pCruz HA™ Mammalian Expression Vector: *sc-5045*

INTRODUCTION

Santa Cruz Biotechnology offers the new pCruz series of mammalian expression vectors, a group of unique and effective vectors suitable for production of tagged recombinant fusion proteins. pCruz mammalian expression vectors are available with seven different fusion protein tags: the Myc tag, polyhistidine tag, hemagglutinin (HA) tag, Green Fluorescent Protein (GFP) tag, and the novel CruzTag series of fusion protein tags. The pCruz vectors are designed to be used together with Santa Cruz Biotechnology's fusion protein tag antibodies, which are suitable for detection and immunoprecipitation of recombinant fusion proteins encoded by the respective pCruz vectors.

SYSTEM COMPONENTS

• pCruz HA mammalian expression vector supplied in three reading frames, A, B and C, at 20 µg each in 20 µl volume, to allow subcloning in-frame with the amino terminal fusion protein tag.

• pCruz HA mammalian expression vector with 3 kb LacZ insert provided as a positive control.

• Antibodies are available separately for detection and purification of HA-tagged recombinant fusion proteins encoded by pCruz HA. Please inquire about catalog numbers sc-7392 and sc-805.

product	cat. #	fusion protein tag	amount
pCruz 09™	sc-5040	CruzTag 09™	20 µg each
pCruz 22™	sc-5041	CruzTag 22™	20 µg each
pCruz 41™	sc-5042	CruzTag 41™	20 µg each
pCruz Myc™	sc-5043	Myc Tag	20 µg each
pCruz His™	sc-5044	His-Probe	20 µg each
pCruz HA™	sc-5045	HA Tag	20 µg each
pCruz GFP™	sc-5046	EGFP Tag	20 µg each

DESCRIPTION OF VECTOR

The pCruz HA mammalian expression vector features: the cytomegalovirus (CMV) mammalian expression promoter; amino terminal HA fusion protein tag (flanked by unique Bam H1 and Eco R1 restriction sites); flexible multiple cloning site; poly A signal; Neomycin resistance gene for selection in stable mammalian expression systems; Kanamycin resistance gene for selection in *E. coli*; ori origin of replication for growth in *E. coli*. Vector map and multiple cloning site sequence are shown in Figure 1 below. Reading frames A, B and C are illustrated in Figure 2 below. Full sequence of coding region is illustrated in Figure 3 on page 2.

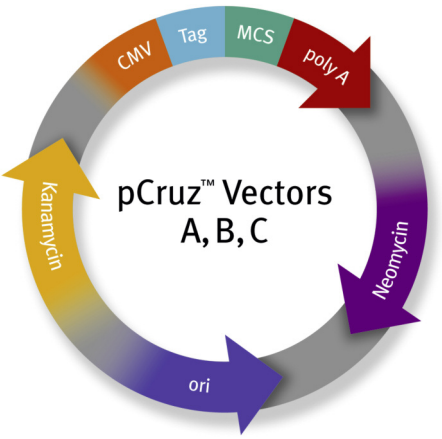

Figure 1. Vector map and multiple cloning site sequence for pCruz vectors. Unique restriction sites are indicated. Note: the Bgl II site may be methylated in some cell lines.

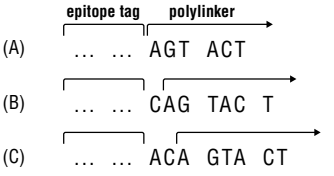

Figure 2. Reading frames A, B and C for pCruz vectors.

METHODS FOR USE

I Propagation of vectors

Prepare stock solutions of each vector and transform *E. coli* according to standard protocols. Select transformants on LB plates containing 30 μg/ml Kanamycin. Prepare glycerol stocks of transformed strains for long-term storage at -80° C.

II Cloning of target protein into pCruz vector

The target cDNA should be cloned into the multiple cloning site, using the restriction map provided to clone in-frame with the epitope tag, with no intervening in-frame stop codons. The target protein will be fused to the C-terminus of the tag protein.

III Transfection of mammalian cells with pCruz vector

Transfect into mammalian cells using a standard transfection method, such as calcium phosphate, electroporation, or liposome-based transfection. For transient transfection, allow cells to grow for 3 - 4 days, harvest, and check for protein expression. For stable transfection, allow cells to grow for 1 - 2 days (approximately to confluence) prior to Neomycin addition. Stable transformants may be selected using 100 - 800 μg/ml Neomycin, depending on the cell line.

STORAGE

Store pCruz vectors at -20° C. Spin sample briefly before pipetting. Avoid repeated freeze/thaw cycles.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

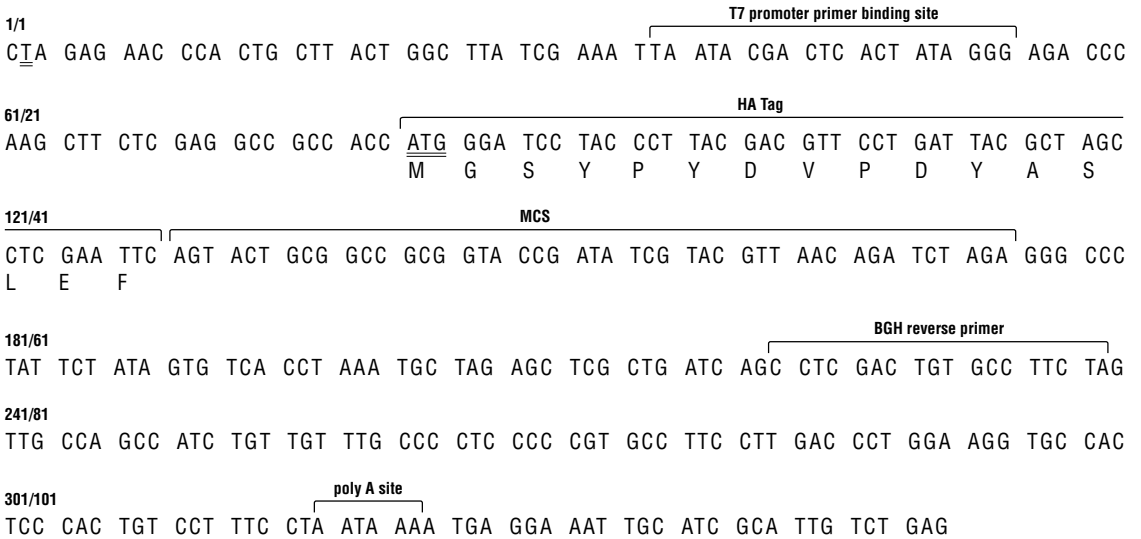

Figure 3. DNA sequence of pCruz HA, reading frame A. Transcription start site (T) and translation start site (ATG) are underlined. Brackets indicate sequences of the T7 promoter primer binding site HA Tag, multiple cloning site (MCS), bovine growth hormone (BGH) reverse primer site, and poly A signal.