Chlamydomonas adapted GFP (CrGFP)

Plasmid pCrGFP

Sequence of the CrGFP

In the following sequence, all amino acids which have been altered from the wildtype GFP from *Aequorea victoria* are printed in bold face. The nucleotides which have been exchanged for codon adaptation are also printed in bold face. The first amino acids in italic (up to *...EDPP*) are encoded by the multiple cloning site (MCS) of the expression wildtype and are thus not identical with the wildtype amino acid sequence. The expression in *E. coli* or *Chlamydomonas* has not been affected by this, however.
There are only six nucleotides missing compared to the wildtype gene (at positions 52 to 57), with the sequence "ATG GCC", which code for methionine and alanine. These six nucleotides have been replaced by the nucleotides 1 to 57 in the sequence below due to ligation into the expression vector.

```
  1 ATG ACC ATG ATT ACG CCA AGC TTG CAT GCC TGC AGG TCG ACT CTA GAG 48
    M   T   M   I   T   P   S   L   H   A   C   R   S   T   L   E

 49 GAT CCC CCC AAG GGC GAG GAG CTG TTC ACC GGT GTG GTC CCC ATC CTG 96
    D   P   P   K   G   E   E   L   F   T   G   V   V   P   I   L

 97 GTG GAG CTG GAC GGC GAC GTG AAC GGC CAC AAG TTC TCC GTC TCC GGC 144
    V   E   L   D   G   D   V   N   G   H   K   F   S   V   S   G

145 GAG GGT GAG GGT GAC GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC 192
    E   G   E   G   D   A   T   Y   G   K   L   T   L   K   F   I

193 TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTG GTC ACC ACC 240
    C   T   T   G   K   L   P   V   P   W   P   T   L   V   T   T

241 CTG ACC TAC GGT GTG CAG TGC TTC TCC CGC TAC CCC GAC CAC ATG AAG 288
    L   T   Y   G   V   Q   C   F   S   R   Y   P   D   H   M   K

289 CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAG GGC TAC GTG CAG GAG 336
    Q   H   D   F   F   K   S   A   M   P   E   G   Y   V   Q   E

337 CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG 384
    R   T   I   F   F   K   D   D   G   N   Y   K   T   R   A   E

385 GTC AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC 432
    V   K   F   E   G   D   T   L   V   N   R   I   E   L   K   G

433 ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGC CAC AAG CTG GAG TAC 480
    I   D   F   K   E   D   G   N   I   L   G   H   K   L   E   Y

481 AAC TAC AAC TCC CAC AAC GTG TAC ATC ATG GCC GAC AAG CAG AAG AAC 528
    N   Y   N   S   H   N   V   Y   I   M   A   D   K   Q   K   N

529 GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC TCC 576
    G   I   K   V   N   F   K   I   R   H   N   I   E   D   G   S

577 GTG CAG CTG GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAT GGC 624
    V   Q   L   A   D   H   Y   Q   Q   N   T   P   I   G   D   G

625 CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG TCC ATC CAG TCC GCC CTG 672
    P   V   L   L   P   D   N   H   Y   L   S   I   Q   S   A   L
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```
 673 TCC AAG GAC CCC AAC GAG AAG CGC GAC CAC ATG GTC CTG CTG GAG TTC 720
      S   K   D   P   N   E   K   R   D   H   M   V   L   L   E   F

 721 GTC ACC GCT GCC GGC ATC ACC CAC GGC ATG GAC GAG CTG TAC AAG TAA 768
      V   T   A   A   G   I   T   H   G   M   D   E   L   Y   K   *
```

After the stop codon follows a 3' untranslated region (3'-UTR) with the sequence:

```
 769 GGA TCC CCG CTC CGT GTA AAT GGA GGC GCT CGT TGA TCT GAG CCT TGC 816
 817 CCC CTG ACG AAC GGC GGT GGA TGG AAG ATA CTG CTC TCA AAG TGC TGA 864
 865 AGC GGT AGC TTA GCT CCC CGT TTC GTG CTG ATC AGT CTT TTT CAA CAC 912
 913 GTA AAA AGC GGA GGA GTT TTG CAA TTT TGT TGG TTG TAA CGA TCC TCC 960
 961 GTT GAT TTT GGC CTC TTT CTC CAT GGG CGG GCT GGG CGT ATT GAA GCG 1008
1009 GGT ACC GAG CTC GAA TTC                                       1026
```

