Promega

Technical Bulletin

Monster Green® Fluorescent Protein phMGFP Vector

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1. Description

Green fluorescent protein (GFP) is commonly used to monitor gene expression and protein trafficking within intact cells. GFP fusion proteins are easily visualized by standard fluorescence microscopy to track real-time subcellular localization of a protein of interest.

The phMGFP Vector[a–d] contains the open reading frame for the Monster Green® Fluorescent Protein cloned into a mammalian expression vector. The Monster Green® Fluorescent Protein is encoded by an improved synthetic version of the green fluorescent protein gene originally cloned from *Montastrea cavernosa* (Great Star Coral). The synthetic gene (hMGFP) expresses a 26kDa protein that shows improved fluorescence intensity compared to the native gene. Furthermore, the hMGFP gene has been codon optimized and cleared of most consensus transcription factor binding sites to ensure reliability and high expression levels.

The spectral properties of the Monster Green® Fluorescent Protein are slightly red-shifted compared to other commercially available GFPs. Peak excitation occurs at 505nm, with a shoulder at 480nm; peak emission occurs at 515nm. We recommend using standard fluoroisothiocyanate (FITC) filters to visualize hMGFP fluorescence. Fluorescent microscopy analysis of hMGFP expression may be performed using an excitation filter of 470±20nm (470/40nm) and an emission filter of 515nm (long pass). For FACS® analyses, we recommend using a laser at 488nm for excitation and filters of 530±15nm (530/30nm) for emission.

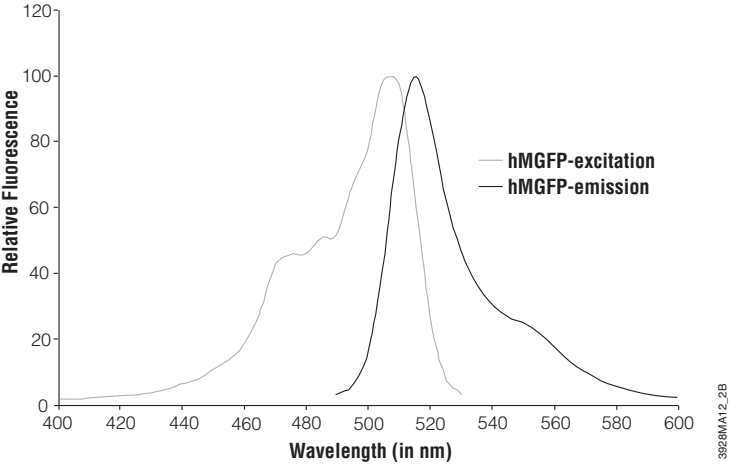

Figure 1. Excitation and emission spectra of the Monster Green® Fluorescent Protein. CHO cells were transfected with the phMGFP Vector. Twenty-four hours after transfection, cells were lysed in Glo Lysis Buffer, 1X (Cat.# E2661), and the excitation and emission spectra were generated using the Spex FluoroLog®-2 spectrofluorometer. The emission spectrum was collected on cell lysates excited at 480nm. Excitation data were collected at 400–530nm with an emission wavelength of 540nm.

2.. Product Components and Storage Conditions

Product	Size	Cat. #
Monster Green® Fluorescent Protein phMGFP Vector	20µg	E6421

Storage Conditions: Store the Monster Green® Fluorescent Protein phMGFP Vector at –20°C or below.

3. General Considerations

The native GFP gene originally cloned from *Montastrea cavernosa* expresses a protein that photobleaches and is very dim, making it unsuitable as a reporter. To improve the fluorescent properties of the native GFP, random mutagenesis was performed to generate MGFP. The resulting clone expresses a green fluorescence protein that is resistant to photobleaching and brighter than the native gene. The MGFP gene was further modified (codon optimization and removal of most consensus sequences for transcription factor binding) to generate the hMGFP gene.

Translation of the MGFP gene involves codons that are not frequently used in mammalian cells, thereby reducing its expression efficiency in mammalian cells. To improve expression levels, the synthetic hMGFP gene utilizes the highest-usage mammalian codons where possible. Low-usage codons from *E. coli* have been eliminated where possible to support expression in *E. coli* when the gene is cloned into a bacterial expression vector. Table 1 provides a comparison of codon usage between the synthetic hMGFP and the MGFP genes. To further increase mammalian expression efficiency, the Kozak sequence for translation initiation has been added to the beginning of the gene.

