



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 victoria* (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 emission wavelength: 507–509 nm.

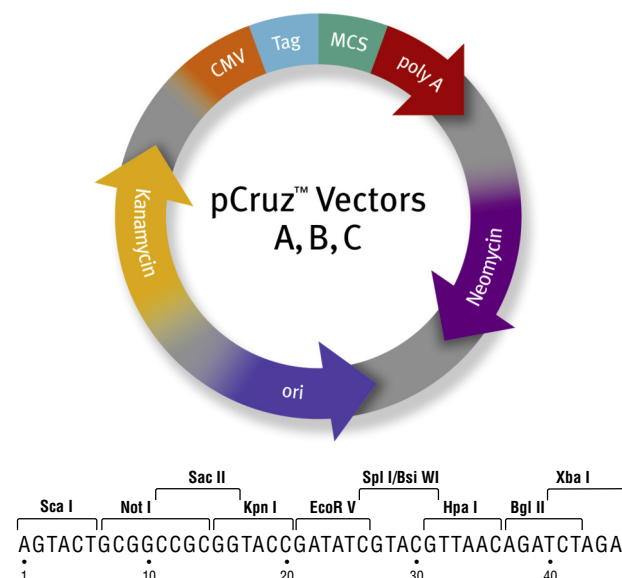


Figure 1. Vector map and multiple cloning site sequence for pCruz vectors. Unique restriction sites are indicated. Note: the Bgl II site may be methylated in some cell lines.

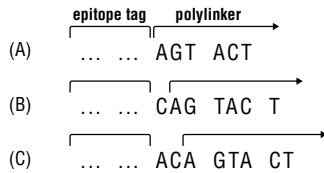


Figure 2. Reading frames A, B and C for pCruz vectors.

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 forceps, 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 Na₂S₂O₄ or 2 mM FeSO₄). 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% H₂O₂, 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.

REFERENCES

1. Prasher, D.C., Eckenrode, V.K., Ward, W.W., Prendergast, F.G., and Cormier, M.J. 1992. Primary structure of the *Aequorea victoria* green-fluorescent protein. *Gene* **111**: 229-233.
2. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. 1994. Green fluorescent protein as a marker for gene expression. *Science* **263**: 802-805.
3. Inouye, S. and Tsuji, F.I. 1994. *Aequorea* green fluorescent protein. Expression of the gene and fluorescence characteristics of the recombinant protein. *FEBS Letts.* **341**: 277-280.
4. Cormack, B.P., Valdivia, R.H., and Falkow, S. 1996. FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* **173**: 33-38.
5. Rizzuto, R., Brini, M., De Giorgi, F., Rossi, R., Heim, R., Tsien, R.Y., and Pozzan, T. 1996. Double labelling of subcellular structures with organelle-targeted GFP mutants *in vivo*. *Curr. Biol.* **6**: 183-188.
6. Tsien, R.Y. 1998. The green fluorescent protein. *Ann. Rev. Biochem.* **67**: 509-544
7. Inouye, S. and Tsuji, F.I. 1994. Evidence for redox forms of the *Aequorea* green fluorescent protein. *FEBS Letts.* **351**: 211-214.
8. Patterson, G.H., Knobel, S.M., Sharif, W.D., Kain, S.R., and Piston, D.W. 1997. Use of the green fluorescent protein and its mutants in quantitative fluorescence microscopy. *Biophys. J.* **73**: 2782-2790.
9. Robart, F.D. and Ward, W.W. 1990. Solvent perturbations of *Aequorea* green fluorescent protein. *Photochem. Photobiol.* **51**: 92s.

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1/1
CTA GAG AAC CCA CTG CTT ACT GGC TTA TCG AAA TTA ATA CGA CTC ACT ATA GGG AGA CCC
T7 promoter primer binding site

61/21
 AAG CTT CTC GAG GCC GCC ACC ATG GGA TCC GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG
GFP Tag
 M G S V S K G E E L F T G

121/41
 GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC
 V V P I L V E L D G D V N G H K F S V S

181/61
 GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC
 G E G E G D A T Y G K L T L K F I C T T

241/81
 GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC
 G K L P V P W P T L V T T L T Y G V Q C

301/101
 TTC AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA
 F S R Y P D H M K Q H D F F K S A M P E

361/121
 GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC
 G Y V Q E R T I F F K D D G N Y K T R A

421/141
 GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC
 E V K F E G D T L V N R I E L K G I D F

481/161
 AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC
 K E D G N I L G H K L E Y N Y N S H N V

541/181
 TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC
 Y I M A D K Q K N G I K V N F K I R H N

601/201
 ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC
 I E D G S V Q L A D H Y Q Q N T P I G D

661/221
 GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC
 G P V L L P D N H Y L S T Q S A L S K D

721/241
 CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT
 P N E K R D H M V L L E F V T A A G I T

781/261
GFP Tag MCS
 CTC GGC ATG GAC GAG CTG TAC AAG GAA TTC AGT ACT GCG GCC GCG GTA CCG ATA TCG TAC
 L G M D E L Y K E F

841/281
 GTT AAC AGA TCT AGA GGG CCC TAT TCT ATA GTG TCA CCT AAA TGC TAG AGC TCG CTG ATC

901/301
BGH reverse primer
 AGC CTC GAC TGT GCC TTC TAG TTG CCA GCC ATC TGT TGT TTG CCC CTC CCC CGT GCC TTC

961/321
poly A site
 CTT GAC CCT GGA AGG TGC CAC TCC CAC TGT CCT TTC CTA ATA AAA TGA GGA AAT TGC ATC

1021/341
 GCA TTG TCT GAG

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.