# 06. (June) 2019

Project: iGEM\_Munich2019 Shared Project

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## Cloning of V8

### Restriction of V4 to generate backbone

The MCP protein was cut out form plasmid V4 by digestion with EcoRI-HF and Mull-HF according to the Restriction digest protocol and purified with the Monarch® DNA Gel Extraction Kit.



### Building of insert out of primers 1 & 2

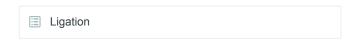
Primers 1 and 2 were diluted from 10  $\mu$ M to 20 nM in MQ-H<sub>2</sub>O. 10  $\mu$ I of each dilution were given together in a PCR tube. The solution was warmed up to 95 °C and cooled down to 45 °C over 5 min in the thermocycler.

This creates a dsDNA fragment, which serves as the insert in the upcoming ligation reaction. The primers were designed to contain the coding sequence of a coiled coiled domain and result in a dsDNA with the appropriate overhangs after dimerization.

#### Ligation: EcoRI-HF/Mull-HF digested V4 and insert from primers 1 & 2

6 μl of the DNA insert (0.06 pmol) and 0.02 pmol digested backbone were used for the ligation.

The standard ligation protocol was used.



#### Transformation of V8

NEB® Stable Competent *E. coli* cells were transformed with 2 µl ligation product according to protocol and plated on LB agar with ampicillin (100 µg/ml).



## Transformation of V1, V4, and V5

NEB® Stable Competent *E. coli* cells were transformed with V1, V4, and V5 according to protocol and plated on LB agar with ampicillin (100 μg/ml).

∷	Chemical Transformation

file:///tmp/tmpRD4EEK.html