# Mammalian Optimized GFP Strand 11 Plasmid

Product Number 22004003

**FOR RESEARCH USE ONLY.** ***Not for Diagnostic Use***

## RESEARCH PRODUCT INSERT

## INTENDED USE

### Description

pCMV-mGFP Cterm S11 Neo Kan vector encodes a mammalian-codon optimized version of the 16 amino-acid engineered split GFP S11 detector fragment (amino acids 215 to 230 of the 238 amino acid GFP) for tagging proteins with a C-terminal strand 11 of GFP "GFP S11". These GFP S11-tagged proteins can be detected using the GFP 1-10 "detector" fragment (GFP amino acids 1 to 214), a highly engineered variant of strands 1-10 of green fluorescent protein (GFP 1-10 D7 optimum). Engineering and characteristics of the bacterial codon optimized version of this optimized split GFP system was originally described in (1). GFP S11 contains 3 amino acid substitutions and truncation of the last 8 amino acids of GFP (listed on page 2), which improve the solubility of the protein and increase the rate of fluorescence formation when complemented with GFP 1-10. 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 24 h to appear after co-transfection of the GFP 1-10 and GFP S11 constructs (2). To overcome this limitation yet retain the improved folding properties of the GFP S11 (1), the amino acid sequence of the GFP S11 expressed by pCMV-mGFP Cterm S11 Neo Kan 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 test proteins cloned into the MCS of pCMV-mGFP Cterm S11 Neo Kan and co-transfected with pCMV-mGFP 1-10 Hyg Amp (which contains the mammalian codon optimized GFP 1-10, available from Sandia Biotech) are up to 50-fold brighter than mammalian cells expressing the bacterial codon versions of the split GFP fragments (*see Fig. 2 below*). When GFP 1-10 mammalian-optimized is expressed in mammalian cell cultures co-expressing GFP S11, green-emitting cells can be detected by either fluorescence microscopy or flow cytometry within 6 to 8 h after transfection. 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 S11 gene is positioned downstream of a multiple cloning site and linker just after the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$). As a result, cells transfected with this vector will constitutively express the proteins of interest as POI-linker-GFP S11. SV40 polyadenylation signals downstream of the GFP S11 gene direct proper processing of the 3' end of the POI-linker-GFP S11 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 neomycin-resistance cassette ($Neo^r$) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

## Use

pCMV-mGFP Cterm S11 Neo Kan vector is designed as: (1) a source of the mammalian-codon optimized GFP S11 coding sequence and (2) to produce proteins of interest with C-terminal fused GFP S11 tagging peptide in mammalian cells. These can be detected by GFP 1-10 either *in vivo* by co-transformation with pCMV-mGFP 1-10 Hyg Amp (expressing the mammalian codon optimized GFP 1-10, available from Sandia Biotech), or by adding the GFP 1-10 detector reagent protein (available as a ready-to-use solution from Sandia Biotech). GFP fluorescence can be detected by fluorescence microscopy, providing direct visual evidence of complementation of GFP 1-10 and POI-linker-GFP S11 (*see Fig. 2*). After cotransfection with pCMV-mGFP 1-10 Hyg Amp vector 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 Cterm S11 Neo Kan vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4).

