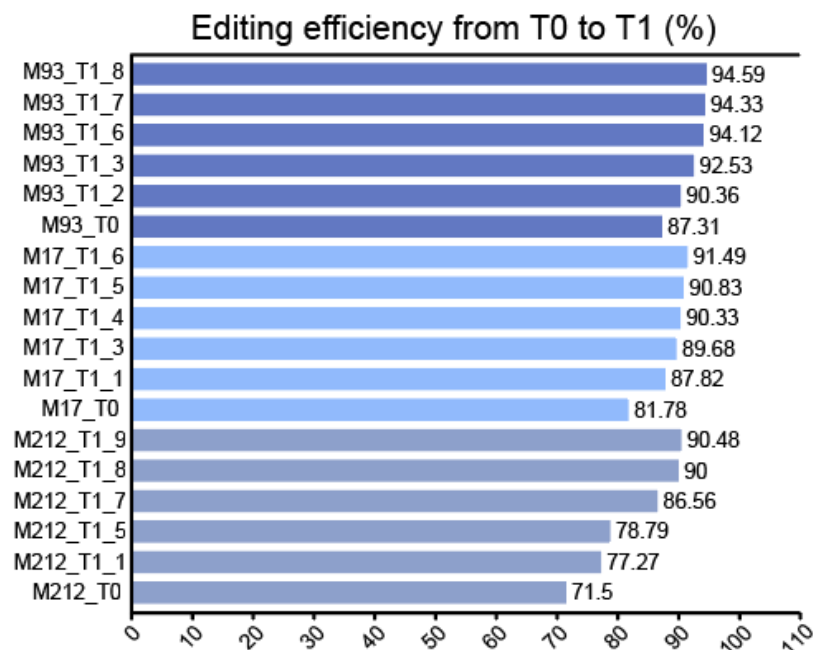


结果：靶标特异性和遗传稳定性

(E)



(H)



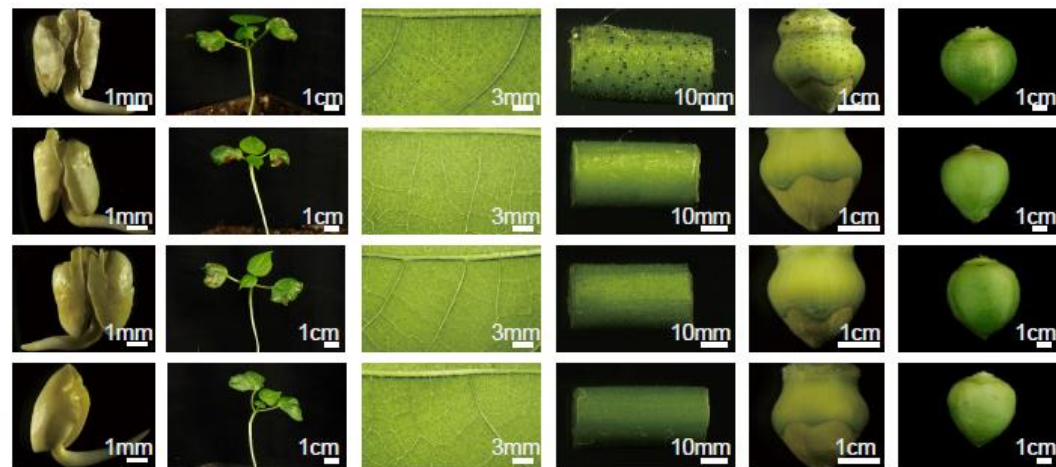
- 在预测的所有脱靶位点都没有检测到脱靶突变；
- 植株中编辑位点的基因型忠实地从T0遗传到T1株系。

图 1. (E) 通过全基因组测序技术评估潜在的脱靶编辑 (H) 利用NGS测定T1株系中GhPGF-crRNA1靶标位点的编辑效率



结果

(F)



(G)

