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Description of plasmids provided

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Plasmids

pART**7** is a synthetic cloning vector from the Andrew Gleave lab (Gleave 1992) harbouring a CaMV 35S promoter and a multi cloning site for inserting genes intended to be transiently expressed in plants. Two NotI restriction sites allow to excise the cloned gene for subcloning into a binary vector.
pART7 is a derivative of pGEM-9zf$^-$ (GenBank X65312).

pART**27** is the binary vector corresponding to pART7. It harbours a transfer DNA gene cassette (tDNA) between the left and right borders to be integrated into the genome of higher plants by agrobacterium-mediated gene transfer. A single NotI restriction site allows to integrate a NotI-restricted gene cassette from pART7 into the tDNA of pART27.
pART27 is a derivative of pMON530 (Rogers et al. 1987 DOI: 10.1016/0076-6879(87)53058-0)

pART7::CLOMELEON is a pART7 vector with the CLOMELEON gene (see below) integrated between EcoRI and HindIII restriction sites.

pART27::CLOMELEON is a binary pART27 vector harbouring the NotI-fragment with gene cassette subcloned from pART7::CLOMELEON.

pQE30::CLOMELEON:Strep is a vector for bacterial expression of the CLOMELEON protein with N-terminal 6xHis-tag and C-terminal Strep-tag (WSHPQFEK). The gene coding for CLOMELEON with Strep-tag was introduced into the commercial expression vector pQE30 (Qiagen) between KpnI and HindIII sites.

Historical Context: CLOMELEON - A FRET-based fluorescent anion indicator

CLOMELEON is a chloride indicator to quantify chloride ion concentrations in living cells (details in Kuner & Augustine 2000). It is a fusion of the cyano-green fluorescent protein variant (CFP: K26R, F64L, S65T, Y66W, N146I, M153T, V163A, N164H, H231L) and a variant of yellow-green fluorescent protein (YFP: S65G, S72A, K79R, T203Y, H231L). The linker between both is short (24 AAs) and carries a TEV-protease recognition site (ENLYFQG). YFP fluorescence is quenched in presence of chloride ions while CFP fluorescence is not. Thus, efficient Förster-resonance between both occurs only in absence of chloride. This effect makes CLOMELEON a ratiometric indicator. Monitoring fluorescence emission at two emission wavelengths (480 nm and 530 nm) allows the calculation of fluorescence intensity ratios which correlate with anion concentrations. The EC_{50} for chloride is at around $[Cl^-]$ = 150 mM.

For expression of CLOMELEON in plants the pART7/pART27 vector system from the Andrew Gleave lab was used (Lorenzen et al. 2004). The gene coding for CLOMELEON, kindly provided by the George Augustine lab, was subcloned into pART7 to bring it under the control of a cauliflower mosaic virus promoter (35S-CaMV). With NotI restriction, the whole gene cassette was then subcloned into the binary vector pART27 to allow for agrobacterium-mediated transfer of the cassette into the plant genome by floral dip method (Clough & Bent 1998).

Note #1: For selection, the binary vector (pART27) confers spectinomycin resistance in bacteria and kanamycin resistance in plants. The latter is important to note because a successful transfer of T-DNA into plants needs to be selected on kanamycin and cannot be selected on spectinomycin.
Note #2: Arabidopsis thaliana expressing CLOMELEON in the cytoplasm have been produced (Lorenzen et al. 2004; Saleh & Plieth 2013). Seeds from transgenic *Arabidopsis* expressing CLOMELEON in the cytoplasm are available with Nottingham Arabidopsis stock centre (https://arabidopsis.info/BasicForm; NASC-IDs N9404 to N9415)

References

Clough S.J. & Bent A.F. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. The Plant Journal 16(6):735-743. PubMed: 10069079; DOI: 10.1007/BF00028910

Gleave, A.P. 1992. A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Molecular Biology 20(6):1203-7. PubMed: 1463857; DOI: 10.1007/BF00028910. (GenBank X69707)

