Plasmids

What to do when you generate a new plasmid

1) Give it a unique plasmid name, such as pLJ# (p stands for plasmids, and LJ is the initial. # should be in numeric order), use your own initials for the plasmids you made.

2) Write details about how you create this plasmid

- a. Include primers and template you used.
- b. Write down the vector backbone.
- c. Indicate whether it has been sequenced (which you always should) and a summary of sequencing result (such as whether it is 100% wt, or contains silent mutations). Indicate where the sequencing result is stored.

3) Enter into the database on wiki and indicate the antibiotic resistance.

4) Draw a plasmid map using SNAPgene software

Example: you generate a plasmid which is lite-1p::YFP
Example of details: Primer pair xxx and xxx are used to amplify lite-1 promoter from N2 genomic DNA (3kb). Primer pair xxx and xxx are used to amplify YFP from plasmid pMS1 (0.7kb). They are cloned into the vector xxx through Gibson Assembly. Primers xxx are used for sequencing. lite-1 promoter region is 100% correct, while there is a silent mutation at position xxx for YFP. Sequencing result can be found at xxx.

Lab Plasmids Database

plasmid #	**Name**	**Description**	**Primer used**	**Status**	**Where**	**Source**	insert size	Comments	Backbone	Backbone size	Frozen	Ref
pMS1	odr-1p::cGAL-N::let-858 3'UTR	split gal-4 cGAL-N expressed in gcy-10(odr-1) promoter expressing in I1 and others (AWC, AWB). DBD				derived from pHW530	1.8kb			3.7kb		
pMS2	ehs-1p::cGAL-C::let-858 3'UTR	split gal-4 cGAL-C expressed pan-pharyngeal neurons. AD				derived from pHW522	62bp	might be issues with the ehs-1 promoter		3.3kb		
pMS3	ehs-1p::his24::wrmScarlett	should be red in all pharyngeal neurons.				derived from PJ20 Anuj		no expression when injected				
pMS4	ehs-1p::his24::GCaMP7s	should be GCaMP in all pharyngeal neurons.				derived from PJ20 Anuj						
pMS5	pHW522	Prab-3::NLS::gp41-1-C-intein::cGAL(AD)::let858 3'UTR						NA		3.3kb		
pMS6	pHW530	Prab-3::NLS::cGAL(DBD)::gp41-1-N-intein::let-858 3′UTR						NA		3.7kb		
pMS7	pHW393	Prab-3::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR					1.2kb			3.6kb		
pMS8	pBX (pha-1 rescue)											
pMS9	rab-3P::2NLS::GFP(65C)											
pMS10	pAS1-his-24::GCaMP7s											
pMS11	pAS1-his-24::tagBFP	should be rab-3 promoter										
pMS12	pAS1-his-24::tagRFP	should be rab-3 promoter										
pMS13	pAS1-his-24::mNeptune	should be rab-3 promoter										
pMS14	pAS1-his-24::mCherry	should be rab-3 promoter						Addgene 124348				
pMS15	pAS1-his-24::linker::wrmScarlet	should be rab-3 promoter										
pMS16	pAS1-his-24::CyOFP::egl-13NLS	should be rab-3 promoter										
pMS17	pPHA2::GFP-F	GFP expressed in I4				from Marc Pilon			pPD95.69			
New plasmids												

