

**Table 1. Spectral characterization of YFP variants<sup>a</sup>**

Name	Substitutions	$\lambda_{\text{abs}}$ ( $\epsilon$ )	$\lambda_{\text{em}}$ ( $\Phi$ )	pKa	$K_d$ for Cl <sup>-</sup> (mM)	$K_{\text{fold}}$ ( $10^{-2} \text{ s}^{-1}$ )	$K_{\text{ox}}$ ( $10^{-3} \text{ s}^{-1}$ )	Relative fluorescence at 37°C
EYFP	–	515 (80.4)	528 (0.61)	6.9	110	0.39	2.53	1
EYFP-F46L	F46L	515 (78.7)	528 (0.61)	6.9	145	1.94	3.87	20
SEYFP	F64L/M153T/V163A/S175G	515 (101)	528 (0.56)	6.0	>10 <sup>4</sup>	6.60	2.36	3
SEYFP-F46L (Venus)	F46L/F64L/M153T/V163A/S175G	515 (92.2)	528 (0.57)	6.0	>10 <sup>4</sup>	5.62	8.04	30

<sup>a</sup>The extinction coefficients ( $\epsilon$ ) (in the units of  $10^3 \text{ M}^{-1}\text{cm}^{-1}$ ) and quantum yields ( $\Phi$ ) were determined as described<sup>4</sup>. For pH titration, all buffers contained 35 mM Cl<sup>-</sup>, with adjustment of the ionic strength to 150 mM using potassium D-gluconate. Chloride titration was performed in 10 mM 4-morpholinepropanesulfonic acid (pH 7.0) containing specified [Cl<sup>-</sup>] ranging from 0 to 400 mM, and the ionic strength was adjusted to 400 mM with potassium D-gluconate. Relative fluorescence intensities in *Escherichia coli* at 37°C were calculated by adjustment of the fluorescence measurement by OD at 600 nm.