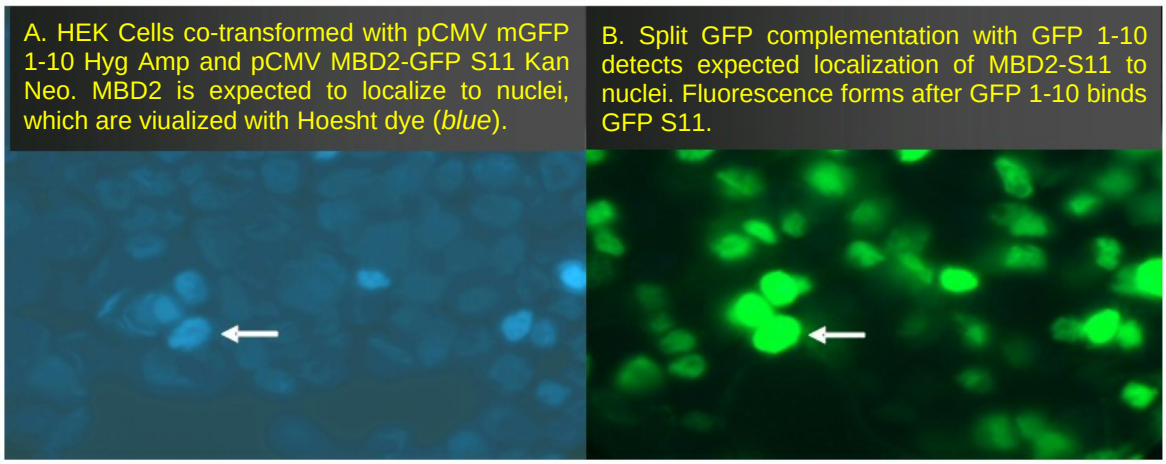


**Figure 1. Using the split GFP mammalian system to follow nuclear localization of MBD2.** HEK cells were co-transfected with pCMV MBD2-GFP Cterm S11 Kan Neo and pCMV-mGFP 1-10 Hyg Amp. GFP S11 is the small 16 amino acid strand 11 peptide of GFP that is detected by the GFP 1-10 by complementation to 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 direct full-length GFP fusion. Nuclear fluorescence is bright and non-punctate as expected.

# REAGENTS

## Components Supplied:

**Mammalian optimized split-GFP Strand 11 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
- Kanamycin
- LB growth media and plates
- Restriction enzymes
- Ligation materials
- Plasmid Isolation Reagents
- Neomycin

- 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 ≥20µl 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).
    - It is recommended that all selected transformed plasmids under go verification testing such as by restriction enzyme digest prior to sub-cloning of your gene of interest (GOI).
3. The Mammalian optimized strand-11 plasmid features a multiple cloning site (MCS) that offers several unique, conveniently arranged restriction sites for insertion of the transcription factor activation domain sequence. Expression of the fusion protein driven by the CMV promoter, a strong promoter that allows high-level constitutive expression in a variety of cell lines and the neomycin-resistance gene, which facilitates selection of stable cell lines that express the fusion protein of interest.

## B. Cloning

Notes: The pCMV-mGFP s11 vector contains a stop codon at the end of the reporter gene so they are most suitable for cloning your gene of interest upstream of the reporter gene (i.e. C-terminal fusion). **The inserted gene (POI or GOI) should include the initiating ATG codon.** Make sure that your gene of interest does not contain a stop codon at its end if you desire to obtain a fusion with the reporter gene downstream.

1. Prepare protein gene of interest by adding restriction enzyme sites on both ends of GOI fragment; this can be done by PCR or by digesting GOI fragment out of a plasmid with restriction enzyme sites compatible with the pCMV-mGFP s11 fusion vector.
2. Digest the pCMV-mGFP s11 fusion vector with the chosen restriction enzyme(s). Make sure that enzyme sites are not too close to each other to avoid digestion interference, and that enzyme sites are not blocked by methylation.
3. Ligate the GOI fragment into the linearized pCMV-mGFP s11 fusion vector using the ligation enzyme supplier's recommendations.

## C. Transformation

1. To ensure that you have a renewable source of your customized fusion protein, transform the plasmid in a *E.coli* host strain.
    - It is recommended that bacterial frozen stocks be prepared of all transformed plasmids using standard molecular biology techniques.
2. Purify plasmid DNA for mammalian transfection using Plasmid Preparation kits or other techniques (not included).
    - It is recommended that all selected transformed plasmids under go verification testing such as by restriction enzyme digest prior to sub-cloning of your gene of interest (GOI).

## D. 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 s11 vector is designed to be co-transfected with pCMV-mGFP s1-10 vector or used with GFP 1-10 Fold n' Glow detector solution for detection of the fusion protein by florescence.

## SEQUENCE INFORMATION

- Detailed sequence information is available on request.

