

08. (August) 2019

Project: iGEM_Munich2019 Shared Project

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MONDAY, 26/8/2019

Johanna:

cell culture:

Medium exchange T75 flask VLP: qPCR and Purification

- take out Medium, add 15 mL new, warm medium
- finished at 9:30 a.m.
- use the old Medium (VLP containing medium) for biotin purification

Biotin Purification

- start 2 overnight-incubations
 - prepare 2 columns with 500 µL Biotin-Agarose
 - protocol **XXXXXX**
 - transfer the Biotin-PBS mixture to 2 new falcons
 - centrifuge the harvested medium (2000g, 10 min, 4 °C)
 - add 2 mL of the centrifuged supernatant to the Biotin-PBS mixture
 - incubate at 4 °C, shaking
 - finished at 10:00 a.m.
- prepare a Biotinbuffer
 - 50 mM in 100 mL
 - use D-Biotin (MW: 244,31 g/mol), NaOH (1 molar), pH-meter, agitator, heating plate (30-40 °C)
 - biotin is only soluble in basic conditions --> use NaOH

Alejandro

Cloning of V33

- V33 ≈ Munich iGEMs 2018's BBa_K2170002 ORF in the pCAG backbone
- Backbone: V4 digestion with Mlu1-HF and Pax1 (37 °C for 20 min)

restriction digest V33		
	A	B
1	DNA	1 µg
2	10X CutSmart Buffer	5 µl (1X)
3	Restriction Enzyme (Pax1 and Mlu1-HF)	each 1 µl
4	ddH2O	to 50 µl
5	Total	50 µl

- Insert generation via PCR:

Insert generation V33 PCR		
	A	B
1	2X Q5 MasterMix	12.5 µL
2	primer 57	1.25 µL
3	primer 58	1.25 µL
4	BBa_K217000 2	0.1 µL (5 ng)
5	ddH2O	10 µL

■ thermocycler:

PCR Insert generation for V33		
	°C	sec
1	98	30
2	98	10
3	67	20
4	72	60
5	go back to 2 (32x)	
6	72	120
7	4	till End

- both fragments were gel purified (1 % agarose, 120 V, 30 min, NEB gel extraction kit)
- Yield: Pac1-V4-Mlu1: 23 ng/µL; Gibson-BB2016: 70 ng/µL
- fragments were frozen @-70 °C

Gibson assembly of V33

- 1:2 vector:insert
- Gibson assembly with the HiFi DNA Assembly Master Mix from NEB (standard protocol followed)
- Vector: 4800 bp; Pac1-Mlu1-digested V4: 3 µL, 69 ng, 0.023 pmol
- Insert: 2800 bp; BBa_K2170002 for Gibson PCR amplicon: 1.13 µL, 79.6 ng, 0.046 pmol
- + 10 µL HiFi Mastermix + 6 µL MQ-H2O -> 20 µL total volume -> 15 min @ 50 °C

Johanna

Prepare new Biotin-Purification

- use the 72h-after transfection medium from the T75 flask, centrifugation (2000 g, 10 min)
- prepare 1 biotin-agarose column
 - 500 µL Biotinagarose (1:1 in PBS) -> transfer it with 1 mL PBS to a flacon
 - add 2 mL SN
 - incubate @ 4°C, shaking; time: 11:30
- prepare 1 Eppi with 50 µL magnetic biotin beads, equilibrate 2x with 100 µL PBS
 - add 400 µL SN

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- incubate @ 4°C, rotating, time: 11:30
- caution: origin SN for HiBit was warmed up to 37 °C for 1 hour with DNase

cell culture:

- splittin: Passage 29 with 800 µL