Bacterial GFP Strand 11 Plasmid

Product Number 21004003

PRODUCT INSERT

INTENDED USE: *FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES*

Bacterial GFP strand 11 plasmid is a component of the "Fold 'n' Glow" Protein Solubility Assay and contains a small (16 aa) GFP fragment for the fusion of the peptide or protein of interest via a flexible linker. Preparation of the s11 plasmid for use in the assay involves generating the s11 fusion protein by cloning the appropriate DNA insert into the S11 vector at the N-terminal. If folded properly or not in the aggregated form, the fusion protein is specifically bound by the s1-10 detection complementation fragment, forming a fluorophore. This allows for the quantitation of the amount of protein that is properly folded in a given sample as the folding reporter gives a signal directly proportional to the amount of correctly folded protein[1]. Misfolding or aggregation of the fusion protein renders the GFP tag inaccessible and prevents complementation, thus preventing fluorescence. Accordingly, misfolded or aggregated proteins are not included in the quantification of the protein of interest. Expressed separately, neither the fusion protein of interest nor the GFP detector (S1-10) is fluorescent.

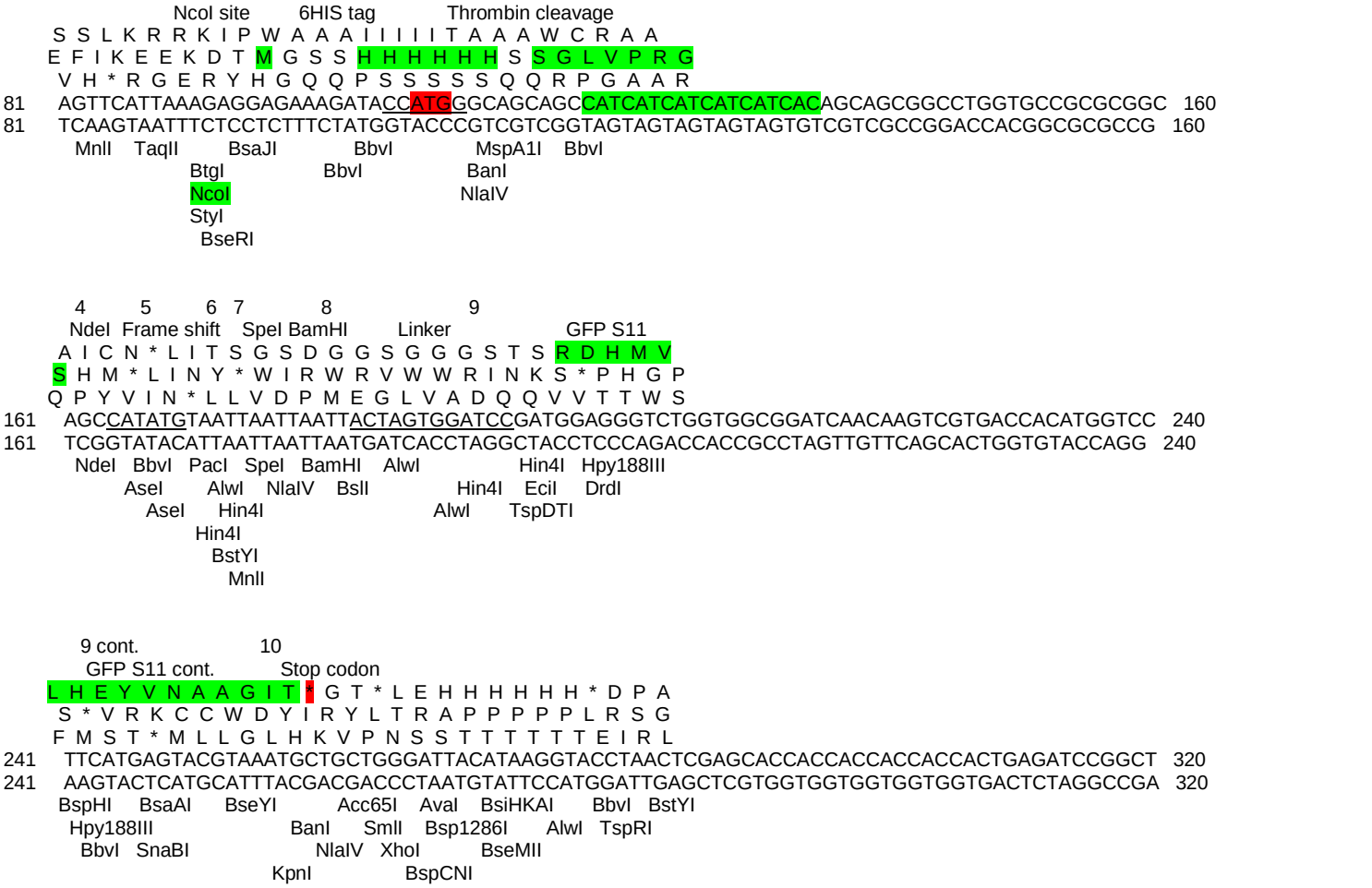
REAGENT

Plasmid (S11) : Kanamycin resistance (Kan^R). *BamHI* and *NdeI* restriction sites. Supplied as a 50 µL volume at 2 ηg/µL.

