**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 \((\text{Cplasmid} \times 2\text{ul}=3\text{ng/ul} \times \text{x} \rightarrow \text{x}=\text{ul}=2\text{ul undiluted plasmid + rest ul MQ})\) 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 IN.

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:

<table>
<thead>
<tr>
<th>Plate Type</th>
<th>96 WELL PLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eject plate on completion</td>
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<tr>
<td>Set Temperature</td>
<td>Setpoint 25°C</td>
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<tr>
<td></td>
<td>Preheat before moving to next step</td>
</tr>
<tr>
<td>Shake</td>
<td>Fast, 0:10 (MM:SS)</td>
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<tr>
<td>Read</td>
<td>Cell Density</td>
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<tr>
<td></td>
<td>Absorbance Endpoint</td>
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<tr>
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<td>Full Plate</td>
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<tr>
<td></td>
<td>Wavelengths: 600</td>
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<tr>
<td></td>
<td>Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 8</td>
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<tr>
<td>Read</td>
<td>Fluorescence Endpoint</td>
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<tr>
<td></td>
<td>Full Plate</td>
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<tr>
<td>Filter Set</td>
<td>Excitation</td>
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</tr>
<tr>
<td>1</td>
<td>395/20,0</td>
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<tr>
<td>2</td>
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<td>3</td>
<td>395/20,0</td>
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<table>
<thead>
<tr>
<th>Read Speed</th>
<th>Normal, Delay: 100 msec, Measurements/Data Point: 10</th>
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<tbody>
<tr>
<td>Read Height</td>
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