

Table S1. Relationship between luciferase substrate requirements and fluorophore photon production

BRET Molecule Name	Luciferase Partner	Luciferase QY ^a	Substrate	Molecules Substrate Photon ⁻¹	Fluorophore Partner	Fluorophore QY	Excitation Photons Emission Photon ⁻¹	^b Molecules Substrate Emission Photon ⁻¹	Reference
LumiFluor - CeNLuc	NLuc	Unknown	FZ	Unknown	mCerulean3	0.87	1.15	3.38 ^c	
LumiFluor - GpNLuc	NLuc	Unknown	FZ	Unknown	eGFP	0.60	1.75	5.16 ^c	(Schaub et al., 2015)
N/A	OLuc	0.34	CLZ	2.94	N/A	N/A	N/A	N/A	(Shimomura et al., 1978)
eBAF-Y	RLuc	0.02	CLZ	50	Venus	0.57	1.75	87.72	(Saito et al., 2012)
Nano-lantern	RLuc8	0.05	CLZ	20	Venus	0.57	1.75	35.09	(Saito et al., 2012)

Abbreviations: BRET, bioluminescence resonance energy transfer; QY, quantum yield; CLZ, coelenterazine; FZ, furimazine;

RLuc, Renilla; OLuc, Oplophorus; NLuc, NanoLuc

^aLuciferase QY = number of photons emitted per luciferase molecule during a single catalytic cycle

^bMolecules Substrate Emission Photon⁻¹ = (Excitation Photons Emission Photon⁻¹) x (Molecules Substrate Photon⁻¹)

^cCalculated based on known QY of OLuc