Aachen Collaboration

Week	Date	Jobs
	16.08.17	Gene of interest (ATCLCc) was synthezised by IDT, gene was flanked by 3b overhangs for eventual golden gate cloning -since the gene of interest still includes BsmBI cutsides, it was ligated into level 0 backbone -backbone of the toolboxpart 32 was digested with BsaI -gelextraction of 1666 bp band (backbone)
	17.08.17	-gene of interest was digested with BsaI in order to generate sticky 3b ends -heat kill was performed in order to deactivate enzyme activity -ligation of backbone (16.08.17) and digested gene of interest -transformation of ligation product into competent *E. coli* cells
	18.08.17	-8 colonies were picked and inoculated into LB media containing chloramphenicol
	19.08.17	-plasmid preparation was performed successfully
	20.08.17	-restriction digest of all 8 plasmids of level 0 construct including ATCLCc gene with PvuII and NcoI -> all 8 plasmids appear to be correct according to restriction digest
	21.08.17	-Level 1 golden gate was prepared trying to create four different plasmids (genome-integration plasmid with Gal1 promotor, plasmid with Gal1 promotor, plasmid with Sac6 promotor, plasmid with RPL18B promotor) -products were transformed into competent *E. coli* cells
	22.08.17	-8 colonies were picked from each construct and inoculated into LB media containing ampicillin
	23.08.17	-plasmid preparation was performed successfully -test restriction was performed, results appeared to be negative ->presumably because of contamination in one of the used toolbox parts
	24.08.17	-since the first golden gate attempt was negative, new golden gate level 1 was performed -products were transformed in competent *E. coli* cells
	25.08.17	-8 colonies were picked from each plasmid and inoculated into LB media containing ampicillin
	26.08.17	-plasmid preparation was performed successfully -test restriction was performed, results appeared to be negative ->the reason may be that we used media plates for transformation which did not include ampicillin

	27.08.17	-new golden gate level 1 was performed using new toolbox parts, additionally we increased the used volume of BsaI enzyme since we needed to ligate 9 parts in our golden gate reaction -transformation into competent *E. coli* cells
	28.08.17	-8 colonies were picked from each plasmid and inoculated into LB media containing ampicillin
	29.08.17	-plasmid preparation was performed successfully -restriction digest was conducted for each colony ->positive result for each plasmid ->plasmids were constructed successfully and therefore delivered to iGEM team Aachen