Base composition of the coding region:

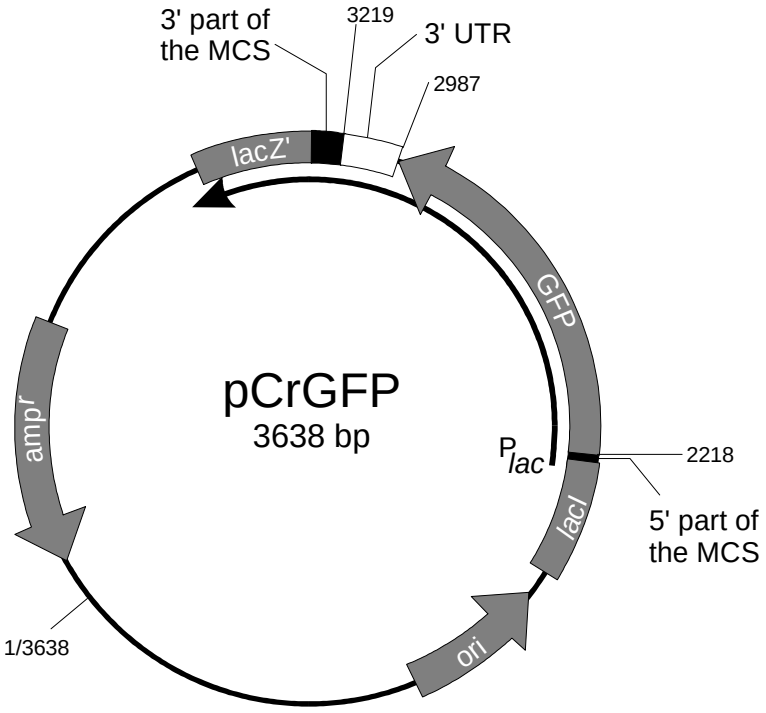
A	23,05 %	GC	61,33 %
C	34,64 %	AT	38,67 %
G	26,69 %		
T	15,63 %		

Amino acid exchanges from the wildtype gene from *Aequorea victoria*

Two amino acids have been exchanged in the gene, F64L and S65T. The exchanges have effects on three properties of the protein: The fluorescence intensity at an excitation wavelength of 480 nm is increased 30fold; the formation of the chromophore is accelerated 10 to 20fold; and the protein shows only one excitation maximum at 489 nm versus two maxima (at 395 nm and 470 nm) in the wildtype. The reduction to one maximum suites the protein for confocal microscopy, because most laser-scanning confocal microscops (LSCM) work with lasers of 488 nm emission wavelength.

The plasmid pCrGFP

The algal CrGFP is carried by the plasmid pCrGFP. This plasmid is based on the vector pUC19. The CrGFP and a 3' untranslated region have been ligated into the MCS of pUC19. This region is the 3'-UTR of the rbcS2 gene from *Chlamydomonas reinhardtii*, which codes for a sub unit of the RUBISCO.



The 5' part of the MCS lies immediately downstream of P_{lac}. As the detailled map of the MCS shows, genes can be ligated immediately in front of the CrGFP by using the existing restriction sites, in order to verify their expression using the GFP fluorescence.

Map of the restriction sites of the vector

Enzymes that do not cut in pCrGFP:

AflII	ApaI	AscI	AvrII	BaeI	BaeI	BbsI
BglII	Bpu10I	BsaBI	BsmI	BspEI	BssHII	BstZ17I
Bsu36I	ClaI	DrdII	EagI	Eco47III	EcoRV	FseI
HpaI	MluI	MscI	MunI	NheI	NotI	NruI
NsiI	NspV	PacI	PmeI	PmlI	PshAI	RsrII
SacII	SanDI	SexAI	SfiI	SgfI	SmaI	SmiI
SnaBI	SpeI	SrfI	Sse8647I	StuI	SunI	Tth111I
UbaDI	UbaEI	XhoI	XmaI			

