

06. (June) 2019

Project: iGEM_Munich2019 Shared Project

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Restriction digest

of V8 and amplicon Nhe1 - CC11 - Mlu1

Restriction Digest 17.6.		
	Component	Volume
1	10x CutSmart Buffer	10ul
2	Pac1	2ul
3	Mlu1-HF	2ul
4	DNA	1,1ul
5	Milli-Q	84,9ul

Purification of Nhe1 - CC11 - Mlu1 (digested) with the NEB 5ug DNA/PCR purification kit.

Purification of Nhe1 - V8 - Mlu1 and Pac1 - V4 - Mlu1 via 1% agarose gel (150V, 20min) and the NEB purification kit.

Purification Concentrations 17.6.			
	Fragment	Concentration (ng/ul)	Volume
1	Nhe1 - CC11 - Mlu1	40	8
2	Nhe1 - V8 - Mlu1	100	30
3	Pac1 - V4 - Mlu1	40	15

Cryostock of V8

- 750ul bacterial preculture of V8#6 were mixed with 750ul glycerol and stored at -80°C.
- 100ul of the same bacterial preculture were used to inoculate 150ul LB-Amp to do a Maxiprep on the next day. (-> 37°C).