# **Nature Methods**

# Molecular evolution approaches to design advanced red fluorescent proteins

Fedor V Subach, Kiryl D Piatkevich & Vladislav V Verkhusha

**Supplementary Figure 1.** Alignment of amino acid sequences of derivatives of RFPs with different phenotype.

**Supplementary Table 1.** Overview of suboptimal properties of current monomeric red fluorescent proteins.

**Supplementary Table 2.** Current red fluorescent proteins exhibiting the best specific property or maximally optimized for the specific imaging application.

**Supplementary Table 3.** Properties of the major chromophore structures observed in fluorescent proteins.

**Supplementary Table 4.** Amino acid residues in the supporting positions responsible for different phenotypes and properties of red fluorescent proteins.

**Supplementary Note** 

**Supplementary Figure 1.** Alignment of amino acid sequences of derivatives of RFPs with different phenotype.

(A) TagBFP-like phenotype, (B) Fluorescent timer-like phenotype, (C) Photoactivatable-like phenotype, (D) Photoswitchable-like phenotype, (E) Far-red-shifted-like phenotype, (F) Large Stokes shift-like phenotype; and properties: (G) High quantum yield, (H) High photostability, (I) High pH-stability, (J) Fast maturation, (K) Monomeric state. Residues in key positions are selected with red, green, or blue colors to denote which of them are responsible for the respective phenotype independently, in concerted manner, or either of these two ways, respectively. Amino acid residues in the supporting positions are highlighted with yellow color.

#### (A) TagBFP-like phenotype

		10	20	30	40	50	
CED	MORO						
GFP Dlue 100	MSKG	LELFIGVVPI	LVELDGDVNGF	IKFSVSGEGEG	DAIIGKLILI	CFICIIGK	
Bluel02	MVSKGEEDN	MALIKEFMRF	KVHMEGSVNGF	IEFETEGEGEG	RPYEGTQTAP	KLKVT – KGGE	LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL
Blue124	MVSKGEEDN	MALIKEF'MRF'	KVHMEGSVNGF	IEF.ETEGEGEG	RPYEGTQTAP	KLKV'I'KGGF	LPF'AWD
mTagBFP	MS	EELIKENMHM	KLYMEGTVDNF	HFKCTSEGEG	KPYEGTQTM	RIKVVEGGE	PLPFAFD
	60				1 0 0	110	
	60		80	90	TOO	110	
		***					-
GFP	TLVT	TFSYGVQCFS	RYPDHMKQHDF	FKSAMPEGYV	QERTIFFKDI	DGNYKTRAEVK	FEGDT
Blue102	ILSP	QF <mark>L</mark> YGS <mark>R</mark> AYV	KHPADIPDY	( <mark>W</mark> KLSFPEGFK	WERVMNFEDO	GVVTVTQDSS	SLQDGE
Blue124	ILSP	QF <mark>M</mark> YGS <mark>R</mark> AYV	KHPADIPDY	( <mark>W</mark> KLSFPEGFK	WERVMNFEDO	GVVTVTQDSS	SLQDGE
mTagBFP	ILAT	SF <mark>L</mark> YGS <mark>K</mark> TFI	NHTQGIP—-DF	" <mark>F</mark> KQSFPEGFT	WERVTTYEDO	GVLTATQDTS	SLQDGC
	120	130	140	150	160	170	
GFP	LVNR	IELKGIDFKE	DGNILGHKLEY	NYNSHNVYIM	ADKQKNGIK	/NFKIRHNIED	GSVQL
Blue102	FIYK	VKLRGTNFPS	DGPVM-QKKTM	IGWEA <mark>I</mark> SERMY	PEDGALKO	GE <mark>I</mark> KORLKLKI	GGHYD
Blue124	FIYK	VKLRGTNFPS	DGPVM-OKKTM	IGWEA <mark>I</mark> SERMY	PEDGALK	JE <mark>I</mark> KORLKLKI	OGGHYD
mTaqBFP	LIYN	VKIRGVNFTS	NGPVM-ÕKKTI	.GWEA <mark>F</mark> TETLY	PADGGLE	GR <mark>N</mark> DMALKLVO	GSHLI
2			~				
	180	190	200	210	220	230	
GFP	ADHYOO	NTPIGD-GPV	LLPDNHYLST	SALSKOPNEK	RDHMVLLEF	TAAGITHGMI	DELYK
Blue102	AEVKTT	YKAKKPV	QLPGAYNVN <mark>I</mark> K	LDITSHNEDY	T-IVEQYER	AEGRHSTGGMI	DELYK
Blue124	ae <mark>v</mark> ktt	YKAKKPV	QLPGAYNVN <mark>I</mark> K	LDITSHNEDY	T-IVEQYER	AEGRHSTGGMI	DELYK
mTagBFP	<mark>a</mark> n <mark>i</mark> ktt	YRSKKPAKNL	KMPGVYYVD <mark>Y</mark> F	RLERIKE-ANN	IETYVEQHEV#	AVARYCDLPSK	LGHKLN

# (B) Fluorescent timer-like phenotype

		10	20	30	40	50	
GFP	MSKGE	ELFTGVVPIL	VELDGDVNGH	IKFSVSGEGEG	DATYGKLTLK	KFICTTG	KLPVPWP
Fast	MVSKGEEDNM	AIIKEFMRFK	VH <mark>V</mark> EGSVNGH	HEFEIEGEGKO	GRPYEGTQTAK	LKVTKGG	PLPFAWD
Medium	MVSKGEEDNM	AIIKEFMRFK	VH <mark>L</mark> EGSVDGH	HEFEIEGEGEG	GRPYEGTQSAK	LKVTKGG	PLPFAWD
Slow	MVSKGEEDNM	AIIKEFMRFK	VH <mark>V</mark> EGSVNGH	HEFEIVGEGEG	GRPYEGTQTAK	LKVTKGG	PLPFAWD
	60	70	80	90	100	110	
		***	_   _				_
GFP	TLVTT	FSYGVQCFSR	YPDHMKQHDB	FFKSAMPEGYV	QERTIFFKDI	)GNYKTRAEV	KFEGDT
Fast	ILSPQ	FMYGS <mark>R</mark> AYVK	HPADIPDY	( <mark>W</mark> KLSFPEGFK	WERVMNFEDG	GV <mark>V</mark> TVTQDS	SLQDGE
Medium	ILSPQ	FMYGS <mark>R</mark> AYVK	HPADIPDY	( <mark>W</mark> KLSFPEGFK	WERVMNFEDG	GV <mark>V</mark> TVTQDS	SLQDGE
Slow	ILSPQ:	FMYGS <mark>R</mark> AYVK	HPADIPDY	( <mark>W</mark> KLSFPEGFK	WERVMNFEDG	GV <mark>V</mark> TVTQDS	SLQDGE
	1.0.0	1.0.0		1 = 0	1.50	1 - 0	
	120	130	140	150	160	170	
GFP	LVNRI	ELKGIDFKED	GNILGHKLEY	NYNSHNVYIM	IADKQKNGIKV	/NFKIRHNIE	DGSVQL
Fast	FIYKV.	KLRGTNFPSD	GPVM-QKKTN	IGWEASTERMY	PEDGALKO	GEIKQRLKLK.	DGGHYD
Medium	FIYKV.	KLRGTNFPSD	GPVM-QKKTN	IGWEASSER <mark>I</mark> Y	PEDGALKO	GEIKQRLKLK.	DGGHYD
Slow	FIYKV.	KLRGTNFPSD	GPVM-QKKTN	IGWEASSER <mark>M</mark> Y	PEDGALKO	SEIKQRLKLK	DGGHYD
	100	100	200	210	220	220	
	190	190	200	210	220	230	
CED							
GFP		IPIGD-GPVL		ZSALSKDPNER			DELIK
rasi		AAAA = -PVQ	LPGAINVN Lr			ECHUCTCOM	DELVK
		KAKKPVL	LPGAINVNL			EGHHSIGGM.	DELIK
STOM	VEVKITY.	KAKKPVQ	LPGAINVN <mark>I</mark> r	UT I SHNEDA	ŢŢŦŢŎĔŎĬĔŔ <mark>ħ</mark>	LGKHSTGGM	υвцік