Enzymes that cut, with position of restriction site:

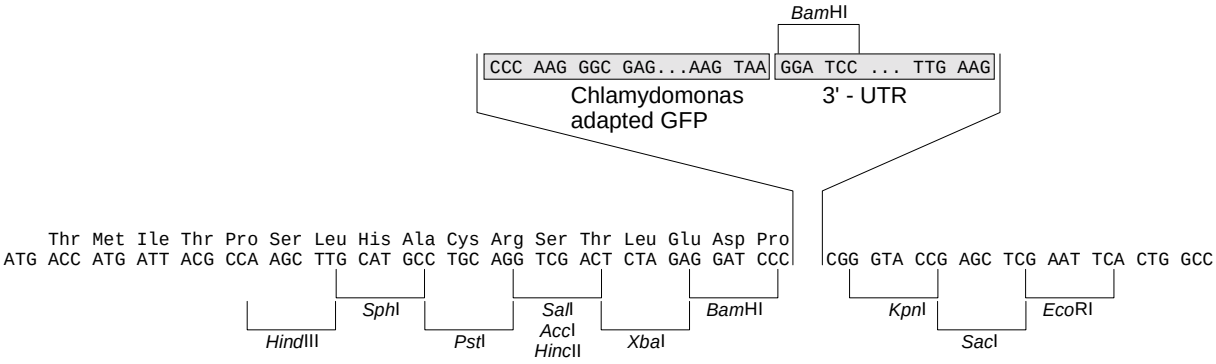
AatII	1	AgeI	1	AhdI	1	AlwNI	1
BcgI	1	BcgI	1	BclI	1	BplI	1
Bpu1102I	1	BsaI	1	BspGI	1	BspLU11I	1
BspMI	1	BstAPI	1	BstEII	1	BstXI	1
DraIII	1	EcoNI	1	EcoRI	1	HindIII	1
KpnI	1	NarI	1	NcoI	1	NdeI	1
NgoAIV	1	Pfl1108I	1	PstI	1	RleAI	1
SacI	1	SalI	1	SapI	1	SbfI	1
ScaI	1	SgrAI	1	SphI	1	SspI	1
XbaI	1	XcmI	1	XmnI	1	AclI	2
BamHI	2	BciVI	2	BfiI	2	BglI	2
BsbI	2	BseRI	2	BsrDI	2	BsrGI	2
FspI	2	HgiEII	2	PflMI	2	PvuI	2
ApaLI	3	BsaXI	3	BsmBI	3	DraI	3
DrdI	3	EarI	3	PvuII	3	RcaI	3
TaqII	3	TaqII	3	VspI	3	Bce83I	4
BpmI	4	BsgI	4	Bsp24I	4	Bsp24I	4
BsrBI	4	BssSI	4	Eco57I	5	EciI	6
AceIII	7						

Enzyme	Position
AatII	70
AgeI	2294
AhdI	989
AlwNI	1468
BcgI	449
BcgI	485
BclI	3111
BplI	2787
Bpu1102I	3091
BsaI	923
BspGI	2323
BspLU11I	1877
BspMI	2245
BstAPI	3457
BstEII	2449
BstXI	2314
DraIII	2716
EcoNI	2891