The MGFP gene contains a multitude of consensus transcription factor binding sites, which can result in nonspecific transcriptional activation under certain experimental conditions and therefore compromise the reliability of MGFP as a reporter. To improve reliability of protein expression, the number of consensus transcription factor binding sites has been reduced from 67 in the MGFP gene to 3 in the synthetic hMGFP (Figure 2). In addition, the synthetic hMGFP gene has been modified to minimize other undesirable elements, including eukaryotic polyadenylation signals (AATAAA).

The number of restriction sites that are commonly used in cloning has been minimized within the hMGFP gene. For cloning convenience, a non-unique NcoI and a unique XbaI restriction site have been added to the beginning and end of the hMGFP gene, respectively. EcoRV, SmaI and NaeI restriction sites have been added to allow convenient creation of protein fusions. The EcoRV and SmaI sites were added to the vector before the starting ATG codon of hMGFP. The NaeI site was added to the hMGFP open reading frame just before the stop codon. Each of these sites, when digested, results in blunt-ended fragments that preserve the translational reading frame of hMGFP.

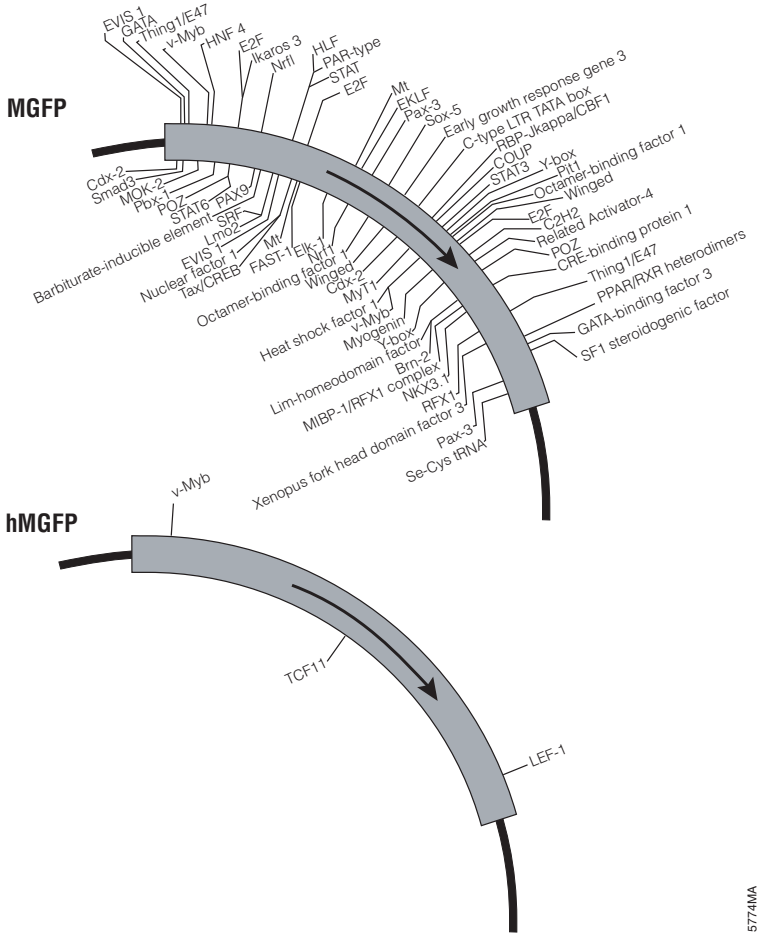

Figure 2. Transcription factor binding sites in the MGFP and synthetic hMGFP genes. Most (96%) of the transcription factor binding sites in the MGFP gene have been removed to create the synthetic hMGFP gene.

Table 1. Comparison of Codon Usage Between the MGFP and Synthetic hMGFP Genes.

Note: To introduce the Kozak sequence, a serine residue in the MGFP gene was changed to a glycine residue in the synthetic hMGFP gene. To create the unique NaeI site, a lysine residue in the MGFP gene was changed to a glycine residue.

Amino Acid	Codon	Number of Codons in MGFP	Number of Codons in hMGFP	Percent Use in Human Cells
Ala	GCT	5	3	28.0
	GCC	5	7	41.6
	GCA	1	1	20.0
	GCG	0	0	10.3
Arg	CGT	2	1	8.9
	CGC	1	5	21.4
	CGA	3	1	10.2
	CGG	0	2	19.7
	AGA	0	0	18.8
	AGG	3	0	21.0
Asn	AAC	6	5	57.5
	AAT	1	2	42.3
Asp	GAT	6	18	42.8
	GAC	13	1	57.2
Cys	TGT	2	1	40.6
	TGC	1	2	59.4
Gln	CAA	0	0	24.8
	GAG	6	6	75.2
Glu	GAA	8	1	39.3
	GAG	5	12	60.7
Gly	GGT	5	3	15.8
	GGC	6	13	35.8
	GGA	4	3	24.1
	GGG	4	2	24.4
His	CAT	5	0	39.6
	CAC	2	7	60.4
Ile	ATT	5	1	33.1
	ATC	3	11	54.0
	ATA	4	0	12.9

Table 1. Comparison of Codon Usage Between the MGFP and Synthetic hMGFP Genes (continued).

