

FLUORESCENT PROTEIN TAGS

INTRODUCTION

In this document is summary information about fluorescent tags that can be added to heterogeneously expressed proteins.

The green fluorescent protein (GFP) was first purified from *Aequorea victoria* in the 1960s and 70s (1), but it was not until the nucleotide sequence was reported in the 1990s that the full potential of GFP as a tool was realised (2, 3). Structures of GFP revealed the basis of the fluorescent chromophore as being formed by the maturation of a triplet of residues serine 65, tyrosine 66 and glycine 67 into an imidazolinone ring system (4). When heterologously expressed, GFP folds and the chromophore forms correctly in the absence of co-factors or chaperones, meaning that the sequence can be fused to other proteins to fluorescently tag them. This ability is useful in numerous experimental techniques including but not limited to fluorescent resonance energy transfer (FRET), super resolution microscopy localisation studies, flow cytometry etc. In addition, fluorescent tags can be extremely useful in the process of protein expression and purification optimisation, especially for challenging targets such as membrane proteins.

The wild-type GFP sequence has been modified with point mutations in order to modify specific properties. For example, the fluorescence intensity has been increased, the protein has been given the ability for folding in an extracellular environment, or a wide array of changes to the excitation and emission spectra have been achieved in order to create fluorescent proteins in the blue, cyan and yellow spectrum. Furthermore, fluorescent proteins from other marine species, for example *Discosoma striata* that has a red colour, have also been added and modified in a similar fashion to provide coverage of the whole colour spectrum.

COMMONLY USED FLUORESCENT PROTEIN TAGS

Wild-type GFP (wtGFP)	
Excitation (nm)	395
Emission (nm)	509
Sequence	
MSKGEELFTGVVPILEVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTFSYGVQCFSR YPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEKEDTLVNRIELKGIKEDGNIILGHKLEYNYN SHNVYIMADKQKNGIKVNFKIRHNIEDGSQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHVM LLEFVTAAGITHGMDELYK	

Enhanced GFP (eGFP)

Excitation (nm)	488
Emission (nm)	507
Sequence difference from wtGFP	F64L, S65T, H231L
Sequence	
<p>MVSKGEELFTGVVPIILVELDGDVNGHKFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWPTLVTTLTLYGVQCFS RYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNY NSHNVIYIMADKQKNGIKVNFKIRHNIEDGSQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHM VLLEFVTAAGITLGMDELYK</p>	

Superfolder GFP (sfGFP)

Excitation (nm)	485
Emission (nm)	510
Sequence difference from wtGFP	S30R, Y39N, F64L, N105T, Y145F, I171V, A206V

Yellow fluorescent protein (YFP)

Excitation (nm)	513
Emission (nm)	527
Sequence difference from wtGFP	S65G, V68L, S72A, T203Y, H231L

Red fluorescent protein (DsRED)

Excitation (nm)	558
Emission (nm)	583
Sequence	
<p>MRSSKNVIKEFMRFKVRMEGTVNGHEFEIEGEGEGRPYEGHNTVKLKVTKGGPLPFAWDILSPQFQYGSKVYV KHPADIPDYKKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGCFIYKVKFIGVNFPSDGPVMQKKTMGWEAS TERLYPRDGV LKGEIHKALKLKDGGHYLVEFKSIYMAKKPVQLPGYYYVDSKLDITSHNEDYTIVEQYERTEG RHHLFL</p>	

mCherry	
Excitation (nm)	587
Emission (nm)	610
Sequence	
MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYG SKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPDGPVMQKKTM GWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQY ERAEGRHSTGGMDELYK	

EXCITATION, EMISSION AND BRIGHTNESS OF FLUORESCENT PROTEIN VARIANTS

Protein(Acronym)	Excitation Maximum (nm)	Emission Maximum (nm)	Relative Brightness (% of EGFP)
GFP (wt)	395/475	509	48
Green Fluorescent Proteins			
EGFP	484	507	100
Emerald	487	509	116
Superfolder GFP	485	510	160
Azami Green	492	505	121
mWasabi	493	509	167
TagGFP	482	505	110
TurboGFP	482	502	102
AcGFP	480	505	82
ZsGreen	493	505	117
T-Sapphire	399	511	79
Blue Fluorescent Proteins			
EBFP	383	445	27
EBFP2	383	448	53
Azurite	384	450	43
mTagBFP	399	456	98

Protein(Acronym)	Excitation Maximum (nm)	Emission Maximum (nm)	Relative Brightness (% of EGFP)
Cyan Fluorescent Proteins			
ECFP	439	476	39
mECFP	433	475	39
Cerulean	433	475	79
mTurquoise	434	474	75
CyPet	435	477	53
AmCyan1	458	489	31
Midori-Ishi Cyan	472	495	73
TagCFP	458	480	63
mTFP1 (Teal)	462	492	162
Yellow Fluorescent Proteins			
EYFP	514	527	151
Topaz	514	527	169
Venus	515	528	156
mCitrine	516	529	174
YPet	517	530	238
TagYFP	508	524	118
PhiYFP	525	537	144
ZsYellow1	529	539	25
mBanana	540	553	13
Orange Fluorescent Proteins			
Kusabira Orange	548	559	92
Kusabira Orange2	551	565	118
mOrange	548	562	146
mOrange2	549	565	104
dTomato	554	581	142
dTomato-Tandem	554	581	283
TagRFP	555	584	142
TagRFP-T	555	584	99
DsRed	558	583	176
DsRed2	563	582	72

Protein(Acronym)	Excitation Maximum (nm)	Emission Maximum (nm)	Relative Brightness (% of EGFP)
DsRed-Express (T1)	555	584	58
DsRed-Monomer	556	586	10
mTangerine	568	585	34
Red Fluorescent Proteins			
mRuby	558	605	117
mApple	568	592	109
mStrawberry	574	596	78
AsRed2	576	592	8
mRFP1	584	607	37
JRed	584	610	26
mCherry	587	610	47
HcRed1	588	618	1
mRaspberry	598	625	38
dKeima-Tandem	440	620	21
HcRed-Tandem	590	637	19
mPlum	590	649	12
AQ143	595	655	11

REFERENCES

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