Enzyme	Position						
EcoRI	3239						
HindIII	2236						
KpnI	3231						
NarI	3401						
NcoI	3199						
NdeI	3453						
NgoAIV	2948						
Pfl1108I	970						
PstI	2252						
RleAI	2726						
SacI	3237						
SalI	2254						
SapI	1997						
SbfI	2252						
ScaI	508						
SgrAI	2294						
SphI	2246						
SspI	184						
XbaI	2260						
XcmI	3206						
XmnI	389						
AclI	387	760					
BamHI	2266	2987					
BciVI	152	1679					
BfiI	948	3280					
BglI	871	3391					
BsbI	3127	3519					
BseRI	2297	3156					
BsrDI	755	927					
BsrGI	2718	2976					
FspI	766	3381					
HgiEII	1295	3453					
PflMI	2689	2965					
PvuI	620	3362					
ApaLI	317	1563	3458				
BsaXI	2023	2347	2704				
BsmBI	2359	3590	3636				
DraI	411	1103	1122				
DrdI	1775	2921	3544				
EarI	193	1997	3343				
PvuII	2057	2800	3331				
RcaI	44	149	1157				
TaqII	455	642	1981				
TaqII	287	302	2972				
VspI	814	2049	2108				
Bce83I	385	1253	1492	1792			
BpmI	920	2336	2711	2951			
BsgI	2393	2492	2567	2816			
Bsp24I	89	1178	1356	2850			
Bsp24I	52	1215	1393	2887			
BsrBI	147	1948	2189	2995			
BssSI	17	320	1704	3655			
Eco57I	323	1333	2420	2663	3100		
EciI	833	1661	1807	2527	2771	2884	
AceIII	689	1929	2277	2310	2631	2964	3585

The multiple cloning site of pCrGFP

The CrGFP has been inserted in frame with the MCS, so that the protein can be expressed in *E. coli* immediately just by transformation.



The two *BamH*I sites allow the excission of the CrGFP without the 3'-UTR.

Transformation of *E. coli* with pCrGFP

To transform *E. coli* with pCrGFP in its original or a modified version, you need competent *E. coli* cells. You find detailled instructions to prepare such cells for electrical or chemical transformation in "Current Protocols in Molecular Biology" (Ausubel et al., 1990) or in "Molecular Biology: A Laboratory Manual" (Sambrook et al. 1989).

Map of the restriction sites of the CrGFP gene

This map contains all restriction sites which cut inside the CrGFP gene including the 3'-UTR. The gene starts at nucleotide 2218 of the plasmid and ends at nucleotide 2987. Then follows the 3'-UTR, which ends at nucleotide 3219.

Enzymes that do not cut in CrGFP:

AatII	AclI	AflII	AhdI	AlwNI	ApaI	ApaLI
AscI	AvrII	BaeI	BaeI	BbsI	Bce83I	BcgI
BcgI	BciVI	BfiI	BglI	BglII	Bpu10I	BsaI
BsaBI	BsmI	BspEI	BspLU11I	BsrDI	BssHII	BssSI
BstAPI	BstZ17I	Bsu36I	ClaI	DraI	DrdII	EagI
EarI	Eco47III	EcoRV	FseI	FspI	HgiEII	HpaI
MluI	MscI	MunI	NarI	NdeI	NheI	NotI
NruI	NsiI	NspV	PacI	Pfl1108I	PmeI	PmlI
PshAI	PvuI	RsrII	SacII	SanDI	SapI	ScaI
SexAI	SfiI	SgfI	SmaI	SmiI	SnaBI	SpeI
SrfI	Sse8647I	SspI	StuI	SunI	TaqII	Tth111I
UbaDI	UbaEI	VspI	XhoI	XmaI	XmnI	