# (C) Photoactivatable-like phenotype

		10	20	30	40	50	
GFP	MSKGE	ELFTGVVPI	LVELDGDVNGH	KFSVSGEGEG	DATYGKLTL	KFICTTGK	LPVPWP
PAmCherryl	MVSKGEEDNM	AIIKEFMRFI	KVH <mark>M</mark> EGSVNGH	VFEIEGEGEG	RPYEGTQTA	KLKVTKGGF	PLPF <mark>T</mark> WD
PAmCherry2	MVSKGEEDNM	AIIKEFMRFI	KVH <mark>L</mark> EGSVNGH	EFEIEGEGEG	RPYEGTQTA	KLKVTKGGF	PLPF <mark>A</mark> WD
PAmCherry3	MVSKGEEDNM	AIIKEFMRFI	KVH <mark>L</mark> EGSVNGH	EFEIEGEGEG	RPYEGTQTA	KLKVTKGGF	PLPF <mark>T</mark> WD
PATagRFP	MS	ELIKENMHM	kly <mark>m</mark> egtvnnh	HFKCTSEGEG	KPYEGTOTM	RIKVVEGGE	PLPF <mark>A</mark> FD
PAmKate	MS	ELIKENMHM	kly <mark>m</mark> egtvnnh	HFKCTSEGEG	KPYEGTOTM	RIKVVEGGE	PLPF <mark>A</mark> FD
	60	70	80	90	100	110	
		***					
GFP	TLVTT	FSYGVOCFS	RYPDHMKOHDF	FKSAMPEGYV	OERTIFFKDI	OGNYKTRÁEVK	FEGDT
PAmCherrv1	ILSPO	FMYGS <mark>Ñ</mark> AYV	KHPADIPDY	FKLSFPEGFK	WERVMKFEDO	GVVTVTODSS	SLODGE
PAmCherrv2	ILSPO	FMYGS <mark>N</mark> AYV	KHPADIPDY	FKLSFPEGFK	WERVMNFEDO	GVVTVTODSS	LODGE
PAmCherrv3	ILSPO	FMYGSNAYV	KHPADIPDY	FKLSFPEGFK	WERVMNFEDO	GVVTVTODSS	LODGE
PATagRFP	ILATS	FMYGS <mark>S</mark> TFI	NHTOGIPDF	WKOSFPEGFI	WERVTTYEDO	GVLTATODTS	SLODGC
PAmKate	TLATS	FMYGSKTETI	VHTOGTPDF	WKOSEPEGET	WERVTTYED	GVITATODTS	SLODGC
1 marca e e	11110						
	120	130	140	150	160	170	
					_ = = =	_ / J	
GFP		ELKGIDEKEI		NYNSHNVYTM	ADKOKNGTK	/NFKTRHNTET	GSVOL
PAmCherrv1	FTYKV	KLRGTNFPSI		GWEALSERMY	PEDGALK	F. VKPRVKLKT	CGHYD
PAmCherry2	FTYKV	KLRGTNEPSI	DGPVM-OKKTM	GWETLSERMY	PEDGALK	FIKARTKLKT	GGHYD
PAmCherry3	FTYKV	KLRGTNFPSI	DGPVT-OKKTM	GWDALSERMY	PEDGALK	FIKARIKIKI	GGHYE
PATagRFP	T.TYNV	KIRGVNEPSI	NGPVM-KKKTI	GWEPSTEKLK	PADGGLE		GGHLT
PAmKate	T.TYNV	KIRGVNEPSI	NGPVM-OKKTI	GWEANTEMLY	PADGGLE		CCHLT
I I Millio CC							
	100	190	200	210	220	230	
	180			210	212112	230	
	180	1 1	200				
GFP		TPIGD-GPV		SALSKDPNEK		 /TAAGITHGMI	)ET'AK
GFP PAmCherry1	ADHYQQN	TPIGD-GPV	LLPDNHYLSTQ	SALSKDPNEK	RDHMVLLEF	 /TAAGITHGMI	)ELYK
GFP PAmCherry1 PAmCherry2	180   ADHYQQN AEVKTTY TEVKTTY	TPIGD-GPVI KAKKPV(	LLPDNHYLSTQ QLPGAYNVNRK	 SALSKDPNEK LD <mark>I</mark> TSHNEDY	TT-IVEQYER	 /TAAGITHGME AEGRHSTGGME AEGLHSTGGME	DELYK DELYK DELYK
GFP PAmCherry1 PAmCherry2 PAmCherry3	180   ADHYQQN AEVKTTY TEVKTTY AEVKTTY	 TPIGD-GPV KAKKPV KAKKPV KAKKPV	LLPDNHYLSTQ QLPGAYNVNRK QLPGAYNVNRK DLPGAYNVNPK	 SALSKDPNEK LDITSHNEDY LDITSHNEDY	RDHMVLLEF T-IVEQYER T-IVEQYER T-IVEQYER	 /TAAGITHGMI AEGRHSTGGMI AEGLHSTGGMI AEGRHSTGGMI	DELYK DELYK DELYK
GFP PAmCherry1 PAmCherry2 PAmCherry3 PATagRFP	ADHYQQN AEVKTTY EVKTTY AEVKTTY AEVKTTY	TPIGD-GPVI KAKKPV KAKKPV KAKKPV	LLPDNHYLSTQ QLPGAYNVNRK QLPGAYNVNRK QLPGAYNVNRK MPGYYYVDPR	 SALSKDPNEK LDITSHNEDY LDITSHNEDY LDITSHNEDY	RDHMVLLEF T-I <mark>V</mark> EQYER T-I <mark>V</mark> EQYER T-I <mark>V</mark> EQYER T-IVEQYER	 VTAAGITHGMI AEGRHSTGGMI AEGRHSTGGMI AEGRHSTGGMI	DELYK DELYK DELYK LCHKLN
GFP PAmCherry1 PAmCherry2 PAmCherry3 PATagRFP PAmKate	ADHYQQN AEVKTTY TEVKTTY AEVKTTY CNFKTTY	TPIGD-GPVI KAKKPV KAKKPV KAKKPV RSKKPAKNLI PSKKDAKNLI	LLPDNHYLSTQ QLPGAYNVNRK QLPGAYNVNRK QLPGAYNVNRK XMPGVYYVDRR	 SALSKDPNEK LDITSHNEDY LDITSHNEDY LEIIKEADKE	RDHMVLLEFY T-I <mark>V</mark> EQYER T-IVEQYER T-IVEQYER T-YWEQHEV T-YWEQHEV	 VTAAGITHGMI AEGRHSTGGMI AEGRHSTGGMI AVARYSDLPSK AVARYCDLPSK	DELYK DELYK DELYK LGHKLN

# (D) Photoswitchable-like phenotype

		10	20	30	40	50	
GFP	MSKGI	EELFTGVVPII	VELDGDVNGH	KFSVSGEGEG	GDATYGKLTLK	FICTTG	-KLPVPWP
rsCherryRev	MVSKGEEDN	MAIIKEFMRFF	<b>VHMEGSVNGH</b>	EFEIEGEGE	GRPYEGTOTAK	LKVTKG	GPLPFAWD
rsCherry	MVSKGEEDNN	AIIKEFMRFF	VHMEGSVNGH	EFEIEGEGEG	GRPYEGTOTAK	LKVTKG	GPLPFAWD
rsTaqRFP	MVSKGI	CELIKENMHMK	LYMEGTVNNH	HFKCTSEGE	GKPYEGTOTMR	IKVVEG	GPLPFAFD
KFP1	MZ	ASLLTETMPFK	TTIEGTVNGH	CFKCIGKGE	GNPFEGTOEMK	IEVIEG	GPLPFAFH
IrisFP	HHN	ISAIKPDMKIN	ILRMEGNVNGH	HFVIDGDGT	GKPFEGKŐSMD	LEVKEG	GPLPFAFD
					~		
	60	70	80	90	100	110	
		***				_	
GFP	TLVT	rfsygvocfsf	YPDHMKOHDF	FKSAMPEGY	VOERTIFFKDD	GNYKTRAE	VKFEGDT
rsCherrvRev	TLSP	FMYGSKAYVK	HPADTPDY	TKLSEPEGER	XWERVMNFEDG	GVVTVTOD	SSLODGE
rsCherry	TLSP	)FMYGSKAYVK	HPADTPDY	LKLSFPEGE	KWERVMNFEDG	GVVTVTOD	SSLODGE
rsTagRFP		SEMYGSRTEIN	IHTOGIPDF	WKOSEPEGE	TWERVTTYEDG	GVUTATOD	
KFD1		SCMVGSKTFIK	VVSGTDDV	FKOSEDECE	TWERTTTVEDC	GFLTAHOD	
TrieFD		LEHYCNRVEDE	VDHIODV	FKOSEDKCV	SWERSLTEEDC	GTCTARND	TTMEGDT
TITOTI				I RODI I ROIL	JWERGETFEDO	OICIARD.	IIIIIIIIII
	120	130	140	150	160	170	
	120	130	110	100	100	1	
CFD		ן זידאידרבאינידי	I CNTLCHKLFV		NADKOKNGTKV	NEKTRHNTI	FDGSVAL
rgCherryRey	ETVKI	KI.RGTNFDSI			VDFDGALKC	F <mark>S</mark> KMRT.KT.I	KDGGHVD
rgChorry	ETVEL	INT DOTMEDO	CDVM_OKKTM		VDEDCALKC		ZDCCUVD
raTagPFD			ICDVM_OKKTI				
rstagnrr ved1		INTERGUNEDAT	CDVM_ONKVC				
TricED			ICDVM_OKKTI				FGGKHLI
IIISFP	FINK	/KF HG V NF PAN	GPVM-QKKIL		IVKDGVLIG		LGNATIK
	190	190	200	210	220	220	
	100	190	200	210	220	230	
CED				ן מאד פארסתעדע	ן זיזייד דידיאינירס איני	 תאמרדיינורו	ארדיע
GFF		VIPIGD-GPVI		JALSKUPNEI I DITCUNEDI	VT TVEOVEDA	FCDUCTCCI	
raChorry		WARKPVQ	U DCAVITATI		UT TUPOVEDA	EGKUSIGGI	NDEI VK
TRUTELLA		IRARRPVQ	VID CIVE VID	TEDITISHNED:			
rsragkry	CNLKTT	KSKNPAKNLF		LERIKEADKI	TT-TAROMEAN	VARYCDLP	SKLGHKLN
KEPI	CHLHTT	RSKKPASALK	MPGFHFEDHR	TETMEEVEK(	JK-CYKQYEAA	VGRYCDAAI	PSKLGHN
ITISFP	CD <mark>S</mark> R <mark>T</mark> TY	KAKEKGVF	CLPGYHLVD <mark>H</mark> C	TETTSHDKD	ΥΝ-ΚVΚLΥΈΗΑ	VAHSGLPDI	NARR

## (E) Far-red-shifted-like phenotype

		10	20	30	40	50	
CED	MCKCET				 ע דייי דערערייג רי	 הדמייימצ	ם מסעת ד
mNentune	MSNGM	ETTKENMH <mark>M</mark> K		HHEKCTSEGEG	KPYEGTOT <mark>G</mark> F	TKWVEGGE	
TagRFP657	MSI	ELITENMHMK	LYMEGTVNN	HHFKCTSEGEG	KPYEGTOTOF	IKVVEGGP	LPFAFD
E2-Crimson	MDSTEN	NVIKPFMR <mark>F</mark> K	VHMEGSVNG	HEFEIEGVGEG	KPYEGTOTAK	LOVTKGGF	LPFAWD
eqFP650	MGEDSI	elisenmh <mark>m</mark> k	LYMEGTVNG	HHFKCTSEGEG	kpyegtõt <mark>a</mark> k	IKVVEGGF	LPFAFD
eqFP670	MGEDSI	ELISENMH <mark>T</mark> K	LYMEGTVNG	HHFKCTSEGEG	KPYEGTQT <mark>C</mark> K	IKVVEGGP	LPFAFD
mPlum	MVSKGEENNMA	AIIKEFMR <mark>F</mark> K	EHMEGSVNG	HEFEIEGEGEG	RPYEGTQT <mark>A</mark> F	LKVTKGGF	LPFAWD
mRouge	MVSKGEEDNMA	AIIKEFMR <mark>F</mark> K	THMEGSVNG	HEFEIEGEGEG	RPYEGTQT <mark>a</mark> k	LKVTKGGF	LPFAWD
RFP639	MNS	SLIKENMR <mark>M</mark> M	<mark>V</mark> VMEGSVNG	YQFKCTGEGDG	NPYMGTQT <mark>M</mark> F	RIKVVEGGF	LPFAFD
	60	70	80	90	100	110	
GFP		SIGVQCFSR	IPDHMKQHD	FFKSAMPEGIV	VERITFFRDL	GNIKIRAEVK	
			HIQGIPD	F FRQSF PEGF I			
E2-Crimson		TEVGSKAVIK	HPADTPD	YLKOSEPECEK	WERVMNFEDO	GVUTUTODSS	ILQDGC
eaFP650		MYGSKTFIN	HTOGIPD	FEKOSEPEGET	WERTTTYED	GVLTATODTS	
eqFP670	ILATS	MYGSKTFIN	HTOGIPD	FFKOSFPEGFT	WERITTYEDO	GVLTATODTS	LONGC
mPlum	ILSPOI	loygs <mark>k</mark> ayvk	HPADIPD	Y <mark>L</mark> KLSFPEGFK	WERVMNFEDO	GVVTVTÕDSS	LODGE
mRouge	ILSPQ	MYGS <mark>K</mark> AYVK	HPADIPD	Y <mark>l</mark> klsfpegfk	WERVMNFEDG	GVVTVTQDSS	LQDGE
RFP639	ILAT <mark>SI</mark>	7MYGS <mark>K</mark> TFIK	HTKGIPD	F <mark>F</mark> KQSFPEGFT	WERVTRYEDO	GVFTVMQDTS	LEDGC
				_			
	120	130	140	150	160	170	
GFP	LVNRI	ELKGIDFKED	GNILGHKLE	YNYNSHNVYIM	ADKQKNGIKV	NFKIRHNIED	GSVQL
mueptune		CIRGVNFPSN ZIRGVNFPSN	GPVM-QKKT	LGWEASTETLY	PADGGLEG		
TagRFP057		CIRGVNFPSN KEICVNEDSD	GPVM-QKKI	LGWEAR IEMLI	PRDGGLEG		CCHVI.
earp650		TNCVNFDSN	GPVM-OKKT	LGWEPSIERNI I.GWEDSTERNI.V			CCVLH
eqFP670	T.TYNVI	TNGVNFPSN	GPVM-OKKT	LGWEANTEMLY	PADSGLRG		GGYLH
mPlum	FIYKV	VRGTNFPSD	GPVM-OKKT	MGWEASTERMY	PEDGALKC	EMKMRLRLKC	GGHYD
mRouge	FIYKV	KLRGTNFPSD	GPVM-OKKT	MGWEA <mark>C</mark> SERMY	PEDGALKO	EMKMRLKLKE	GGHYD
RFP639	LVYHAP	KVTGVNFPSN	GAVM-QKKT	KGWEP <mark>S</mark> TEMLY	PADGGLRG	y <mark>c</mark> q <mark>m</mark> alnvdg	GGYLF
	180	190	200	210	220	230	
<b>275</b>							
GF'P	ADHYQQN'	PIGD-GPVL	LPDNHYLS'I'	QSALSKDPNEK		TAAGITHGML	ELYK
mveptune	CNLKTTYF	KSKKPAKNLK.	MPGVYFVDR	RLERIKEADKE			LGHKLN
IAGKEP05/	CINFRITIY	KOKKPAKNLK.		KDEKIKEADKE VIDITCUMEDV	I - I V E Q H E V F	VARICULPSK	.пенкти
		MARKEN KNIK	MDGEVENDD	KI'EB IKEVUKE VIITI I SUNFDI		WARVCDI.Dev	TCHC
		SCKKDVKNI'K.	MPGFYFVDR	KIESIKEVDKE		VARYCUI.DCK	LGHS
mPlum		AKKPVO	LPGAYKTDI	KLDITSHNEDY	T-IVEOYER	EGRHSTGGM	ELYK
mRouge	AEVKTTYF	(AKKPVO	LPGAYNTNY	KLDITSHNEDY	T-IVEOYERN		ELYK
RFP639	CS <mark>F</mark> ETTYF	RSKKTDENFK	MPGFHF <mark>V</mark> DH	RLERLEESDKE	M-FVVQHEH <mark>Z</mark>	VAKFCDLPSK	LGRL

