06. (June) 2019

Project: iGEM_Munich2019 Shared Project
Authors: Johanna Wallner

TUESDAY, 18/6/2019

Transformation for V9&V10 cloning

Ligation of V9

	Fragment	Volume (ul)	Comment
1	Nhe1 - V8 - Mlu1	2.125	6406bp; 85ng; ~20fmol
2	Nhe1 - CC10 - Mlu1	1.25	111bp; 5ng; ~60fmol; Volume from 1:10 dilution
3	T4 10x Buffer	2	
4	MQ-H2O	13	
5	T4 Ligase	1	
6	*Total*	20	

- Incubated at RT for 10min
- Heat inactivation at 65°C for 10min
- Transformation into NEB stable competent E. coli (50ul) with 4ul ligation assay.

Gibson Assembly of V10

	Fragment	Volume (ul)	Comment
1	Pac1 - V4 - Mlu1	2	4817bp; 200ng; 63fmol
2	CC10 Gibson	3.63	179bp; 37ng; 315fmol
3	L7Ae Gibson	1.62	418bp; 35ng; 126fmol
4	MQ-H2O	2	
5	HiFi NEB Master Mix	10	
6	*Total*	20	

- Incubate at 50°C for 15min.
- Put on ice.
- Transform NEB stable competent E.coli (50ul) with 2ul Gibson assembly assay.

Note: The cells were incubated for 60min in the recommended medium at 37°C and not 30°C!

Primer shipment

Primer numbers 15, 24, 25, 27, 32 & 33 were received, diluted to 100uM and also diluted again to 10uM in seperate Eppis for use in PCRs.

Maxiprep of Plasmid V8

- spin 4 Falcons (45ml each) (4000rpm, 15min)
- Join all 4 pallets in 1 Falcon and resuspend in 8ml Buffer P1
- follow instructions on "Qiagen Plasmid Plus Maxi Kit"
    - choose high-yield protocol

change to step 13+14: 13000 instead of 9700 rpm, 300 instead of 400yl Milli-Q
note: Filter blocked after 80% volume => 20% waste

concentration: 1208,3ng/ul (260/280 = 1,88; 260/230 = 2,31)