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FLUORESCENCE LOSS ASSAY

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Day 1 (=Transformation -> ONC in 96-well plate)

1 50ul of chemically competent E. coli DH10B_gfp cells were transformed (heat-shock: 42°C, 30sec) with 3ng plasmid (Cplasmid*2ul=3ng/ul*x -> x=...ul=2ul undiluted plasmid + rest ul MQ) and recovered in 450 ul LB for 1h at 37°C.
(PC = E. coli DH10B_gfp + pACYC184)
(NC = E. coli DH10B + pACYC184)

2 2ul of recovered cells were inoculated in 198ul M9TG+Cam15. [1 Masterblock] (B = Blank, just medium)

3 Incubate at 37°C under shaking (900rpm) for 21-22 hours.

Day 2 (=Dilution 1/100 NO INDUCERS -> Dilution 1/100 WITH INDUCERS -> ONC in triplicates 96-well plates)

4 2ul of each preculture were inoculated in 198ul M9TG+Cam15. [1 Masterblock]

5 Shake the plate on the thermoblock (900rpm)

6 2ul of each dilution were inoculated in 198ul M9TG+Cam15+inducer. [4 Masterblocks=triplicates + control plate] Plate 1,2,3 (triplicates)

7 Incubate at 37°C under shaking (900rpm) for 21-22 hours.

Day 3 (=SPECTROPHOTOMETRY: 1/5 DILUTION -> 100ul)

8 Mix at thermoblock the preculture -> 40ul of each sample were inoculated in 160ul of 1xPBS [4 normal 96-well plates]

9 Transfer 100ul in 4 black plates with transparent bottom

10 Procedure Details:

Plate Type	96 WELL PLATE
Eject plate on completion	
Set Temperature	Setpoint 25°C
	Preheat before moving to next step
Shake	Fast, 0:10 (MM:SS)
Read	Cell Density
	Absorbance Endpoint
	Full Plate
	Wavelengths: 600
	Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 8
Read	Fluorescence Endpoint
	Full Plate
	Filter Set 1
	Excitation: 395/20,0, Emission: 508/20,0
	Optics: Top, Gain: 50
	Filter Set 2
	Excitation: 395/20,0, Emission: 508/20,0
	Optics: Top, Gain: 75
	Filter Set 3
	Excitation: 395/20,0, Emission: 508/20,0
	Optics: Top, Gain: 100
	Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10
	Read Height: 8 mm