# (F) Large Stokes shift-like phenotype

		10	20	30	40	50	
GFP	MSKGEELF	TĠVVPILV	ELDGDVNGH	KFSVSGEGEGD	ATYĠKLTLKF	'ICTTGKLPVP'	WP
LSSmKate1	MSELT	TENMHMKI	YMEGTVNNH	HEKCTSEGEGK	PYEGTOTMRT	KVVEGGPLPFA	FD
LSSmKate2	MSFLT	TENMHMET	VMFCTVNNH	HEKCTSEGEGK	DVFCTOTMRT	KWVFCCDLDFA	 הח
$mV_{ata} / 159C / 160E$	MORT	TENMUMUT		UEVOTOEOEOK		YUN ECCDIDEN	
mKate/130G/100E	MGELI			HF KCISEGEGK	PIEGIQIMAI		F D TTD
mkate/143D/158V/160S	MSELI	KENMHMKL	YMEGTVNNH.	HFKCTSEGEGK	PYEGTQTMRI	.KVVEGGPLPFA	FD
mNeptune/158D	MSELI	KENMHMKL	YMEG'I'VNNH.	HFKCTSEGEGK	DAFG.I.Ö.I.GKT	.KVVEGGPLPFA	F'D
mCherry/158E/160A SKG	GEEDNMAII	KEFMRFKV	HMEGSVNGH	EFEIEGEGEGR	PYEGTQTAKL	KVTKGGPLPFA	WD
mStrawberry/158D VSK	GEENNMAII	KEFMRFKV	RMEGSVNGH	EFEIEGEGEGR	PYEGTQTAKL	KVTKGGPLPFA	WD
mOrange/158D/160G SK	GEENNMAII	KEFMRFKV	RMEGSVNGH	EFEIEGEGEGR	PYEGFQTAKL	KVTKGGPLPFA	WD
mKO/158E	MVSVI	KPEMKMRY	YMDGSVNGH	EFTIEGEGTGR	PYEGHQEMTL	RVTMAKGGPMPFA	FD
mKeima	MVSVI	AKQMTYKV	YMSGTVNGH	YFEVEGDGKGK	PYEGEQTVKL	TVTKGGPLPFA	WD
	60	70	80	90	100	110	
	**	*					
GFP	TLVTTFSY	GVOCFSRY	PDHMKOHDF	FKSAMPĖGYVO	ERTIFFKDDG	NYKTRAEVKFEGD	Т
LSSmKatel	ILATSFMY	GS <mark>Y</mark> TFINH	TOGIPDF	FKOSFPEGFTW	ERVTTYEDGG	VLTATODTSLODG	С
LSSmKate2	TLATSFMY	GSYTEINH	TOGTPDF	FKOSEPEGETW	ERVTTYEDGG	VI TATODTSLODG	C
$mK_{ate}/158G/160F$	TLATCEMV	COTITING	TOCIP-DF	FKOSEDECETW	FRUTTVFDCC		C C
$mV_{2} = 0.112D / 150U / 160C$	TIATOPMY	CONTETNU	TQGIP DP.	FROGREGETW	ERVITIEDGC		C C
$m_{1} = \frac{1}{1} = \frac{1}{1$	TLAISPMI	GONTEINE	TQGIP-DF.	FROGEDECETW	ERVITIEDGG		a
	LAICFMI	GSKIFINH	IQGIPDF	FRQSFPEGFIW	ERVIIIEDGG		
mCherry/158E/160A	TT2bdf.wa	GSKAYVKH	PADIPDY.	LKLSFPEGFKW	ERVMNFEDGG	WVTVTQDSSLQDG	E:
mStrawberry/158D	ILTPNFTY	GSKAYVKH	PADIPDY	LKLSFPEGFKW	ERVMNFEDGG	;vvtvtqdsslqdg	E
mOrange/158D/160G	ILSPQFTY	'GS <mark>K</mark> AYVKH	PADIPDY	FKLSFPEGFKW	ERVMNFEDGG	VVTVTQDSSLQDG	Ε
mKO/158E	LVSHVFCY	GH <mark>R</mark> PFTKY	PEEIPDY	FKQAFPEGLSW	ERSLEFEDGG	SASVSAHISLRGN	Т
mKeima	ILSPQLQY	GS <mark>I</mark> PFTKY	PEDIPDY	FKQSFPEGYTW	ERSMNFEDGA	VCTVSNDSSIQGN	С
-	120	130	140	150	160	170	
CFD							LА
GLI	LVNRIELK	GIDFKEDG	NILGHKLEY	NYNSHNVYLMA	DKQKNGIKVN	IFKIRHNIEDGSVQ	
LSSmKatel	LVNRIELK LIYNVKIR	GIDFKEDG GVNFTSNG	NILGHKLEY PVM-QKKTL	NYNSHNVYIMA Gwea <mark>g</mark> temlyp	DKQKNGIKVN ADGGLEGR	IFKIRHNIEDGSVQ SDEALKLVGGGHL	IC
LSSmKate1 LSSmKate2	LVNRIELK LIYNVKIR LIYNVKIR	GIDFKEDG GVNFTSNG GVNFTSNG	NILGHKLEY PVM-QKKTL PVM-OKKTL	NYNSHNVYIMA GWEA <mark>G</mark> TEMLYP GWEAGTEMLYP	DKQKNGIKVN ADGGLEGR ADGGLEGR	IFKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL	IC IC
LSSmKate1 LSSmKate2 mKate/158G/160E	LVNRIELK LIYNVKIR LIYNVKIR	GIDFKEDG GVNFTSNG GVNFTSNG GVNFPSNG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-OKKTL	NYNSHNVYIMA Gwea <mark>g</mark> temlyp Gwea <mark>g</mark> temlyp Gwea <mark>s</mark> temlyp	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL	IC IC IC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR	GIDFKEDG GVNFTSNG GVNFTSNG GVNFPSNG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL	NYNSHNVYIMA GWEAGTEMLYP GWEAGTEMLYP GWEASTEMLYP GWEADTEMLYP	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL GDEALKLVGGGHL	IC IC IC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptupe/158D	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR	GIDFKEDG GVNFTSNG GVNFTSNG GVNFPSNG GVNFPSNG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL	NYNSHNVYIMA GWEA <mark>G</mark> TEMLYP GWEAGTEMLYP GWEASTEMLYP GWEADTEMLYP GWEASTETLYD	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL GDEALKLVGGGHL VDSALKLVGGGHL	IC IC IC IC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR	GIDFKEDG GVNFTSNG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL	NYNSHNVYIMA GWEAGTEMLYP GWEAGTEMLYP GWEASTEMLYP GWEADTEMLYP GWEASTETLYP	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL GDEALKLVGGGHL VDSALKLVGGGHL SDMALKLVGGGHL	IC IC IC IC IC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKLR	GIDFKEDG GVNFTSNG GVNFTSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL	NYNSHNVYIMA GWEAGTEMLYP GWEAGTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL VDSALKLVGGGHL SDMALKLVGGGHL KARLKLKDGGHY	IC IC IC IC DA
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKLR FIYKVKLR	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM	NYNSHNVYIMA GWEAGTEMLYP GWEAGTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DDALKLVGGGHL SALKLVGGGHL KARLKLKDGGHY DKMRLKLKDGGHY	IC IC IC IC DA DA
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKLR FIYKVKLR FIYKVKLR	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DDALKLVGGGHL DMALKLVGGGHL KARLKLKDGGHY DKMRLKLKDGGHY	IC IC IC IC DA DA TS
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GVNFPADG	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE EDGALKGE	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DDALKLVGGGHL NDSALKLVGGGHL KARLKLKDGGHY DKMRLKLKDGGHY DKGRLKLKDGGHY	IC IC IC IC DA DA TS KC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR FIYNVKIR FIYKVKLR FIYKVKLR FYHKSKFT FYHKSKFT	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GVNFPADG GVNFPADG	PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTQ	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTERLFA	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DDALKLVGGGHL SALKLVGGGHL KARLKLKDGGHY DKMRLKLKDGGHY DKMRLKLEGGGNH IDYMALKLEGGGHY	IC IC IC IC DA DA TS KC LC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR FIYNVKIR FIYKVKLR FIYKVKLR FYKVKLR FYHKSKFT FIYNVKIS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190	PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PIM-QNQSV PVM-QKKTQ 200	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTERIFA 210	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN 220	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DMALKLVGGGHL KARLKLKDGGHY DKMRLKLKDGGHY DKMRLKLKDGGHY DKJKLKLEGGGNH DYMALKLEGGGHY 230	IC IC IC DA DA TS KC LC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKIR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190	PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM 200 	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTERIFA 210	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN 220	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DMALKLVGGGHL KARLKLKDGGHY SKARLKLKDGGHY SKARLKLKDGGHY DKORLKLKDGGHY 200 FTMYLKLEGGGNH 230	IC IC IC DA DA TS KC LC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR FIYNVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30   DHYQQNTP	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM 200 LPDNHYLST	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTERIFA 210	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN 220   RDHMVLLEFV	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DMALKLVGGGHL KARLKLKDGGHY SKRLKLKDGGHY DKGRLKLKDGGHY 20 KGRLKLEGGGNH MALKLEGGGHY 230   TAAGITHGMDELY	IC IC IC DA DA TS KC LC
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR IIYNVKIR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30   DHYQQNTP NLK <mark>S</mark> TYRS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM 200 LPDNHYLST VPGVYYVDR	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTERLFA 210   QSALSKDPNEK RLERIKEADKE	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DMALKLVGGGHL KARLKLKDGGHY SKRLKLKDGGHY DKGRLKLKDGGHY 230   TAAGITHGMDELY VARYCDLPSKLGH	IC IC IC IC DA DA TS KC LC K KLN