pLJ1		Pceh-28::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ17/18	sequenced		Jun	2.5kb	M4 specific	pHW393	3.6kb	yes	PMID: 20713707 (primer sequence not provided)
pLJ2		Podr-1 long::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ27/28	sequenced		Jun	1.7kb	I1, AWB, AWC	pHW393	3.6kb	yes	primer sequence provided by Nikhil Bhatla, who says they are from PMID: 16547101
pLJ3		Podr-1 short::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ27/29	sequenced		Jun	0.7kb	putative I1	pHW393	3.6kb	yes	Educated guess, based on information from Jihye Yeon, Sengupta Lab
pLJ4		Pceh-34::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ25/26	sequenced		Jun	3.6kb	pan pharyngeal neruons	pHW393	3.6kb	yes	PMID: 19879847
pLJ5		Pceh-2::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ45/46	sequenced		Jun	1.2kb	I2, I3, NSM	pHW393	3.6kb	yes	NeuroPAL
pLJ6		Ptph-1::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ47/48	sequenced		Jun	0.2kb	NSM specific	pHW393	3.6kb	yes	PMID: 30580965
pLJ7		Ptrxr-1::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ39/40	sequenced		Jun	0.8kb	M2 specific	pHW393	3.6kb		PMID: 22836943 (primer sequence not provided)
pLJ8		Plin-44::NLS-tagBFP::let-858 3'UTR	oLJ51/52 for Plin-44 and oLJ53/54 for NLS-tagBFP	sequenced			2.6kb for Plin-44 and 0.9kb for NLS-BFP		pHW393 without GAL4 (FseI+KpnI)	2.7kb	yes	PMID: 32188430
pLJ9		Pins-10::NLS::cGAL(DBD):: cGAL(AD)::let-858 3′UTR	oLJ43/44	sequenced			5.4kb		pHW393	3.6kb	yes	PMID: 24806678
pLJ10		Podr-1 long::NLS::gp41-1-C-intein-cGAL(AD)::let-858 3′UTR	subcloned from pLJ2 (odr-1p) and pJL081 (backbone, similar to pHW522)	sequenced			1.7kb	for split GAL4, C-term	equivalent to pHW522	3.3kb		No.
pLJ11		Pceh-34::NLS::cGAL(DBD)::gp41-1-N-intein::let-858 3′UTR	subcloned from pLJ4 (ceh-34p) and pJL080 (backbone, similar to pHW530)									

Gift Plasmids Database

Plasmid name	**Description**	**Notes**	**antibiotics**	**Source**	**Plasmid location**	**-80**
pMS17	pPHA2::GFP-F	GFP expressed in I4	amp	Marc Pilon. They did not give it a name, and suggested us to name it by ourselves, so this plasmid appears in both this and our lab databases.	B4	yes
pCFJ90	Pmyo-2::mCherry::unc-54utr		amp		A1	yes
pGH8	pRAB-3::mCherry::unc-54utr	do not use.	amp	very likely wrong after sequencing	B5, B6	
Addgene_104483	pGP-CMV-jGCaMP7f		Kan		C1, C2	yes

Plasmid name	**Description**	**Notes**	**antibiotics**	**Source**	**Plasmid location**	**-80**
pPD96.48	myo-2 promoter + unc-54 3’utr	cloning vector with myo-2 promoter	Amp	Gift from Ian Chin-Sang	A2	yes
pPD95.75	promoterless + NLS-GFP, unc-54 3'utr		Amp	Gift from Ian Chin-Sang	A3	yes
pBSII SK+	cloning vector		Amp	Gift from Ian Chin-Sang	A5	yes
odr-1p::rfp	odr-1 promoter driving rfp, co-injection marker		Amp	Gift from Ian Chin-Sang	A4	yes
pJLR11	[tph-1(short NSM fragment)::del-7-sl2-GFP]		Amp	Gift from Steven Flavell	B3	yes
pLJ080	unc-17p sGAL4sk gp41 N intein		Amp	Gift from Paul Sternberg	A6	yes
pJL081	ceh-19bp sGAL4sk gp41 C-intein.ape		Amp	Gift from Paul Sternberg	A7	yes
pHW530	Prab-3::cGAL-N::let-858 3'UTR		Amp	Gift from Paul Sternberg	A8	yes
KP1368	Pmyo-2::nls::mCherry	co-injection marker	Amp	Gift from Paul Sternberg	A9	yes
pENTR-lin-44p	pENTR-lin-44		Kan	Gift from Yuichi Iino	B1	yes
ser-2p NLS-tagBFP	pG-ser2(prom2)p_svNLS2-TagBFPsyn		Amp	Gift from Yuichi Iino	B2	yes