EXPERIMENT 5: EVALUATION STRAINS.VERSION1

EZ RICH MEDIUM

50 mL EZ rich:

- EZ (5x): 10 mL
- ACGU (10x): 5 mL
- MOPS (10x): 5 mL
- Phosphate (100x): 0,5 mL
- Glucose (20%): 5 mL
- mQ: 24.5mL

PRECULTURE

1. Inoculate 4 colonies from each strain in 5mL LB+Amp/Spec: Grow for 8h @ 37°C
2. Inoculate reference strain in 5mL LB+Amp/Spec: Grow for 8h @ 37°C

MICROTITERPLATE

1. Sterilise microtiter plate (MTP)
2. Prepare EZ rich medium + Amp/Spec
   - 3 repeats/colony = 594 µl in eppendorf
3. Add 6µL of preculture
4. VORTEX!
5. Add 170µL in 3 wells of MTP
6. Reference strain: add 170µL in 3 wells of MTP
   Blank: add 170µL in 3 wells of MTP
7. Attach breathing sticker to MTP
8. Put plate in FLUOstar OPTIMA
9. Start script iGEM_GFP_OD

- Shaking: 9min (540sec), orbital, amplitude of 2 nm, wait time before measurement of 5 sec
- Measurement: Kinetic measurement
  1) duration: 24h (maximal)
  2) interval time: 10 min
  3) 2 measurements: Fluorescence and Absorbance
- Fluorescence Top Reading:
  1) ex/em wavelength: 555nm/584nm
  2) ex/em bandwidth: 9nm/20nm
  3) Gain: 1700
  4) Number of flashes: 5
  5) Integration time: 20 microsec
  6) lag-time en settle-time: 0s
- Absorbance:
  1) wavelength: 600nm
  2) bandwidth: 9nm
  3) number of flashes
  4) settle time: 5