**pCMV-mGFP Cterm S11 Neo Kan Vector Information** **Catalog Number 22004003**

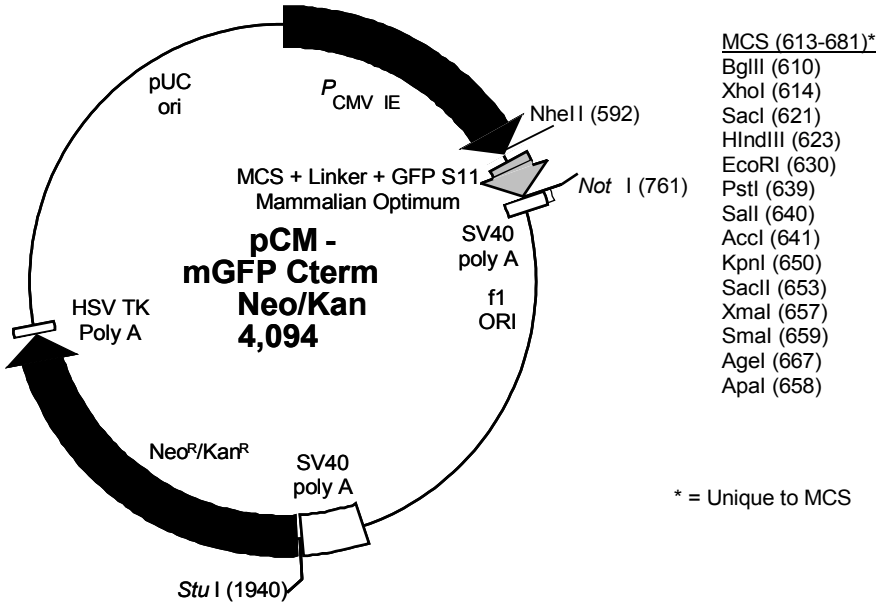


**Figure 2. Feature Map of pCMV-mGFP Cterm S11 Neo Kan.** Unique restriction sites shown flanking key modules. The *Not* I site follows the mGFP S11 stop codon. Genes are cloned with initiator codons just after *Nhe* I in the multiple cloning site (MCS) and in-frame with the downstream linker and GFP S11 module. The structure of expressed proteins of interest (POI) is POI-linker-GFP S11.

```
                                        Begin MCS

561     ******************************GCTAGCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGT   640
561     ******************************CGATCGCGATGGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCA   640
                                     NheI             BglII                EcoRI
                                                    XhoI   HindIII

                                        Linker                                GFP S11
641     CGACGGTACCGCGGGCCCGGGATCCACCGGTCGCCACCATGGGCGACGGCGGCAGCGGCGGCGGCAGC************   720
641     GCTGCCATGGCGCCCGGGCCCTAGGTGGCCAGCGGTGGTACCCGCTGCCGCCGTCGCCGCCGCCGTCG************   720
          KpnI           BamHI

                  GFP S11 cont.'d       STOP (TAA)
721     ************************************TAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTT   800
721     ************************************ATTTCGCCGGCGCTGAGATCTAGTATTAGTCGGTATGGTGTAAA   800
                                                    XbaI
```

### Location of features

• Human cytomegalovirus (CMV) immediate early promoter: 1–589

Enhancer region: 59–465; TATA box: 554–560

Transcription start point: 538

C→G mutation to remove *Sac* I site: 569

- Multiple cloning site: 613-681

  Linker GDGGSGGGS: 682-708

- GFP S11 mouse codon-optimized (GFP amino acids 215-230)

  GFP S11: 709-756

  Stop codon: 757-759

- SV40 early mRNA polyadenylation signal

  Polyadenylation signals: 918-918 & 942-947

  mRNA 3' ends: 951 & 963

- f1 single-strand DNA origin: 1010-1465

  (Packages noncoding strand of POI-linker-GFP S11.)

- Ampicillin resistance (β-lactamase) promoter

  –35 region: 1527-1532; –10 region: 1550-1555

  Transcription start point: 1562

- SV40 origin of replication: 1806-1941

- SV40 early promoter

  Enhancer (72-bp tandem repeats): 1639-1710 & 1711-1782

  21-bp repeats: 1786-1806, 1807-1827 & 1829-1849

  Early promoter element: 1862-1868

  Major transcription start points: 1858, 1896, 1902 & 1907

- Kanamycin/neomycin resistance gene

  Neomycin phosphotransferase coding sequences:

  Start codon (ATG): 1990-1992; stop codon: 2781-2784

- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

  Polyadenylation signals: 3020-2025 & 3033-3038

- pUC plasmid replication origin: 3369-401

## Propagation in *E. coli*

- Suitable host strains: DH5α, 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 kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

## Related Materials

• pCMV-mGFP 1-10 Hyg Amp Vector Catalog Number 22004005

• 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.
4. Gorman, C. (1985) In *DNA cloning: A Practical Approach, Vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.


