Cell Lysis, General

1. Inoculate 10mL of LB (+appropriate antibiotics) with the bacterial strain of choice and incubate overnight at 37ºC, 200rpm.
2. The next day, measure the OD_{600} and spin the culture down at 4,700x g for 12 minutes.
3. Add 8µL of Lysozyme mix (50mg/mL) and 8µL of DNase mix to the pellet.
4. Resuspend the pellet in 400µL B-PER™ Bacterial Protein Extraction Reagent and transfer the solution to a microcentrifuge tube.
5. Incubate the tube for 15 minutes at room temperature.
6. Centrifuge at 15,000x g for 5 minutes.

Cell Lysis, for the Study of Split Proteins

1. Inoculate 10mL of LB (+appropriate antibiotics) with the bacterial strain of choice and incubate overnight at 37ºC, 200rpm.
2. The next day, measure the OD_{600} and spin the culture down at 4,700x g for 12 minutes.
3. To the pelleted cells, add 8µL of Lysozyme mix (50mg/mL) and dilute EDTA-Free Protease Inhibitor Cocktail to a final concentration of 1x.
4. Resuspend the pellet in 400µL B-PER™ Bacterial Protein Extraction Reagent and transfer the solution to a microcentrifuge tube.
5. Incubate the tube for 15 minutes at room temperature.

Measuring Cell Lysates and Calculating Quantum Yields

1. Inoculate 10mL of LB (+appropriate antibiotics) with the bacterial strain of choice and incubate overnight at 37ºC, 200rpm.
2. To 5mL of LB (+appropriate antibiotics), containing 0.2% arabinose, add such a volume of overnight culture that the final OD_{600} is 0.1.
3. Incubate the new culture at 37ºC, 200rpm for 3 hours.
4. Afterwards, leave the cultures overnight at 4ºC, to allow for protein maturation.
5. Lyse samples according to the protocol(s) above and measure 100µL from three dilutions (1:1, 1:10 and 1:100, dilute with MQ) of the supernatants in the Synergy Mx™ Microplate Reader.
   - Measure absorbance from 340nm to 750nm in intervals of 10nm.
   - Measure the fluorescence for the fluorescent proteins were measured using the maximum excitation value described in the table below. Emissions were recorded from 340nm to 750nm in intervals of 10nm.

Protein:	Excitation:	Emission:
mRFP	584nm	607nm
mVenus	510nm	530nm
mCerulean	433nm	475nm
sfGFP	485nm	510nm
eYFP	510nm	530nm

6. Calculate the quantum yield, using the following formula:

$$QY = QY_{ST} * \frac{grad}{grad\_st}$$

QY: Quantum Yield
QY_{ST}: Standardized Quantum Yield
grad: Gradient, arbitrary units
$grad_{ST}$: Standardized gradient, arbitrary units

"Grad" refers to the linear gradient that correlates absorbance and integrated fluorescence (area of the emission spectrum). The range of absorbance will be achieved with the different dilutions used in the fluorescence measurement (1:1, 1:10 and 1:100).

Measuring Fluorescence Generation Over Time

1. Inoculate 10mL of M9 medium (+appropriate antibiotics) with the bacteria containing the plasmids coding for full/split proteins and incubate overnight at 37ºC, 200rpm.
2. To 5mL of M9 (+appropriate antibiotics), add such a volume of overnight culture that the final OD_{600} is 0.1.
3. In a 96-wells plate, mix 50µL of culture with 50µL of M9 media, containing the following final concentrations of arabinose: 0%, 0.004%, 0.04% and 0.4%.
4. Measure the plate in a Synergy Mx™ Microplate Reader during 18-24 hours at 30ºC, with fast shaking enabled. Make a measurement every 30 minutes, using the maximum values for excitation and emission for each protein.

Protein:	Excitation:	Emission:
mRFP	584nm	607nm
mVenus	510nm	530nm
mCerulean	433nm	475nm
sfGFP	485nm	510nm
eYFP	510nm	530nm

Measuring the Effect of Temperature

1. Inoculate 10mL of LB medium (+appropriate antibiotics) with the bacteria expressing full/split proteins and incubate overnight in 37ºC, 200rpm.
2. To 5mL of LB (+appropriate antibiotics), containing 0.2% arabinose, add such a volume of overnight culture that the final OD_{600} is 0.1.
3. Incubate at 37ºC, with shaking, for 2 hours.
4. Spin the culture down at 4,700x g for 12 minutes and discard the supernatant.
5. Resuspend the pellet in 5mL MQ and transfer to a 96-wells plate.
6. Incubate the 96-wells plate overnight at the following temperatures: 4ºC, 10ºC, 20ºC, 30ºC, 45ºC and 60ºC.
7. Use the Synergy Mx™ Microplate Reader to measure the fluorescence, using the maximum excitation and emission values for each protein.

Protein:	Excitation:	Emission:
mRFP	584nm	607nm
mVenus	510nm	530nm
mCerulean	433nm	475nm
sfGFP	485nm	510nm
eYFP	510nm	530nm

Calculation of Maturation Rates

1. Inoculate 10mL of M9 medium (+appropriate antibiotics) with the bacteria expressing full/split proteins and incubate overnight in 37ºC, 200rpm.
2. To 5mL of LB (+appropriate antibiotics), containing 0.2% arabinose, add such a volume of overnight culture that the final OD_{600} is 0.1.
3. Incubate at 37ºC, with shaking, for 2 hours.
4. Transfer cultures to a 96-wells plate and add geneticin until a final concentration of 50µM.
5. Measure in a Synergy Mx™ Microplate Reader for 3 hours, using the following settings:
    - Take measurement every 5 minutes of the OD_{600} and the fluorescence, using maximum excitation and emission wavelengths for each protein as displayed in the table below.
6. Calculate the maturation rate, following the signal generation time formula.