



基本信息

平台编号	bio-82481
启动子:	Lac/lac promoter
复制子:	ColE1 ori
质粒大小:	2686bp
质粒标签:	LacZ
原核抗性:	氨苄青霉素 Amp
克隆菌株:	DH5 α
培养条件:	37°C, 有氧, LB
5'测序引物:	M13F:TGTAAAACGACGGCCAGT
3'测序引物:	M13R:CAGGAAACAGCTATGACC

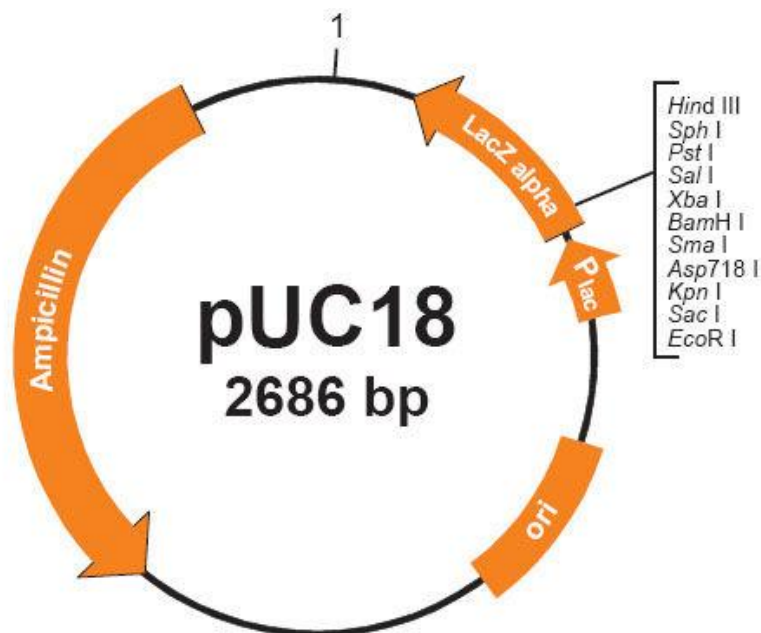
质粒简介

pUC18 是适合于双脱氧法 DNA 测序的载体, 通常运用于重组 dna 的分子克隆中, 首先我们制备大肠杆菌的感受态细胞 (能接受外来重组的 dna), 这种感受态菌株必须不同外来 DNA 分子发生遗传重组, 通常是 rec 基因缺陷型的突变体, 同时它们必须是限制系统缺陷或限制与修饰系统均缺陷的菌株, 即具有这三种缺陷 (rk mk rec), 同时对氨苄青霉素敏感 (ap)。

puc18 载体自身带有抗氨苄青霉素基因, 而外源片段不具有, 这样只有带有 puc18 的转化子的菌株才能在氨苄青霉素平板上存活, 而外源片段自身环化的不能存活, 这称为抗性筛选。

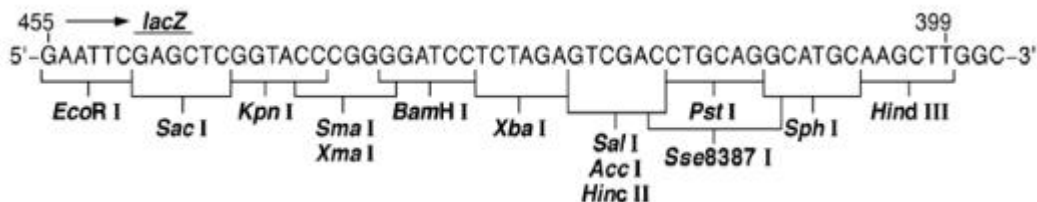
pUC18 上带有 β -半乳糖苷酶基因(lacZ)的调控序列和 β -半乳糖苷酶 N 端 146 个氨基酸的编码序列, 在这个编码区中插有一个多克隆位点, 且不影响正常功能, DH5 α 感受态菌株带有 β -半乳糖苷酶 C 端部分序列的编码信息, 当 puc18 载体在正常情况下同感受态菌株融合后, 互补表达有活性的 β -半乳糖苷酶, 称为 α -互补现象, 当有外源片段插入多克隆位点后, 不能产生互补, 即细菌不能产生 β -半乳糖苷酶活性。在呈色底物 X-gal (5-溴-4-氯-3-吲哚- β -半乳糖苷酶) 和诱导物 IPTG (异丙基- β -D-硫代半乳糖苷) 的存在下, 互补产物菌落呈现蓝色, 不互补产物菌落呈白色, 很容易鉴别, 这种筛选方法称为 α -互补现象筛选(蓝白斑筛选)。由上可见, puc18 载体除了能重组导入外源片段外, 在筛选表达过程中还具有重要的作用。