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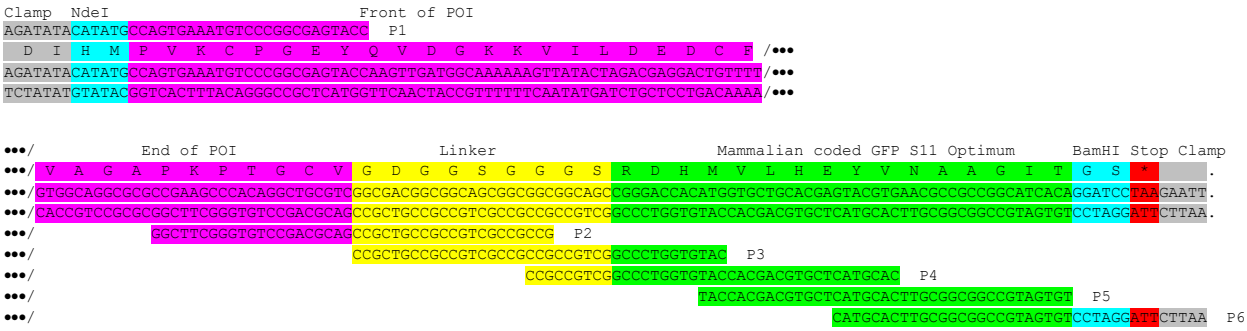
## Appendix A. Examples / alternate cloning techniques

### Using PCR to Extend GFP S11 tag with mammalian codon usage onto target proteins of interest.

pCMV-mGFP Cterm S11 Neo Kan vector, available from Sandia Biotech, encodes a mammalian-codon optimized version of the 16 amino-acid engineered split GFP S11 detector fragment for tagging proteins with a C-terminal strand 11 of GFP "GFP S11". These GFP S11-tagged proteins can be detected using the so-called GFP 1-10 "detector" fragment, a highly engineered variant of strands 1-10 of green fluorescent protein. pCMV-mGFP Cterm S11 Neo Kan vector contains a the Neo Kan selection marker and so can be co-transfected with pCMV-mGFP 1-10 Hyg Amp Vector and co-transformants can be selected using neomycin and hygromycin in eukaryotes.

It is also possible to add GFP S11 mammalian coding sequence for user-specific constructs and plasmids via PCR assembly and restriction cassette cloning, as described in the following examples.

### Example 1. Attaching GFP S11 to C-terminus of gene of interest (GOI) via flexible linker, and adding 5' *Nde* I site and 3' *BamH* I site to assembled module.