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate1 LSSmKate2	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR FIYNVKIR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30   DHYQQNTP NLKSTYRS NLKSTYRS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTO 200 LPDNHYLST VPGVYYVDR	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTEKITA 210   QSALSKDPNEK RLERIKEADKE	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL DMALKLVGGGHL KARLKLKDGGHY SKRLKLKDGGHY 20KRLKLKDGGHY 230   TAAGITHGMDELY VARYCDLPSKLGH	IC IC IC IC DA TS KC LC K KLN KLN
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate1 LSSmKate2 mKate/158G/160	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR FIYNVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30 DHYQQNTP NLKSTYRS NLKSTYRS NLKTTYRS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM 200 LPDNHYLST VPGVYYVDR MPGVYYVDR	NYNSHNVYIMA GWEAGTEMLYP GWEAGTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTEKITA 210   QSALSKDPNEK RLERIKEADKE RLERIKEADKE	DKQKNGIKVN - ADGGLEGR - ADGGLEGR - ADGGLEGR - ADGGLEGR - ADGGLEGR - EDGALKGE - EDGALKGE - EDGALKGE - EDGALKGE - SDGVLKGD - RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL SALKLVGGGHY SARLKLKDGGHY CKRLKLKDGGHY 230   TAAGITHGMDELY VARYCDLPSKLGH VARYCDLPSKLGH	IC IC IC DA DA TS KC LC K KLN KLN KLN
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate1 LSSmKate2 mKate/158G/160 mKate/143D/158V/160S	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR FIYNVKIR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30 DHYQQNTP NLKSTYRS NLKSTYRS NLKTTYRS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTO 200 LPDNHYLST VPGVYYVDR MPGVYYVDR	NYNSHNVYIMA GWEAGTEMLYP GWEAGTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA 210   QSALSKDPNEK RLERIKEADKE RLERIKEADKE RLERIKEADKE	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLKDGGHY CDEARLKLKDGGHY CDEARLKLKDGGHY 230   TAAGITHGMDELY VARYCDLPSKLGH VARYCDLPSKLGH	IC IC IC IC DA DA TS KC LC K KLN KLN KLN KLN
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate1 LSSmKate2 mKate/158G/160 mKate/143D/158V/160S mNeptune/158D	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR IIYNVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30 DHYQQNTP NLKSTYRS NLKSTYRS NLKTTYRS NLKTTYRS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GTNFPSDG GVNFPADG EENFPPNG 190   IGD-GPVL KKPAKNLK KKPAKNLK KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTO 200 LPDNHYLST VPGVYYVDR MPGVYYVDR MPGVYYVDR	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA 210 QSALSKDPNEK RLERIKEADKE RLERIKEADKE RLERIKEADKE	DKQKNGIKVN - ADGGLEGR - ADGGLEGR - ADGGLEGR - ADGGLEGR - ADGGLEGR - EDGALKGE - EDGALKGE - EDGALKGE - EDGALKGE - SDGVLKGD - RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SALKLVGGGHL SARLKLKDGGHY SKRLKLKDGGHY 230   TAAGITHGMDELY VARYCDLPSKLGH VARYCDLPSKLGH	IC IC IC IC DA DA TS KC LC K KLN KLN KLN KLN KLN
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate1 LSSmKate2 mKate/158G/160 mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30 DHYQQNTP NLKSTYRS NLKSTYRS NLKTTYRS NLKTTYRS SNLKTTYRS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK KKPAKNLK KKPAKNLK KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTQ 200 LPDNHYLST VPGVYYVDR MPGVYYVDR MPGVYYVDR MPGVYYVDR 1.PGAYNVNT	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA 210 QSALSKDPNEK RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE EDGALKGE SDGVLKGD -RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHY CDEARLKLKDGGHY COMPLEXCOG COMPLEX COMPLE	IC IC IC IC DA DA TS KC LC K KLN KLN KLN KLN KLN KLN
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate1 LSSmKate2 mKate/158G/160 mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30 DHYQQNTP NLKSTYRS NLKSTYRS NLKTTYRS NLKTTYRS SNLKTTYRS	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK KKPAKNLK KKPAKNLK KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTQ 200 [ LPDNHYLST VPGVYYVDR MPGVYYVDR MPGVYYVDR LPGAYTVCT	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSER GWEASSER GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSER GWE	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE EDGALKGE SDGVLKGE RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLVGGGHL CDEALKLKDGGHY CDETMYLKLEGGGNH CHARLKLEGGGNH CHARCOLPSKLGH VARYCDLPSKLGH VARYCDLPSKLGH VARYCDLPSKLGH VARYCDLPSKLGH CARYCDLPSKLGH	IC IC IC IC DA DA TS KC LC K KLN KLN KLN KLN KLN K K K K K K K
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate2 mKate/158G/160 mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30 DHYQQNTP NLKSTYRS NLKSTYRS NLKTTYRS NLKTTYRS NLKTTYRS EVKTTYKA	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK KKPAKNLK KKPAKNLK KKPAKNLK KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTQ 200 LPDNHYLST VPGVYVVDR MPGVYVVDR MPGVYVVDR LPGAYIVGI LPGAYIVGI	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTETLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA GWEPSTEKITA 210 QSALSKDPNEK RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE EDGALKGE SDGVLKGE SDGVLKGE RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SALKLVGGGHL SALKLVGGGHL SKARLKLKDGGHY SKRLKLKDGGHY 230   TAAGITHGMDELY VARYCDLPSKLGH VARYCDLPSKLGH VARYCDLPSKLGH VARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH	IC IC IC IC DA TS KC LC K KLN KLN KLN KLN KLN K KLN K K K K
LSSmKate1 LSSmKate2 mKate/158G/160E mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G mKO/158E mKeima 18 GFP LSSmKate1 LSSmKate1 LSSmKate2 mKate/158G/160 mKate/143D/158V/160S mNeptune/158D mCherry/158E/160A mStrawberry/158D mOrange/158D/160G	LVNRIELK LIYNVKIR LIYNVKIR LIYNVKIR LIYNVKIR FIYKVKIR FIYKVKLR FIYKVKLR FIYKVKLR FYHKSKFT FIYNVKIS 30 DHYQQNTP NLKSTYRS NLKTTYRS NLKTTYRS NLKTTYRS NLKTTYRS EVKTTYKA EVKTTYKA	GIDFKEDG GVNFTSNG GVNFPSNG GVNFPSNG GVNFPSNG GTNFPSDG GTNFPSDG GTNFPSDG GVNFPADG GENFPPNG 190   IGD-GPVL KKPAKNLK KKPAKNLK KKPAKNLK KKPAKNLK KKPAKNLK KKPAKNLK	NILGHKLEY PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTL PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTM PVM-QKKTQ 200 LPDNHYLST VPGVYVVDR MPGVYVVDR MPGVYVVDR LPGAYIVGI LPGAYIVGI LPGAYIVGI	NYNSHNVYIMA GWEAGTEMLYP GWEASTEMLYP GWEASTEMLYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP GWEASSERMYP DWEPSTEKITA 210 QSALSKDPNEK RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE RLERIKEADKE	DKQKNGIKVN ADGGLEGR ADGGLEGR ADGGLEGR ADGGLEGR EDGALKGE EDGALKGE EDGALKGE EDGALKGE SDGVLKGD RDGMLIGN 220   RDHMVLLEFV T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-YVEQHEVA T-IVEQYERA T-IVEQYERA T-IVEQYERA	FKIRHNIEDGSVQ SDEALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SDDALKLVGGGHL SALKLVGGGHL SALKLVGGGHL SKARLKLKDGGHY SKRLKLKDGGHY 230   TAAGITHGMDELY VARYCDLPSKLGH VARYCDLPSKLGH VARYCDLPSKLGH VARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH SARYCDLPSKLGH	IC IC IC IC DA TS KC LC K KLN KLN KLN KLN KLN K K K K K K K K K
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# (G) High quantum yield

