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 MulI-HF according to the Restriction digest protocol and purified with the Monarch® DNA Gel Extraction Kit.

Gel Extraction (Monarch® DNA Gel Extraction Kit)

Restriction Digest

Building of insert out of primers 1 & 2

Primers 1 and 2 were diluted from 10 µM to 20 nM in MQ-H_2O. 10 µl 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/MulI-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.

Ligation

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).

Chemical Transformation

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