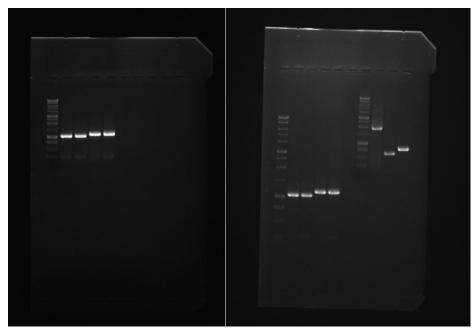
08.07

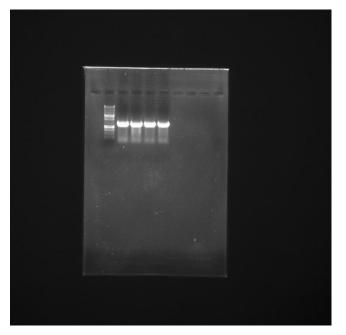
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080717b



09.07 We prepared competent cells and ran a PCR

# 21.07



- 07.08 competent cells DH5α, ligase reaction, transformation
- 08.08 competent cells DH5α, ligase reaction, transformation
- 09.08 medium cell stock MP6
- 10.08 PCR electrophoresis
- 11.08 preparing TSS Buffer, competent cells, electrophoresis, transformation
- 12.08 competent cells, miniprep of pSB1A3, electrophoresis, ligase reaction, transformation
- 13.08 -----
- 14.08 miniprep of MP6, preparing competent cells, electrophoresis
- 15.08 competent cells, miniprep, electrophoresis
- 16.08 -----
- 17.08 -----
- 18.08 One of our teammates presented to us a scientific article on topic Clonetegration
- 19.08 -----
- 20.08 -----
- 21.08 For the first time we tried clonetegration. Then ran a PCR and electrophoresis
- 22.08 We repeated the clonetegration. We made competetent cells, electrophoresis, extraction
- 23.08 electrophoresis, restriction with Pst1 and EcoR1, ligase reaction, transformation
- 24.08 We made a restriction with Pst1 and EcoR1, electrophoresis
- 25.08 This day we had a seminar
- 26.08 -----
- 27.08 -----
- 28.08

29.08 clonetegration vectors: KP, KO, KL, KH, KT, restriction with EcoR1 and Pst1, electrophoresis, competent cells, transformation

30.08 restriction, gel extraction, electrophoresis, ligase reaction

31.08 competent cells, clonetegration, electrophoresis, gel extraction, electrophoresis, ligase reaction, transformation in DH5 $\alpha$ 

- 01.09 We were working on wiki and planning new activities all day
- 02.09 One of our teammates held a presentation about MutL, MutH and MutS genes

03.09 -----

04.09 Inoculation of overnight DH5a culture

05.09 Running an overlap PCR, electrophoresis, gel extraction, medium cell stocks with different concentrations, autoclave, overnight PCR

06.09 We left E.coli cells on petri dishes with different antibiotic concentrations to grow for a day at a room temperature

07.09 We checked the results and ran an electrophoresis of overlap PCR

08.09 miniprep pOSIP\_CH, restriction with EcoR1 and Pst1, electrophoresis, gel extraction, ligase reaction, overnight DH5 $\alpha$  cells with pOSIP TH vector (tetracycline resistance)

09.09 miniprep pOSIP\_TH, restriction, electrophoresis,

10.09 We watched and discussed one of our teammates' presentation about Methods for in vivo mutagenesis

11.09 electrophoresis for pOSIP\_TH and quantification, wiki tools

12.09 colony PCR with Phusion polymerase, restriction with EcoR1 and Pst1, electrophoresis, overnight cells

13.09 colony PCR of iGEM constructs, electrophoresis

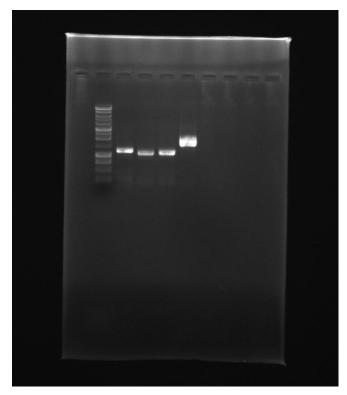
14.09 colony PCR of iGEM constructs with Taq polymerase, electrophoresis

15.09 -----

16.09 miniprep, PCR with Phusion polymerase

17.09 electrophoresis, restriction of GFP and RFP with Xba1 and Pst1, restriction of vector with tetracycline promotor Spe1 and Pst1, electrophoresis





18.09 -----

19.09 -----

20.09 miniprep of RFP and GFP, restriction of RFP and GFP, restriction of fragment tetR generator, electrophoresis, extraction, electrophoresis, writing a project for sponsorship21.09 PCR with previous days' results, extraction, ligase reaction of GFP and RFP, transformation. We spend the rest of the day writing a project for sponsorship.