GFP MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPI rsTagRFP MVSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKVVEGGPLPFAI KFP1 MASLLTFTMPEKTTIFGTVNGHCFKCICKGEGNPEFGTOFMKIFVIEGGPLPFAI	
GFP MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPI   rsTagRFP MVSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKVVEGGPLPFAI   KFP1 MASLLTFTMPEKTTIFGTVNGHCFKCICKGEGNPEFGTOFMKIFVIEGGPLPFAI	
rsTagRFP MVSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKVVEGGPLPFAI	٧P
	FD
	FH
asFP595_70A/148S MASFLKKTMPFKTTIEGTVNGHYFKCTGKGEGNPFEGTQEMKIEVIEGGPLPFA	FH
HcRed1 MVSGLLKESMRIKMYMEGTVNGHYFKCEGEGDGNPFAGTQSMRIHVTEGAPLPFAJ	FD
mCherry MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA	ND
mKate2 MVSELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKAVEGGPLPFAJ	FD
mOrange MVSKGEENNMAIIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGFQTAKLKVTKGGPLPFA	ND
TagRFP MVSKGEELIKENMHMKLYMEGTVNNHHFKCTSEGEGKPYEGTQTMRIKVVEGGPLPFAJ	FD
60 70 80 90 100 110	
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GFP TLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGJ	ЭT
rsTagRFP ILATSFMYGSRTFINHTQGIPDFWKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDG	ЭС
KFP1 ILSTSCMYGSK <mark>T</mark> FIKYVSGIPDYFKQSFPEGFTWERTTTYEDGGFLTAHQDTSLDGJ	C
asFP595_70A/148S ILSTSCMYGSKAFIKYVSGIPDYFKQSFPEGFTWERTTTYEDGGFLTAHQDTSLDG	C
HcRed1 ILAPCCEYGSRTFVHHTAEIPDFFKQSFPEGFTWERTTTYEDGGILTAHQDTSLEGI	NC
mCherry ILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD(	ΞE
	ЭС
IIIAISEMIGSKIFINHIQGIPDEEKQSEPEGEIWERVIIIEDGGVLIAIQDISLQDO	
mOrange ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVUTVTQDSSLQDC	ΞE
mOrange ILATSFMYGSKTFINHTQGIPDFFKQSFPEGFIWERVIIYEDGGVLIATQDISLQD TagRFP ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQD	JE JC
mkate2   ILAISFMYGSKIFINHIQGIPDFFKQSFPEGFIWERVIIYEDGGVLIAIQDISLQD     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQD	JE JC
mixacte2   ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVIIYEDGGVLIAIQDISLQD/     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD/     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDISLQD/     120   130   140   150   160   170	∃E }C
mkate2   ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVIIYEDGGVLIAIQDISLOD     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDISLQD     120   130   140   150   160   170	GE GC
mkate2   ILAISFMYGSKIFINHIQGIPDFFKQSFPEGFIWERVIIYEDGGVLIAIQDISLQD     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQD     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ	GE GC )L
mkate2   ILAISFMYGSKIFINHIQGIPDFFKQSFPEGFIWERVIIYEDGGVLIAIQDISLQD     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQD     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ   rstagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAA   TEMLYPADGGLEGRGDMALKLVGGGHI	JE JC JL
mkate2   ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVIIYEDGGVLIAIQDISLQD(     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD(     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQD(     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV(   rstagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     KFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI	GE GC JL JI
mkate2   ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVTIYEDGGVLTATQDISLQD(     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD(     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDISLQD(     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     KFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRQSLMALKCPGGRHI     asFP595_70A/148s   LVYKVKILGNNFPADGPVM-QNKAGRWEPGTEIVYEVDGVLRQSLMALKCPGGRHI	GE GC JL JI JT
mkate2   ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVTIYEDGGVLTATQDISLQD     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFIWERVTTYEDGGVLTATQDISLQD     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFIWERVTTYEDGGVLTATQDISLQD     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     KFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     asFP595_70A/148s   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     HcRed1   LIYKVKVLGTNFPADGPVM-KNKSGGWEPSTEVYP-ENGVLCGRNVMALKV-GDRHI	GE GC JL JT JT JT
mNrate2   ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVTIYEDGGVLTATQDISLQDC     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDC     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDISLQDC     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     KFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRQQSLMALKCPGGRHI     asFP595_70A/148s   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRQQSLMALKCPGGRHI     HcRed1   LIYKVKVLGTNFPADGPVM-KNKSGGWEPSTEVYPENGVLCGRNVMALKV-GDRHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHY	GE GC JL JI JI JI JI (D
ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVTIYEDGGVLTATQDISLQDC     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFIWERVTIYEDGGVLTATQDISLQDC     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFIWERVTTYEDGGVLTATQDISLQDC     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     KFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     HcRed1   LIYKVKVLGTNFPADGPVM-QKKTMGWEASSERMYPENGVLCGRNVMALKV-GDRHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHY     mKate2   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI	GE GC LL LT LT LT LT LT LT
ILAISFMYGSKTFINHIQGIPDFFKQSFPEGFIWERVTIYEDGGVLTATQDISLQD(     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD(     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDISLQD(     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV(     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     kFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     HcRed1   LIYKVKVLGTNFPADGPVM-QKKTMGWEASSERMYPENGVLCGRNVMALKV-GDRHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     mKate2   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKMRLKLKDGGHI	GE GC LL LT LT LT LT LT LT
ILATSFMIGSKTFINHIQGIPDFFKQSFPEGFIWERVTIYEDGGVLIATQDISLQD(     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD(     TagRFP   ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDISLQD(     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV(     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     kFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     hCred1   LIYKVKULGTNFPADGPVM-QNKAGRWEPSTEIVYPENGVLCGRNVMALKV-GDRHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     mKate2   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     TagRFP   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI	GE GC LL LT LT LT LT LT LT LT LT LT
ILAISFMYGSKIFINHIQGIPDFFKQSFPEGFIWERVITYEDGGVLTAIQDISLOD     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFIWERVITYEDGGVLTAIQDISLQD     TagRFP   ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFIWERVTTYEDGGVLTATQDTSLQD     120   130   140   150   160   170                                       GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV(     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     KFP1   LVYKVKILGNNFPADGPVM-QNKAGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     HCRed1   LIYKVKVLGTNFPADGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     mKate2   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     TagRFP   LIYNVKIRGVNFPSNGPVM-QKKTLGWEANTEMLYPADGGLEGRSDMALKLVGGGHI	GE GC JL JT JT JI JI JI JI
ILATSFMYGSKIFTNHIGGTPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDX     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDX     TagRFP   ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDX     120   130   140   150   160   170                                       GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV(     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKAGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     hcred1   LIYKVKVLGTNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKORLKLKDGGHI     mKate2   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     TagRFP   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     180   190   200   210   220   230	GE GC L L L L L L L L L L L L L L L L L L
ILAISFMIGSKIFINHIGGIPDFFKQSFPEGFIWERVIITEDGGVLIAIODISLOD     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDO     TagRFP   ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDO     120   130   140   150   160   170                                       GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     ASFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     hCred1   LIYNVKVLGTNFPADGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     morange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI     morange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     180   190   200   210   220   230     180   190   200   210   220   230	GE GC LL LT LT LT LT LT LT LT
IILATSFMYGSKIFTINHTQGIPDFFKQSFPEGFTWERVTTIEDGGVITATQDISLQDX     mOrange   ILSPQFTYGSKAYVKHPADIPDFFKQSFPEGFTWERVTTYEDGGVUTVTQDSSLQDX     TagRFP   ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDX     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNYYIMADKQKNGIKVNFKIRHNIEDGSVG     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHJ     KFP1   LYYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRQSLMALKCPGGRHJ     asFP5955_70A/1485   LYYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRQSLMALKCPGGRHJ     mCherry   FIYKVKLRGTNFPADGPVM-QKKTMGWEASSERMYPENGVLCGRNVMALKV-GDRHJ     mCharry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHJ     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASTETIYPADGGLEGRADMALKLVGGGHJ     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASTETIYPADGGLEGRADMALKLVGGGHJ     mOrange   FIYKVKLRGVNFPSNGPVM-QKKTLGWEANTEMLYPADGGLEGRSDMALKLVGGGHJ     180   190   200   210   220   230                                   GFP   ADHYQQNTPIGD-GPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDEI	GE GC L L L L L L L L L L L L L L L L L L
ILAISFMIGSKIFINHUGIPDFRQSFPEGFIWERVITIEDGGVLIAIDDISLDOK     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDOK     TagRFP   ILAISFMIGSKIFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDOK     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVG     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEATEMLYPADGGLEGRGDMALKLVGGGHJ     KFP1   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHJ     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHJ     HcRed1   LIYKVKVLGTNFPADGPVM-QNKAGRWEPSTEIVYEDGALKGEIKQRLKLKDGGHJ     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTGWEASSERMYPEDGALKGEIKQRLKLKDGGHJ     mKate2   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHJ     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPADGGLEGRADMALKLVGGGHJ     TagRFP   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPADGGLEGRSDMALKLVGGGHJ     GFP   ADHYQQNTPIGD-GPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDEJ     rstagRFP   LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPADGGLEGRSDMALKLVGGGHJ	GE GC L L L L L L L L L L L L L L L L L L
ILAISPMIGSKIPINELGIPDYFKQSFPEGFIWERVITIEDGGVLTATQDISLQDA     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDA     TagRFP   ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDA     120   130   140   150   160   170                                   GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV(     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     asFP595_70A/148S   LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     hCRed1   LIYKVKVLGTNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRGQSLMALKCPGGRHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKQRLKLKDGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     morange   FIYKVKLRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMALKLVGGGHI     morange   FIYKVKLRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     GFP   ADHYQQNTPIGD-GPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDEI     rstagRFP	GE GC L L L L L L L L L L L L L L L L L L
ILATSFMIGSKIFINHIGGEPDFFKQSFPEGFIMEKVITTEDGGVITATQDISLQDA mOrange ILSPQFTYGSKAYVKHPADIPDFFKQSFPEGFIMERVITTEDGGVITATQDISLQDA TagRFP ILATSFMIGSKIFINHIQGIPDFFKQSFPEGFIMERVITTEDGGVITATQDISLQDA 120 130 140 150 160 170 120 120 120 120 160 170 120 120 120 160 170 120 120 120 160 170 120 120 120 160 170 120 120 120 170 120 120 120 160 170 120 120 120 170 120 120 120 170 120 120 120 170 120 120 120 220 230 120 120 220 230 120 120 220 230 130 190 200 210 220 230 130 190 200 210 220 230 140 1 1 1 140 1 1 1 157 agRFP LIYNVKIRGVNFPSNGPVM-QKKTIGWEASSERMYPEDGALKGEIKMRLKLKDGGHI 180 190 200 210 220 230 10 1 1 1 1 1 10 1 1 10 1 1 10 1 1 10	GE GC L L L L L L L L L L L L L L L L L L
ILATSPMIGSKIFINNTOGREFINNTOGREFINERVERVERVERVERVERVERVERVERVERVERVERVERVE	GE GC L L L L L L L L L L L L L L L L L L
ILAISPMIGSKIPINHUGSKP-DFFKQSFPEGFIWERVITTEDGGVITATQDTSLQDA     mOrange   ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDA     TagRFP   ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVITATQDTSLQDA     120   130   140   150   160   170                                       GFP   LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV(     rsTagRFP   LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI     kFP1   LVYKVKILGNNFPADGPVM-QKKTGRWEPGTEIVYEVDGVLRGQSLMALKCPGGRHI     asFP595_70A/1485   LVYKVKLGTNFPADGPVM-QKKTGWEASSERMYPEDGALKGEIKORLKLKDGGHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKORLKLKDGGHI     mCherry   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKORLKLKDGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     mOrange   FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     morange   FIYKVKLRGTNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     morange   FIYKVKLRGTNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     morange   FIYKVKLRGTNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHI     morange   FIYKVKLRGTNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMARLKLKDGGNI	GE GC L L L L L L L L L L L L L L L L L L
ILAISFMIGSALEINHIQGIPDFFAQSFPEGFINERVIIHEDGGVLIAIQDISLQM mOrange ILSPQFTYGSKAYVKHPADIPDFFKQSFPEGFINERVITYEDGGVLIAIQDISLQM TagRFP ILAISFMYGSRTFINHTQGIPDFFKQSFPEGFINERVITYEDGGVLIAIQDISLQM 120 130 140 150 160 170                   GFP LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV( rsTagRFP LIYNVKLRGVNFPSNGPVM-QKKTLGWEAATEMLYPADGGLEGRGDMALKLVGGGHI asFP5955_70A/148S LVYKVKILGNNFPADGPVM-QNKVGRWEFGTEIVYEVDGVLRGQSLMALKCPGGRHI hcRed1 LIYKVKVLGNNFPADGPVM-QNKVGRWEFGTEIVYEVDGVLRGQSLMALKCPGGRHI mCherry FIYKVKLRGTNFPADGPVM-QKKTMGWEASSERMYPEDGALKGEIKORLKLKDGGHT mKate2 LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKORLKLKDGGHT mGrange FIYKVKLRGTNFPSDGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHT TagRFP LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHT mStagFP LIYNVKIRGVNFPSNGPVM-QKKTLGWEASSERMYPEDGALKGEIKMRLKLKDGGHT 180 190 200 210 220 230               GFP ADHYQQNTPIGD-GPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDEI rsTagRFP CNLKTYRSKNPAKNLKMPGYFVDHRLERIKEADKET-YVEQHEVAVARYCDLPSKLK KFP1 CHLHTTYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKI HCRed1 CHHYTSYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKI HCRed1 CHHYTSYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKI HCRed1 CHHYTSYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKI HCRed1 CHHYTSYRSKKPAKNLKMPGVYYVQRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG mCherry AEVKTTYKSKKPAKNLKMPGVYYVRRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG MCherry AEVKTTYKSKKPAKNLKMPGVYVVRRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG MCherry AEVKTTYKSKKPAKNLKMPGVYVVRRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG MCherry AEVKTTYKSKKPAKNLKMPGVYVVRRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG MCherry AEVKTTYKSKKPAKNLKMPGVYVVRRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG MCherry AEVKTTYKSKKPAKNLKMPGVYVVRRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG MCherry AEVKTTYKSKKPAKNLKMPGVYVVRRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG	GE GC QL LT LT LT LT LT LT LT LT LT LT LT LT LT
ILAISFMIGSARITINHIGGIPDFFAQSFPEGFIREAVIIADGSVLIAADDSLQD mOrange ILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFIREAVITYEDGGVLIAADDSLQD TagRFP ILATSFMYGSRTFINHTQGIPDFFKQSFPEGFIRERVTTYEDGGVLTATQDTSLQD 120 130 140 150 160 170               GFP LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVG rsTagRFP LIYNVKLRGVNFPSNGPVM-QKKTLGWEATTEMLYPADGGLEGRGDMALKLVGGGHI KFP1 LVYKVKILGNNFPADGPVM-QNKVGRWEPGTEIVYEVDGVLRQQSLMALKCPGGRHI asFP595_70A/148S LVYKVKILGNNFPADGPVM-QNKAGRWEPSTEIVYEVDGVLRQQSLMALKCPGGRHI HCRed1 LIYKVKVGGTNFPADGPVM-QNKKTGWEASSERMYPEDGALKGEIKORLKLKDGGHT mKate2 LIYNVKIRGVNFPSNGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI mOrange FIYKVKLRGTNFPSDGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI TagRFP LIYNVKIRGVNFPSNGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI MCTange FIYKVKLRGTNFPSDGPVM-QKKTLGWEASTETLYPADGGLEGRADMALKLVGGGHI 180 190 200 210 220 230               GFP ADHYQQNTPIGD-GPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGTHGMDEI rsTagRFP CNLKTTYRSKNPAKNLKMPGVYFVDHRLERIKEADKET-YVEQHEVAVARYCDLPSKLG KFP1 CHLHTYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKI asFP595_70A/148S CHLHTYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKI mCherry AEVKTTYKAKKPVQLPGAYIVQRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG mCherry AEVKTTYKAKKPVQLPGAYIVGIKLDITSHNEDYT-IVEQYERAEGRHSTGGMDEI MCate2 CNLKTTYRSKKPAKNLKMPGVYFVDRRLERIKEADKET-YVEQHEVAVARYCDLPSKLG mOrange SEVKTTYKAKKPVQLPGAYIVGIKLDITSHNEDYT-IVEQYERAEGRHSTGGMDEI	GE GC QL LT LT LT LT LT LT LT LT LT LT LT LT LT