Note: The stability of this plasmid is approximately 6 months when stored at -20°C. When stored properly, this product is stable until the date indicated either on the box or each component. Depending on the particular usage requirements, it may be practical to re-aliquot reagents to smaller working volumes to avoid repeated freeze-thawing or repeated pipetting from the same vial.

1 NcoI site CCATGG has initiator methionine
2 6HIS tag CATCATCATCATCATCAC amino acids HHHHHH
3 Trombin site amino acids SGLVPPRGS
4 Cloning site NdeI CATATG
5 Frame shift stuffer TAATTAATTAATT
Note that this is a +1 frame shift to make sure S11 doesn't express unless in-frame construct between NdeI/SpeI or NdeI/BamHI
Typically user can digest NdeI + SpeI and clone that way, or NdeI/BamHI and clone that way. See our Nature methods papers for guidelines on primer design Cabantous S, Waldo GS (2006) In vivo and in vitro protein solubility assays using split GFP. Nature Methods 3: 845-854.
6 SpeI ACTAGT (expressed as amino acids TS)
7 BamHI GGATCC (expressed as amino acids GS)
8 Linker amino acids DGGSGGGSTS
9 GFP S11 tag
10 stop codon

1 2 3



Materials required, but not supplied:

- Competent expression cells (BL2/DE3)
- Kanamycin
- IPTG (Isopropyl β-D-1-thiogalactopyranoside)
- LB growth media and plates
- *NdeI* And *BamHI* restriction enzymes
- Ligation materials
- Metal affinity column
- TNG Buffer (50mM Tris pH 7.4, 0.1M NaCl, 10% glycerol)
- Bovine serum albumin
- Plasmid Isolation Reagents
- 96 well plate
- 4°C refrigerator
- -20°C freezer
- Incubator
- Centrifuge(s) and appropriate size tubes
- Sonicator
- Microplate fluorescence reader
- Vortex mixer
- Water bath
- Graduated cylinders and assorted beakers
- Pipettes and tips
- Disposable gloves

A. Preparation of insert DNA

Prior to performing the assay, carefully read all instructions.

1. Perform plasmid prep and/or PCR. Use standard materials/protocol not included.
2. Use *NdeI* (5') and *BamHI* (3') restriction sites to perform a restriction digest that generates overhangs on the DNA insert. *Optional:* Purify digest fragment from agarose gel.

B. Preparation of S11 Vector

1. To ensure that you have a renewable source of plasmid DNA, transform the plasmid vector in an *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 cloning using Plasmid Preparation kits or other techniques (not included).
3. Perform restriction enzyme digest of the S11 vector using *Nde1* and *BamH1*to prepare the plasmid for the insert DNA. Follow the manufacturer's instructions for use of the enzymes. Leave sticky ends for over hangs in preparation for ligation.
4. *Optional:* Dephosphorylate the digest to decrease non-recombinant background. Use molecular grade calf intestinal or shrimp alkaline phosphatase according to the manufacturer's directions.
5. Perform ligation reaction according to manufacturers' instructions.
6. Store vector at –20^{o}C until used.

C. Clone DNA insert as an N-terminal fusion into S11 vector

1. Ligate the DNA insert with the digested S11 using standard DNA ligation protocol and manufacturer's protocol resulting in a S11 fusion plasmid.
2. Transform the S11-fusion plasmid in an expression host for high yields of quality plasmid. Use standard methods based on the screening host used.
3. Identify the positive clones by lysing the cells and detecting with S1-10 reagent
4. Perform plasmid DNA purification, sequence to verify reading frame, or use *in vitro* transcription/translation.

D. Preparation of S11 fusion protein

1. Prepare cells and extract *soluble* S11 fusion proteins.
    a. Grow a 200ml culture of bacteria, transformed S11 fusion protein, in LB growth medium and Kanamycin [20-50 μg/mL] to log phase at OD_{600nm} of 0.5-0.8.
    b. Induce with 1mM IPTG for 4hr at 37^{o}C.
    c. Pellet cells by centrifugation and re-suspend in 2 mL TNG buffer.
    d. Sonicate to disrupt bacterial cell walls to release soluble proteins.
2. *Optional*:
    a. Purify *soluble* S11 fusion protein by metal-affinity column.
    b. Determine the purity and quantify protein.
3. Store the fusion proteins at -20^{o}C until *In vitro* complementation assay is performed.

REFERENCES

1. Waldo et al. "Rapid protein-folding assay using green fluorescent protein," *Nature Biotechnology* 17, 691 - 695, July 1999.

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