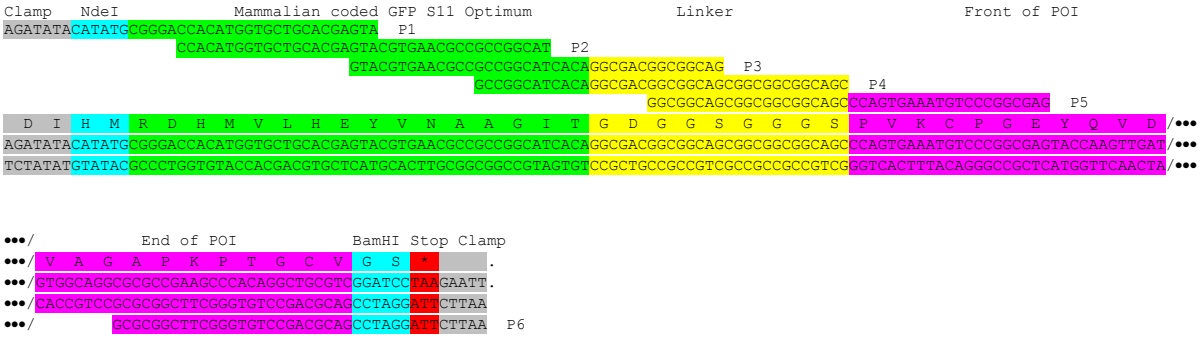

### Considerations

- Terminal primers that contain restriction sites also have 5' extensions or "clamps" to facilitate restriction enzyme binding to cognate restriction site. Such extensions are required by many restriction enzymes for efficient cutting.
- In this example, five sequential PCRs can be performed to obtain the final full-length product. A first PCR is performed using the primers P1 and P2, and the template protein of interest. The product is cleaned and used as a template for a second PCR with primers P1 and P3, and the product is cleaned and used as the template for a PCR using primers P1 and P4, etc. After the first PCR, fewer cycles (ca. 15 extensions) can be performed using a larger amount of template.
- Alternatively, a first PCR can be performed using a mixture of primers P1, P2, P3, P4, P5, P6 in the ratio 5:1:1:1:1:5 (i.e., the outside primers P1 and P6 are used in ca. 5-fold molar excess compared to each interior primer P2-p5. The product is cleaned and then used as a template for a PCR with primers P1 and P6, to ensure that the product is fully extended.
- Primers are generally ordered for synthesis in the 5'-3' orientation. Be sure to order reverse complements for lower primers P2-P6.

| | |
|---|---|
| AGATATACATATGCCAGTGAAATGTCCCGGCGAGTACC | P1 as ordered |
| GCCGCCGCTGCCGCCGTCGCCGACGCAGCCTGTGGGCTTCGG | P2 as ordered |
| CATGTGGTCCCGGCTGCCGCCGCCGCTGCCGCCGTCGCC | P3 as ordered |
| CACGTACTCGTGCAGCACCATGTGGTCCCGGCTGCCGCC | P4 as ordered |
| TGTGATGCCGGCGGCGTTCACGTACTCGTGCAGCACCAT | P5 as ordered |
| AATTCTTAGGATCCTGTGATGCCGGCGGCGTTCACGTAC | P6 as ordered |

- If several different proteins of interest (POI) and restriction sites are to be constructed with linker-GFP S11 tags, the same linker and GFP S11 coding primers can be used throughout as long as they do not containing POI or restriction site sequences. For example, P3, P4, P5 can be ordered once and used for many different cloning experiments.

**Example 2.** Attaching GFP S11 to N-terminus of gene of interest via flexible linker, and adding 5' *Nde* I site and 3' *BamH* I site to assembled module.




**Considerations.**

- Terminal primers that contain restriction sites also have 5' clamp extensions to facilitate restriction enzyme binding to cognate restriction site. Such extensions are required by many restriction enzymes for efficient cutting.
- In this example, five sequential PCRs can be performed to obtain the final full-length product. For example, a first PCR is performed using the primers P5 and P6, and the template protein of interest. The product is cleaned and used as a template for a second PCR with primers P4 and P6, and the product is cleaned and used as the template for a PCR using primers P3 and P6, etc. After the first PCR, fewer cycles (ca. 15 extensions) can be performed using a larger amount of template.
- Alternatively, a first PCR can be performed using a mixture of primers P1, P2, P3, P4, P5, P6 in the ratio 5:1:1:1:1:5 (i.e., the outside primers P1 and P6 are used in ca. 5-fold molar excess compared to each interior primer P2-P5. The product is cleaned and then used as the template for a PCR with primers P1 and P6, to ensure that the product is fully extended.
- Primers are generally ordered for synthesis in the 5'-3' orientation. Be sure to order reverse complements for lower primer P6.

| | |
|---|---|
| AGATATACATATGCGGGACCACATGGTGCTGCACGAGTA | P1 as ordered |
| CCACATGGTGCTGCACGAGTACGTGAACGCCGCCGGCAT | P2 as ordered |
| GTACGTGAACGCCGCCGGCATCACAGGCGACGGCGGCAG | P3 as ordered |
| GCCGGCATCACAGGCGACGGCGGCAGCGGCGGCGGCAGC | P4 as ordered |
| GGCGGCAGCGGCGGCGGCAGCCCAGTGAAATGTCCCGGCGAG | P5 as ordered |
| AATTCTTAGGATCCGACGCAGCCTGTGGGCTTCGGCGCG | P6 as ordered |

- If several different proteins of interest (POI) and restriction sites are to be constructed with linker-GFP S11 tags, the same linker and GFP S11 coding primers can be used throughout as long as they do not containing POI or restriction site sequences. For example, P2, P3, P4 can be ordered once and used for many different cloning experiments.

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