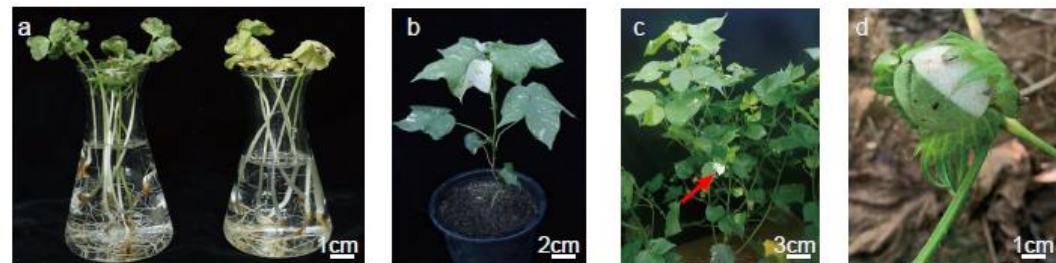
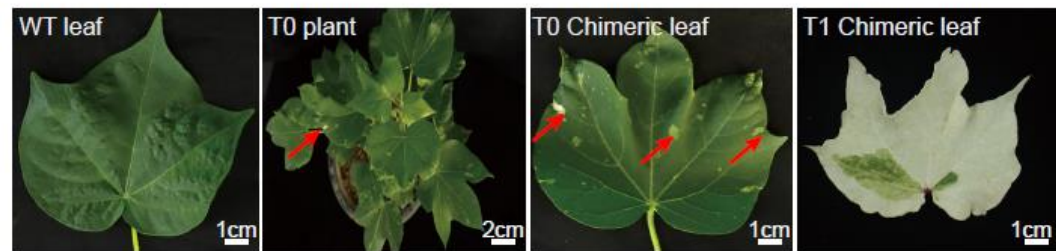


图 1. (F) *GhPGF*-crRNA1位点T1株系的表型特征 (G) *GhCLA1*-crRNA2位点突变的T1棉花植株在不同环境下出现漂白现象

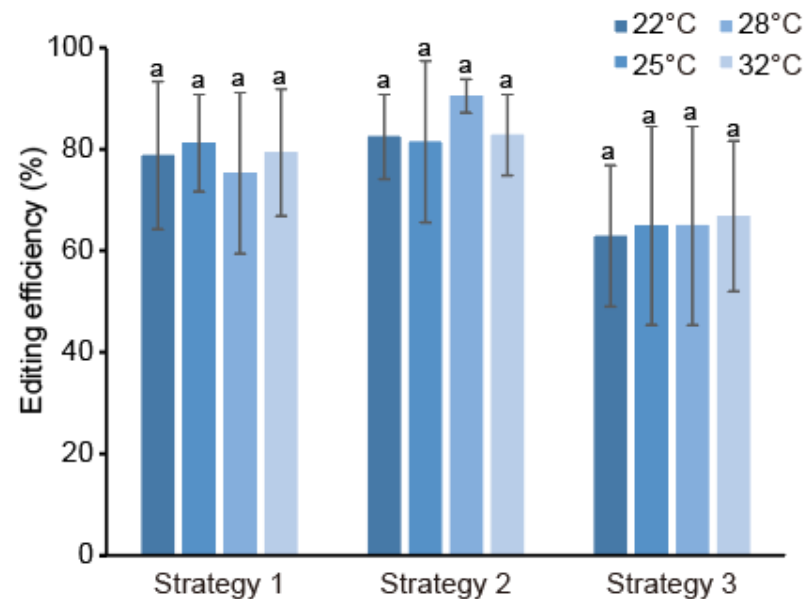


结果：广泛的温度适应性

(A)