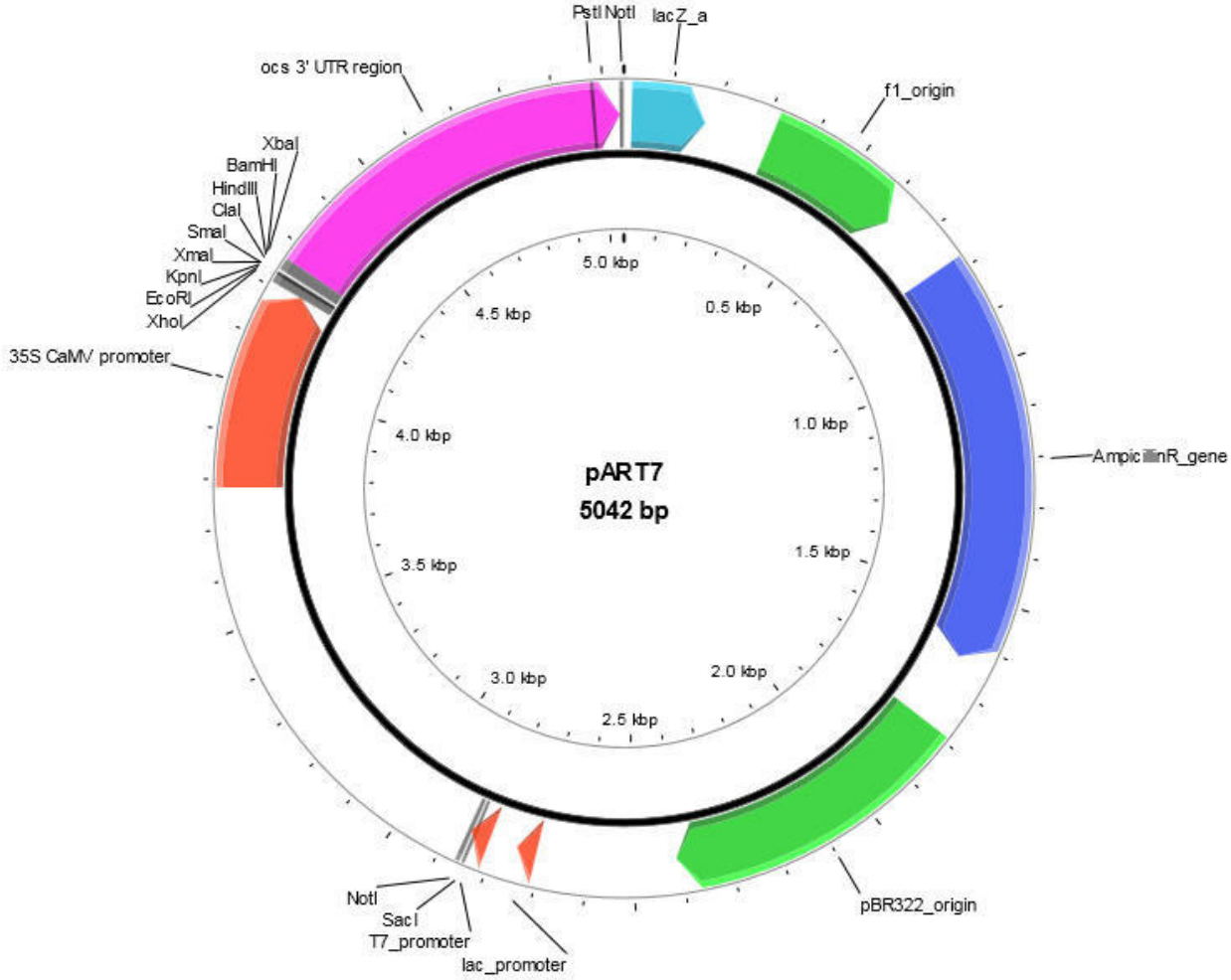
Kuner, T. & Augustine, G.J. 2000. A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons. Neuron 27(3):447-59. PubMed: 11055428; DOI: 10.1016/S0896-6273(00)00056-8

Lorenzen, I., Aberle, T. & Plieth, C. 2004. Salt stress-induced chloride flux: a study using transgenic *Arabidopsis* expressing a fluorescent anion probe. The Plant Journal 38(3):539-44. PubMed: 15086798; DOI: 10.1111/j.0960-7412.2004.02053.x.

