Plasmid preparation for transformation into *S.epidermidis*

Materials:

- epiFlex p1 backbone
- epiFlex p0 plasmids containing parts of interest
- epiFlex colony PCR primers
- Taq polymerase PCR kit
- NEB BsaI-HFV2 enzyme
- T4 DNA Ligase Buffer
- T4 DNA Ligase
- Nuclease free dH20
- Competent *E.coli* Top 10
- Competent *dam-/dcm- E.coli*
- LB + Kanamycin agar plates
- LB Media + Kanamycin
- Plasmid Miniprep Kit

Methods:

- Prepare golden gate reaction according to the NEB guidelines and transform into *E.coli* TOP10 cells using the heatshock method.
- Screen for successful transformants using Taq polymerase PCR kit and 10µM epiFlex primers, run reaction at the following cycling parameters:

initial denaturation	95	3 min	1
Denaturation	93	30 sec	
Annealing	56	45 sec	
Elongation	72	1 sec	
Final Elongation	72	10 min	1
Store	4		

- Inoculate successful colonies for overnight culture in 5mL LB + Kanamycin media
  and simultaneously streak a corresponding fresh plate. Leave plates to incubate at 37°C and liquid culture to incubate at 37°C with shaking at 250rpm.
- Miniprep overnight cultures using miniprep kit of choice, check DNA concentration and quality.
- Transform *dam-/dcm- E.coli* with miniprepped plasmid using the heatshock method as before.
- Inoculate transformed colonies for overnight culture in 100mL LB + Kanamycin media incubate at 37°C with shaking at 250rpm.
- Collect cells and mini-prep DNA as before, elute with 25-30 µL of nuclease free water (want DNA to be as concentrated as possible!)
- Check DNA concentration and purity, and note it for future electroporations.