pCruz GFP™ Expression Vector: *sc-5046*

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.

pCruz GFP is used for fusing heterologous proteins to the C-terminus of EGFP, for the expression of EGFP-tagged recombinant proteins in a mammalian system. The green fluorescent protein (GFP) was originally identified as a protein involved in the bioluminescence of the jellyfish *Aequorea victora* (1). GFP cDNA produces a fluorescent product when expressed in prokaryotic and eukaryotic cells, without the need for exogenous substrates or cofactors, making GFP a useful tool for monitoring gene expression and protein localization *in vivo* (2,3). Several GFP mutants have been developed including enhanced green fluorescent protein (EGFP), which fluoresce more intensely than the wild type GFP and have shifted excitation maxima (4), making them useful for FACS and fluorescence microscopy as well as double-labeling applications (5). GFP is widely used in expression vectors as a fusion protein tag, allowing expression and monitoring of heterologous proteins fused to GFP (6). Numerous heterologous proteins have been shown to retain their biological function and localization when fused to GFP and expressed in cells (6).

SYSTEM COMPONENTS

• pCruz GFP 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 GFP mammalian expression vector with LacZ insert provided as a positive control.

• Antibodies are available separately for detection and purification of GFP-tagged recombinant fusion proteins encoded by pCruz GFP. Please inquire about catalog numbers sc-5384, sc-5385, sc-8334 and sc-9996. Antibodies are reactive with GFP and GFP mutant fusion proteins.

DESCRIPTION OF VECTOR

The pCruz GFP Mammalian Expression Vector features: the cytomegalovirus (CMV) mammalian expression promoter; amino terminal EGFP 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 on page 2. Full sequence of cloning region is illustrated in Figure 3 on page 4.

The pCruz GFP vector contains the full length EGFP cDNA, encoding the enhanced green fluorescent protein (239 amino acids, 27 kDa monomer). EGFP is a red-shifted variant of GFP which is optimized for brighter fluorescence and greater expression in mammalian systems. Maximal excitation wavelength: 488 nm. Maximal emisson wavelength: 507–509 nm.

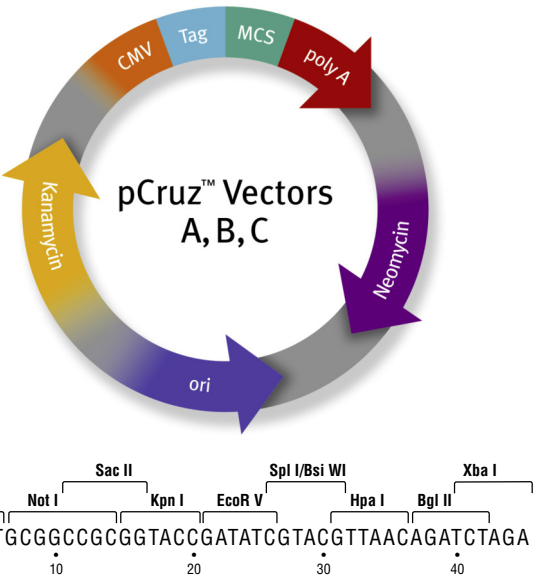

Figure 1. Vector map and multiple cloning site sequence for pCruz vectors. Unique restriction sites are indicated. Note: the Bgl II site may be methylated in some cell lines.

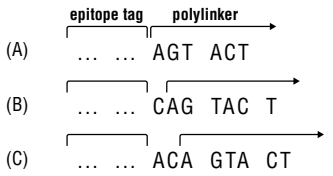

Figure 2. Reading frames A, B and C for pCruz vectors.

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.

IV Detection of EGFP

EGFP expression is detectable by fluorometer, fluorescence microscope, or FACS analysis, in most cases within 24–72 hours of transfection. EGFP has a maximal excitation wavelength of 488 nm and can thus be detected with a FITC filter.

Procedure for fluorescence microscopy:

• Sterilize glass coverslips and place in sterile cell culture plate.

• Plate and transfect mammalian cells on sterile glass coverslip(s) in cell culture plate.

• After growth and selection of cells, remove medium and wash cells 3 times with PBS.

• To fix cells, add 2 ml freshly made PBS/4% paraformaldehyde onto coverslip and incubate for 30 minutes at room temperature. Wash cells twice with PBS. (For non-fixed cells, omit this step.)

• Carefully remove coverslip from cell culture plate with foreceps, noting which side contains the cells.

• Place a small drop of PBS on a glass microscope slide and slowly lower the coverslip into the PBS on the slide, with the cells on the coverslip facing the slide.

• Carefully remove excess PBS from edges by aspiration. Seal coverslip with either rubber cement or molten agarose. (Do not use nail polish.) Allow to dry for 30 minutes.

• Slides may be examined immediately or stored for up to 2–3 weeks at 4° C. For optimal visual detection, view slides in a dark room, after adjusting eyes to the dark for 10 minutes.

• It is advisable to include a mock-transfected control plate, to identify any cellular autofluorescence that may obscure the EGFP fluorescent signal.

NOTES ON GFP FLUORESCENCE

• GFP must be in an oxidized state to fluoresce. GFP will not fluoresce in the presence of strong reducing agents (e.g. 5 mM $Na_2S_2O_4$ or 2 mM $FeSO_4$). Fluorescence is restored upon exposure to atmospheric oxygen (7).

• EGFP fluorescence is stable within a pH range of 7.0 and 11.5 (8).

