

Materials List for:

Transient Gene Expression in Tobacco using Gibson Assembly and the Gene Gun

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URL: <https://www.jove.com/video/51234>

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Materials

Name	Company	Catalog Number	Comments
Oligonucleotide primers	IDT	(custom)	Design specifically for construct
GeneBlocks	IDT	(custom)	500 bp oligonucleotides
ApE software	U Utah	(download)	http://biologylabs.utah.edu/jorgensen/wayned/ape/
MinElute kit	Qiagen	28004	Used to purify PCR products
QIAprep spin miniprep kit	Qiagen	27104	Used to prepare cloning-appropriate amounts of plasmid
Phusion Master Mix with GC Buffer	NEB	M0532S	Used to PCR-amplify gene of interest
Gibson assembly reaction mix	NEB	E2611L	Master mix of the following 9 ingredients
1 M Tris-HCl pH7.5	Teknova	T1075	Gibson assembly mix
1 M MgCl ₂	G Biosciences	82023-086	Gibson assembly mix
dNTP mix	Fermentas	R0192	Gibson assembly mix
1 M DTT	Fermentas	R0861	Gibson assembly mix
PEG-8000	Affymetrix	19966	Gibson assembly mix
NAD	Applchem	A1124,0005	Gibson assembly mix
T5 exonuclease	Epicentre	T5E4111K	Gibson assembly mix
Phusion polymerase	NEB	F530S	Gibson assembly mix
Taq DNA ligase	NEB	M0208L	Gibson assembly mix
Gibthon.org			Website to simplify calculations
Plasmid PLUS Maxi kit	Qiagen	12963	Used to prepare DNA for gene gun bullets
Gene Gun system	BioRad	165-2451	Includes all parts necessary
Nicotiana benthamiana	(n/a)	(n/a)	Gift of Jen Sheen, MGH
Confocal microscope	Leica	SP5 X MP	Imaging of resultant cells
Deep well slides	Electron Microscopy Sciences	71561-01	Used for confocal imaging
A Plasmid Editor (ApE)	University of Utah		http://biologylabs.utah.edu/jorgensen/wayned/ape
Gibthon:Ligation calculator			http://django.gibthon.org/tools/ligcalc/