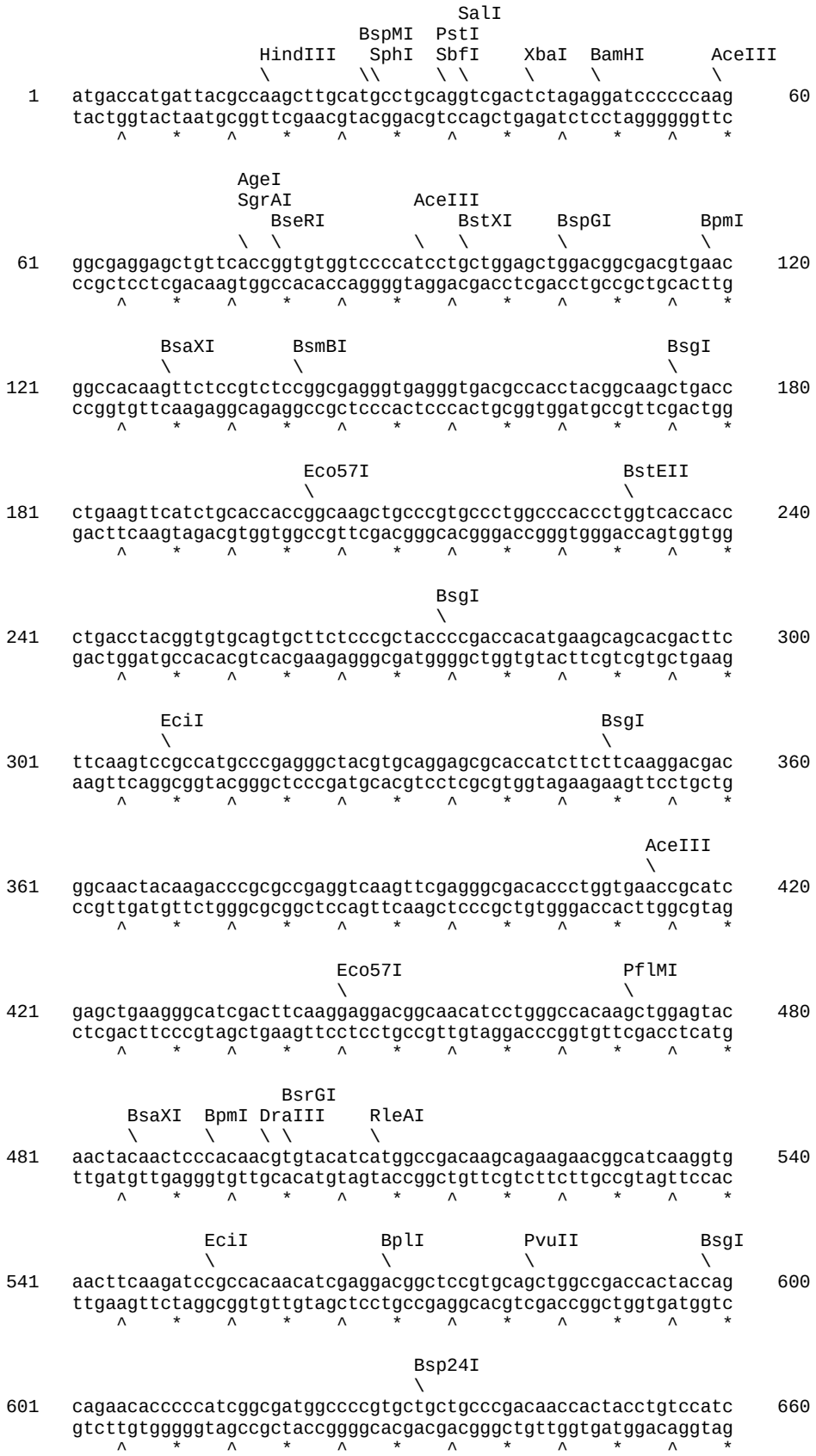
Enzymes that cut, with position of restriction sites:
The positions refer to the position of the gene in the plasmid. The restriction map below starts with nucleotide 1 of the gene (the A of the start codon), which lies at position 2218 in the plasmid.

AgeI	1	BclI	1	BplI	1	Bpu1102I	1
BsbI	1	BsmBI	1	Bsp24I	1	Bsp24I	1
BspGI	1	BspMI	1	BsrBI	1	BstEII	1
BstXI	1	DraIII	1	DrdI	1	EcoNI	1
HindIII	1	NcoI	1	NgoAIV	1	PstI	1
PvuII	1	RleAI	1	SalI	1	SbfI	1
SgrAI	1	SphI	1	TaqII	1	XbaI	1
XcmI	1	BamHI	2	BsaXI	2	BseRI	2

