

Dear Customer!

Welcome to dual-parameter bioluminescence *in vivo* imaging beyond the absorption range of blood! In this free info pack you will find the protein sequences of first generation infraluciferases (ilucs) along with the sequences of control enzymes such as wild-type *Photinus pyralis* firefly luciferase (Fluc), x11 Fluc and x11 Fluc colour mutants ('FLuc green' and 'FLuc red') used in Stowe *et al.*, 2019 (Elife 8: e45801). The table below contains the sequences and some relevant descriptions of their properties with infraluciferin. The iluc1 variants are described more fully in a 2022 paper in Frontiers Bioengineering and Biotechnology: <https://www.frontiersin.org/articles/10.3389/fbioe.2022.897272/full>.

**How to get the DNA:** It is recommend to codon optimise the sequences for your organism(s) of choice using the online codon optimisation tool by IDT DNA (Coralville, IA, USA) and purchasing the genes synthesised as dsDNA gblocks directly from them with your restriction sites of choice added. Then simply clone into your vector of choice. For more information, or further advice please email [customerservices@bioflares.com](mailto:customerservices@bioflares.com).

In the table below, good colour pairs for dual parameter (>600nm) imaging with infraluciferin are indicated by the similar shading of adjacent boxes containing sequences for the blue-shifted ('g') and red-shifted ('r') variants of each type of enzyme. 'g' stands for green and 'r' for red (the colours of their emission with natural luciferin) and g and r variants listed next to each other and shaded the same in the table will pair together for dual parameter imaging *in vivo* with infraluciferin. Δ means this is a deletion mutation of the residue. \* The peak wavelengths listed are the approximate peaks as measured in the Photon Imager Optima small animal spectral imager (30nm passband) (Biospace Labs, Paris, France).

Fluc/ mutant	Protein sequence	Mutations relative to WT	Peak wavelength *
WT	MEDAKNIKKGPAPPFYLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSNSLQFFMPVLGALFIGVAVAPANDIYNE RELLNSMNISQPTVVVFSKGLQKILNVQKLPPIIQKIIIMDSKTDYQGFSMYTFVT SHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPI FGNQIIPDTAILSVPFHGGFMFTTLGYLICGFRVVLMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLT ETTSAILITPEGDDKPGAVGKVVPFPEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRKSLIKYKGYQVAPAELE	-	Variable (unstable): 672-697

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	SILLQHPNIFDAGVAGLPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKK LRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGK		
x11	MEDAKNIKGPAPRYPLEDGTAGEQLHKAMKRYAQPVGITAFDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE RELLNSMNIQSPTVVVFSKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPHHGFGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGRQGYGLT ETTSAILITPRGDYKPGAVGKVPVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKLSLIYKGYQVAPAELE SILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKK LRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	F14R, L35Q, A105V, V182K, T214C, I232K, D234G, E354R, D357Y, S420T, F465R	697
iluc1  (Second brightest iluc1)	MEDAKNIKGPAPRYPLEDGTAGEQLHKAMKRYAQPVGITAFDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE RELLNSMNIQSPTVVVFSKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPHHGFGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGRQGYGLT ETTSAILITPHGDAKPGAVGKVPVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKLSLIYKGYQVAPAELE SILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKK LRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	F14R, L35Q, A105V, V182K, T214C, I232K, D234G, E354H, D357A, S420T, F465R	697
x11g  (‘FLuc_gr een’ in Stowe et al., 2019)	MEDAKNIKGPAPRYPLEDGTAGEQLHKAMKRYAQPVGITAFDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE RELLNSMNIQSPTVVVFSKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPHHGFGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGRQGYGLT ETTSAILITPRGDYKPGAVGKVPVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKLSLIYKGYQVAPAELE SILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKK LRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	F14R, L35Q, A105V, V182K, T214C, I232K, D234G, V241I, G246A, F250S, E354R, D357Y, S420T, F465R	672
x11r2  (‘FLuc_re d’ in Stowe et al., 2019)	MEDAKNIKGPAPRYPLEDGTAGEQLHKAMKRYAQPVGITAFDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE RELLNSMNIQSPTVVVFSKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPHHGFGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGRQGYGLT ETTSAILITPRGDYKPGAVGKVPVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKLSLIYKGYQVAPAELE SILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKK LRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	F14R, L35Q, A105V, V182K, T214C, I232K, D234G, S284T, E354I, D357Y, S420T, F465R	722
iluc1g3  (Stable blueshift)	MEDAKNIKGPAPRYPLEDGTAGEQLHKAMKRYAQPVGITAFDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE RELLNSMNIQSPTVVVFSKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPHHGFGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGRQGYGLT ETTSAILITPHGDAKPGAVGKVPVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKLSLIYKGYQVAPAELE SILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKK LRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	F14R, L35Q, A105V, V182K, T214C, I232K, D234G, V241I, G246A, F250S, E354H, D357A, S420T, F465R	672
iluc1r  (Stable red)	MEDAKNIKGPAPRYPLEDGTAGEQLHKAMKRYAQPVGITAFDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE RELLNSMNIQSPTVVVFSKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPHHGFGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGRQGYGLT ETTSAILITPHGDAKPGAVGKVPVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKLSLIYKGYQVAPAELE SILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKK LRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	F14R, L35Q, A105V, V182K, T214C, I232K, D234G, S284T, E354H, D357A, S420T, F465R	722
iluc1g2	MEDAKNIKGPAPRYPLEDGTAGEQLHKAMKRYAQPVGITAFDAHIEVNITYAEY FEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE	F14R, L35Q,	672

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(Brightest iluc1 but less stable blueshift)	RELLNSMNISQPTVVVFSKKGKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPFHHGFGMSTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLT ETTSAILITPHGDAKPGAVGKVPFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMS GYVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKSLIKYKGYQVAPAELE SILQHPNIRDAGVAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKK RGGVVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	A105V, V182K, T214C, I232K, D234G, F250S, E354H, D357A, S420T, F465R	
x11r3  (Bright red)	MEDAKNIKKGPAPRYPLEDGTAGEQLHKAMKRYAQVPGTIAFTDAHIEVNITYAEY FEMSURLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPVNDIYNE RELLNSMNISQPTVVVFSKKGKGLQKILNVQKLPPIQKIIIMDSKTDYQGFQSMYTFVT SHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRCACVRFSHARDPI FGNQIKPGTAILSVVPFHHGFGMSTTLGYLICGFRVVMYRFEELFLRSLQDYKIQS ALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLT ETTSAILITPRGDYKGA VGVKVPFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSG YVNNPEATNALIDKDGWLHTGDIAYWDEDEHFFIVDRKSLIKYKGYQVAPAELESI LLQHPNIRDAGVAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKL RGGVVVFVDEVPKGLTGKLDARKIREILIKAKKGGK	F14R, L35Q, A105V, V182K, T214C, I232K, D234G, S284T, E354R, D357Y, ΔP359, S420T, F465R	722

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Yours Sincerely,

Dr. Amit P. Jathoul (Director of Bioflares Ltd).