LUCERNA™

***E. coli* cell imaging protocol**

1. Transform BL21 Star (DE3) or similar *E. coli* competent cells with 40 ng of plasmid DNA expressing the tRNA-Spinach™ chimeras in a bacterial expression vector under the control of a T7 promoter (e.g. pET28c).
2. After overnight growth, pick single colonies for inoculation in Luria Broth containing kanamycin (LB-Kan).
3. At OD_{600} = 0.4, induce culture with 1 mM of IPTG and continue shaking at 37°C for more 2 hours.
4. During this time, coat glass-bottom dishes with 0.1 mg/mL poly-L-lysine (PLL) for at least 2 hours in 37°C. Wash the dishes at least twice with ddH_2O to remove free PLL. (Dishes can also be coated overnight at 4°C)
5. Remove and spin down 100 μL of culture, then resuspend in 2 mL of pH 6.0 M9 minimal media.
6. Plate 200 μl aliquot of resuspended culture on poly-L-lysine (PLL)-coated glass-bottom dishes and incubate for 45 minutes at 37°C.
7. Wash adherent cells twice with M9 media and then incubate with 200 μM DFHBI in pH 6.0 M9 minimal media for 45 min at 37°C.
8. Take live fluorescence images with a 60X oil objective using a FITC filter.

M9 minimal media:

1X M9 salts

2 mM MgSo4

0.1 mM CaCl2

0.4% glucose

5X M9 salts:

64 g $NaHPO_4 \cdot 7H_20$

15 g KH_2PO4

2.5 g NaCl

5 g NH_4Cl

Add water to 1 L and autoclave