BsrGI	2	PflMI	2	BpmI	3	EciI	3
Eco57I	3	AceIII	4	BsgI	4		

Enzyme	Position			
AgeI	2294			
BclI	3111			
BplI	2787			
Bpu1102I	3091			
BsbI	3127			
BsmBI	2359			
Bsp24I	2887			
Bsp24I	2850			
BspGI	2323			
BspMI	2245			
BsrBI	2995			
BstEII	2449			
BstXI	2314			
DraIII	2716			
DrdI	2921			
EcoNI	2891			
HindIII	2236			
NcoI	3199			
NgoAIV	2948			
PstI	2252			
PvuII	2800			
RleAI	2726			
SalI	2254			
SbfI	2252			
SgrAI	2294			
SphI	2246			
TaqII	2972			
XbaI	2260			
XcmI	3206			
BamHI	2266	2987		
BsaXI	2347	2704		
BseRI	2297	3156		
BsrGI	2718	2976		
PflMI	2689	2965		
BpmI	2336	2711	2951	
EciI	2527	2771	2884	
Eco57I	2420	2663	3100	
AceIII	2277	2310	2631	2964
BsgI	2393	2492	2567	2816

Map of the CrGFP gene:



```
                 EcoNI
          EciI   TaqII
             Bsp24I                              DrdI
            \  \   \                             \
 661   cagtccgccctgtccaaggaccccaacgagaagcgcgaccacatggtcctgctggagttc    720
       gtcaggcgggacaggttcctggggttgctcttcgcgctggtgtaccaggacgacctcaag
           ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

            NgoAIV          AceIII      BsrGI
               BpmI          PflMI  TaqII          BamHI   BsrBI
            \  \            \\      \   \          \       \
 721   gtcaccgctgccggcatcacccacggcatggacgagctgtacaagtaaggatccccgctc    780
       cagtggcgacggccgtagtgggtgccgtacctgctcgacatgttcattcctaggggcgag
           ^    *    ^    *    ^    *    ^    *    ^    *    ^    *



 781   cgtgtaaatggaggcgctcgttgatctgagccttgccccctgacgaacggcggtggatgg    840