• GFP loses fluorescence in 100% ethanol, thus it is not suitable for use in paraffin embedded tissues and other procedures that require dehydration.

• Some organic solvents may shift the absorption maxima of GFP (9).

• GFP fluorescence is sensitive to nail polishes that are used to seal coverslips (2).

• Fluorescence is irreversibly destroyed by 1% H_2O_2, 1 mM DTNB, and other sulfhydryl reagents (7).

• Fluorescence is not affected by mild denaturants and fixatives (e.g. 1% SDS, 8 M urea, glutaraldehyde or formaldehyde fixation), but full denaturation will eliminate fluorescence.

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.

NOTICE

GFP products are sold under license from Columbia University. Rights to use this product are limited to research use only; NOT FOR DIAGNOSTIC OR THERAPEUTIC USE IN HUMANS OR ANIMALS. No other rights are conveyed. Inquiry into the availability of a license to broader rights or the use of this product for commercial purposes should be directed to Columbia Innovation Enterprise, Columbia University, Engineering Terrace-Suite 363, Mail Code 2206, 500 West 120th Street, New York, New York 10027, USA. The GFP technology is covered by U.S. Patent No. 5,491,084 assigned to Columbia University.

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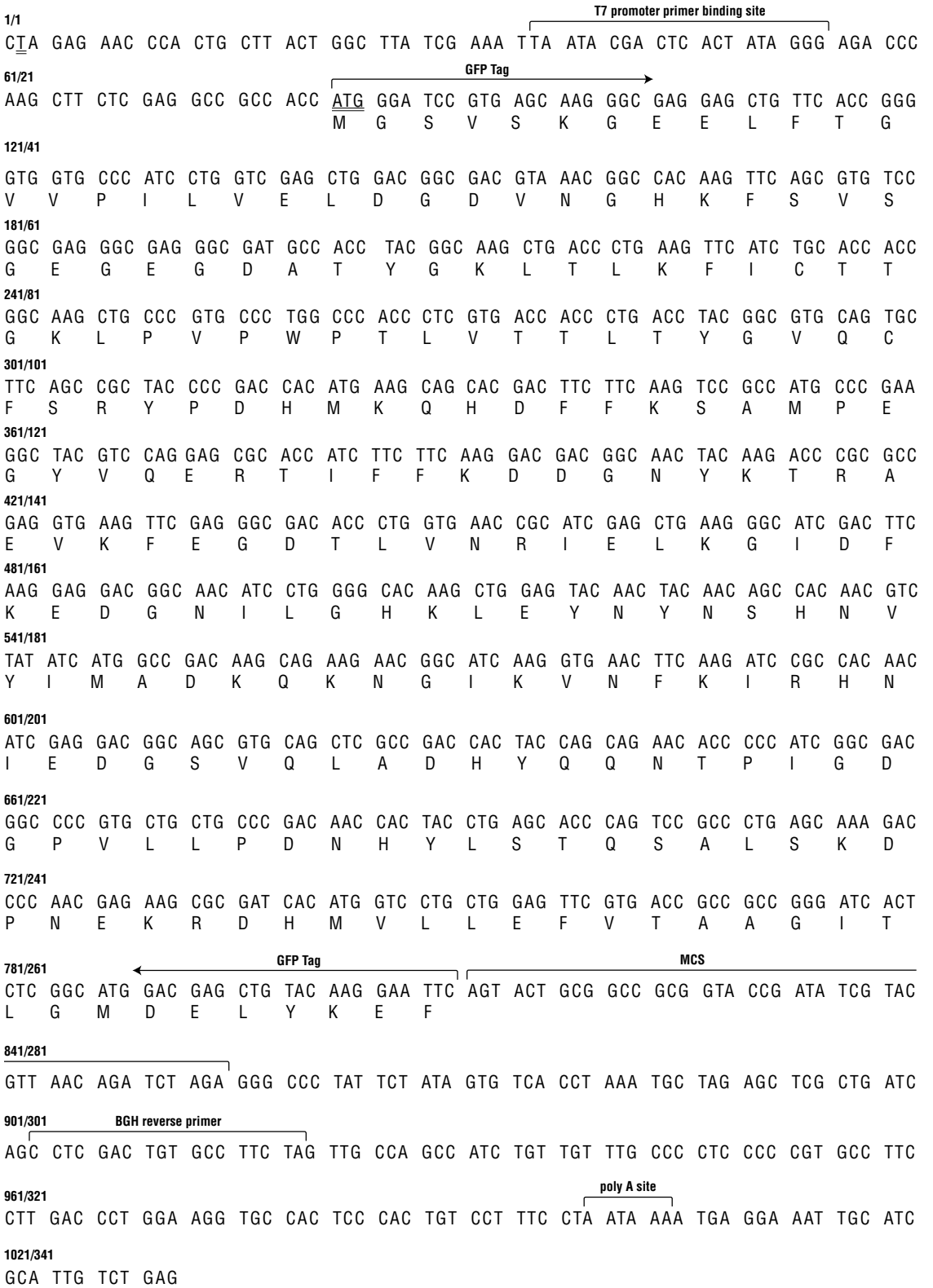

Figure 3. DNA sequence of pCruz GFP, reading frame A. Transcription start site (T) and translation start site (ATG) are underlined. Brackets indicate sequences of the T7 promoter primer binding site, EGFP Tag, multiple cloning site (MCS), bovine growth hormone (BGH) reverse primer site, and poly A signal.