OCT 01, 2019

FLUORESCENCE LOSS ASSAY

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iGEM Wageningen 2019

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OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.7mfhk3n

Protocol Citation: Alexander Niederau, Despoina Trasanidou 2019. FLUORESCENCE LOSS ASSAY. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.7mfhk3n>

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Protocol status: In development
 We are still developing and optimizing this protocol

Created: Sep 23, 2019

Last Modified: Oct 01, 2019

PROTOCOL integer ID:
 28039

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.)

- 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
	Absorban ce Endpoint
	Full Plate
	Waveleng ths: 600
	Read Speed: Normal, D elay: 100 msec, Me asuremen ts/Data Point: 8
Read	Fluoresce nce Endpoint
	Full Plate
	Filter Set 1
	Excitati on: 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