



Mammalian Optimized GFP Strand 1-10 Plasmid

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RESEARCH PRODUCT INSERT

INTENDED USE

Description

pCMV-mGFP 1-10 Hyg Amp encodes a mammalian-codon optimized version of the engineered split GFP 1-10 detector fragment, used to detect proteins tagged with strand 11 of GFP “GFP S11”. GFP 1-10 is a highly engineered variant of strands 1-10 of green fluorescent protein. Engineering and characteristics of the bacterial codon optimized version was originally described in (1). GFP 1-10 contains 16 amino acid substitutions (listed on page 3), which improve the solubility of the protein and increase the rate of fluorescence formation when complemented with GFP S11. Using the *in vivo* bacterial optimized two-plasmid system described in (1) to express GFP S11-tagged proteins, GFP fluorescence is easily detectable within 15 minutes after induction of the GFP 1-10 detector strand in *E. coli* cells. Transfer of these bacterial codon sequences to mammalian expression vectors gives detectable fluorescence, but can take up to 24h to appear after co-transfection of the GFP 1-10 and GFP S11 constructs. To overcome this limitation yet retain the improved folding properties of the GFP 1-10 D7 (1), the amino acid sequence of the GFP 1-10 expressed by pCMV-mGFP 1-10 Hyg Amp is the same as the one described in (1), but the coding sequence is mouse codon-optimized for high expression in mammalian cells. Consequently, the fluorescence of mammalian cells co-transfected with pCMV-mGFP 1-10 Hyg Amp and a compatible plasmid expressing GFP S11 is up to 50-fold brighter than mammalian cells expressing the bacterial codon version. When GFP 1-10 is expressed in mammalian cell cultures co-expressing GFP S11, green-emitting cells can be detected by either fluorescence microscopy or flow cytometry within 8 h after transfection (*Fig. 2 below*). GFP from complemented GFP 1-10 + GFP S11 has excitation and emission maxima = 488 nm and 525 nm, respectively. Complemented GFP 1-10 + GFP S11 is stable and most likely forms the same structure as full-length GFP.

The mGFP 1-10 gene is positioned just downstream of the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$). As a result, cells transfected with this vector will express the GFP 1-10 protein constitutively. SV40 polyadenylation signals downstream of the GFP 1-10 gene direct proper processing of the 3' end of the GFP 1-10 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A hygromycin resistance gene (Hyg^r) allows stably transfected eukaryotic cells to be selected using hygromycin. This cassette consists of the SV40 early promoter, the hygromycin resistance gene, and polyadenylation signals from the SV40 polyadenylation signals. A bacterial promoter upstream of the ampicillin resistance gene (Amp^r) expresses ampicillin resistance protein in *E. coli*.

Use

pCMV-mGFP 1-10 Hyg Amp vector is designed primarily as a source of the mammalian-codon optimized GFP 1-10 D7 coding sequence and to produce the GFP 1-10 D7 protein in mammalian cells for detecting the presence of proteins bearing the GFP S11 tag. GFP fluorescence can be detected by fluorescence microscopy, providing direct visual evidence of complementation of GFP 1-10 and GFP S11. After cotransfection with pCMV-mGFP 1-10 Hyg Amp along with a construct expressing a GFP S11-tagged protein of interest, cells can also be sorted by flow cytometry (FACS) to enrich for transfected cells, or observed by microscopy to monitor GFP 1-10 and GFP S11-tagged protein expression, interaction, translocation, or to label structures and organelles. pCMV-mGFP 1-10 Hyg Amp vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using hygromycin (4).

