

Additional File 9. Expression clones and their building parts (For simplicity, terminators are not presented).

i) Expression vectors and parts for their assembly

Construct Number	JBEI ICE Registry ID	Construct Name	Cloning Method	Construct Backbone	Integrated Piece(s)
-	JBx_042298	pUBQ10	Acquired from JBEI Registry (Shih et al., 2016)	-	-
-	JBx_092437	pTKan-p35S::attR1-GW-attR2	Acquired from JBEI Registry (Gonzalez et al., 2015)	-	-
C11	JBx_092439	pTKan-pNST3::attR1-GW-attR2	Acquired from JBEI Registry	-	-
C50	JBx_092441	pTKan-pNOS::DsRed-p35S:: attR1-GW-attR2	Infusion	pTKan-p35S::attR1-GW-attR2, ApaI digest	pNOS:: DsRed
C381	JBx_092443	pTKan-pNOS::DsRed-p35S::PcoCAS9-AmpR- attR1-GW-attR2	Infusion	C50, AvrII digest	PcoCAS9 and AmpR
C382	JBx_092445	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR- attR1-GW-attR2	Infusion	C381, XhoI+AvrII digest	pUBQ10

ii) Expression clones and parts for their assembly

Construct Number	JBEI ICE Registry ID	Construct Name	Cloning Method	Construct Backbone	Integrated Piece(s)
C267	JBx_082113	pTKan-p35S::HCT::2A::Cys4-pNOS::cogGFP	Multisite Gateway	pTKan-p35S::attR1-GW-attR2	pDONR221-attL1-pU6:: HCT::2A::Cys4-attL4, pDONR221-attR4-pNOS-attR3, pDONR221-attL3-cogGFP-attL2
C349	JBx_092449	pTKan-pNST3::CAS9-pU6::HCT_gRNA12	Infusion	C11, AvrII+ HindIII digest	CAS9-pU6::HCT_gRNA12
C350	JBx_092451	pTKan-pNST3::CAS9-pU6::HCT_gRNA13	Infusion	C11, AvrII+ HindIII digest	CAS9-pU6::HCT_gRNA13
C351	JBx_092453	pTKan-pNST3::CAS9-pU6::HCT_gRNA14	Infusion	C11, AvrII+ HindIII digest	CAS9-pU6::HCT_gRNA14
C378	JBx_092455	pTKan-p35S::GONST2::2A::Cys4-pNOS::cogGFP	Infusion	C267, AscI digest	GONST2 (Jing et al., 2018)
C384	JBx_092457	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-U6::Gonst2_gRNA1	Gateway	C382	pDONR/Zeo-attL1-pU6:: Gonst2_gRNA1-attL2
C385	JBx_070930	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-U6::Gonst2_gRNA2	Gateway	C382	pDONR/Zeo-attL1-pU6:: Gonst2_gRNA2-attL2
C409	JBx_076699	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA1	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA1-attL2
C410	JBx_076701	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA2	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA2-attL2
C412	JBx_076705	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA3	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA3-attL2
C413	JBx_076707	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA4	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA4-attL2
C414	JBx_076159	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA5	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA5-attL2
C415	JBx_076709	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA6	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA6-attL2
C416	JBx_076711	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA7	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA7-attL2
C417	JBx_076161	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA8	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA8-attL2
C418	JBx_076163	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA9	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA9-attL2
C419	JBx_076165	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA10	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA10-attL2
C420	JBx_076167	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA11-attL2

		pU6::HCT_gRNA11			
C421	JBx_076169	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA12	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA12-attL2
C422	JBx_076171	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA13	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA13-attL2
C423	JBx_076713	pTKan-pNOS::DsRed-pUBQ10::PcoCAS9-AmpR-pU6::HCT_gRNA14	Gateway	C382	pDONR/Zeo-attL1-pU6::HCT_gRNA14-attL2

- GONZALEZ, T. L., LIANG, Y., NGUYEN, B. N., STASKAWICZ, B. J., LOQUÉ, D. & HAMMOND, M. C. 2015. Tight regulation of plant immune responses by combining promoter and suicide exon elements. *Nucleic Acids Research*, 43, 7152-7161.
- JING, B., ISHIKAWA, T., SOLTIS, N., INADA, N., LIANG, Y., MURAWSKA, G., ANDEBERHAN, F., PIDATALA, V., YU, X., BAIDOO, E., KAWAI-YAMADA, M., LOQUE, D., KLIEBENSTEIN, D., DUPREE, P. & MORTIMER, J. 2018. GONST2 transports GDP-Mannose for sphingolipid glycosylation in the Golgi apparatus of *Arabidopsis*. *bioRxiv*.
- SHIH, P. M., VUU, K., MANSOORI, N., AYAD, L., LOUIE, K. B., BOWEN, B. P., NORTHERN, T. R. & LOQUE, D. 2016. A robust gene-stacking method utilizing yeast assembly for plant synthetic biology. *Nat Commun*, 7, 13215.