SuperFolder GFP Plasmid

Product Number 23004006

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

RESEARCH PRODUCT INSERT

INTENDED USE

SuperFolder GFP is a highly engineered robustly folded version of GFP that shows greater tolerance to chemical denaturants and extreme temperatures with improved folding kinetics. The SuperFolder GFP plasmid allows a test protein to be expressed as an N-terminal fusion with SuperFolder GFP. SuperFolder GFP fluorescence is unaffected by the fusion partner misfolding or solubility and is directly proportional to the amount of expressed protein.[1]

REAGENTS

Components Supplied:

SuperFolder GFP 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 expression cells (BL2/DE3)
- Kanamycin
- IPTG (Isopropyl β-D-1-thiogalactopyranoside)
- LB growth media and plates
- *NdeI* And *BamH1* 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 *Nde1* (5') and *BamH1* (3') restriction sites to perform a restriction digest that generates overhangs on the DNA insert.
3. *Optional:* Purify digest fragment from agarose gel.

B. Preparation of SuperFolder 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 SuperFolder 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 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°C until used.

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

1. Ligate the DNA insert with the digested SuperFolder using standard DNA ligation protocol and manufacturer's protocol resulting in a SuperFolder fusion plasmid.
2. Transform the SuperFolder-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 using standard methods. Note: IPTG / X-gal screening is effective in the first 24 hours post plating as the T7 promoter is highly active and absorbs resources from the LacZ gene.
4. Perform plasmid DNA purification, sequence to verify reading frame, or use *in vitro* transcription/translation.

D. Preparation of SuperFolder fusion protein

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

E. Detection

1. SuperFolder fluoresces at 490nm excitation with emission at 510nm.
2. Live cultures can be directly observed, by direct fluorescence of colonies, by microscopy or by flow cytometry.
3. Purified protein fusions can be detected by fluorimiter, a fluorescent plate reader or by fluorescence spectrometer.

SEQUENCE INFORMATION

- Detailed sequence information is available on request.

Flanking Sequences of SuperFolder GFP C6HIS and internal restriction sites.
Engineering and characterization of SuperFolder GFP is described in: Pedelacq JD, Cabantous S, Tran T, Terwilliger TC, Waldo GS **(2006)** Engineering and characterization of a SuperFolder green fluorescent protein. *Nature Biotechnology* **24: 79-88.**

