



基本信息

启动子:	Trc/lac
平台编号:	bio-82478
复制子:	PBR322 ori
终止子:	rrnB T2
质粒分类:	大肠杆菌载体; pTrc 系列表达质粒
质粒大小:	4414bp
质粒标签:	N-His, N-EK, N-Xpress
原核抗性:	氨苄青霉素 Amp
克隆菌株:	DH5α
培养条件:	37°C, 有氧, LB
表达宿主:	大肠杆菌
诱导方式:	IPTG 或乳糖及其类似物
5'测序引物:	pTrcHis-F: GAGGTATATATTAATGTATCG
3'测序引物:	pTrcHis-R:GATTTAATCTGTATCAGG
备注:	pTrc 表达质粒

质粒简介

pTrcHis 载体是由 pBR322 质粒衍生而来的表达载体,设计用来在大肠杆菌中进行重组蛋白的高效表达和纯化。载体上的 Trc 启动子和 rrnB 抗终止区使得高水平蛋白表达成为可能。Trc 启动子由 Trp 启动子的-35 区域和 Lac 启动子的-10 区域共同组成。pTrcHis 载体同时包含有一个 LacIq 基因, 该基因编码了 Lac 启动子的抑制蛋白。LacIq 基因的存在使得宿主菌是 LacIq⁺还是 LacIq⁻都可以高效抑制插入基因的转录。进行蛋白表达时, 大肠杆菌需要生长到对数生长期, 然后加入 1mM IPTG, 去除阻遏蛋白, 诱导表达。载体上 pTrcHis 载体是由 pBR322 质粒衍生而来的表达载体,设计用来在大肠杆菌中进行重组蛋白的高效表达和纯化。载体上的 Trc 启动子和 rrnB 抗终止区使得高水平蛋白表达成为可能。Trc 启动子由 Trp 启动子的-35 区域和 Lac 启动子的-10 区域共同组成。pTrcHis 载体同时包含有一个 LacIq 基因, 该基因编码了 Lac 启动子的抑制蛋白。LacIq 基因的存在使得宿主菌是 LacIq⁺还是 LacIq⁻都可以高效抑制插入基因的转录。进行蛋白表达时, 大肠杆菌需要生长到对数生长期, 然后加入 1mM IPTG, 去除阻遏蛋白, 诱导表达。载体上的迷你顺反子结构能够增强插入基因的转录水平, 从而高水平表达目的蛋白。DNA 插入片段位于载体表达片段下游, 在其上游有一个 N-端融合表达多肽序列, 该序列包含有 ATG 起始密码子, 6xHis 标签, T7 噬菌体 10 号基因的翻译增强子, Xpress 抗原表位, 肠激酶酶切识别位点的迷你顺反子结构, 能够增强插入基因的转录水平, 从而高水平表达目的蛋白。DNA 插入片段位于载体表达片段下游, 在其



