

Materials List for

Microinjection of Corn Planthopper, *Peregrinus maidis*, Embryos for CRISPR/Cas9 Genome Editing

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Citation

Klobasa, W., Chu, F.C., Huot, O., Grubbs, N., Rotenberg, D., Whitfield, A.E., Lorenzen, M.D. Microinjection of Corn Planthopper, *Peregrinus maidis*, Embryos for CRISPR/Cas9 Genome Editing. *J. Vis. Exp.* (169), e62417, doi:10.3791/62417 (2021).

Date Published

March 26, 2021

DOI

10.3791/62417

URL

jove.com/video/62417

Materials

Name	Company	Catalog Number	Comments
1 oz Containers	Dart	P100N	Adult container for egg-laying setup
15 mL Conical Tubes	Olympus	Genesee 28-103	Serves as collection tube on vacuum aspirator setup
15 mL Conical Tubes	Olympus	Genesee 28-106	For making 10% sucrose solution and for holding adults when chilling before screening
Aspirator	Bioquip	1135A	For handling planthoppers
Vacuum Aspirator	Fischer Technical	LAV-3	Vacuum for aspirating larger numbers of insects
Blue Spectrum LED Lights	Home Depot	GLP24FS/19W/LED	Grow lights for potted corn plants hoppers are feeding on
Cas9	TrueCut Cas9 Protein v2	A36498	Endonuclease for cutting planthopper genes
Clear Vinyl Tubing	Home Depot	3/8 in. I.D. x 1/2 in. O.D. x 10 ft.	Connects collection tube to pump on vacuum aspirator setup
Corn planthoppers	North Carolina State University	N/A	Request from Dr. Anna Whitfield's lab
Cotton balls	Genessee	51-101	Serves as a filter/insect catcher in collection tube on vacuum aspirator setup
Double sided tape	Scotch Double Sided Tape	NA	Holding eggs for microinjection
Early Sunglow corn	Park Seed Company	05093-PK-N	Corn for rearing planthoppers
epTIPS Microloader Tips	Eppendorf	C2554691	Backfilling needle loading tips
Femtojet Microinjection System	Eppendorf	5247	Controls injection pressure (12-20 psi, depending on needle bore size)
Nutri-Fly Drosophila Agar	Genessee	66-103	Substrate for everything except egg-laying dish
Fine forceps	Bioquip	4731	Egg handling
General Purpose LE Agarose	Apex	20-102	Substrate inn egg-laying dish (oviposition medium)

Guide RNA 1 - GGUUCAUCGCAAAUAGCAG	Synthego	CRISPRvolution sgRNA EZ Kit (1.5 nmol)	RNA guides for targeting planthopper <i>white</i> gene
Guide RNA 2 - UCUGAAAUCACUGGCCAAUA	Synthego	CRISPRvolution sgRNA EZ Kit (1.5 nmol)	RNA guides for targeting planthopper <i>white</i> gene
Guide RNA 3 - GAGGGCAGAGUCGCUUUCUU	Synthego	CRISPRvolution sgRNA EZ Kit (1.5 nmol)	RNA guides for targeting planthopper <i>white</i> gene
Humidifier	Homedics	UHE-CM45	For providing humidity in humidified hood
Humidity chamber	Billups-Rothenberg	MIC-101	For holding injected embryos until hatching
Insect rearing cages	Bioquip (special order)	Close to 1450 L (has plastic front and mesh fabric sides)	Cage for planthoppers on corn
Laser-based Micropipette Puller	Sutter Instruments	P-2000/G	For making injection needles / Heat = 700, FIL = 4, VEL = 40, DEL = 170, PUL = 160
Leica M165 FC Fluorescence Stereomicroscope	Leica	M165 FC	Planthopper screening
Microinjection Scope	Leica	MZ12-5	Microinjection scope outfitted with an XY stage
Micromanipulator	Narishige	MN-151	For positioning microinjection needle
Micropipette beveler	Sutter Instruments	FG-BV10-D	For beveling injection needles / Used 'fine' graded plate at 20° angle
Microscope Stage	AmScope	GT100 X-Y Gliding Table	For positioning and moving embryos under microscope
Miniature Paint Brush	Testor #2 8733	Sold in 3 pack 281206	Fine paintbrushes for embryo handling
Needle Holder	Narishige	HI-7	For holding the microinjection needle
Percival Incubator	Percival	I41VLH3C8	Rearing injectees until hatch
Petri Dishes (100 x 15 mm)	VWR	89038-968	Making agar dish for egg-lay
pGEM-T Easy Vector System I cloning kit	Promega	A1360	Cloning <i>Pm white</i> target site
Phenol Red	Sigma	143-78-8	Microinjection buffer
Plain Microscope Slides or coverslip	Fisher Scientific	12-549-3	Hold eggs for microinjection
Plasmid DNA Midi Kit	Zymo	D4200	Purification of injection-ready plasmid DNAs
Plastic paraffin film	Pechiney Plastic Packaging	PM-996	Roll size 4 in. x 125 ft
Plastic wrap	Glad ClingWrap Plastic Wrap	NA	Wrap the entire egg-laying chamber
Primer - PmW CRISPR check F1 - AAGGAATTTCTGGAGGTGAAA	IDT	25 nmole DNA Oligo	First-round Primer for amplifying across target site within the <i>Pm white</i> gene
Primer - PmW CRISPR check R1 - GATTCCTCGCTGTTGGGT	IDT	25 nmole DNA Oligo	First-round Primer for amplifying across target site within the <i>Pm white</i> gene
Primer - PmW CRISPR check F3 - TCACAGACCCTGGTGCTAATC	IDT	25 nmole DNA Oligo	Second-round Primer for amplifying across target site within the <i>Pm white</i> gene
Primer - PmW CRISPR check R3 - GTCCACAATCCACACTTCTGA	IDT	25 nmole DNA Oligo	Second-round Primer for amplifying across target site within the <i>Pm white</i> gene
Quartz capillaries	Sutter Instruments	QF100-50-10	For making microinjection needles / O.D. 1 mm, I.D. 0.7 mm, 10 cm length
Screen (White Organza Fabric)	Joann Fabrics	16023889	For covering the adult container
Sparkleen	Fisher Scientific	04-320-4	Wash dishes
Sucrose	Fisher Scientific	BP220-1	To make 10% sucrose solution