# 06. (June) 2019

**Project:** iGEM_Munich2019 Shared Project
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Sequencing results :

- V10 : #3,#5,#8 were positive -> continue with V10#5
- 500mL LB medium + 500µL Amp

restriction :

- Insert DNA creation from primers 22 and 23 / primers 32 and 33 according to standard restriction protocol
- restriction digest of V10#5 (backbone) and V4 (insert)

**scheme**

| | A | B | C |
|---|---|---|---|
| 1 | DNA | 0.5µL | 3.3µL |
| 2 | 10xCutSmartBuffer | 5µL | 5µL |
| 3 | NheI-HF | 1µL | 1µL |
| 4 | Mlu-HF | 1µL | 1µL |
| 5 | MilliQ | 42.5µL | 39.7µl |

- incubate at 37°C for 15min
- both samples were mixed with 15 µL DNA loading dye and put on  an electrophoresis gel for 20min
- V4 was discarded, because there were 2 bands visible and we already ampified the insert via PCR

ligation :

- T4 Buffer 10x : 2µL
- T4 Ligase : 1µL
- Vector DNA (V10) : 50ng
- Insert DNA (V4) :  37.5ng
- nuclease free water : 17.04µL

restriction digest :

- DNA (Nhe-MCP-Mlu1) : 7.3µL
- 10x Cut Smart Buffer : 5µL
- Nhe1-HF : 1µL
- Mlu1-Hf : 1µL
- MiiliQ : 35.7µL

purification :

- cleanup of restriction digest using NEB Monarch PCR DNA cleanup kit
- ligation on digested V10 backbone and MC-fragment to form V11 (see above)
    - incubate for 10min at room temperature
    - heat inactivation at 65°C for 10min

transformation :

- according to standard transformation protocol started with overnight culture

cell culture :

- splitting of motherplates
- 6 wells of a 6 well plate a 500.000 cells/well
- 30 wells of a 96 well plate a 20.000 cells per well
- new DMEM medium prepared