微生物菌种查询网

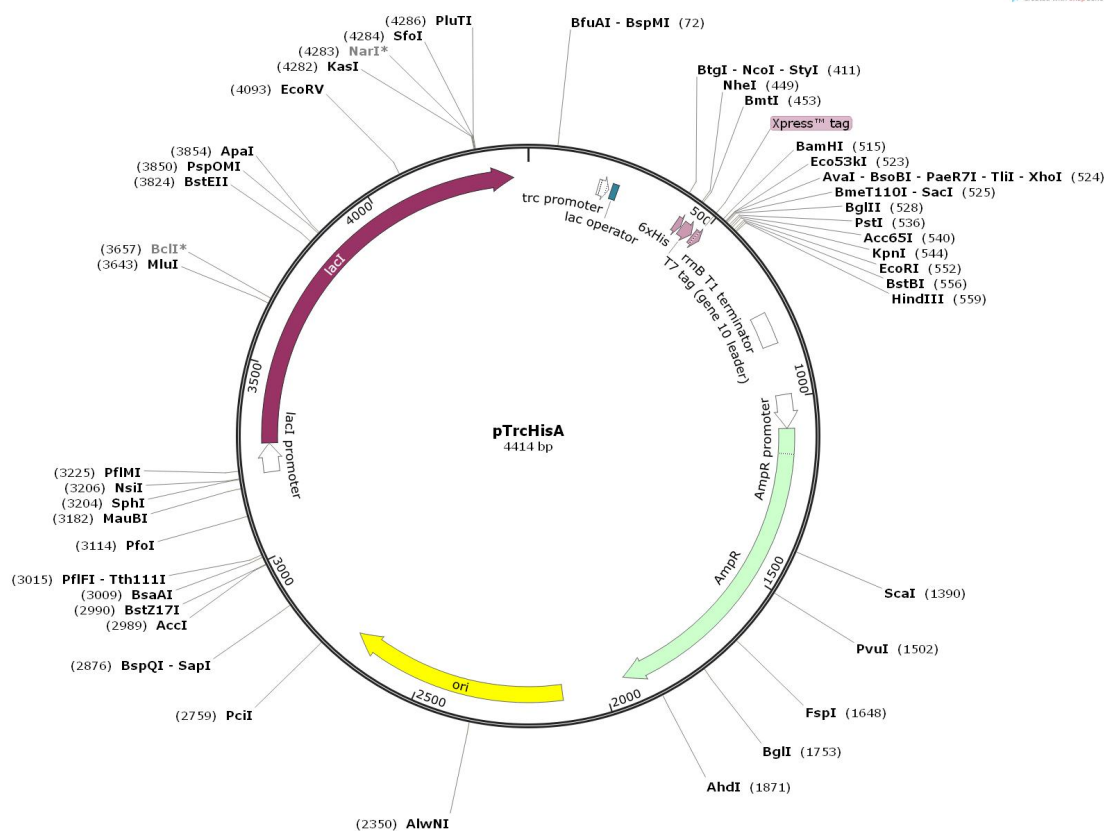
上游有一个 N-端融合表达多肽序列, 该序列包含有 ATG 起始密码子, 6xHis 标签, T7 噬菌体 10 号基因的翻译增强子, Xpress 抗原表位, 肠激酶酶切识别位点。

Multiple Cloning Site of pTrcHis A

Below is the multiple cloning site for pTrcHis A. Restriction sites are labeled to indicate the actual cleavage site. Boxed nucleotides indicate the variable region. Sequencing and functional testing have confirmed the multiple cloning site. The complete sequence of pTrcHis A is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 14). For a map and description of the features of pTrcHis, refer to pages 11-12.

pTrcHis forward primer		Mini cistron	
RBS			
361	AAAATTAAG AGGTATATAT TA	ATG TAT CGA TTA AAT AAG GAG GAA TAA ACC	
		Met Tyr Arg Leu Asn Lys Glu Glu ***	
Polyhistidine (6xHis) region			
413	ATG GGG GGT TCT CAT CAT CAT CAT CAT CAT	GGT ATG GCT AGC ATG ACT	
	Met Gly Gly Ser His His His His His His	Gly Met Ala Ser Met Thr	
gene 10 leader		Xpress™ Epitope	
461	GGT GGA CAG CAA ATG GGT CGG ACT CTG TAC	GAC GAT GAC GAT AAG GAT	EK cleavage site
	Gly Gly Gln Gln Met Gly Arg Thr Leu Tyr	Asp Asp Asp Asp Lys Asp	
BamHI		XhoI	
509	GGA TGG GGA TCC GAG CTC GAG ATC TGC AGC	TGG TAC CAT ATG GGA ATT	
	Arg Trp Gly Ser Glu Leu Glu Ile Cys Ser Trp Tyr His Met Gly Ile		
HindIII			
557	CGA AGC TTG GCT GTT TTG GCG GAT GAG AGA AGA TTT TCA GCC TGA		
	Arg Ser Leu Ala Val Leu Ala Asp Glu Arg Arg Phe Ser Ala ***		

质粒图谱



质粒序列



微生物菌种查询网

LOCUS Exported 4414 bp ds-DNA circular SYN 29-七月-2016

DEFINITION synthetic circular DNA

ACCESSION .

VERSION .

KEYWORDS pTrcHisA

SOURCE synthetic DNA construct

 ORGANISM synthetic DNA construct

REFERENCE 1 (bases 1 to 4414)

AUTHORS .

