

Materials List for:

# High-throughput CRISPR Vector Construction and Characterization of DNA Modifications by Generation of Tomato Hairy Roots

Thomas B. Jacobs<sup>1</sup>, Gregory B. Martin<sup>1,2</sup>

<sup>1</sup>Boyce Thompson Institute for Plant Research

<sup>2</sup>Section of Plant Pathology & Plant-Microbe Biology, School of Integrative Plant Science, Cornell University

Correspondence to: Thomas B. Jacobs at [tbj26@cornell.edu](mailto:tbj26@cornell.edu)

URL: <https://www.jove.com/video/53843>

DOI: [doi:10.3791/53843](https://doi.org/10.3791/53843)

## Materials

Name	Company	Catalog Number	Comments
NEBuilder® (HiFi DNA assembly mix)	New England Biolabs	E5520	
p201N:Cas9	Addgene	59175	The p201H:Cas9 plasmid (59176) is also compatible with the reported overlaps and enzymes.
pUC gRNA Shuttle	Addgene	47024	
SwaI	New England Biolabs	R0604S	
SpeI	New England Biolabs	R0133S	
Zymo clean and concentrator-5 column purification	Zymo Research	D4003	
NEB Buffer 2.1	New England Biolabs	B7202S	
NEB CutSmart (Buffer 4)	New England Biolabs	B7204S	
NEB Buffer 3.1	New England Biolabs	B7203S	
EconoSpin Mini Spin Column (plasmid prep)	Epoch Life Sciences	1910-050/250	
EcoRV-HF®	New England Biolabs	R3195S	
StyI-HF®	New England Biolabs	R3500S	
MS Salts + Gamborg Vitamins	Phytotechnology Laboratories	M404	
Phytigel™ (gellan gum)	Sigma Aldrich	P8169	
GA-7 Boxes	Sigma Aldrich	V8505	
Micropore™ surgical tape	3M	1535-0	
Timentin® (Ticarcillin/Clavulanic acid)	Various	0029-6571-26	
Primers 5' → 3'			
SwaI_MtU6F	<b>GATATTAATCTCTTCGATGA</b> <b>AATTTATGCCTATCTTATAT</b> GATCAATGAGG		
MtU6R	AAGCCTACTGGTTCGCTTG AAG		
ScaffoldF	GTTTTAGAGCTAGAAATAGC AAGTT		
SpeI_Scaffold R	<b>GTCATGAATTGTAATACGACTC</b> <b>AAAAAAAAGCACCGACTCGGTG</b>		
StUbi3P218R	ACATGCACCTAATTTCACTA GATGT		
ISceI R	GTGATCGATTACCCTGTTAT CCCTAG	Cannot be used for Sanger sequencing since there is a second binding site on the plasmid	

UNS1_Scaffold R	GAGAATGGATGCGAGTAATGAA AAAAAGCACCGACTCGGTG		
UNS1_MtU6 F	CATTACTCGCATCCATTCTCAT GCCTATCTTATATGATCAATGAGG		
p201R	CGCGCCGAATTCTAGTGATCG		
Bolded sequences denote 20-nt overlaps with linearized p201N:Cas9.			