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DEFINITION .

ACCESSION .

VERSION .

KEYWORDS pMY304

SOURCE synthetic DNA construct

ORGANISM synthetic DNA construct

REFERENCE 1 (bases 1 to 8244)

AUTHORS ZN

TITLE Direct Submission

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<http://www.snappgene.com>

FEATURES Location/Qualifiers

source 1..8244
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/mol_type="other DNA"

misc_feature 1..6510
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 /note="cauliflower mosaic virus 35S promoter with a duplicated enhancer region"
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promoter complement(8139..8157)
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 /note="RB T-DNA repeat"
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ORIGIN

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//

Output	Vector	Insertion	Annotation
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pAP	pAB, XbaI/SpeI	>anneal oligo pAB-FP: ctagagAGATCTCGCGGATCCTa pAB-RP: ctagtaGGATCCGCGAGATCTct >ligate with vector and transform E.Coli	Entry vector: EcoRI-XbaI-BglII-BamHI-SpeI-PstI
pAB-linker1	pAP, BglII/BamHI	>anneal oligo linker1-FP: GATCTGGTGGAGGTG linker1-RP: GATCCACCTCCACCA >ligate with vector and transform E.Coli	GGGS linker
pAP-YC-A	pAP, BglII/BamHI	>PCR by AP-YC-A: AGATCTGGAACAATGGACAAGCAGAAGAAC AP-GFP-R2: GGATCCCTTGTACAGCTCGTCC from pC131-eYFP (Kept by XL lab) >cut by BamHI/BglII	YC155 fragment with ATG, no stop codon.
pAB059	pAP-linker1, BamHI/PstI	>pAP-YC-A, BglII/PstI	linker1-YC
pAB060	pAP-linker1, BamHI/PstI	>pAB059, BglII/PstI	[linker1]2-YC
pAP-myc-A	pAP, BamHI/BglII	>anneal oligo myc-A-FP: GATCTGGAACAATGGCTGAACAAAAGTTGATTTCTGAAGAAGATTTGG myc-A-RP: GATCCCAAATCTTCTTCAGAAATCACTTTTGTTCAGCCATTGTTCCA >ligate with vector and transform E.Coli	Myc with ATG
pAB061	pAP-myc-A, BamHI/PstI	>pAB060, BglII/PstI	Myc-[linker1]2-YC
pAB-MCSrp	pAB as PCR template	>PCR by AB-MCSrp-F1: GGAcccgggAACgtcgacgctagcagatctactagtagcgccgctgc	MCSrp

		<p>AB-MCSrp-R1: ggtaccTCCgagctcctcgaggctagcggatcctctagaagcggccgcgaa >T4 PNK treatment and self-ligate</p>	
pAB-MCSrp2	pAB-MCSrp, BamHI	<p>>anneal oligo ZN0164: gatctTGACTGACTGAg ZN0165: gatccTCAGTCAGTCAa</p>	MCSrp2: MCSrp with tandem stop codon
pAB-HSPter	pAB, XbaI/SpeI	<p>>PCR by AB-Thsp-F1: TCTAGAATATGAAGATGAAGATGAAA AB-Thsp-R1: ACTAGTCTTATCTTTAATCATATTCC from Col-0 gDNA</p>	HSP18.2 terminator with HindIII/BglII/PstI mutation
pAB046	pAB-MCSrp2, SpeI/PstI	>pAB-HSPter, XbaI/PstI	MCSrp2-HSPter
pAB-HSPutr	pAB, XbaI/SpeI	<p>>PCR by HSP18-2-5UTR-FP: TATTCTAGAcctaaatcccgcacagt HSP18-2-5UTR-RP: GCGACTAGTgtcgtagatcggaaa from Col-0 gDNA</p>	HSP18.2 5'UTR to enhance protein expression
pAB-d35Spro	pAB, XbaI/SpeI	<p>>PCR by d35S-FP: CGGTCTAGATGAGACTTTTCAACAA d35S-RP: CAGACTAGTTCAGCGTGCCTCTCCA from pC131-eYFP</p>	d35S promoter
pAB080	pAB-HSPutr, EcoRI/XbaI	>pAB-d35Spro, EcoRI/SpeI	d35Spro-HSPutr
pAB081	pAB046, EcoRI/XbaI	>pAB080, EcoRI/SpeI	d35Spro-HSPutr-MCSrp2-HSPter
pAB106	pAB081, BglII	>pAB061, BamHI/BglII	d35Spro-HSPutr-MCSrp2-Myc-[linker1]2-YC-HSPter
pMY301	pER8, HindIII/EcoRI	<p>>anneal oligo HiT7E-FP: agctcTAATACGACTCACTATAGGGtctagaagcttgaattcactagtc HiT7e-RP: aattgactagtggaattcaagcttttctagaCCCTATAGTGAGTCGTATTAg</p>	Backbone vector: RB-(T7-XbaI-HindIII-EcoRI-SpeI)-LB
pMY304p1	pMY301, EcoRI/XbaI	>pAB106, EcoRI/SpeI	(SpeI-EcoRI-XbaI)-d35Spro-HSPutr-MCSrp2-Myc-[linker1]2-YC-HSPter
pMY304p2	pMY304p1,	>self-ligation	LB-(d35Spro-HSPutr-MCSrp2-Myc-[linker1]2-YC-HSPter)-R

	XbaI/SpeI		B
pMY304p3	pMY304p2, XhoI/KpnI	>anneal oligo ZN0216: tcgagtctagagaattcggagagctcggaggtag ZN0217: ctccgagctctccgaattctctagac	modified MCSrp2
pMY304	pMY304p3, KpnI/SalI	>anneal oligo ZN0218: tcgacactagtaagcttTCCctgcagggtcccgggtccggtag ZN0219: cggacccgggaacctgcagGGAagcttactagtg	LB-(d35Spro-HSPutr-MCSp2-Myc-[linker1]2-YC-HSPter)-RB