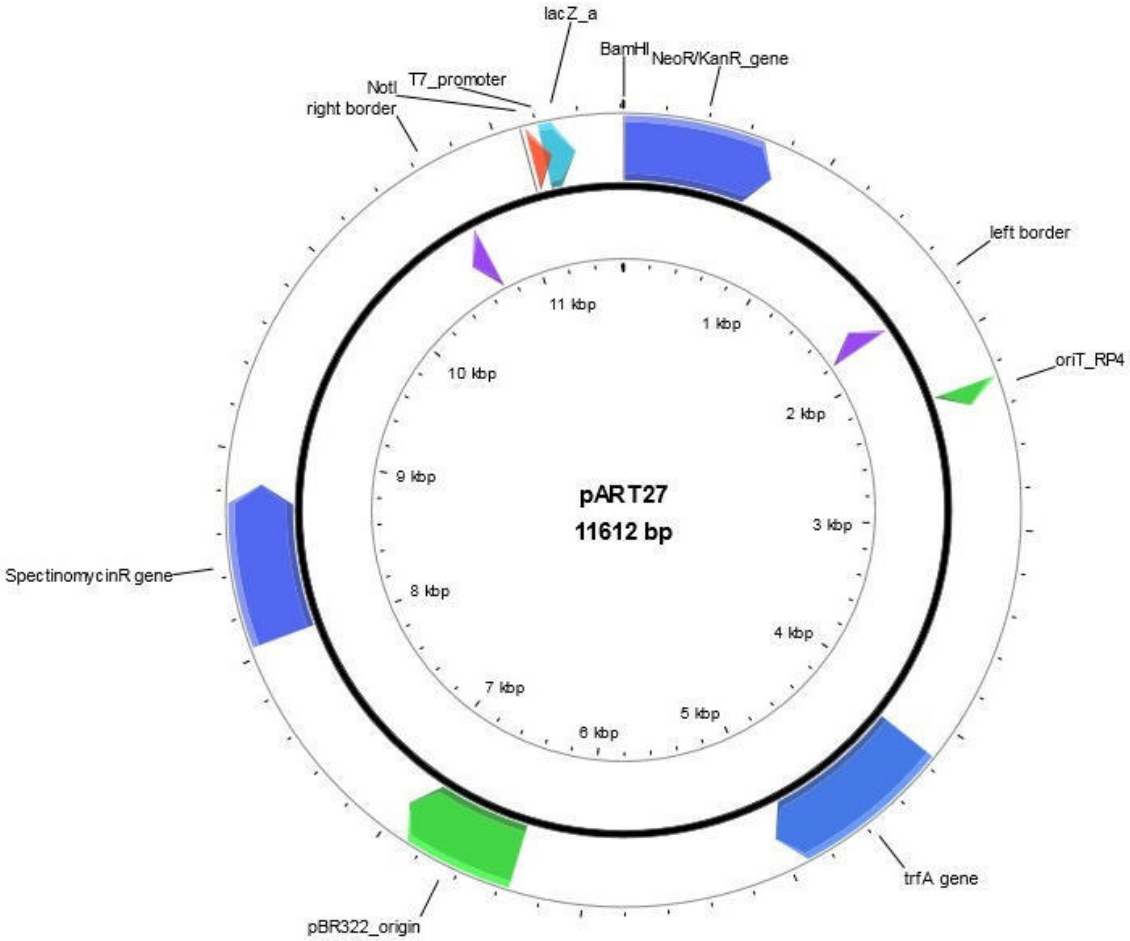
Rogers, Stephen G., et al. 1987. Improved vectors for plant transformation: Expression cassette vectors and new selectable markers. Methods in Enzymology. 153: 253-277 DOI: 10.1016/0076-6879(87)53058-0

Saleh, L., & Plieth, C. (2013). A9C sensitive Cl^- - accumulation in *A. thaliana* root cells during salt stress is controlled by internal and external calcium. Plant Signaling & Behavior, 8(6). PubMed 23603974; DOI: 10.4161/psb.24259