#### (H) High photostability

		10	20	30	40	50	
GFP	MSKGE	ELFTGVVPIL	VELDGDVNGH	KFSVSGEGEG	DATYGKLTLK	FICTTGK	LPVPWP
TagRFP-T	MVSKGE	ELIKENMHMK	LYMEGTVNNH	HFKCTSEGEG	KPYEGTQTMR	IKVVEGGP	LPFAFD
mOrange2	MVSKGEENNM	IAIIKEFMRFK	VRMEGSVNGH	EFEIEGEGEG	RPYEGFQTAK	LKVTKGGP	LPFAWD
mKate2	MVS	SELIKENMHMK	LYMEGTVNNH	HFKCTSEGEG	KPYEGTQTMR	IKAVEGGP	LPFAFD
	60	70	80	90	100	110	
		***	_ ! _				_
GFP	TLVTI	FSYGVQCFSR	YPDHMKQHDF	FKSAMPEGYV	QERTIFFKDD	GNYKTRAEVK	FEGDT
TagRFP-T	ILATS	FMYGSRTFIN	HTQGIPDF	FKQSFPEGFT	WERVTTYEDG	GVLTATQDTS	LQDGC
mOrange2	ILSPE	F"I'YGSKAYVK	HPADIPDY	FKLSFPEGFK	WERVMNYEDG	GVVTVTQDSS	LQDGE
mKate2		F'MYGSK'I'F'IN	H.I.ÖĞT D – D.F.	FKQSFPEGFT	MERAIL <b>A</b> EDG	GVLTATQDTS	LQDGC
	120	130	140	150	160	170	
	1					_ / 0	
GFP	LVNRI	ELKGIDFKED	GNILGHKLEY	NYNSHNVYIM	ADKOKNGIKV	NFKIRHNIED	GSVOL
TagRFP-T	LIYNV	KIRGVNFPSN	GPVM-QKKTL	G <mark>WEA</mark> NTEMLY	PADGGLEG	R <mark>T</mark> DMALKLVG	GGHLI
mOrange2	FIYKV	KLRGTNFPSD	GPVM-QKKTM	IG <mark>WEA</mark> SSERMY	PEDGALKG	K <mark>I</mark> KMRLKLKD	GGHYT
mKate2	LIYNV	KIRGVNFPSN	GPVM-QKKTL	G <mark>WEA</mark> STETLY	PADGGLEG	r <mark>a</mark> dmalklvg	GGHLI
	180	190	200	210	220	230	
GFP	ADHYQQN	TPIGD-GPVL	LPDNHYLSTQ	SALSKDPNEK	RDHMVLLEFV	TAAGITHGMD	ЕГАК
TagRFP-T	CNFKTTY	RSKKPAKNLK	MPGVYYVDHR	LERIKEADKE	T-YVEQHEVA	VARYCDLPSK	LGHKLN
mOrange2	SEVKTTY	KAKKPVQ	LPGAYIVDIK	LDITSHNEDY	T-IVEQYERA	EGRHSTGGMD	ЕГАК
mKate2	CNLKTTY	RSKKPAKNLK	MPGVYYVDRR	LERIKEADKE	'I'-YVEQHEVA	VARYCDLPSK	LGHKLN

#### (I) High pH-stability

		10	20	30	40	50 I	
GFP	MSKGE	I ELFTGVVPII	LVELDGDVNGH	I IKFSVSGEGEG	I DATYGKLTLK	FICTTGK	LPVPWP
mTaqBFP	MSE	ELIKENMHMK	<b>LYMEGTVDNH</b>	IHFKCTSEGEG	KPYEGTOTMR	IKVVEGGP	LPFAFD
DsRed2	MASSE	NVITEFMRFK	<b>VRMEGTVNGH</b>	IEFEIEGEGEG	RPYEGHNTVK	LKVTKGGP	LPFAWD
	60	70	80	90	100	110	
		* * *					
GFP	TLVTI	FSYGVQCFS	RYPDHMKQHDF	FKSAMPEGYV	QERTIFFKDD	GNYKTRAEVK	FEGDT
mTagBFP	ILATS	FLYGSKTFIN	NHTQGIPDF	FKQSFPEGFT	WE <mark>R</mark> VTTYEDG	GVLTATQDTS	LQDGC
DsRed2	ILSPQ	FQYGSKVYV	KHPADIPDY	KKLSFPEG <mark>F</mark> K	WERVMNFEDG	GVATVTQDSS	LQDGC
	120	130	140	150	160	170	
GFP	LVNRI	ELKGIDFKEI	DGNILGHKLEY	NYNSHNVYIM	ADKQKNGIKV	NFKIRHNIED	GSVQL
mTagBFP	LIYNV	KIRGVNFTSN	IGPVM-QKKTI	LG <mark>WEA</mark> FTETLY	PADGGLEG	RNDMALKLVG	GSHLI
DsRed2	FIYKV	KFIGVNFPSI	DGPVM-QKKTM	1G <mark>WEA</mark> STERLY	PRDGVLKG	ETH <mark>K</mark> ALKLKD	GGHYL
						·	
	180	190	200	210	220	230	
GFP	ADHYQQN	TPIGD-GPVI	LPDNHYLSTQ	SALSKDPNEK	RDHMVLLEFV	TAAGITHGMD	ELYK
mTagBFP	ANIKTTY	RSKKPAKNLK	KMPGVYYVDYF	RLERIKEANNE	T-YVEQHEVA	VARYCDLPSK	LGHKLN
DsRed2	VEFKSIY	MAKKPVÇ	) LPGYYYVDAK	LDITSHNEDY	T-IVEQYERT	'EGRHHLFL	
		-					

## (J) Fast maturation

		10	20	30	40	50	
GFP	MSKGE	ELFTGVVPI	LVELDGDVNGH	IKFSVSGEGEC	JDATYGKLTLI	KFICTTGK	LPVPWP
mKate2	MVS	ELIKENMHM	KLY <mark>M</mark> EG <mark>T</mark> VNNH	IHFKCTSEGEO	GKPYEGT <mark>Q</mark> T <mark>M</mark> I	RIK <mark>A</mark> VEGGP	LPFAFD
mCherry	MVSKGEEDNM	AIIKEFMRF	KVH <mark>M</mark> EG <mark>S</mark> VNGH	IEFEIEGEGEC	GRPYEGT <mark>O</mark> TAI	KLK <mark>V</mark> TKGGP	LPFAWD
SlowFT	MVSKGEEDNM	AIIKEFMRF	KVH <mark>V</mark> EG <mark>S</mark> VNGH	IEFEIVGEGEC	GRPYEGT <mark>O</mark> TAI	KLK <mark>V</mark> TKGGP	LPFAWD
mRuby	MN	SLIKENMRM	kvv <mark>l</mark> eg <mark>s</mark> vngf	IQFKCTGEGEC	GNPYMGT <mark>O</mark> TMI	RIK <mark>V</mark> IEGGP	LPFAFD
	60	70	80	90	100	110	
		***					
GFP	TLVTT	FSYGVQCFS	RYPDHMKQHDF	FKSAMPEGY	QERTIFFKD	OGNYKTRAEVK	FEGDT
mKate2	ILATS	FMYGS <mark>K</mark> TFI	NHTQGIPDF	FKQSFPEGF	TWERVTTYED	GGV <mark>L</mark> TATQDTS	LQDGC
mCherry	ILSPQ	FMYGS <mark>K</mark> AYV	KHPADIPDY	LKLSFPEGF	WERVMNFED	GV <mark>V</mark> TVTQDSS	LQDGE
SlowFT	ILSPQ	FMYGS <mark>R</mark> AYV	KHPADIPDY	WKLSFPEGF	(WERVMNFED)	GV <mark>V</mark> TVTQDSS	LQDGE
mRuby	ILATS	FMYGS <mark>R</mark> TFI	KYPKGIPDF	FKQSFPEGF	TWERVTRYED	GGV <mark>I</mark> TVMQDTS	LEDGC
	120	130	140	150	160	170	
					I		
GFP	LVNRI	ELKGIDFKE	DGNILGHKLEY	NYNSHNVYIN	ADKQKNGIK	VNFKIRHNIED	GSVQL
mKate2	LIYNV	KIRGVNFPS	NGPVM-QKKTI	LG <mark>WEA</mark> STET <mark>L</mark> S	(PADGGLE)	GR <mark>A</mark> DMALKLVG	GGHLI
mCherry	FIYKV	KLRGTNFPS	DGPVM-QKKTM	IG <mark>WEA</mark> SSER <mark>M</mark> Y	PEDGALK	GE <mark>I</mark> K <mark>Q</mark> RLKLKD	GGHYD
Slow	FIYKV	KLRGTNFPS	DGPVM-QKKTM	IG <mark>WEA</mark> SSER <mark>M</mark> Y	PEDGALK	GE <mark>I</mark> K <mark>Q</mark> RLKLKD	GGHYD
mRuby	LVYHA	QVRGVNFPS	NGAVM-QKKTF	(G <mark>WEP</mark> NTEM <mark>M</mark> )	PADGGLR	GY <mark>T</mark> H <mark>M</mark> ALKVDG	GGHLS
	100	100	000	010		0.2.0	
	180	190	200	210	220	230 I	
CED							ET VV
GFP mKato2		IPIGD-GPV		ZALSKDPNEI IVI PALSKDPNEI			T CU <mark>R</mark> T N
mCherry		K 7 K K – – – D/1 V 9 V V – V N U			UT-TVFOVFP	AFCRHSTCCMD	TT.VK
SlowFT						FCPUSTCCMD	
mPuby		DCKKTUCNT			T TARATER	WAKEACI.CCC	
uncuby		VOVVI AGNT	KPIPGINAVD <mark>H</mark> P	чвклеезриг		VANT AGLIGGG	ngan <mark>u</mark> ak

