

Construction of Homozygous Mutants of Migratory Locust using CRISPR/Cas9 Technology

Qiang Yan^{*1}, Yingying He^{*2,3}, Yang Yue^{2,3}, Luyao Jie¹, Tingmei Wen^{2,3}, Yumiao Zhao^{2,3}, Min Zhang², Tingting Zhang²

¹State Key Laboratory of Cotton Biology, Key Laboratory of Plant Stress Biology, School of Life Sciences, Henan University ²Research Institute of Applied Biology, Shanxi University ³School of Life Science, Shanxi University

*These authors contributed equally

Corresponding Authors

Min Zhang

minzhang@sxu.edu.cn

Tingting Zhang

zhangyanqiu3520@sxu.edu.cn

Citation

Yan, Q., He, Y., Yue, Y., Jie, L., Wen, T., Zhao, Y., Zhang, M., Zhang, T. Construction of Homozygous Mutants of Migratory Locust using CRISPR/Cas9 Technology. *J. Vis. Exp.* (181), e63629, doi:10.3791/63629 (2022).

Date Published

March 16, 2022

DOI

10.3791/63629

URL

jove.com/video/63629

Materials

Name	Company	Catalog Number	Comments
10×NEBuffer r3.1	New England Biolabs	B7030S	The buffer of <i>in vitro</i> Cas9 cleavage assays
2xEs Taq MasterMix (Dye)	Cwbio	CW0690	For gene amplification
2xPfu MasterMix (Dye)	Cwbio	CW0686	For gene amplification
CHOPCHOP			Online website for designing sgRNAs, http://chopchop.cbu.uib.no/ .
CRISPOR			Online website for designing sgRNAs, http://crispor.org .
CRISPRdirect			Online website for designing sgRNAs, http://crispr.dbcls.jp/ .
Electrophoresis power supply	LIUYI BIOLOGY	DYY-6D	Separation of nucleic acid molecules of different sizes
Eppendorf Tube	Eppendorf	30125177	For sample collection, etc.
Fine brushes	Annigoni	1235	For cleaning and isolating eggs. Purchased online.
Flaming/brown micropipette puller	Sutter Instrument	P97	For making the microinjection needles
Gel Extraction Kit	Cwbio	CW2302	DNA recovery and purification
Gel Imaging Analysis System	OLYMPUS	Gel Doc XR	Observe the electrophoresis results
GeneTouch Plus	Bioer	B-48DA	For gene amplification
Glass electrode capillary	Gairdner	GD-102	For making injection needles with a micropipette Puller
Incubator	MEMMERT	INplus55	For migratory locust embryo culture
Metal bath	TIANGEN	AJ-800	For heating the sample
Micro autoinjector	Eppendorf	5253000068	Microinjection of embryos early in development
Micro centrifuge	Allsheng	MTV-1	Used for mixing reagents
Microgrinder	NARISHIGE	EG-401	To ground the tip of injection needle

Microloader	Eppendorf	5242956003	For loading solutions into the injection needles.
Micromanipulation system	Eppendorf	TransferMan 4r	An alternative manipulation system for microinjection
Microscope	cnoptec	SZ780	For microinjection
Motor-drive Manipulator	NARISHIGE	MM-94	For controlling the position of the micropipette during the microinjection procedure
Multi-Sample Tissue Grinder	jingxin	Tissuelyser-64	Grind and homogenize the eggs
oviposition pot	ChangShengYuanYi	CS-11	Filled with wet sterile sand for locust ovipositing in it. The oocysts are collected from this container. Purchased online.
Parafilm	ParafilmM	PM996	For wrapping the petri dishes.
pEASY-T3 Cloning Kit	TransGen Biotech	CT301-01	For TA cloning
Petri dish	NEST	752001	For culture and preservation of the eggs.
Pipettor	Eppendorf	Research plus	For sample loading
plastic culture cup			For rearing locusts separately and any plastic cup big enough (not less than 1000 mL in volume) will do. Purchased online.
Precision gRNA Synthesis Kit	Thermo	A29377	For sgRNA synthesis
Primer Premier	PREMIER Biosoft	Primer Premier 5.00	For primer design
SnapGene	Insightful Science	SnapGene®4.2.4	For analyzing sequences
Steel balls	HuaXinGangQiu	HXGQ60	For sample grinding.Purchased online.
Tips	bioleaf	D781349	For sample loading
Trans DNA Marker II	TransGen Biotech	BM411-01	Used to determine gene size
TrueCut Cas9 Protein v2	Thermo	A36496	Cas9 protein
UniversalGen DNA Kit	Cwbio	CWY004	For genomic DNA extraction
VANNAS Scissors	Electron Microscopy Sciences	72932-01	For cutting off the antennae
Wheat			To generate wheat seedlings as the food for locusts. Bought from local farmers.
ZiFiT			Online website for designing sgRNAs, http://zifit.partners.org/ZiFiT/ChoiceMenu.aspx .