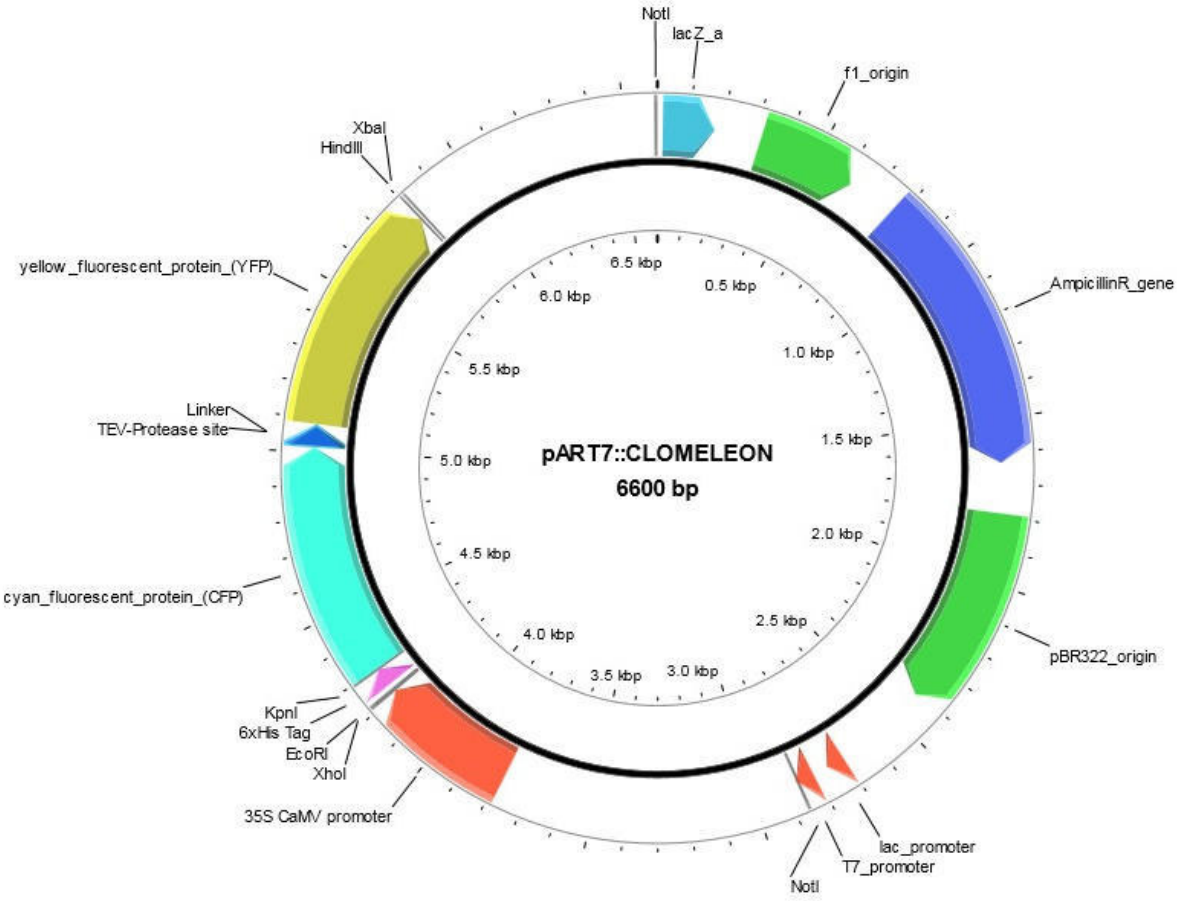
Qiagen www.qiagen.com/us/resources/download.aspx?id=26cbf325-e982-482f-a09e-14e73a814c23&lang=en