TITLE Direct Submission

JOURNAL Exported 2016年7月29日 from SnapGene Viewer

2.8.1FEATURES Location/Qualifiers

 source 1..4414

 /organism="synthetic DNA construct"

 /mol_type="other DNA"

 misc_feature 193..222

 /note="trc promoter"

 /note="strong E. coli promoter; hybrid between the trp and

 lac UV5 promoters"

 protein_bind 230..246

 /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)."

 misc_feature 263..332

 /note="rrnG antiterminator"

 /note="antiterminator from the E. coli rrnG leader region

 (Berg et al., 1989)"

 CDS 383..409

 /codon_start=1

 /product="synthetic cistron containing a ribosome binding

 site (Shine-Dalgarno sequence), for enhancing the bacterial

 expression of a downstream cistron (Schoner, 1997)"

 /note="minicistron"

 /note="This first cistron should terminate 3 bp upstream of

 the ATG for the second cistron."

 /translation="MYRLNKEE"

 CDS 425..442

 /codon_start=1

 /product="6xHis affinity tag"



	/note="6xHis"
	/translation="HHHHHH"
CDS	446..478
	/codon_start=1
	/product="leader peptide from bacteriophage T7 gene 10"
	/note="T7 tag (gene 10 leader)"
	/note="promotes efficient translation in E. coli"
	/translation="MASMTGGQQMG"
CDS	482..505
	/codon_start=1
	/product="Xpress(TM) epitope tag, including an enterokinase recognition and cleavage site"
	/note="Xpress(TM) tag"
	/translation="DLYDDDDK"
misc_feature	515..564
	/note="MCS"
terminator	767..853
	/gene="Escherichia coli rrnB"
	/note="rrnB T1 terminator"
	/note="transcription terminator T1 from the E. coli rrnB gene"
terminator	945..972
	/note="rrnB T2 terminator"
	/note="transcription terminator T2 from the E. coli rrnB gene"
promoter	992..1083
	/gene="bla"
	/note="AmpR promoter"
CDS	1084..1944
	/codon_start=1
	/gene="bla"
	/product="beta-lactamase"
	/note="AmpR"
	/note="confers resistance to ampicillin, carbenicillin, and related antibiotics"
	/translation="MSIQHFRVALIPFFAAFCCLPVFAHPETLVKVKDAEDQLGARV
GYI	ELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLV
EYS	PVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRW	EPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQQLIDWMEADKVAGPLL



微生物菌种查询网

RSA

LPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEI

GAS

LIKHW"

rep_origin

2115..2703

/direction=RIGHT

/note="ColE1 ori"

/note="high-copy-number ColE1/pMB1/pBR322/pUC origin of replication"

misc_feature

2889..3029

/note="bom"

/note="basis of mobility region from pBR322"

promoter

3215..3292

/gene="lacI"

/note="lacI promoter"

CDS

3293..4375

/codon_start=1

/gene="lacI"

/product="lac repressor"

/note="lacI"

/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)."

/translation="MKPVTLYDVAEYAGVSYQTVSRVNVQASHVSAKTRKVEAAM

AEL

NYIPNRVAQQLAGKQSLIGVATSSLALHAPSQIVAAIKSRADQLGASVVVSMVER

SGV

EACKAAVHNLLAQRVSGLIINYPLDDQDAIAVEAACTNVPALFLDVSDQTPINSII

FSH

EDGTRLGVEHLVALGHQQIALLAGPLSSVSARLRLAGWHKYLTRNQQIPIAEREGD

WSA

MSGFQQTMQMLNEGIVPTAMLVANDQMALGAMRAITESGLRVGADISVVGYYDDTED

SSC

YIPPSTTIKQDFRLLGQTSVDRLLQLSQGQAVKGNQLLPVSLVKRKTTLAPNTQTA

SPR

ALADSLMQLARQVSRLESQ"

