Resource Summary Report

Generated by RRID on Aug 19, 2026

pSAC5.02

RRID:Addgene_16077
Type: Plasmid

Proper Citation

RRID:Addgene_16077

Plasmid Information

URL: http://www.addgene.org/16077

Proper Citation: RRID:Addgene_16077

Insert Name: soluble adenylyl cyclase

Organism: Rattus norvegicus

Bacterial Resistance: Kanamycin

Defining Citation: PMID:9874775

Vector Backbone Description: Backbone Marker:Stratagene; Backbone Size:4500; Vector Backbone:pBK-CMV; Vector Types:Mammalian Expression; Bacterial Resistance:Kanamycin

Comments: Original library clone from Lambda-Zap rat testis library. CTCT repeats inserted between EcoRI sites in 5' UTR. Clone starts at nucleotide 241 of sAC sequence. EcoRI/XhoI digest: 0.7, 1.1, 1.5, 2.0, 4.5 kb. pSAC5.02 is derived from an original library clone obtained in the cloning of rat sAC. The isolation of this cDNA clone seems outdated now, but it was still a current method back in 1999, when we cloned sAC. We obtained peptide sequences from purified sAC protein, and designed PCR primers to those peptides. These resulted in isolation of a 1 kb fragment encoding a portion of the sAC cDNA, but there was open reading frame on each end of this PCRd clone. We then screened a rat testis library we generated in the lamdba zap cloning system. We screened the library by hybridization using the 1 kb PCR fragment as probe and we identified three, independent cDNA clones. THe lambda zap cloning system that we used enabled isolation of plasmids from the lambda phage library using helper phage. THe resultant plasmids contained library inserts (in our case, a sAC cDNA) in the pBK-CMV cloning vector. In this cloning vector, the cDNAs were inserted directionally, meaning that the vectors were already suitable for bacterial expression using the T7 promoter. We were unable to get sufficient protein via bacterial expression, so we deleted the T7 promoter region by digesting with NheI-SpeI and reclosing the vector; this brought the CMV

promoter closer to the inserted sAC cDNA and facilitated high level expression in mammalian cells (i.e., HEK293 cells). This is how plasmid psAC5.02 was generated. THe CTCT repeats are within the 5' UTR of the sAC cDNA - they may have been a cloning artifact during generation of the library, or they may represent some 5' control element. The cDNA clone can not be moved (in its entirety) as an EcoR1 fragment (the pBK-CMV vector has EcoRI sites flanking the inserted cDNA) because there is an internal EcoR1 site within the sAC cDNA. EcoR1 can be used as a diagnostic digest to confirm the validity of the plasmid.

Plasmid Name: pSAC5.02

Record Creation Time: 20220422T221909+0000

Record Last Update: 20260815T080843+0000

Ratings and Alerts

No rating or validation information has been found for pSAC5.02.

No alerts have been found for pSAC5.02.

Data and Source Information

Source: Addgene

Usage and Citation Metrics

We found 1 mentions in open access literature.

Listed below are recent publications. The full list is available at RRID .

Inda C, et al. (2016) Different cAMP sources are critically involved in G protein-coupled receptor CRHR1 signaling. The Journal of cell biology, 214(2), 181.