Oct 01, 2019

# FLUORESCENCE LOSS ASSAY

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

Alexander Niederau[1], Despoina Trasanidou[1]

[1]Wageningen University

iGEM Wageningen 2019

Alexander Niederau





DOI: https://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



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

**Created:** September 23, 2019

**Last Modified:** October 01, 2019

**Protocol Integer ID:** 28039

**Keywords:** fluorescence, loss

## 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)

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

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

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

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 |