ORIGIN

```
1 gtttgacagc ttatcatcga ctgcacggtg caccaatgct tctggcgta ggcagccatc
61 ggaagctgtg gtatggctgt gcaggtcgta aatcactgca taattcgtgt cgctcaaggc
121 gcactcccggt tctggataat gttttttgcg cgcacatcat aacggttctg gcaaatatc
181 tgaaatgagc tgttgacaat taatcatccg gctcgtataa tgtgtggaat tgtgagcgga
```



241 taacaatttc acacaggaac cagcgccgt gagaaaaagc gaagcggcac tgctctttaa
301 caatttatca gacaatctgt gtgggactc gaccggaatt atcgattaac tttattatta
361 aaaattaaag aggtatata taatgtatcg attaaataag gaggaataaa ccatgggggg
421 ttctcatcat catcatcatc atggtatggc tagcatgact ggtggacagc aaatgggtcg
481 ggatctgtac gacgatgacg ataaggatcg atggggatcc gagctcgaga tctgcagctg
541 gtaccatatg ggaattcgaa gcttggtgt tttggcggat gagagaagat tttcagcctg
601 atacagatta aatcagaacg cagaagcggc ctgataaaac agaatttgcc tggcggcagt
661 agcgcggtgg tcccacctga ccccatgccg aactcagaag tgaaacgccg tagcgccgat
721 ggtagtgtgg ggtctcccca tgcgagagta gggaaactgcc aggcatacaa taaaacgaaa
781 ggctcagtcg aaagactggg cctttcgttt tatctgttgt ttgtcgtgta acgctctcct
841 gagtaggaca aatccgccgg gagcggatct gaacgttgcg aagcaacggc ccggaggggtg
901 gcgggcagga cgcccgccat aaactgccag gcatcaaatt aagcagaagg ccatcctgac
961 ggatggcctt tttgcgtttc tacaactct tttgtttat ttttctaatt acattcaaat
1021 atgtatccgc tcatgagaca ataaccctga taaatgcttc aataatattg aaaaaggaag
1081 agtatgagta ttcaacattt ccgtgtgcgc cttattccct tttttggggc attttgccct
1141 cctgtttttg ctcaccaga aacgctgggt aaagtaaaag atgctgaaga tcagtgggtg
1201 gcacgagtg gttacatcga actggatctc aacagcggta agatccttga gagttttcgc
1261 cccgaagaac gttttccaat gatgagcact tttaaagtcc tgctatgtgg cgcggtatta
1321 tcccgtgttg acgcccggca agagcaactc ggtcgccgca tacactatcc tcagaatgac
1381 ttggttgagt actcaccagt cacagaaaag catcttacgg atggcatgac agtaagagaa
1441 ttatgcagtg ctgccataac catgagtgat aacactgcgg ccaacttact tctgacaacg
1501 atcggaggac cgaaggagct aaccgctttt ttgcacaaca tgggggatca tgtaactcgc
1561 cttgatcggt gggaaccgga gctgaatgaa gccataccaa acgacgagcg tgacaccacg
1621 atgcctgtag caatggcaac aacgttgccg aaactattaa ctggcgaact acttactcta
1681 gcttcccggc aacaattaat agactggatg gaggcggata aagttgcagg accacttctg
1741 cgctcgccc ttccggctgg ctggtttatt gctgataaat ctggagccgg tgagcgtggg
1801 tctcgcggta tcattgcagc actggggcca gatggtgaag cctcccgat cgtagttatc
1861 tacacgacgg ggagtcaggc aactatggat gaacgaaata gacagatcgc tgagataggt
1921 gcctcactga ttaagcattg gtaactgtca gaccaagtct actcatatat acttttagatt
1981 gattttaaacc ttcatTTTTA atttaaaagg atctaggtga agatcctttt tgataatctc
2041 atgacaaaaa tcccttaacg tgagttttcg ttccactgag cgtcagaccc cgtagaaaaa
2101 atcaaaggat cttcttgaga tccttttttt ctgcgcgtaa tctgctgctt gcaacaaaaa
2161 aaaccaccgc taccagcggg ggtttgtttg ccggatcaag agctaccaac tctttttccg
2221 aaggtaactg gcttcagcag agcgcagata ccaaatactg tccttctagt gtagccgtag
2281 ttaggccacc acttcaagaa ctctgtagca ccgcctacat acctcgctct gctaactcctg
2341 ttaccagtgg ctgctgccag tggcgataag tcgtgtctta ccgggttgga ctcaagacga
2401 tagttaccgg ataaggcgca gcggtcgggc tgaacggggg gttcgtgcac acagcccagc
2461 ttggagcgaa cgacctacac cgaactgaga tacctacagc gtgagctatg agaaagcgcc
2521 acgcttcccg aaggagagaa ggccgacagg tatccggtaa gcggcagggg cggaacagga
2581 gagcgcacga gggagcttcc agggggaaac gcctggatat tttatagtcc tgtcgggttt
2641 cgccacctct gacttgagcg tcgatttttg tgatgctcgt cagggggggc gagcctatgg
2701 aaaaacgcca gcaacgcggc ctttttacgg ttcttgccct tttgctggcc ttttgcctac



2761 atgttctttc ctgcgttata ccctgattct gtggataacc gtattaccgc ctttgagtga
2821 gctgataccg ctgcgccgag ccgaacgacc gagcgcagcg agtcagtgag cgaggaagcg
2881 gaagagcgcc tgatgcggta ttttctcctt acgcatctgt gcggtatttc acaccgcata
2941 tgggtgactc tcagtacaat ctgctctgat gccgcatagt taagccagta tacactccgc
3001 tatcgctacg tgactgggtc atggctgcgc cccgacaccc gccaacaccc gctgacgcgc
3061 cctgacgggc ttgtctgctc ccggcatccg cttacagaca agctgtgacc gtctccggga
3121 gctgcatgtg tcagaggttt tcaccgtcat caccgaaacg cgcgaggcag cagatcaatt
3181 cgcgcgcgaa ggcgaagcgg catgcattta cgttgacacc atcgaatggc gcaaacctt
3241 tcgcggtatg gcatgatagc gcccggaaga gagtcaattc aggggtggtga atgtgaaacc
3301 agtaacgtta tacgatgtcg cagagtatgc cgggtgtctt tatcagaccg tttcccgct
3361 ggtgaaccag gccagccacg tttctgcgaa aacgcgggaa aaagtggaag cggcgatggc
3421 ggagctgaat tacattccca acccggtggc acaacaactg gcgggcaaac agtcgttgct
3481 gattggcgct gccacctcca gtctggccct gcacgcgcgc tcgcaaatg tcgcggcgat
3541 taaatctcgc gccgatcaac tgggtgccag cgtggtggtg tcgatggtag aacgaagcgg
3601 cgtcgaagcc tgtaaagcgg cggtgcaaa tcttctcgcg caacgcgtca gtgggctgat
3661 cattaactat ccgctggatg accaggatgc cattgctgtg gaagctgcct gcactaatgt
3721 tccggcgtta tttcttgatg tctctgacca gacacccatc aacagtatta ttttctccca
3781 tgaagacggt acgcgactgg gcgtggagca tctggtcgca ttgggtcacc agcaaatcgc
3841 gctgttagcg ggcccatata gttctgtctc ggcgctctg cgtctggctg gctggcataa
3901 atatctcact cgcaatcaaa ttcagccgat agcggaaacg gaaggcgact ggagtgccat
3961 gtccggtttt caacaaacca tgcaaatgct gaatgagggc atcgttccca ctgcgatgct
4021 ggttgccaac gatcagatgg cgtggggcgc aatgcgcgcc attaccgagt cgggctgcg
4081 cgttggtgcg gatatctcgg tagtgggata cgacgatacc gaagacagct catgttatat
4141 cccgccgtca accaccatca aacaggattt tcgcctgctg gggcaaaacca gcgtggaccg
4201 cttgtgcaa ctctctcagg gccaggcggg gaagggcaat cagctgttgc ccgtctcact
4261 ggtgaaaaga aaaaccaccc tggcgcccaa tacgcaaacc gcctctcccc gcgcgttggc
4321 cgattcatta atgcagctgg cacgacaggt ttcccactg gaaagcgggc agtgagcgca
4381 acgcaattaa tgtgagttag cgcgaattga tctg