# (K) Monomeric state

		10	20	30	40	50	
GFP	MSKGE	ELFTGVVPII	LVELDGDVNGH	KFSVSGEGEGI	DATYGKLTLK	FICTTGKI	LPVPWP
PATaqRFP	MS	ELIKENMHM	<b>LYMEGTVNNH</b>	HFKCTSEGEGI	<b>XPYEGTOTMR</b>	IKVVEGGPI	LPFAFD
mNeptune	MS	ELIKENMHM	<b>KLYMEGTVNNH</b>	HFKCTSEGEG	XPYEGTOTGE	IKVVEGGPI	LPFAFD
mCherry	MVSKGEEDNM	ATTKEFMRF	VHMEGSVNGH	EFETEGEGEG	RPYEGTOTAK	T.KVTKGGPI	PFAWD
mRuby	MN	SLIKENMRM	WWI.EGSWNGH	OFKCTGEGEGI	JPYMGTOTMR	TKVI – EGGPI	DFAFD
lincaby	1.114	Olitical					
	60	70	80	9.0	100	110	
	00	/U ***	80	90	100	110	
CED							
GFP DIT DID		FSIGVQCFS		FKSAMPEGYVÇ	2ERIIFFKDL	GNIKIRALVKI	LGDI
PATagRFP	ILA'I'S	FMYGSSTFT	NH.I.QGIPDF	WKQSFPEGFT	NERVI.I.AEDG	GVLTATQDTSI	LQDGC
mNeptune	ILATC	FMYGSKTFI	NHTQGIPDF	FKQSFPEGFT	VERVTTYEDG	GVLTATQDTSI	JQDGC
mCherry	ILSPQ	FMYGSKAYV	KHPADIPDY	LKLSFPEGFKI	VERVMNFEDG	GVVTVTQDSSI	LQDGE
mRuby	ILATS	FMYGSRTFI	KYPKGIPDF	FKQSFPEGFT	VERVTRYEDG	GVITVMQDTSI	LEDGC
	120	130	140	150	160	170	
GFP	LVNRI	ELKGIDFKEI	DGNILGHKLEY	NYNSHNVYIM	ADKQKNGIKV	NFKIRHNIEDO	SSVQL
PATagRFP	LIYNV	KI <mark>R</mark> G <mark>V</mark> NFPSI	IGPVM-KKKTL	GWE <mark>P</mark> STE <mark>K</mark> L <mark>K</mark> I	? <mark>A</mark> DGGL <mark>E</mark> G	RV <mark>D</mark> M <mark>A</mark> LKLVG	GHL <mark>I</mark>
mNeptune	LIYNV	KI <mark>R</mark> G <mark>V</mark> NFPSI	JGPVM-QKKTL	GWE <mark>A</mark> STE <mark>TLY</mark> I	? <mark>A</mark> DGGL <mark>E</mark> G	RC <mark>DMA</mark> LKLVG	GHL <mark>I</mark>
mCherry	FIYKV	KL <mark>R</mark> GTNFPSI		GWEASSERMY	<u></u> דריתד <mark>ד</mark> ר	ETKORIKIKD	GHY <mark>D</mark>
mRuby			JOI VII OILILII				
	LVYHA	.0V <mark>R</mark> G <mark>V</mark> NFPSI	IGAVM-OKKTK	GWEPNTEMMY	P ADGGLRC	YTHMALKVDG	GHLS
1	LVYHA	QV <mark>R</mark> G <mark>V</mark> NFPS1	NGAVM-QKKTK	GWE <mark>P</mark> NTE <mark>M</mark> MYI	P <mark>A</mark> DGGL <mark>R</mark> G	YT <mark>H</mark> MALKVDG0	GHL <mark>S</mark>
1	LVYHA 180	QV <mark>R</mark> GVNFPSI 190	JGAVM-QKKTK	GWE <mark>P</mark> NTE <mark>M</mark> MY 210	220	YT <mark>HMA</mark> LKVDG(	GHL <mark>S</mark>
-	LVYHA 180 I	QV <mark>R</mark> G <mark>V</mark> NFPSI 190 I	IGAVM-QKKTK 200	GWE <mark>PNTEMMY</mark> I 210 I	220	Y <mark>THMA</mark> LKVDG0 230 I	GHL <mark>S</mark>
- CFD	LVYHA 180   ADHYOON	QV <mark>R</mark> GVNFPS1 190   TPIGD-GPVI	JGAVM-QKKTK 200   JEDNHYLSTO	GWEPNTEMMY 210 ALSKDPNEKI	220	YT <mark>HMA</mark> LKVDG0 230   TAAGITHGMDI	GHL <mark>S</mark>
GFP	LVYHA 180   ADHYQQN CNEKTTY	QV <mark>RGV</mark> NFPSI 190   TPIGD-GPVI	JGAVM-QKKTK 200   LLPDNHYLSTQ	GWE <mark>PNTEMMY</mark> I 210   SALSKDPNEKI	220 220 RDHMVLLEFV	YT <mark>HMA</mark> LKVDGO 230   TAAGITHGMDI VARY <mark>S</mark> DLOSKI	GHL <mark>S</mark> ELYK
GFP PATagRFP	LVYHA 180   ADHYQQN C <mark>NFKTT</mark> Y CNI KTTY	QV <mark>RGV</mark> NFPSI 190   TPIGD-GPVI RSKKPAKNLH	JGAVM-QKKTK 200   LLPDNHYLSTQ KMPGVYYVDRR	GWEPNTEMMYI 210   SALSKDPNEKI LEIIKEADKE'	220 220 RDHMVLLEFV F-YWEQHEVA	YT <mark>HMA</mark> LKVDGO 230 TAAGITHGMDI VARY <mark>S</mark> DLPSKI	GGHL <mark>S</mark> ELYK LGHKLN
GFP PATagRFP mNeptune	LVYHA 180   ADHYQQN CNFKTTY CNLKTTY	QV <mark>RGV</mark> NFPSI   TPIGD-GPVI RSKKPAKNLH RSKKPAKNLH	JGAVM-QKKTK 200   LLPDNHYLSTQ KMPGVYYVDRR KMPGVYFVDRR	GWEPNTEMMYI 210   SALSKDPNEKI LEIIKEADKE' LERIKEADKE'	220 220 RDHMVLLEFV F-YWEQHEVA F-YVEQHEVA	YT <mark>HMA</mark> LKVDGO 230   TAAGITHGMDI VARY <mark>S</mark> DLPSKI VARYCDLPSKI	GGHL <mark>S</mark> ELYK LGHKLN LGHKLN
GFP PATagRFP mNeptune mCherry	LVYHA 180   ADHYQQN CNFKTTY CNLKTTY AEVKTTY	QV <mark>RGV</mark> NFPSI   TPIGD-GPVI RSKKPAKNLI RSKKPAKNLI KAKKPVQ	JGAVM-QKKTK 200   LLPDNHYLSTQ (MPGVYYVDRR (MPGVYFVDRR )LPGAYNVNIK	GWEPNTEMMYI 210   SALSKDPNEKI LEIIKEADKE' LERIKEADKE' LDITSHNEDY	220 220 RDHMVLLEFV F-YWEQHEVA F-YVEQHEVA F-IVEQYERA	YT <mark>HMA</mark> LKVDGO 230   TAAGITHGMDI VARYSDLPSKI VARYCDLPSKI EGRHSTGGMDI	GGHL <mark>S</mark> ELYK LGHKLN LGHKLN ELYK

Supplementary Table 1. Overview of suboptimal properties of current monomeric red fluorescent proteins.

Phenotype of RFP	Fluorescent protein	Form and its Excitation/Emission maxima, nm	Suboptimal properties	Ref.
	mKO	548/559	maturation, photoconversion to red spices	1
	mKO2	551/565	photostability	2
	mOrange	548/562	photostability, pH- stability, maturation, photoconversion to far-red species	3
Fluorescent	mOrange2	549/565	pH-stability, maturation, photoconversion to far-red species	4
	TagRFP555/584maturation, photostability, monomeric state		5	
	TagRFP-T555/584maturation, monomeric state		4	
Fluorescent	mRuby	558/605	maturation, photostability	6
	mStrawberry	mStrawberry 574/596 quantum yield, photostability		3
	mRaspberry	574/596	maturation, quantum yield, photostability	7
	mCherry	587/610	quantum yield	3
	mKate2	588/633	pH-stability	8
	mPlum	590/649	maturation, brightness, photostability	7
	eqFP650	592/650	quantum yield, photostability	9
	mNeptune	600/650	quantum yield, maturation, monomeric state, photostability	10
	eqFP670	605/670	quantum yield	9
Large Stokes shift	mKeima	440/620	brightness, pH-stability, monomeric state, maturation, residual red fluorescence with yellow excitation light	11
	LSS-mKate1	463/624	brightness, maturation	12
	LSS-mKate2	460/605	brightness, maturation	
Fluorescent	EastET	Blue, 403/466	photostability	13
Timers	rastr 1	Red, 583/606	quantum yield	1
	MadiumET	Blue, 401/464	photostability	1
	wiediumr i	Red, 579/600	quantum yield	
	SlowFT	Blue, 402/583	photostability	

		Red, 465/604	quantum yield		
		Dark, 404/466	N/A	- 14	
Photoactivatable	PAmCherry1	Red, 564/594	extinction coefficient, pH- stability		
	DATagPFD	Dark, 351/none	N/A	15	
	TATagKIT	Red, 562/595	maturation		
	mEos2	Green, 506/519	photostability, maturation, oligomeric state	16	
Photoconvertable		Red, 573/584	pH stability		
	mKikGP	Green, 505/515	photostability, pH-stability	17	
	IIIKIKOK	Red, 580/591	photostability		
	KEP1	Dark, 580/600	N/A	18	
	KI I I	Red, 580/600	maturation, quantum yield		
		Dark, 440/none	extinction coefficient	10	
	rsTagRFP	Red, 567/585	brightness, pH stability, maturation, contrast	19	
Reversibly		Dark, 572/610	contrast		
photoswitchable	rsCherry	Red, 572/610	brightness, maturation, complex photobehavior during switching,	14 20	
		Dark, 572/608	contrast	17,20	
	rsCherryRev	Red, 572/608	brightness, maturation, photoactivation during switching,		

N/A – not applicable.

**Supplementary Table 2.** Current red fluorescent proteins exhibiting the best specific property or maximally optimized for the specific imaging application.