Amino Acid	Codon	Number of Codons in MGFP	Number of Codons in hMGFP	Percent Use in Human Cells
Leu	TTA	0	0	5.5
	TTG	4	1	11.5
	CTT	2	1	11.2
	CTC	0	2	20.8
	CTA	1	2	6.5
	CTG	5	6	44.4
Lys	AAA	12	5	38.9
	AAG	12	18	61.1
Met	ATG	10	10	100
Phe	TTT	5	1	41.4
	TTC	7	11	58.9
Pro	CCT	4	2	27.3
	CCC	0	9	35.3
	CCA	6	0	25.7
	CCG	2	1	11.6
Ser	TCT	1	0	18.2
	TCC	2	0	24.4
	TCA	0	0	12.8
	TCG	0	1	5.8
	AGT	1	1	13.0
	AGC	2	3	25.8
Thr	ACT	4	1	22.4
	ACC	0	9	40.5
	ACA	6	2	25.4
	ACG	2	0	11.8
Trp	TGG	2	2	100
Tyr	TAT	5	1	39.9
	TAC	8	12	60.1
Val	GTT	5	1	16.4
	GTC	4	3	25.7
	GTA	4	1	9.3
	GTG	5	13	48.7

4. phMGFP Vector Backbone

Some functional regions in the phMGFP Vector backbone are listed below:

CMV Enhancer/Promoter

The CMV enhancer/promoter region present in the phMGFP vector allows strong, constitutive expression in many cell types. The promiscuous nature of the CMV promoter/enhancer has been demonstrated in transgenic mice, where its transcriptional activity was observed in 24 of 28 murine tissues examined (1).

Chimeric Intron

Downstream of the enhancer/promoter region is a chimeric intron composed of the 5´-donor site from the first intron of the human β-globin gene and the branch and 3´-acceptor site from the intron between the leader and body of an immunoglobin gene heavy chain variable region (2). The sequences of the donor and acceptor sites, along with the branch point site, have been changed to match the consensus sequences for splicing (3). Transfection studies have demonstrated that the presence of an intron flanking the cDNA insert frequently increases the level of gene expression (4–7). The intron is located upstream of the hMGFP sequence to minimize the utilization of cryptic 5´-donor splice sites that may reside within the gene (8).

T7 Promoter

A T7 RNA polymerase promoter is located downstream of the chimeric intron and immediately precedes the hMGFP gene. This promoter can be used to synthesize RNA transcripts in vitro using T7 RNA Polymerase (Cat.# P2075). In vitro translation of these transcripts using rabbit reticulocyte lysate-based systems (e.g., the TNT® Coupled Rabbit Reticulocyte Lysate Systems and TNT® Quick Coupled Transcription/Translation Systems) is not recommended because high light-absorbing properties of the hemoglobin present in these lysates can interfere with the detection of GFP fluorescence (9).

Note: Use the T7 EEV Promoter Primer (Cat.# Q6700) to sequence the phMGFP Vector. **Do not** use the T7 Promoter Primer (Cat.# Q5021).

SV40 Late Polyadenylation Signal

Polyadenylation signals cause termination of transcription by RNA polymerase II and signal the addition of approximately 200–250 adenosine residues to the 3´-end of the RNA transcript (10). Polyadenylation has been shown to enhance RNA stability and translation efficiency (11,12). The late SV40 polyadenylation signal is extremely efficient and has been shown to increase the steady-state level of RNA approximately fivefold over levels achieved with the early SV40 polyadenylation signal (13).