GhPGF-crRNA1	Temperature (°C)	Line 86 (%)	Line 67 (%)	Line 71 (%)	Line 200 (%)
Strategy 1	22	54.66	79.98	90.26	90.21
	25	69.65	75.44	94.62	85.12
	28	50.42	72.9	91.18	86.53
	32	59.26	79.27	92.29	86.53
GhPGF-crRNA1		Line 149	Line 70	Line 77	Line 95
Strategy 2	22	92.53	81.73	69.54	86.21
	25	84.67	94.33	54.66	92.33
	28	86.57	94.92	92.26	88.18
	32	92.44	86.37	70.51	82
GhPGF-crRNA1		Line 156	Line 208	Line 212	Line 76
Strategy 3	22	47.5	77.4	50.63	76.15
	25	39.21	67.81	59.16	93.4
	28	35.59	60.17	76.04	88.03
	32	46.67	61.66	71.5	87.47

(B)



(C)

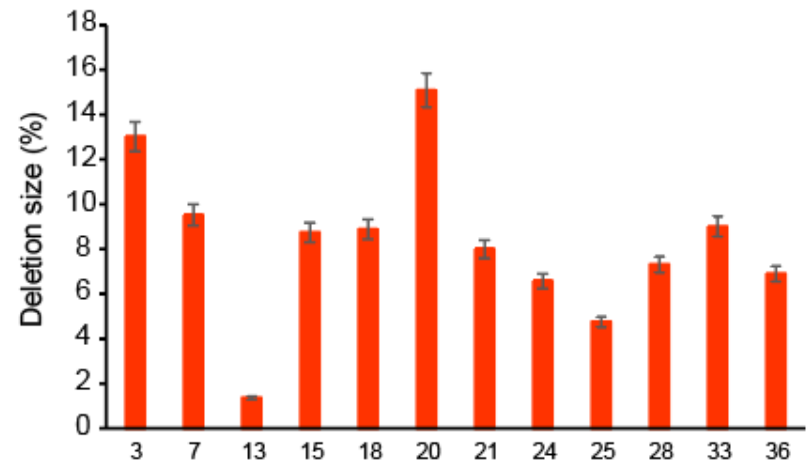
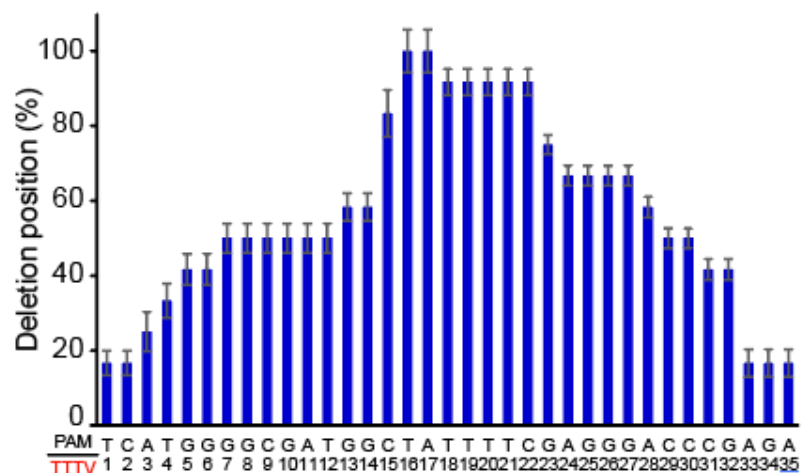
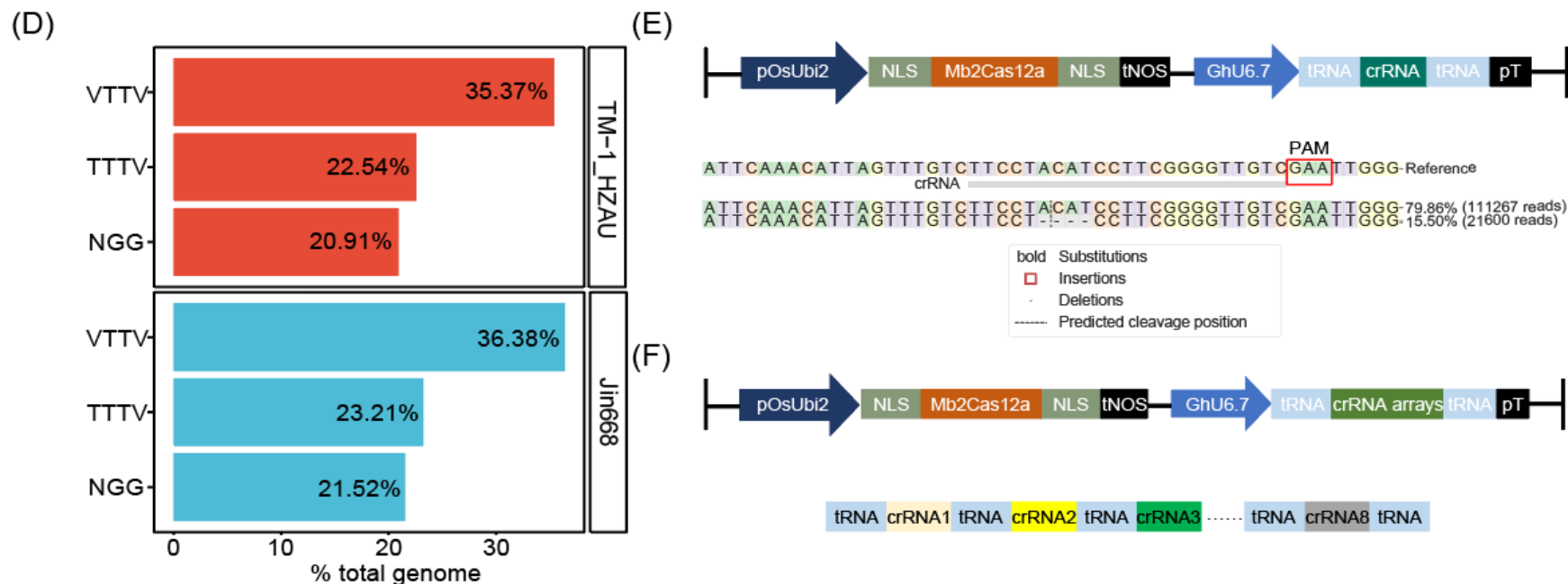


