NEB BioBrick Assembly Kit

- We used the BioBrick Assembly Kit to assemble an upstream part with a downstream part into destination plasmid.
- Digestion Protocoll:
  - Digest upstream part with EcoRI-HF and SpeI
  - Digest downstream part with XbaI and PstI
  - Digest destiantion destination plasmid with EcoRI-HF and PstI
  - 500 ng part DNA
  - 1 µl of each enzyme
  - 5 µl 10x NEBuffer 2.1
  - to 50 µl H_2O
- Incubate all three restriction digest reactions at 37 °C for 10 minutes and then heat inactivate at 80 °C for 20 minutes.
- Dephosphorylation Protocoll
  - Add 1 µl of AP (Antarctic phosphatase) and 5 µl of 10x AP reaction buffer to digested destination plasmid, incubate for 1 h at 37 °C.
- Ligation Protocoll
  - Ligate the upstream and downstream parts into the digested destination plasmid.
    - 2 µl of each part
    - 2 µl 10x T4 DNA Ligase Buffer
    - 1 µl T4 DNA Ligase
    - 11 µl H_2O
  - Incubate at room temperature for 10 minutes and then heat inactivate at 80 °C for 20 minutes.
- Transform 1-2 µl of the ligation product into 50 µl of competent E. coli cells.