质粒图谱



Comments for pUC18 2686 nucleotides

LacZ alpha peptide: bases 149-469 (complementary strand)
lac promoter: bases 470-543 (complementary strand)
 pUC origin: bases 808-1481 (complementary strand)
 Ampicillin (*bla*) resistance gene: bases 1629-2486 (complementary strand)
bla promoter: bases 2487-2585 (complementary strand)



质粒序列

LOCUS	Exported File	2686 bp ds-DNA	circular SYN
02-2-2015			
KEYWORDS	Untitled		
SOURCE	synthetic DNA construct		
ORGANISM	synthetic DNA construct		
REFERENCE	1 (bases 1 to 2686)		
TITLE	Direct Submission		
JOURNAL	Exported 2015-2-2		
FEATURES	Location/Qualifiers		
source	1..2686		
	/organism="synthetic DNA construct"		
	/mol_type="other DNA"		



CDS	complement(146..469) /codon_start=1 /gene="lacZ fragment" /product="LacZ-alpha fragment of?beta-galactosidase" /note="lacZ-alpha" /translation="MTMITNSSSVPGDPLESTCRHASLALAVVLQ RRDWENPGVTQLNR LAAHPPFASWRNSEEARTDRPSQQLRSLNGEWRLMRYFLLTHLCG ISHRIWCTLSTICS DAA"
primer_bind	379..395 /note="M13 fwd" /note="common sequencing primer, one of multiple similar variants"
misc_feature	399..455 /gene="" /note="MCS" /note="pUC18/19 multiple cloning site"
primer_bind	complement(465..481) /note="M13 rev" /note="common sequencing primer, one of multiple similar variants"
protein_bind	489..505 /bound_moiety="lac repressor encoded by lacI" /note="lac operator" /note="The lac repressor binds to the lac operator to inhibit transcription in E. coli. This inhibition can be relieved by adding lactose or isopropyl-beta-D-thiogalactopyranoside (IPTG)."
promoter	complement(513..543) /note="lac promoter" /note="promoter for the E. coli lac operon"
protein_bind	558..579 /bound_moiety="E. coli catabolite activator protein"



微生物菌种查询网

/note="CAP binding site"
/note="CAP binding activates transcription in
the presence
of cAMP."
rep_origin complement(867..1455)
/direction=LEFT
/note="ori"
/note="high-copy-number ColE1/pMB1/pBR322/pUC
origin of
replication"
CDS complement(1626..2486)
/codon_start=1
/gene="bla"
/product="beta-lactamase"
/note="AmpR"
/note="confers resistance to ampicillin,
carbenicillin, and
related antibiotics"
/translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKV
KDAEDQLGARVGYI
ELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLG
RRIHYSQNDLVEYS
PVTEKHLTDGMTVRELCSAAITMSDNTAANLLLLTTIGGPKELTAF
LHNMGDHSVTRLDRW
EPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQIDW
MEADKVAGPLLRSA
LPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQAT
MDERNRQIAEIGAS
LIKHW"
promoter complement(2487..2591)
/gene="bla"
/note="AmpR promoter"
ORIGIN
1 tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg
gagacggtca
61 cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg
tcagcgggtg
121 ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta
ctgagagtgc
181 accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc
atcagggcgc
241 attcgccatt caggctgctc aactgttggg aagggcgatc ggtgcggggc