RFP property	Conventional RFPs	Photoactivatable RFPs	RFP application	Conventional RFPs	Photoactivatable RFPs	
Excitation beyond 610 nm	TagRFP657 <sup>21</sup>	not available	STED	TagRFP657	not applicable	
Emission beyond 670 nm	eqFP670 <sup>9</sup>	not available	Deep tissue imaging	mNeptune and E2- Crimson	not available	
One photon brightness	mKO $\kappa^{22}$ and tdTomato <sup>3</sup>	mEos2 <sup>16</sup>	RESOLFT	not applicable	rsTagRFP	
Two-photon brightness	DsRed2 and dTomato <sup>23</sup>	unknown	PALM	not applicable	PATagRFP and tdEosFP	
Photon counts per molecule	Unknown	tdEosFP <sup>24</sup>	Multicolor DALM	not applicable	green PA-GFP	
pH stability	LSS-mKate2 <sup>12</sup> and TagRFP <sup>5</sup>	PATagRFP <sup>15</sup> and KikGR <sup>25</sup>	MULLICOLOF FALM	not applicable	red PATagRFP	
Maturation rate	mCherry <sup>3</sup> and mKate2 <sup>8</sup>	rsTagRFP <sup>19</sup>	Multicolor imaging	blue TagBFP		
Intracellular half-life and thermodynamic stability	mRFP1 <sup>26</sup>	Dendra2 <sup>27</sup>	with a single excitation wavelength	green T-Sapphire	not available	
Cytotoxicity	DsRed-Express2 <sup>28</sup> and E2-Crimson <sup>29</sup>	unknown	, areiengin	red LSS-mKate2		
Monomeric state and performance in fusions	mCherry <sup>3</sup>	PATagRFP <sup>15</sup> , Dendra2 <sup>30</sup> and mEos2 <sup>16</sup>	FRET-based biosensors	Venus-mCherry <sup>31</sup>	EYFP-rsTagRFP <sup>19</sup>	

**Supplementary Table 3.** Properties of the major chromophore structures observed in fluorescent proteins.

Chemical structure of chromophore	Chromophore name	Number of double bonds in conjugation	Typical absorbance/ emission max, nm	Examples of proteins sharing this chromophore
1	non-cyclized tripeptide X65-Tyr66-Gly67	none	none	majority of FPs
3	aromatic α-enolate	2	350-360/ undetectable	PATagRFP (dark state)
4	TagBFP-like	4	400-420/ 456-466	TagBFP, FTs (blue state), PAmCherry (dark state), mCherry-Blue102
5a, 6a, 8	DsRed-like asFP595-like (anionic)	7, 8	555-610/ 583-650	DsRed, mCherry, PAmCherry ( <i>trans</i> ), TagRFP ( <i>trans</i> ), mKate, PATagRFP, asFP595
5b, 6b	<b>DsRed-like</b> (neutral)	8	440-463/535, 605-624	mKeima, LSS-mKate1, LSS-mKate2, GmKate
9, 10	mOrange-like, zFP538- like, mKO-like (anionic)	7	540-548/ 561-565	mOrange, mKO, E2-Orange, zFP538
7	DsRed-like a) H-bonding b) hydrophobic packing c) π-π stacking	8	>610/ >650	TagRFP657, E2-Crimson, mNeptune, mRouge
11	PSmOrange-like	8	>610/ >650	PSmOrange
12	<b>GFP-like</b> (anionic)	6	484-493/ 502-510	EGFP, TagGFP2, Emerald
13	<b>GFP-like</b> (neutral)	6	399/ 511	wtGFP, Sapphire, Ametrine
14	Kaede-like	9	550-580/ 580-595	Kaede, mEos2, mKikGR, Dendra, mIrisFP

See Figure 2 for chemical structures of the chromophores. Additional characteristics of the listed proteins are summarized in the review papers<sup>32-34</sup>.

Ph (str	enotype/property <i>cucture/transition</i> )	Supporting positions									Ref.			
ſ	TagBFP-like (4)	65 H,L	69 K,R	165 A,I,N	1	.79 A	181 A,I							13,35
Fluorescent timer-like		18	\$	106	1	52	179	20	13	205	224			13,36
	$(4 \rightarrow 5a, 6a)$ V,L,M		,M	Α	Ν	A,I	V,A	S,	J,	M,L	S,A			
Pho	otoactivatable-like	18	58	84	1	48	165	16	67	169	179	207	218	14,15,37,38
	( <i>4</i> → <i>5a</i> , <i>6a</i> )	L,M	T,A	F,W	L,S,N	,H,T,F	V,L,S,A,G,Q	<b>P,A</b> ,	M,G	V,T,L	Т,А,С	I,R	W,V	
Pho	otoswitchable-like	146	150	181	1	.83								19,20,39,40
	(5a↔6a)	V,E	Е	F,L,S		Т								, , , ,
	H-bonding to N-	63	64	165										
nifted-	acylimine oxygen (7a)	C,F	I,F	С										
l-sl ike	Hydrophobic	18	1	201			224							9,10,21,41-45
li	packing (7c)	F,L	,V		T,V		C,N,A							
ar-	$\pi$ stacking (7b)	18	1	201	2	24								
H	<i>n</i> -stacking (70)	F,L	,V	A,T	C,	N,A								
Larg	ge Stokes shift-like	69	)	148 1		65 167		183			11,12,46-48			
(5b,6b)		R,K	,Y	G,S,N	N G,A		A,V,N	S,M,A,G,L,N		T,S				
Hig	gh quantum vield	145	146	147	2	03								3,5,8,41,49-52
-	51 2	W E P,A		Н,	I,K									
High photostability		145 W	140 E	147 P A	1	05 K								4,8,52
High pH-stability		145	146	147										52 54
		W	E	P.A										52-54
Fast maturation		18	21	44	48	106	152	165	167	181	203	205	238	0 12 45 55 57
		V,L,M	T,S	M,A	A,V	I,V, L	M,I,L	A,I,T	M,Q	L,V,F	S,I,R,H	M,L	R	8,13,43,33-37
Monomeric state		128	151	153	1:	57	178	180	184	198	200		229	6 10 15 56 59
		V,T	K,T,R, M	K,Y	А	,Е	I,D,S	N,E,S	Т	V,A,I	Y,F,N	,A	S,C,A	0,10,13,30-38

Supplementary Table 4. Amino acid residues in the supporting positions of red fluorescent proteins.

The supporting residues correspond to those on Figure S1 highlighted with yellow color. Residues' numbering follows that for GFP.

#### **Supplementary Note**

#### Major steps of molecular evolution

A typical process of the development of fluorescent proteins with required spectral and biochemical properties includes a rational design and several steps of directed molecular evolution (**Fig. 1**). The starting points are design of new spectral phenotype of fluorescent protein based on the known mechanisms of chromophore transformations and choosing of an appropriate template gene.

Next step involves the rational design based on the knowledge of relationship between immediate chromophore environment and postranslational chemistry of fluorescent proteins and photochemical properties of the chromophore. At this step, the selected templates are subjected to multiple site-specific amino acid substitutions at the key and supporting positions, in order to generate a library of site-specific mutants. After screening this library typically in a lowthroughput format, several primary clones, possessing the required spectral phenotype, are selected.

Several rounds of directed molecular evolution are then applied to improve the properties of imperfect primary variants. Major challenges to get a successful directed evolution include selecting an optimal biological system for expression, obtaining a sufficient number of clones in a library, and achieving reliable screening conditions. Each round of the directed molecular evolution begins with a generation of a large mutant library, using various stratagies of mutagenesis and recombination<sup>59-62</sup>. A high-throughput screening (HTS) of the library and subsequent evaluation of the selected clones in a low-throughput format then follows. The clones exhibiting prominent characteristics of the required phenotype are then applied to the next round of molecular evolution. After several rounds, the screened properties usually reach a plateau, suggesting that either a natural limit is achieved or some bias, caused by the mutagenesis is typically applied to the selected mutants at amino acid positions found during the rounds of molecular evolution. Finally, testing of advanced variants with optimized characteristics by creating fusion proteins and their imaging in live cells comes before wide usage in the life sciences.

# Historical aspects of the molecular evolution strategy in the development of fluorescent proteins

In the past, fluorescent proteins of new colors such as mFruits have been mainly developed using random mutagenesis on the basis of wild-type protein templates of required weak phenotype<sup>3</sup>. Over time the more structure-to-function information has become available for fluorescent proteins and it resulted into appearance of rational component in the creation of fluorescent proteins. The PAmCherrys<sup>14</sup>, PATagRFP<sup>15</sup>, rsCherrys<sup>20</sup>, rsTagRFP<sup>19</sup>, KikGR<sup>25</sup>, mRouge<sup>41</sup> and series of TagBFP-<sup>35</sup> and LSS-like<sup>46</sup> fluorescent proteins have different colors and photochemical behavior and all of them were developed using rational mutagenesis at amino acids around chromophore to find weak phenotype followed by random mutagenesis for the further improvement of mainly brightness, maturation and photostability.

#### Correlation between residues at key positions and fluorescent protein phenotype

The residues at key positions 84, 148 and 203 for TagBFP-like phenotype mainly sterically hinder Tyr-ring of the chromophore occupies position necessary for the oxidation of

 $C\alpha 2$ - $C\beta 2$  double bond between Tyr-ring and imidazolinone and improve brightness of blue chromophore 4.

The residues at key positions 69 and 84 for FT-like phenotype slow down 4-to-5a,6a transition and increase brightness of blue chromophore 4. The residues at crucial positions 69 and 203 for photoactivatable-like phenotype block 4-to-5a,6a transition. The residues at main positions 148, 165, 167 and 203 for photoswitchable-like phenotype provide space around Tyrring of the chromophore and ensure flexibility of the chromophore for *cis-trans* isomerization of the chromophore to occur.

The far-red shift in RFPs can be attributed by the formation of hydrogen bond between residues at positions 14, 16 and 44 and N-acylimine oxygen, by hydrophobic parking around chromophore provided by hydrophobic residues at positions 84, 148, 165 and 167 and by stacking interactions between aromatic residue at positions 65, 69, 148 and 203 and Tyr-ring of the chromophore.

Large Stokes shift-like phenotype relies on the Glu and Asp acidic amino acids at positions 148, 165 and 167 which stabilize neutral chromophore 5b,6b and support proton transfer in excited state.

High photostability of RFPs mainly depends on amino acids at positions 64, 99 and 165 which provide tight packing around chromophore and block photochemical reactions.

Fast maturation of RFPs is primarily affected by residues at positions 42, 69, 179, and 224 which facilitate 2-to-5a,6a transition.

The A-B and A-C dimerizing interfaces in RFPs are mainly disrupted by charged amino acid residues at positions 126, 162, 166 and 168.

High quantum yield in RFPs depends on the residues at positions 70, 148 and 167 which provide planar configuration and rigidity of the chromophore.

High pH-stability of blue and red chromophores 4 and 5a,6a, respectively, is provided by the residues at positions 96 and 167 which stabilize negative charge on CO-group of imidazolinone and Tyr-ring, respectively.

Maturation rate, pH-stability, quantum yield and photostability may be affected by the width of the water-filled pore which regulated by the residues at positions 145-147.

There are many examples in the world of fluorescent proteins where a particular feature (e.g., a red color in DsRed) is a synergistic effect of a large number of residue substitutions, including many residues that are remote from the active site. How these positions control fluorescence via coupling to each other is poorly understood. This is why the directed evolution approach will be useful for final turning of the residues remote from chromophore with found spectral phenotype.

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