图 2. Mb2Cas12a在棉花中具有广泛的温度适应性和多基因编辑能力



结果：扩展了棉花基因编辑的范围



(G)

Strategy	Target	Gene	crRNA	No. of transgenic events identified	Editing efficiency	No. of events with edited
Strategy 1 with TTTV	crRNA1	<i>GhPGF</i>	TCATGGGGCGATGGCTATTTTCG	79	1.8-39.7%	29 (42.03%)
	crRNA2	<i>GhPGF</i>	TCTTCCTACATCCTTCGGGGTTG	79	4.93-5.47%	2 (2.53%)
	crRNA3	<i>GhCLA1</i>	TCTGCCAACTGCCAATCTCGACG	79	7.46%	1 (1.45%)
	crRNA4	<i>GhTAC1</i>	CTGGAACAAGTGGCACTGGTTGA	79	1.82-17.93%	6 (8.70%)
	crRNA5	<i>GhTAC1</i>	ATGGGACGTGAATCCTCGCCAAC	79	4.08-26.43%	33 (47.83%)
	crRNA6	<i>GhMYB25</i>	GCAAATGAGCCGCAATAGCCGAC	79	1.06-19.92%	28 (40.58%)
	crRNA7	<i>GhMYB25</i>	GTAGCATGAGCCAAACCAACATC	79	10.02-71.47%	59 (85.51%)
	crRNA8	<i>GhFAD</i>	ACTTTCTCCCACAACCCTTTTCC	79	17.12-58.62%	60 (86.96%)



总结

- ❑ CRISPR/Mb2Cas12显著提高了棉花基因组编辑的能力和准确性;
- ❑ Mb2Cas12a 表现出了广泛的温度适应性;
- ❑ Mb2Cas12a 扩大了棉花基因编辑的范围;
- ❑ 基于Mb2Cas12a我们成功的建立了一种有效的多基因编辑系统。

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