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Project: iGEM_Munich2019 Shared Project
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gibson assembly, ligation and Transformation :

- V27, V28, V25
- Inserts : gBlocks from IDT (500ng)
  - spin down
  - add 50µL Milli Q
  - vortex
  - incubate at 50°C 15-20min
- V28 : gibson overlap
- Restriction for V27
  - Backbone : V26 from Jeff
  - insert gBlock PS6L1-TM and CT
  - DNA : 0.42µL (Backbone) / insert (20µL)

Table5

	A	B	C
1	10x Cutsmart Buffer	5µL	5µL
2	BamH1HF	1µL	1µL
3	Mlu1 HF	1µL	1µL
4	MilliQ	41µL	23µL

  - 15min, 37°C
- 1% Agarose Gel
- mix samples with 15µL loading dye no SDS; 10min 150V
- NEB Monarch Gel Extraction kit for gel purification : elute in 10µL H_2O (Backbone) in 14µL H_2O (insert)
  (insert bands were not clearly visible; next time if the insert is a gBlock no gel purification !)
- Nanodrop :
  insert : 20,4 ng/µL
  backbone : 52,3 ng/µL

ligation V27 :

- T4 Buffer: 2µL
- T4 ligase : 1µL
- Vector (backbone) : 1,05µL
- insert : 2,04µL
- H_2O : 13,9µL
- 10min at roomtemperature, 10min at 65°C

gibson assembly V28, V25 :

- V28 :

    - V4 (backbone) : 1.32µL
    - insert (HisGPSGL1) : 1.74µL
    - Mlu : 10µL
    - H_2O : 9.69µL
- V25 :
    - V4 (backbone) : 1.32µL
    - insert (hArcHiBit) : 4.6µL
    - Mlu : 10µL
    - H_2O : 4.08µL
- 15min at 50°C

Transformation :

- V26 : 0.5µL V26 + 500µL H_2O -> 2µL
- V25, V27, V28 : 2µL
  -> NEB C3040 protocol

VLP harvesting and HiBit assay :

- VLP harvesting was carreid out according to the standard protocol
- The HiBit assay was carried out according to the standard HiBit assay protocol
- Notes : sample
    - A11 might be diluted
    - Sample E2 might contain the sample from E3
    - sample C5 might have a higher signal because the cells were suspended much more than in the other wells
    - No PBS washing was carried out for the first time