

Cpf1 digest and gRNA

Aim of the experiment:

Generating of gRNA

Material:

10x TX buffer

rNTP mix

T7 polymerase

DNAse I (2.000 U/mL)

Cpf1 (4000 u/mL)

NEB 3.1 10x NEB buffer

Protocol:

1. Pipette following chemicals together (vol: 100 uL) and incubate for 4h at 37°C
 - a. 10 µl 10x TX buffer
 - b. 16 µl rNTP mix
 - c. 2 µg template DNA
 - d. 8 µl T7 polymerase
 - e. Fill up with water
 - f. 1 ul DNAse I (2.000 U/mL) added and incubated for 10 minutes at 37 °C.
2. Afterwards a phenol chloroform precipitation occurred
3. A cpf1 digest has been carried out
 - a. 0,5 uL cpf1
 - b. 10 uL NEB buffer
4. Incubate for 1h at 37°C and inactivate cpf1 at 65°C for 10 min

Zweites Protovoll

Cfp1 digest: