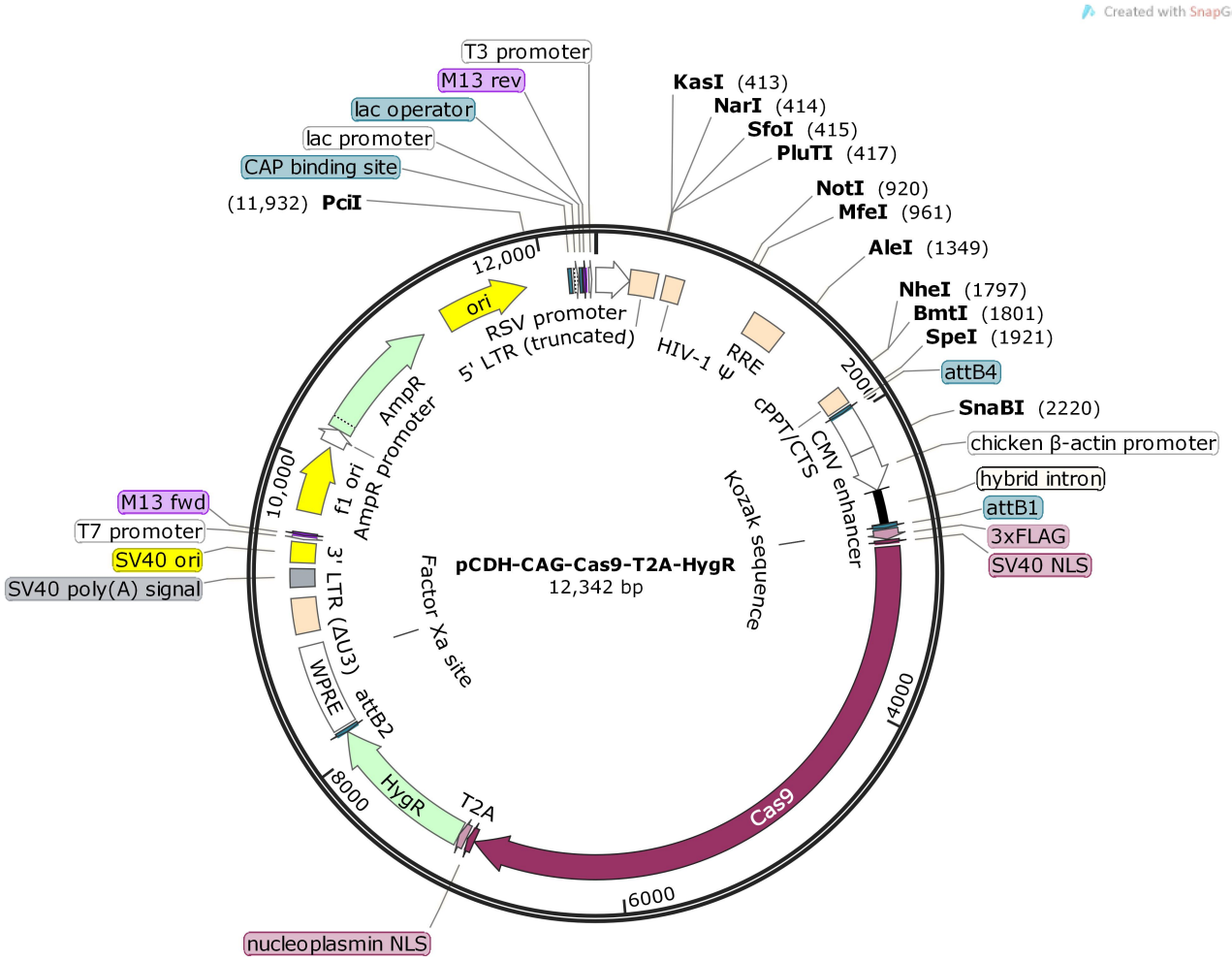
IGEbio
广州艾基生物技术有限公司
标准产业单元三期四栋 301 室
番禺区，国际生物岛
广州市，广东省

pCDH-CAG-Cas9-T2A-HygR Vector Information



载体名称:	pCDH-CAG-Cas9-T2A-HygR
质粒类型:	慢病毒表达载体
表达水平:	低拷贝
启动子:	CAG promoter
克隆方法:	多克隆位点，限制性内切酶
克隆位点:	MCS
载体大小:	12342bp
5’测序引物及序列:	--
3’测序引物及序列:	--
载体标签:	3xFLAG, SV40 NLS
载体抗性:	Amp
筛选标记:	HygR
产品目录号:	---
稳定性:	稳定表达 Stable
组成型/诱导型:	组成型
病毒/非病毒:	慢病毒
克隆菌株:	Stbl3

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LOCUS       Exported 12342bp ds-DNA circular SYN 13-JUL-2018
DEFINITION  pCDH-CAG-Cas9-T2A-HygR.
ACCESSION   .
VERSION     .
KEYWORDS    pCDH-CAG-Cas9-T2A-HygR
SOURCE      synthetic DNA construct
  ORGANISM  synthetic DNA construct
REFERENCE   1  (bases 1 to 12342)
  AUTHORS   .
  TITLE     Direct Submission
  JOURNAL   Exported Monday, June 24, 2019 from SnapGene 3.2.1
            http://www.snapgene.com
FEATURES             Location/Qualifiers
     source          1..12342
                     /organism="synthetic DNA construct"
                     /mol_type="other DNA"
     promoter        3..229
                     /note="RSV promoter"
                     /note="Rous sarcoma virus enhancer/promoter"
     LTR             230..410
                     /note="5' LTR (truncated)"
                     /note="truncated 5' long terminal repeat (LTR) from HIV-1"
     misc_feature    457..582
                     /note="HIV-1 Psi"
                     /note="packaging signal of human immunodeficiency virus
                     type 1"
     misc_feature    1075..1308
                     /note="RRE"
                     /note="The Rev response element (RRE) of HIV-1 allows for
                     Rev-dependent mRNA export from the nucleus to the
                     cytoplasm."
     misc_feature    1803..1920
                     /note="cPPT/CTS"
                     /note="central polypurine tract and central termination
                     sequence of HIV-1"
     protein_bind    1938..1958
                     /gene="mutant version of attB"
                     /bound_moiety="BP Clonase(TM)"
                     /note="attB4"
                     /note="core recombination site for the Gateway(R) BP
                     reaction"
     enhancer        1959..2244
                     /note="CMV enhancer"
                     /note="human cytomegalovirus immediate early enhancer;
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	contains an 18-bp deletion relative to the standard CMV enhancer"
promoter	2246..2523
	/note="chicken beta-actin promoter"
intron	2524..2751
	/note="hybrid intron"
	/note="hybrid between chicken beta-actin (CBA) and minute virus of mice (MMV) introns (Gray et al., 2011)"
protein_bind	2756..2780
	/gene="mutant version of attB"
	/bound_moiety="BP Clonase(TM)"
	/note="attB1"
	/note="recombination site for the Gateway(R) BP reaction"
regulatory	2781..2790
	/regulatory_class="other"
	/note="Kozak sequence"
	/note="vertebrate consensus sequence for strong initiation of translation (Kozak, 1987)"
CDS	2790..2855
	/codon_start=1
	/product="three tandem FLAG(R) epitope tags, followed by an enterokinase cleavage site"
	/note="3xFLAG"
	/translation="DYKDHDGDYKDHDIDYKDDDDK"
CDS	2862..2882
	/codon_start=1
	/product="nuclear localization signal of SV40 (simian virus 40) large T antigen"
	/note="SV40 NLS"
	/translation="PKKKRKV"
CDS	2907..7007
	/codon_start=1
	/product="Cas9 (Csn1) endonuclease from the Streptococcus pyogenes Type II CRISPR/Cas system"
	/note="Cas9"
	/note="generates RNA-guided double strand breaks in DNA"
	/translation="DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKN
	LIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF
	LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF
	RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLE
	NLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQI
	GDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ
	QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
	KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN

SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF
TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQ
LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRH
KPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYL
YYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS
EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLN
AVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTE
ITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKE
SILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNEL
ALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADA
NLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD
ATLIHQSITGLYETRIDLSQLGGD"

CDS 7008..7055
/codon_start=1
/product="bipartite nuclear localization signal from nucleoplasmin"
/note="nucleoplasmin NLS"
/translation="KRPAATKKAGQAKKKK"

CDS 7065..7118
/codon_start=1
/product="2A peptide from Thosea asigna virus capsid protein"
/note="T2A"
/note="Eukaryotic ribosomes fail to insert a peptide bond between the Gly and Pro residues, yielding separate polypeptides."
/translation="EGRGSLLTCGDVEENPGP"

CDS 7119..8144
/codon_start=1
/gene="aph(4)-Ia"
/product="aminoglycoside phosphotransferase from E. coli"
/note="HygR"
/note="confers resistance to hygromycin"
/translation="MKKPELTATSVEKFLIEKFDSVSDLMQLSEGEESRAFSFDVGGRG
YVLRVNSCADGFYKDRYVYRHFASAALPIPEVLDIGEFSESLTYCISRRAQGVTLQDLP
ETELPAVLQPVAEAMDAIAAADLSQTSGFGPFGPQGIGQYTTWRDFICAIADPHVYHWQ
TVMDDTVSASVAQALDELMLWAEDCPEVRHLVHADFGSNNVLTDNGRITAVIDWSEAMF
GDSQYEVANIFFWRPWLACMEQQTRYFERRHPELAGSPRLRAYMLRIGLDQLYQSLVDG
NFDDAAWAQGRCDAIVRSGAGTVGRTQIARRSAAVWTDGCVEVLADSGNRRPSTRPRAK
E"

protein_bind	complement(8145..8169)
	/gene="mutant version of attB"
	/bound_moiety="BP Clonase(TM)"
	/note="attB2"
	/note="recombination site for the Gateway(R) BP reaction"
misc_feature	8187..8775
	/note="WPRE"
	/note="woodchuck hepatitis virus posttranscriptional regulatory element"
CDS	complement(8658..8669)
	/codon_start=1
	/product="Factor Xa recognition and cleavage site"
	/note="Factor Xa site"
	/translation="IEGR"
LTR	8863..9096
	/note="3' LTR (Delta-U3)"
	/note="self-inactivating 3' long terminal repeat (LTR) from HIV-1"
polyA_signal	9168..9289
	/note="SV40 poly(A) signal"
	/note="SV40 polyadenylation signal"
rep_origin	9329..9464
	/note="SV40 ori"
	/note="SV40 origin of replication"
promoter	complement(9485..9503)
	/note="T7 promoter"
	/note="promoter for bacteriophage T7 RNA polymerase"
primer_bind	complement(9513..9529)
	/note="M13 fwd"
	/note="common sequencing primer, one of multiple similar variants"
rep_origin	9671..10126
	/direction=RIGHT
	/note="f1 ori"
	/note="f1 bacteriophage origin of replication; arrow indicates direction of (+) strand synthesis"
promoter	10152..10256
	/gene="bla"
	/note="AmpR promoter"
CDS	10257..11117
	/codon_start=1
	/gene="bla"
	/product="beta-lactamase"
	/note="AmpR"

	/note="confers resistance to ampicillin, carbenicillin, and related antibiotics"
	/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW"
rep_origin	11288..11876
	/direction=RIGHT
	/note="ori"
	/note="high-copy-number ColE1/pMB1/pBR322/pUC origin of replication"
protein_bind	12164..12185
	/bound_moiety="E. coli catabolite activator protein"
	/note="CAP binding site"
	/note="CAP binding activates transcription in the presence of cAMP."
promoter	12200..12230
	/note="lac promoter"
	/note="promoter for the E. coli lac operon"
protein_bind	12238..12254
	/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)."
primer_bind	12262..12278
	/note="M13 rev"
	/note="common sequencing primer, one of multiple similar variants"
promoter	12299..12317
	/note="T3 promoter"
	/note="promoter for bacteriophage T3 RNA polymerase"