       gcacatttacctccgcgagcaactagactcggaacgggggactgcttgccgccacctacc
           ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

                                     Bpu1102I Eco57I     BclI
                                     \        \          \
 841   aagatactgctctcaaagtgctgaagcggtagcttagctccccgtttcgtgctgatcagt    900
       ttctatgacgagagtttcacgacttcgccatcgaatcgaggggcaaagcacgactagtca
           ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

            BsbI                          BseRI
            \                             \
 901   ctttttcaacacgtaaaaagcggaggagttttgcaattttgttggttgtaacgatcctcc    960
       gaaaaagttgtgcatttttcgcctcctcaaaacgttaaaacaaccaacattgctaggagg
           ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

                        NcoI   XcmI                    KpnI  SacI
                        \      \                       \     \
 961   gttgattttggcctctttctccatgggcgggctgggcgtattgaagcgggtaccgagctc   1020
       caactaaaaccggagaaagaggtacccgcccgacccgcataacttcgcccatggctcgag
           ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

       EcoRI
       \
1021   gaattc                                                        1080
       cttaag
           ^    *    ^    *    ^    *    ^    *    ^    *    ^    *
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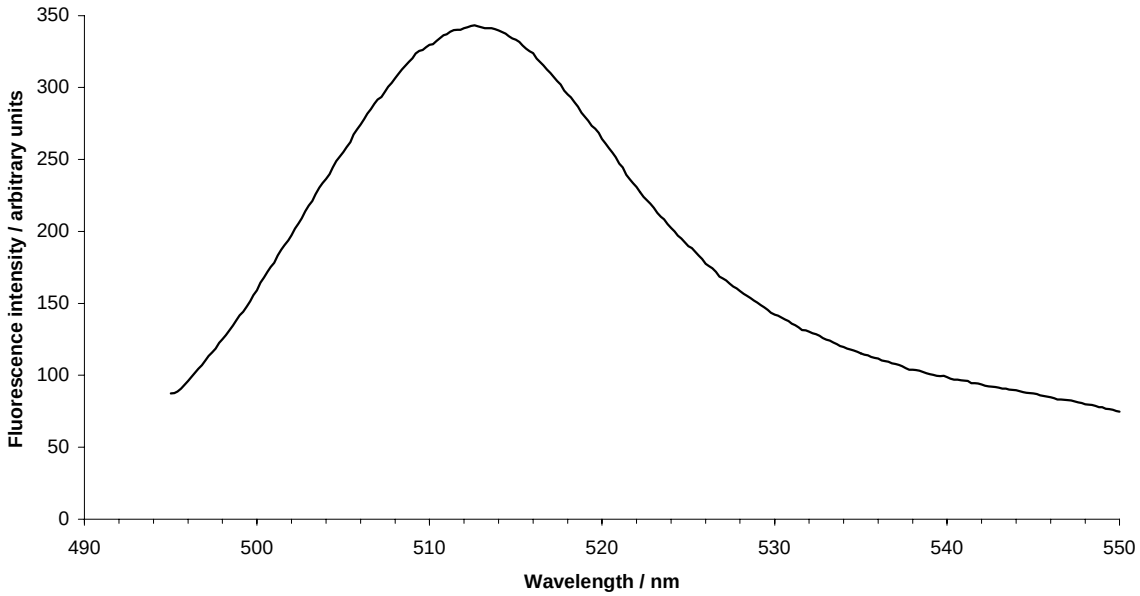
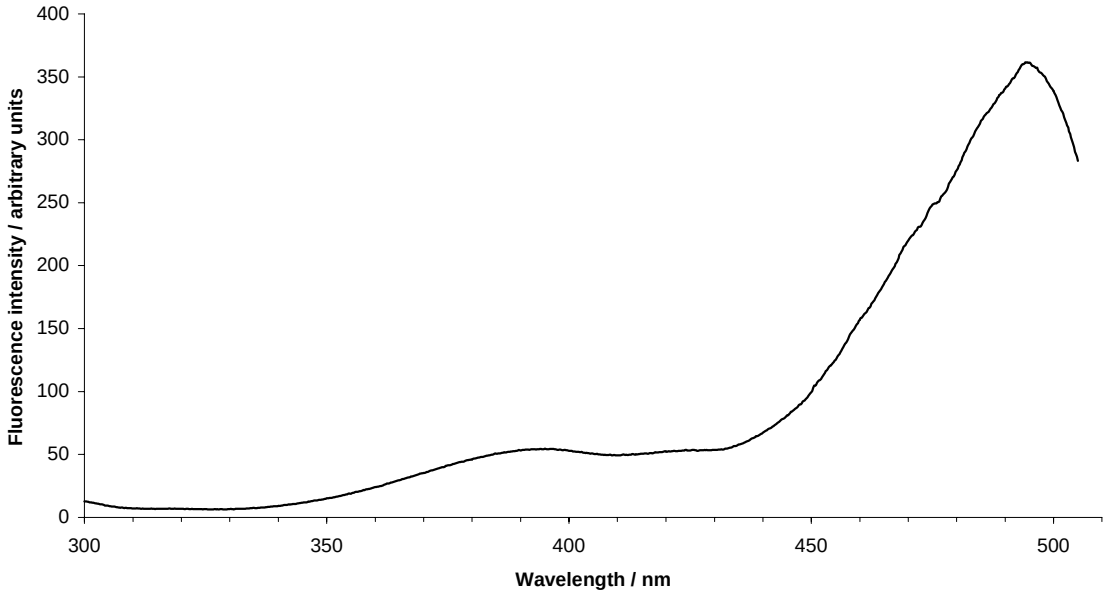
Fluorescence spectra

Emission spectrum with excitation at 488 nm

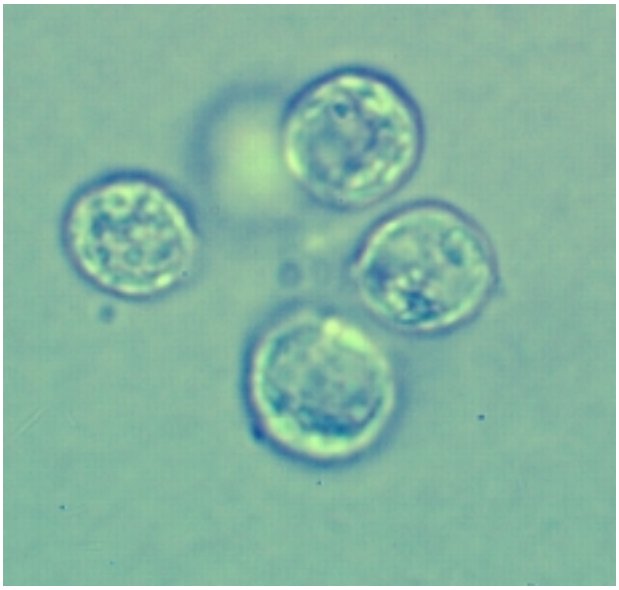