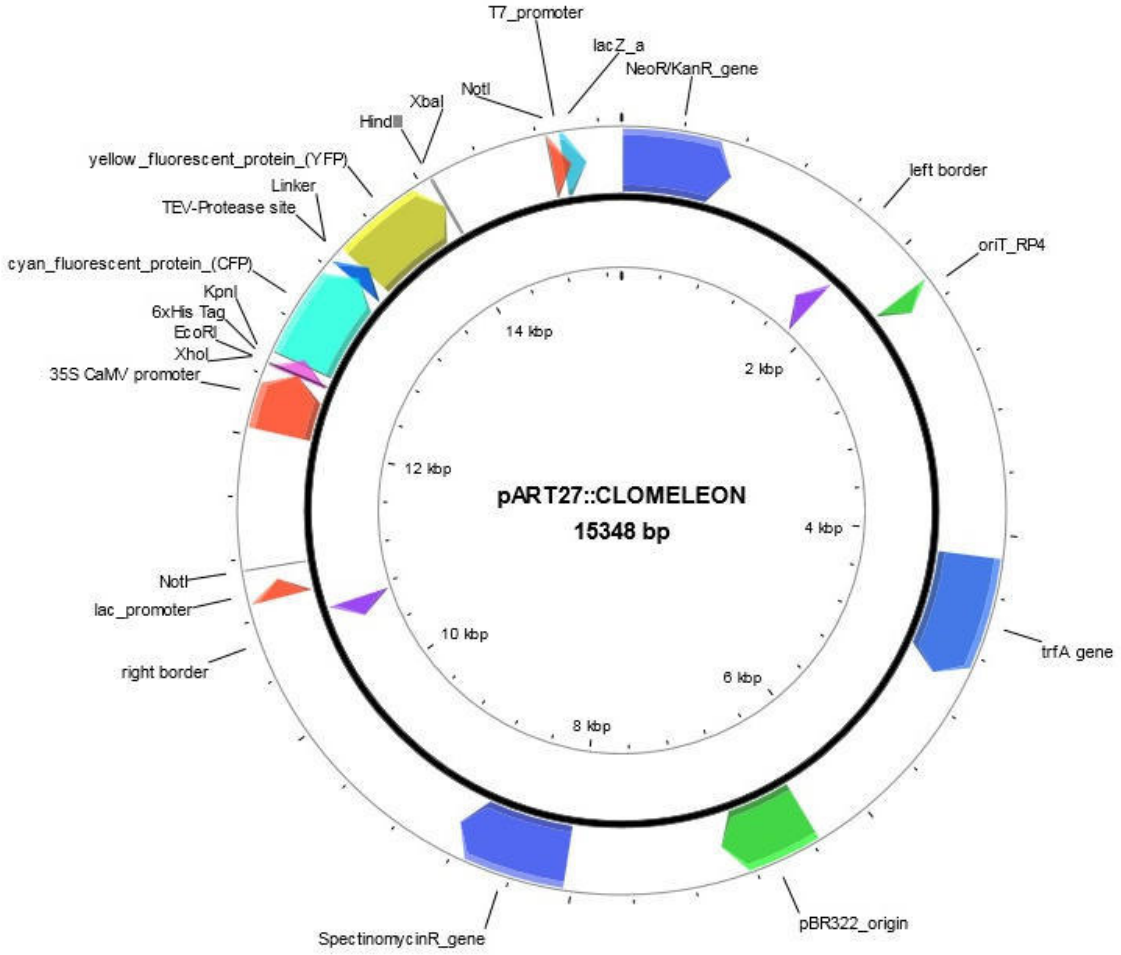
pART7				
	Fragments	Start	End	Category
	35S CaMV promoter	3784	4210	Promoter
	ocs 3' UTR region	4264	5034	Regulatory Sequence
	Selected Restriction Sites cuts once / cuts twice	Location		Sequence
	BamHI	4253		glgatcc
	ClaI	4242		atlcgat
	EcoRI	4218		glaattc
	HindIII	4247		alagctt
	KpnI	4228		ggtaclc
	NotI	2859; 5037		ctgcalg
	PstI	4982		ctgcalg
	SacI /SstI	2851		gagctlc
	XbaI	4259		tlctaga
	XhoI	4212		cltcgag
	XmaI / SmaI	4229 / 4231		clccggg / ccclggg



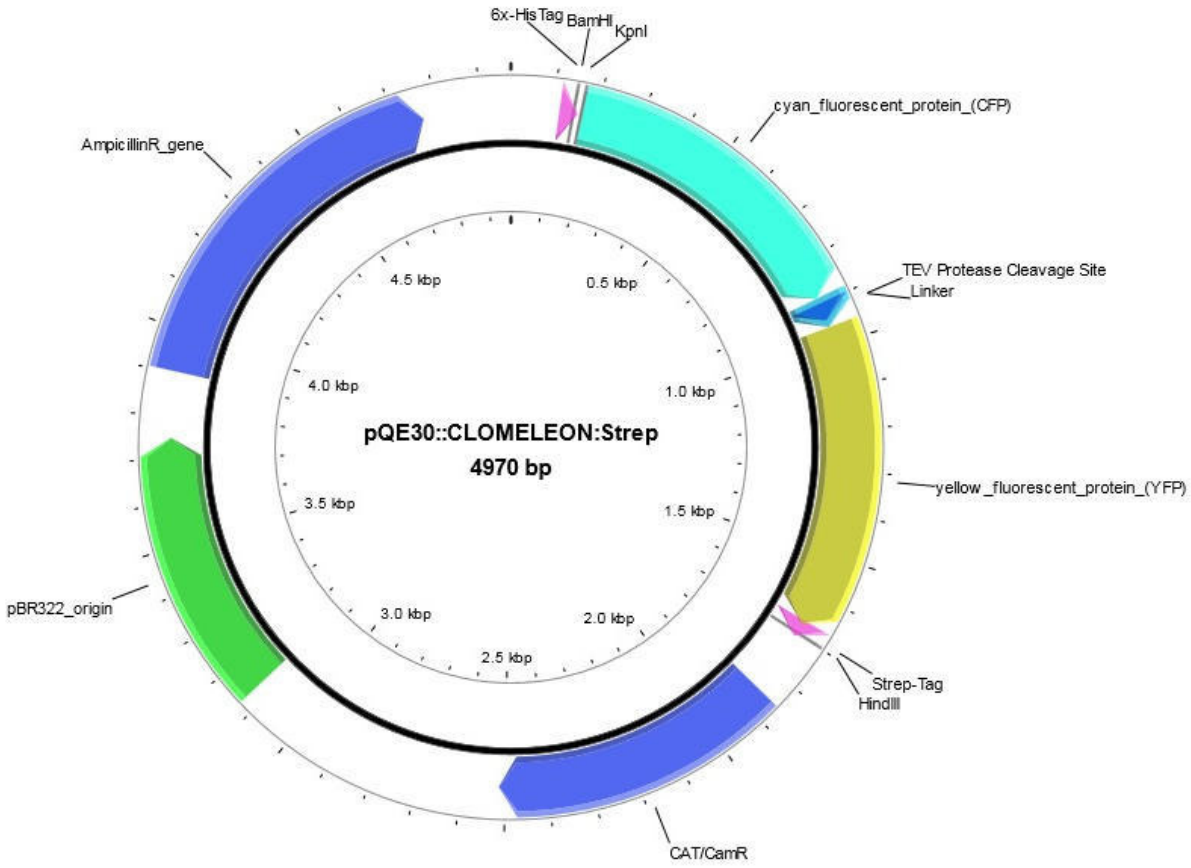
pART27::CLOMELEON				
	Fragments	Start	End	Category
	KanamycinR gene	9	776	Selectable Marker
	Left border	1719	1743	miscellaneous
	Right border	10638	10662	miscellaneous
	trfA gene	4150	5007	Gene
	SpectinomycinR gene	8052	8840	Selectable Marker
	Selected Restriction Sites cuts once / cuts twice	Location		Sequence
	BamHI	2		glgatcc
	NotI	11123		ctgcalg
	SacI /SstI	11102		gagctlc