5. phMGFP Vector Map

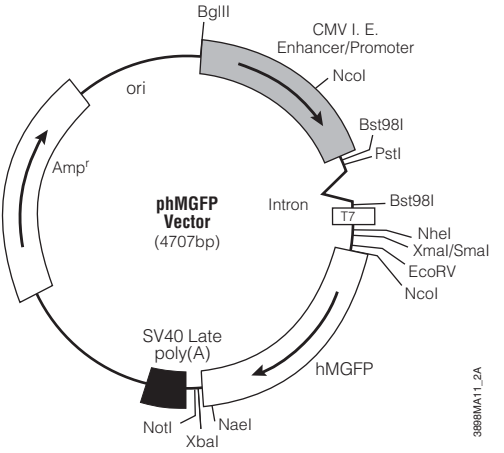

Figure 3. phMGFP Vector map. Additional description: hMGFP, open reading frame of hMGFP; Amp^r, gene conferring ampicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the hMGFP and Amp^r genes indicate the direction of transcription.

phMGFP Vector Sequence Reference Points:

CMV enhancer/promoter	1–742
Chimeric intron	857–989
T7 EEV Promoter Primer binding site	1020–1041
T7 promoter	1034–1052
hMGFP open reading frame	1076–1759
SV40 late polyadenylation signal	1807–2028
β-lactamase (Amp^r) coding region	3015–3875

Note: Use the T7 EEV Promoter Primer (Cat.# Q6700) to sequence the phMGFP Vector. **Do not** use the T7 Promoter Primer (Cat.# Q5021).

6. Appendix

6.A. phMGFP Vector Restriction Sites and Sequence Accession Number

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number **AY218848**) and online at: **www.promega.com/vectors/**

Table 2. Restriction Enzymes That Cut the phMGFP Vector 1–5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	5	278, 331, 414, 600, 2883	**DraI**	4	1998, 3224, 3916, 3935
AccB7I	1	1654	DraII	1	2822
AflII	2	820, 1017	DraIII	2	1654, 2356
Alw44I	3	2633, 3130, 4376	DrdI	4	809, 2400, 2719, 4588
AlwNI	1	4281			
AvaI	2	1058, 1325	DsaI	2	513, 1074
AvaII	5	1195, 1363, 1657, 3438, 3660	EagI	1	1771
			EarI	2	2061, 3003
BalI	2	10, 64	**EclHKI**	1	3802
BanII	3	721, 1506, 2282	Eco52I	1	1771
BbeI	2	1125, 1218	**EcoICRI**	1	719
BbsI	2	928, 1267	**EcoRV**	1	1066
BclI	1	1083	EheI	2	1123, 1216
BglII	1	4702	Fs*p*I	2	2102, 3579
BsaI	2	882, 3736	**HaeII**	5	1125, 1218, 2198, 2206, 4450
BsaOI	5	1774, 2083, 3284, 3433, 4356	**HincII**	3	669, 1453, 1937
BsaAI	3	493, 1525, 2353	HindII	3	669, 1453, 1937
BsaBI	1	2039	**HindIII**	1	748
BsaMI	2	1858, 1951	**HpaI**	1	1937
BsmI	2	1858, 1951	KasI	2	1121, 1214
BspHI	3	2857, 2962, 3970	**NaeI**	1	1753
BspMI	1	844	**NarI**	2	1122, 1215
BsrGI	2	96, 1428	**NcoI**	2	513, 1074
BssSI	4	1336, 2826, 3133, 4517	**NdeI**	2	387, 2628
			NgoMIV	1	1751
Bst98I	2	820, 1017	**NheI**	1	1052
BstEII	1	1265	**NotI**	1	1771
BstXI	1	1627	NspI	1	2777
BstZI	1	1771	PflM1	1	1654
Cfr10I	2	1751, 3717	PspAI	1	1058
ClaI	1	2043	**PstI**	1	830

Note: The enzymes listed in boldface type are available from Promega.

Table 2. Restriction Enzymes That Cut the phMGFP Vector 1–5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
PvuI	2	2083, 3433	**SspI**	4	5, 52, 2561, 2997
SacI	1	721	**StyI**	3	513, 1074, 1290
ScaI	2	1030, 3321	TfiI	1	1371
SinI	5	1195, 1363, 1657, 3438, 3660	**Tth111I**	1	1448
			VspI	2	160, 3627
SmaI	1	1060	**XbaI**	1	1764
SnaBI	1	493	**XmaI**	1	1058
SpeI	1	152	**XmnI**	1	3202

Table 3. Restriction Enzymes That Do Not Cut the phMGFP Vector.