Excitation spectrum with emission at 512 nm

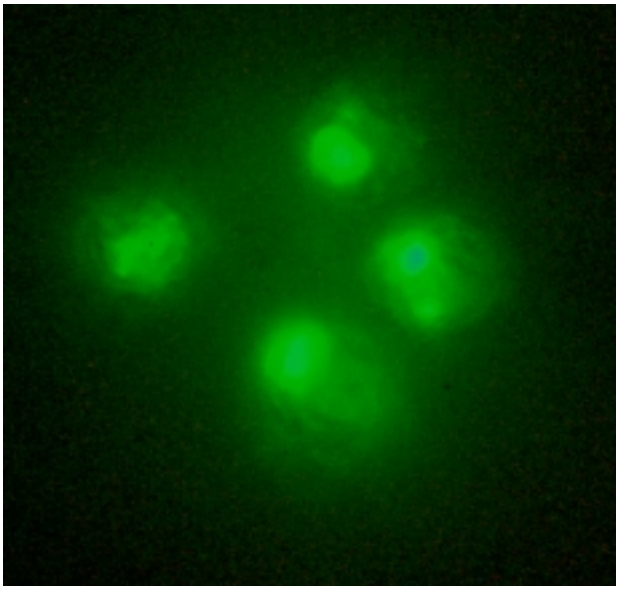


Microphotographs

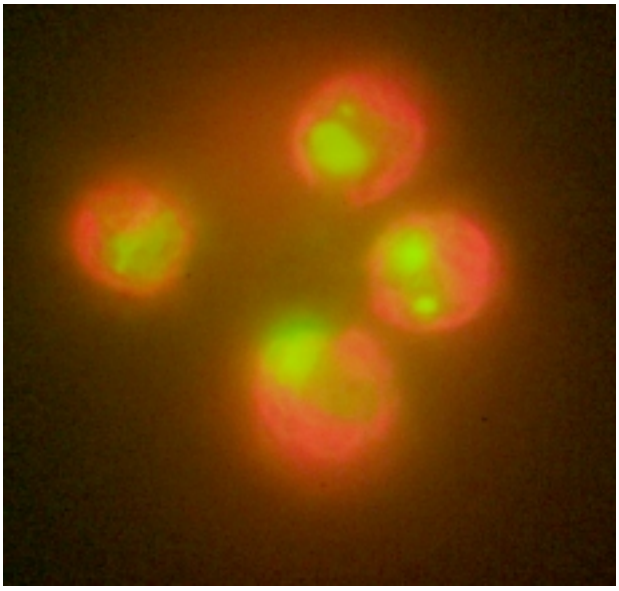
Coloured versions of the photographs shown here can be found on our website under *www.entelechon.com*.



This photo shows cells of the single-celled alga Clamydomonas reinhardtii as seen under the light icroscope. These cells have been transformed with a construct of the algal GFP and a resistence gene. The resistence gene binds to DNA, so that one can expect to find the GFP-resistence gene hybrid to be enriched in the nucleus.



Here, you can see the fluorescence of the cells shown above in the transmission at an excitation of 395 nm, with a bandpass emission filter of 510-520 nm. The emission filter is used to block the red chlorophyll fluorescence. You can clearly see the green fluorescence of the GFP, localized in a spherical, central region inside the cells.



In this picture, you see the fluorescence at an excitation of 330 nm, using a 520 nm longpass emission filter. The nucleus of the cells has been stained with ethidium bromide. There is red fluorescence of the chlorophyll from the chloroplast, as well as the yellowish fluorescence of ethidium bromide. The chloroplast has the typical cup-shaped outline, whereas the nucleus lies inside the chloroplast cup. The localization of GFP and ethidium bromide matches perfectly, showing that the GFP-resistance gene construct is located in the nucleus.

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