pART7::CLOMELEON				
	Fragments	Start	End	Category
	35S CaMV promoter	3784	4210	Promoter
	6xHis-Tag	4256	4273	Affinity Tag
	CFP	4298	5014	Reporter Gene
	Linker	5015	5086	Miscellaneous
	TEV-Protease Site	**5039**	**5059**	**Gene**
	YFP	5087	5803	Reporter Gene
	Selected Restriction Sites cuts once / cuts twice	Location		Sequence
	BamHI	4275, 5811		glgatcc
	EcoRI	4218		glaattc
	HindIII	5805		alagctt
	KpnI	4297		ggtaclc
	NotI	2859; 6595		ctgcalg
	PstI	6540		ctgcalg
	SacI /SstI	2851; 4291		gagctlc
	XbaI	5817		tlctaga
	XhoI	4212		cltcgag



pART27::CLOMELEON				
	Fragments	Start	End	Category
	KanamycinR gene	9	776	Selectable Marker
	Left border	1719	1743	miscellaneous
	Right border	10638	10662	miscellaneous
	trfA gene	4150	5007	Gene
	SpectinomycinR_gene	8052	8840	Selectable Marker
	35S CaMV promoter	12049	12475	Promoter
	6xHis-Tag	12520	12537	Affinity Tag
	CFP	12562	13278	Reporter Gene
	Linker	13279	13350	Miscellaneous
	TEV-Protease Site	13303	13323	Gene
	YFP	13351	14067	Reporter Gene
	Selected Restriction Sites cuts once / cuts twice	Location		Sequence
	EcoRI	12482		glaattc
	HindIII	14069		alagctt
	KpnI	12561		ggtaclc
	NotI	11123; 14859		ctgcalg
	SacI /SstI	11102; 12555		gagctlc
	XbaI	14081		tlctaga
	XhoI	12476		cltcgag



pQE30::CLOMELEON:Strep				
	Fragments	Start	End	Category
	6xHis-Tag	127	144	Affinity Tag
	CFP	172	885	Reporter Gene
	Linker	886	957	Miscellaneous
	TEV-Protease Site	910	930	Gene
	YFP	958	1674	User defines
	Strep-Tag	1675	1698	Affinity Tag
	AmpicillinR gene	3905	4765	Selectable Marker
	Selected Restriction Sites (cuts once)	Location		Sequence
	BamHI	146		glgatcc
	HindIII	1697		alagctt
	KpnI	168		ggtaclc
	EcoRI	89		glaattc
	SacI /SstI	162		gagctlc
	XbaI	2673		tlctaga
	XhoI	2		cltcgag