09. (September) 2019

Project: iGEM_Munich2019 Shared Project
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THURSDAY, 12/9/2019

Johanna

Restriction for Cloning V25-41 and E19

- according to NEB-Protocoll:
- Set up reaction as follows:

Restriction 12/09/19

	A	B
1	DNA	1 µg
2	10X CutSmart Buffer	5 µl (1X)
3	Restriction Enzyme	1 µl
4	ddH2O	to 50 µl
5	Total	50 µl

- calculate µL DNA with concentrations from 13/09/2019
- Incubate at 37°C for 1 hour.
- Purification
  - alpha, gamma, delta, I: purification with PCR/DNA Clan up kit (NEB, Monarch)
    - concentrations: alpha: 71 ng/µL, gamma: 81 ng/µL, delta: 77 ng/µL, I: 75 ng/µL
  - III, IV, V, VII: purification with Gel
    - mix with 15 µL Loading Dye, no SDS
    - Agarose gel (1 %)
    - NEB Gel extraction kit

Johanna

cell culture

- 13:00-14:00
- splitting: passage 34 with 300 µL
- seeding
  - 1 6-well plate for qPCR: 750000 cells in 2 mL Medium/well

Alejandro:

cloning:

- constructs V35-V41 and E19 were created by ligation and Gibson assembly:
  - Gibson:
    - V36: V8 (NheI/MluI): II + beta
    - E19: E17 (NheI/MluI): VII + beta
  - Ligation
    - V35: V10 (NheI): I + PP
    - V37: V10 (PacI/NheI): III + gamma + PP
    - V38: V11 (PacI/NheI): IV + gamma

    - V39: V10 (NheI/MluI): V + delta
    - V40: V4 (PacI/MluI): VI + gamma + delta
    - V41: V8 (NheI/MluI): II + alpha
- NEB stable cells were then transfomred with 2 µL of each construct following the standard protocol and plated on LB + Amp plates