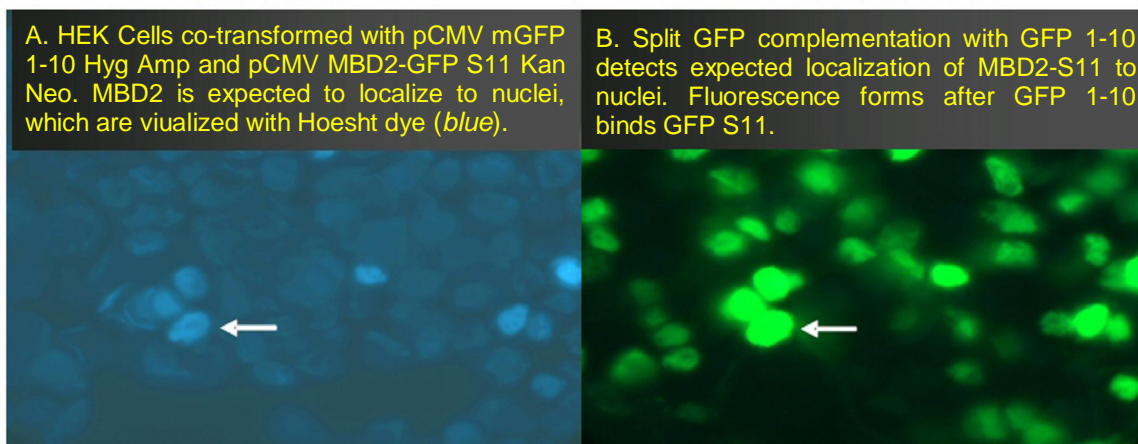


Figure 2. Using the split GFP mammalian system to follow nuclear localization of MBD2. HEK cells were co-transfected with pCMV-mGFP 1-10 Hyg Amp and pCMV MBD2-GFP S11 Kan Neo. GFP S11 is the small 16 amino acid strand 11 peptide of GFP that is detected by the GFP 1-10 by complementation to form fluorescent 11-stranded GFP. MBD2 is known to translocate to nuclei (*Fig 2A*). GFP 1-10 complements the GFP S11 tag, and the resulting GFP fluorescence is translocated to the nuclei (*Fig 2B*). Since MBD2 is expressed with only the short GFP S11 tag, and subsequently complemented with GFP 1-10, there is minimal folding perturbation compared to expressing MBD2-GFP as a full-length fusion. Nuclear fluorescence is bright and non-punctate as expected.

REAGENTS

Components Supplied:

Mammalian optimized Split GFP Strand 1-10 Plasmid: 100ng store at -20°C.

Reagent Storage: Store all reagents between 2° and -20°C as listed on each kit component. When stored properly, the reagents are stable until the date indicated either on the box or each component. Depending on the particular usage requirements, it may be appropriate to re-aliquot reagents to smaller working volumes to avoid repeated freeze-thawing or repeated pipetting from the same vial.

Materials required, but not supplied:

- Competent E.coli
- Ampicillin
- Hygromycin
- LB growth media and plates
- Restriction enzymes
- Ligation materials
- Plasmid Isolation Reagents
- Fluorescence readers for detection

Prior to performing the assay, carefully read all instructions.

A . Plasmid Vector Propagation and Construction of Custom Fusion Protein Vector

1. To ensure that you have a renewable source of plasmid DNA, transform each of the plasmid vectors provided in this kit in an *E.coli* host strain. It is suggested to use $\geq 20\mu\text{l}$ of supplied plasmid for a standard chemically competent *E.coli* bacterial transformation.
 - It is recommended that bacterial frozen stocks be prepared of all transformed plasmids using standard molecular biology techniques.
2. Purify plasmid DNA for cloning using Plasmid Preparation kits or other techniques (not included).

B. Transfection

Plasmid DNA for transfection into mammalian cells must be clean and free of phenol and sodium chloride. Transfection methods include calcium phosphate, cationic lipids, and electroporation techniques (not included). The pCMV-mGFP s1-10 vector is designed to be co-transfected with pCMV-mGFP s11-Fusion Protein plasmids for detection of the fusion protein by fluorescence.

Propagation in *E. coli*

- Suitable host strains: DH5 α , HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to ampicillin (100 $\mu\text{g}/\text{ml}$) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Unique restriction sites within the S1-10 fragment sequence:

Enzyme	Position
NheI	592
BmtI	596
MscI	778
SpeI	784
EcoRV	827

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
 - Enhancer region: 59–465; TATA box: 554–560
 - C→G mutation to remove *Sac* I site: 569
 - Transcription start point: 583
- GFP 1-10 D7 mouse codon-optimized
 - Kozak consensus translation initiation site: 597-607
 - Start codon (ATG): 604-606
 - Stop codon: 1249-1251
- SV40 early mRNA polyadenylation signal
 - Polyadenylation signals: 1412-1417 & 1441-1446
 - mRNA 3' ends: 1450 & 1462
- f1 single-strand DNA origin: 1509-1964
(Packages noncoding strand of GFP 1-10 D7.)
- SV40 origin of replication: 2305-2440
- SV40 early promoter
 - Enhancer (72-bp tandem repeats): 2138-2209 & 2210-222281
 - 21-bp repeats: 2285-2295, 2306-2326, 2328-2348
 - Early promoter element: 2361-2367
 - Major transcription start points: 2357, 2395, 2401 & 2406
- Hygromycin resistance gene
 - Hygromycin coding sequences:
 - Start codon (ATG): 2462-2464; stop codon: 3584-3487
- SV40 early mRNA polyadenylation signals
 - Polyadenylation signals: 3584-3639 & 3663-3668
 - mRNA 3' ends: 3672 & 3684
- Ampicillin resistance (β -lactamase) promoter
 - 35 region: 3834-3839; -10 region: 3857-3862
- Ampicillin resistance (β -lactamase) coding sequence
 - Start codon (ATG): 3904-3906; stop codon: 4762-4764
- pUC plasmid replication origin: 4927-5570

Related Materials

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|--|-------------------------|
| • pCMV-mGFP Cterm Strand 11 Neo Kan Vector | Catalog Number 22004003 |
| • GFP 1-10 Fold'n Glow detector solution | Catalog Number 21004001 |

REFERENCES

1. Cabantous S, Terwilliger TC, Waldo GS (2005) Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. *Nature Biotech.* **23**: 102-107.
2. Chun WJ, Waldo GS, Johnson GVW (2007) Split GFP complementation assay: a novel approach to quantitatively measure aggregation of tau in situ: effects of GSK3 beta activation and caspase 3 cleavage. *Journal of Neurochemistry* **103**: 2529-2539.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

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