AccI	BlpI	EcoNI	PmeI	**SgfI**
AccIII	Bpu1102I	**EcoRI**	PmlI	SgrAI
Acc65I	Bsp120I	FseI	Ppu10I	**SphI**
AflIII	**BssHII**	**I-PpoI**	Ppu I	SplI
AgeI	Bst1107I	**KpnI**	PshAI	SrfI
ApaI	**Bsu36I**	**MluI**	Psp5II	Sse8387I
AscI	**CspI**	**NruI**	**PvuII**	**StuI**
AvrII	**Csp45I**	**NsiI**	RsrII	SwaI
BamHI	**Eco47III**	PacI	**SacII**	XcmI
BbrPI	Eco72I	PaeR7I	**SalI**	**XhoI**
BbuI	Eco81I	PinAI	**SfiI**	

Table 4. Restriction Enzymes that Cut the phMGFP Vector 6 or More Times.

AciI	BsrI	**FokI**	MaeIII	PleI
AcyI	**BsrSI**	**HaeIII**	**MboI**	**RsaI**
AluI	Bst71I	HgaI	**MboII**	**Sau3AI**
Alw26I	**BstOI**	**HhaI**	MnlI	Sau96I
AspHI	BstUI	**HinfI**	MseI	ScrFI
BanI	**CfoI**	**HpaII**	**MspI**	SfaNI
BbvI	**DdeI**	HphI	**MspA1I**	**TaqI**
BglI	**DpnI**	**Hsp92I**	**NciI**	**Tru9I**
BsaHI	DpnII	**Hsp92II**	**NdeII**	**XhoII**
BsaJI	EaeI	MaeI	NlaIII	
Bsp1286I	Fnu4HI	MaeII	NlaIV	

Note: The enzymes listed in boldface type are available from Promega.

6.B. References

1. Schmidt, E.V. *et al*. (1990) The cytomegalovirus enhancer: A pan-active control element in transgenic mice. *Mol. Cell. Biol*. **10**, 4406–11.
2. Bothwell, A.L. *et al*. (1981) Heavy chain variable region contribution to the NP^b family of antibodies: Somatic mutation evident in a gamma 2a variable region. *Cell* **24**, 625–37.
3. Senapathy, P., Shapiro, M.B. and Harris, N.L. (1990) Splice junctions, branch point sites, and exons: Sequence statistics, identification, and applications to genome project. *Methods Enzymol*. **183**, 252–78.
4. Gross, M.K., Kainz, M.S. and Merrill, G.F. (1987) Introns are inconsequential to efficient formation of cellular thymidine kinase mRNA in mouse L cells. *Mol. Cell. Biol*. **7**, 4576–81.
5. Buchman, A.R. and Berg, P. (1988) Comparison of intron-dependent and intron-independent gene expression. *Mol. Cell. Biol*. **8**, 4395–405.
6. Evans, M.J. and Scarpulla, R.C. (1989) Introns in the 3´-untranslated region can inhibit chimeric CAT and β-galactosidase gene expression. *Gene* **84**, 135–42.
7. Huang, M.T. and Gorman, C.M. (1990) Intervening sequences increase efficiency of RNA 3´ processing and accumulation of cytoplasmic RNA. *Nucleic Acids Res*. **18**, 937–47.
8. Huang, M.T. and Gorman, C.M. (1990) The simian virus 40 small-t intron, present in many common expression vectors, leads to aberrant splicing. *Mol. Cell. Biol*. **10**, 1805–10.
9. van Kampen, E.J. and Zijlstra, W.G. (1983) Spectrophotometry of hemoglobin and hemoglobin derivatives. *Adv. Clin. Chem*. **23**, 199–257.
10. Proudfoot, N. (1991) Poly(A) signals. *Cell* **64**, 671–4.
11. Bernstein, P. and Ross, J. (1989) Poly(A), poly(A) binding protein and the regulation of mRNA stability. *Trends Biochem. Sci*. **14**, 373–7.
12. Jackson, R.J. and Standart, N. (1990) Do the poly(A) tail and 3´ untranslated region control mRNA translation? *Cell* **62**, 15–24.
13. Carswell, S. and Alwine, J.C. (1989) Efficiency of utilization of the simian virus 40 late polyadenylation site: Effects of upstream sequences. *Mol. Cell. Biol*. **9**, 4248–58.

6.C. Related Products

Product	Size	Cat.#
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Transfectam® Reagent for the Transfection of Eukaryotic Cells	1mg	E1231
	0.5mg	E1232
Tfx™-50 Reagent	2.1mg	E1811
Tfx™-20 Reagent	4.8mg	E2391
ProFection® Mammalian Transfection System—Calcium Phosphate	40 reactions	E1200
Glo Lysis Buffer, 1X	100ml	E2661
T7 EEV Promoter Primer	2µg	Q6700

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