Flanking sequences:

```
                                          Begining of SuperFolder GFP coding sequence
                                       ↓    (in second frame)
        I  L  F  N  F  K  K  E  I  Y  I  *  A  K  E  K  N  F  S  L  E  L  S  Q  F  L  L
          F  V  *  L  *  E  G  D  I  H  M  S  K  G  E  E  L  F  T  G  V  V  P  I  L  V
           F  C  L  T  L  R  R  R  Y  T  Y  E  Q  R  R  R  T  F  H  W  S  C  P  N  S  C  *
5041    TTTTGTTTAACTTTAAGAAGGAGATATACATATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTG   5120
5041    AAAACAAATTGAAATTCTTCCTCTATATGTATACTCGTTTCCTCTTCTTGAAAAGTGACCTCAACAGGGTTAAGAACAAC   5120
                                     NdeI                 BsmFI       TspRI             BpmI
                                                                MboII                  Eco57MI
                                                                     BsrI

         **********************************************************************************
          End of SuperFolder GFP              6HIS Tag     Stop
        H  M  A  W  M  S  S  T  K  D  P  I  T  I  T  I  T  N  S  S  T  T  T  T  T  E
          H  G  M  D  E  L  Y  K  G  S  H  H  H  H  H  H  *  L  E  H  H  H  H  H  H  *
           T  W  H  G  *  A  L  Q  R  I  P  S  P  S  P  S  L  T  R  A  P  P  P  P  P  L  R
5761    ACATGGCATGGATGAGCTCTACAAAGGATCCCATCACCATCACCATCACTAACTCGAGCACCACCACCACCACCACTGAG   5840
5761    TGTACCGTACCTACTCGAGATGTTTCCTAGGGTAGTGGTAGTGGTAGTGATTGAGCTCGTGGTGGTGGTGGTGGTGACTC   5840
                      BstF5I     BamHI    AlwI              AvaI    BsiHKAI       BbvI   BstYI
                       EcoICRI    BstYI                     SmlI    Bsp1286I       AlwI
                         BanII    HphI   HphI                XhoI          BseMII
                         BsiHKAI    NlaIV                               BspCNI
                         Bsp1286I
                         SacI
                            AlwI
                          FokI
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Enzymes that do not cut:

AarI, AatII, Acc65I, AflII, AgeI, AhdI, AleI, AscI, AvrII, BbvCI, BmgBI, BmtI
BplI, BsaI, BsiWI, BspMI, Bsu36I, EagI, EcoRI, FalI, FseI, HindIII, KpnI, NheI
NotI, PacI, PmeI, PmlI, PsrI, PsrI, PstI, RsrII, SacII, SanDI, SbfI, ScaI, SexAI
SfiI, SnaBI, SpeI, SrfI, StuI, SwaI, ZraI

Restriction Site(s)	Occurrences	Position(s)
XhoI	1	5493
TspRI	1	5102
TspGWI	2	5203
		5585
TspDTI	1	5319
TatI	2	5348
		5497
StyI	1	5238
SmlI	2	5493
		5733
SfaNI	1	5168
SalI	1	5675
SacI	1	5779
PpuMI	1	5383
NlaIV	2	5384
		5594
NdeI	1	5071
NcoI	1	5238
MscI	1	5243
MnlI	2	5154
		5159
MluI	1	5394
MfeI	1	5632
MboII	3	5097
		5478
		5598
Hpy8I	2	5522
		5677
Hpy188III	3	5295
		5324
		5733
HphI	5	5142
		5181
		5187
		5189
		5430
HincII	1	5677
Hin4I	2	5476
		5508
HgaI	1	5402
FokI	1	5784
EcoO109I	1	5383
EcoICRI	1	5777
Eco57MI	2	5119
		5422
Eco57I	1	5422
EaeI	1	5241
DrdI	1	5724
DraI	1	5462
Cac8I	1	5398

Restriction Site(s)	Occurrences	Position(s)
BtgI	1	5238
BstZ17I	1	5522
BstYI	1	5699
BstF5I	1	5777
BstBI	1	5694
BsrI	2	5102
		5225
BsrGI	1	5348
BspEI	1	5294
Bsp1286I	2	5146
		5779
BsmFI	2	5091
		5396
BslI	1	5294
BsiHKAI	1	5779
BseYI	1	5751
BseRI	1	5176
BsaWI	1	5294
BsaJI	1	5238
BpuEI	1	5754
BpmI	1	5119
Bme1580I	1	5146
BceAI	2	5325
		5545
BbvI	1	5735
BanII	1	5779
BamHI	1	5787
BaeI	1	5413
BaeI	1	5446
AvaI	1	5493
ApoI	3	5148
		5205
		5569
AlwI	3	5305
		5694
		5782
AflIII	1	5394
AclI	1	5581
AccI	2	5521
		5676

REFERENCES

1. Pédelacq et. al. “Engineering and characterization of a SuperFolder green fluorescent protein,” *Nature Biotechnology* 24, 79 - 88 (2005)
2. Cava et. al. “Expression and use of SuperFolder green fluorescent protein at high temperatures in vivo: a tool to study extreme thermophile biology.” *J Environmental microbiology* 10(3):605 2008 Mar.
3. Andrews et. al. “The Rough Energy Landscape of SuperFolder GFP Is Linked to the Chromophore,” *Journal of Molecular Biology*, Volume 373, Issue 2, 19 October 2007, Pages 476-490
4. Waldo et al. "Rapid protein-folding assay using green fluorescent protein," *Nature Biotechnology* 17, 691 - 695, July 1999.
5. Cabantous et. al. “New molecular reporters for rapid protein folding assays.” *PLoS ONE* 3(6) 2008

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