Anatomy of a plasmid

Plasmids are autonomously replicating pieces of extrachromosomal DNA.

Plasmids are <u>vectors</u> – DNA molecules, used to transfer foreign genetic material into another cell

promoter elements -

a sequence driving the expression of a gene (such as gene of interest, selection marker or reporter gene)



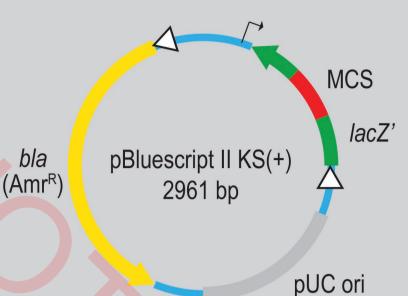
bla

selection marker -

a gene encoding antibiotic resistance, whose expression is required for survival of bacterial cells containing the plasmid

additional elements -

tags, targeting elements, primer sequences, etc



multiple cloning site (MCS) -

a sequence in a plasmid, containing several unique restriction sites allowing to incorporate the *gene of interest*



reporter gene(s) -

a gene, whose expression can be monitored as a marker for the successful uptake of the gene of interest



a sequence in a plasmid, where replication is initiated; defines plasmid's copy number

Origin of replication

vector	copy number	ori
pBR322	15-20	pMB1
pUC	500-700	pMB1'
pET	15-20	pBR322
pBluescript	300-500	CoIE1' / f1

Antibiotic resistance

antibiotic	class	selection marker	
ampicillin	bactericidal	β-lactamase (<i>bla</i>)	
carbenicillin	bactericidal	β-lactamase (<i>bla</i>)	
kanamycin	bactericidal	neomycin phosphotransferase (neo)	
chloramphenicol	bacteriostatic	chloramphenicol acetyltransferase (cat)	

Promoter elements

PROKARYOTIC

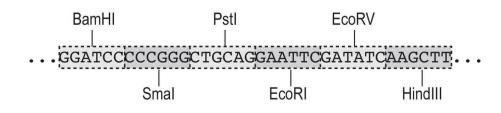
promoter	purpose	regulation
T7	general expression	constitutive, requires
		T7 RNA polymerase
T7lac	protein overexpression	tightly reguated by lac
		operator. Inducible by IPTG
lac	general expression	constitutive without lac
		repressor (lacl or laclq).
		Inducible by IPTG or lactose
trp	protein overexpression	repressible by tryptophan
	T7 T7lac lac	T7 general expression T7lac protein overexpression lac general expression

EUKARYOTIC

promoter	purpose		regulation		
CMV	mammalian expression		strong, constitutive		
EF1a	mammalian expression		constitutive, cell type-independent		
Ubc	mammalian expression		ubiquitous, constitutive		
TRE	mammalian expression		inducible by tetracycline		
UAS*	Drosophila expression		requires Gal4 expression		
polyhedrin	baculovirus/insect cells		strong, constitutive		
	expression				
ADH1	yeast expression		strong; inhibited by ethanol		

^{*} an enhancer element

Multiple cloning site ("polylinker")



Reporter genes