tcttcgctat
301 tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta
acgccagggt
361 tttcccagtc acgacgttgt aaaacgacgg ccagtgccaa gcttgcatgc
ctgcaggctg
421 actctagagg atccccgggt accgagctcg aattcgtaat catggtcata
gctgtttcct
481 gtgtgaaatt gttatccgct cacaattcca cacaacatac gagccggaag
cataaagtg
541 aaagcctggg gtgcctaata agtgagctaa ctacattaa ttgcgttgcg
ctcactgccc
601 gctttccagt cgggaaacct gtcgtgccag ctgcattaat gaatcggcca
acgcgcgggg
661 agaggcggtt tgcgtattgg gcgctcttcc gcttcctcgc tcaactgactc
gctgcgctcg
721 gtcgttcggc tgcggcgagc ggtatcagct cactcaaagg cggtaatacg
gttatccaca
781 gaatcagggg ataacgcagg aaagaacatg tgagcaaaag gccagcaaaa
ggccaggaac
841 cgtaaaaagg ccgcgttgct ggcgtttttc cataggctcc gccccctga
cgagcatcac
901 aaaaatcgac gctcaagtca gaggtggcga aaccgcagag gactataaag
ataccaggcg
961 tttccccctg gaagctccct cgtgcgctct cctgttccga ccctgccgct
taccggatac
1021 ctgtccgcct ttctcccttc gggaagcgtg gcgctttctc aaagctcacg
ctgtaggtat
1081 ctcaattcgg ttaggtcgt tcgctccaag ctgggctgtg tgcacgaacc
ccccgttcag
1141 cccgaccgct gcgccttatc cggtaactat cgtcttgagt ccaaccgggt
aagacacgac
1201 ttatcgccac tggcagcagc cactggtaac aggattagca gagcgaggta
tgtaggcggt
1261 gctacagagt tcttgaagtg gtggcctaac tacggctaca ctagaagaac
agtatttgg
1321 atctgcgctc tgctgaagcc agttaccttc ggaaaaagag ttggtagctc
ttgatccggc
1381 aaacaaacca ccgctggtag cggtggtttt tttgtttgca agcagcagat
tacgcgcaga
1441 aaaaaaggat ctcaagaaga tcctttgatc ttttctacgg ggtctgacgc
tcagtggaac
1501 gaaaactcac gttaagggat tttggatcatg agattatcaa aaaggatctt



cacctagatc
1561 cttttaaatt aaaaatgaag ttttaaatca atctaaagta tatatgagta
aacttggtct
1621 gacagttacc aatgcttaat cagtgaggca cctatctcag cgatctgtct
atttcgttca
1681 tccatagttg cctgactccc cgtcgtgtag ataactacga tacgggaggg
cttaccatct
1741 ggccccagtg ctgcaatgat accgcgagac ccacgctcac cggctccaga
tttatcagca
1801 ataaaccagc cagccggaag ggccgagcgc agaagtggtc ctgcaacttt
atccgcctcc
1861 atccagtcta ttaattgttg ccgggaagct agagtaagta gttcgccagt
taatagtttg
1921 cgcaacgttg ttgccattgc tacaggcatc gtggtgtcac gctcgtcgtt
tggtatggct
1981 tcattcagct ccggttccca acgatcaagg cgagttacat gatccccat
gttgtgcaaa
2041 aaagcggtta gtccttcggt tctccgatac gttgtcagaa gtaagttggc
cgcaagtgtta
2101 tcactcatgg ttatggcagc actgcataat tctcttactg tcatgccatc
cgtaagatgc
2161 ttttctgtga ctggtgagta ctcaaccaag tcattctgag aatagtgtat
gcggcgaccg
2221 agttgctctt gcccggcgtc aatacgggat aataccgcgc cacatagcag
aactttaaaa
2281 gtgctcatca ttggaaaacg ttcttcgggg cgaaaactct caaggatctt
accgctgttg
2341 agatccagtt cgatgtaacc cactcgtgca cccaactgat cttcagcatc
ttttactttc
2401 accagcgttt ctgggtgagc aaaaacagga aggcaaatg ccgcaaaaaa
gggaataagg
2461 gcgacacgga aatgttgaat actcatactc ttcctttttc aatattattg
aagcatttat
2521 cagggttatt gtctcatgag cggatacata tttgaatgta tttagaaaaa
taaacaaata
2581 ggggttccgc gcacatttcc ccgaaaagtg ccacctgacg tctaagaaac
cattattatc
2641 atgacattaa cctataaaaa taggcgtatc acgaggccct ttcgtc
//