22.09 We made overnight cells with GFP-tetR generator and RFP-tetR generator and began preparing for "The European Researchers' Night" where we had a workshop.

23.09 colony PCR with Taq polymerase, transformation with pSB1A3 in DH5 $\alpha$ , electrophoresis, overnight cells GFP-tetR promotor

24.09 stock Tet, competent cells, PCR with Pfu polymerase, electrophoresis, overnight cells of pSB1A3 + Amp

25.09 miniprep of pSB1A3, dilution of CRISPR gRNA vector and primers - BioBrick\_Sulffix-R, BioBrick\_Prefix-F, PCR with Phusion polymerase, electrophoresis, PCR, restriction of pSB1A3 and CRISPR gRNA, electrophoresis, ligase reaction of pSB1A3 and CRISPRgRNA, transformation in DH5α, overnight cultures on petri dishes

26.09 PCR (x2) with Phusion polymerase and Phu polymerase, electrophoresis (x2), extraction of PCR with Phusion, transformation

27.09 miniprep of dCas9 vector, competent cells, transformation of T-vector in DH5 $\alpha$ , inoculating the transformants on petri dishes with Amp, IPTG and X-gal

28.09 miniprep of pSB1K3, overnight cells of the previous day transformants (1 and 2)

29.09 We took part in the event "The European Researchers' Night", miniprep of the overnight's cells (1 and 2), restriction of 1,2 and pSB1K3 with EcoR1 and Pst1, electrophoresis, gel extraction, electrophoresis

30.09 miniprep of 1, restriction with EcoR1 and Pst1, electrophoresis, gel extraction

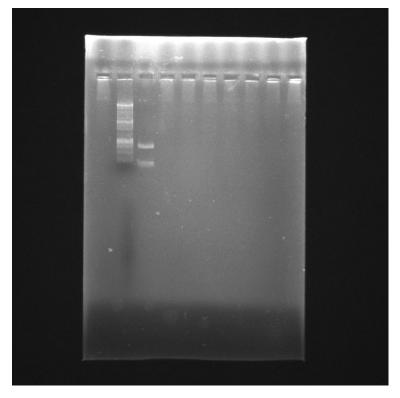
01.10 PCR with Phusion polymerase of the previous day's cultures, electrophoresis, miniprep of 1-4 cultures, wiki design

02.10 PCR with Phusion polymerase of previous day's miniprep, electrophoresis, working on wiki

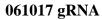
03.10 Colony PCR with Taq polymerase of gRNA, electrophoresis. We diluted CRISPRgRNA, ran an overnight PCR with Taq polymerase

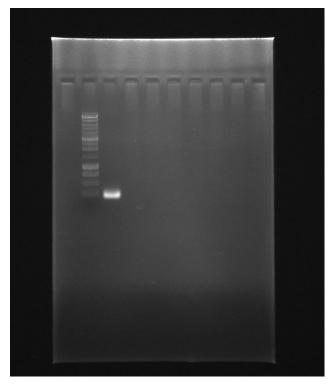
04.10 electrophoresis, competent DH5 $\alpha$  cells, extraction, electrophoresis, quantification, aqua cloning, transformation in DH5 $\alpha$  and inoculation on petri dishes with Chl

05.10 PCR with supernova fragment, extraction, restriction with Xba1 and Pst1, extraction, electrophoresis, overnight cultures from aqua cloning



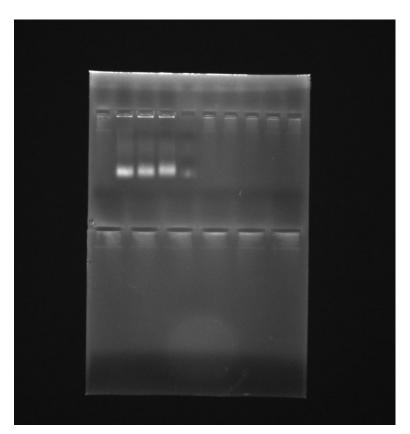
06.10 dilution of Preffix and Sulfix primers with TE buffer, PCR of gRNA with Phusion polymerase, electrophoresis, colony PCR of aqua clones with Taq polymerase, gRNA extraction, restriction of pSB1K3 with EcoR1 and Pst1, electrophoresis, gel extraction, electrophoresis, overnight PCR





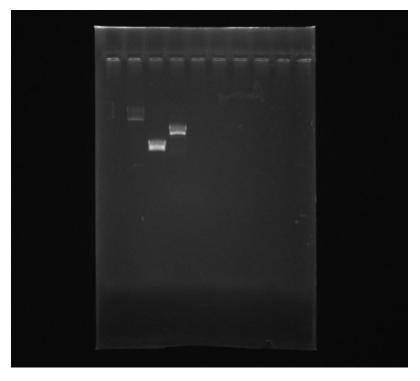
07.10 electrophoresis, PCR with Pfu polymerase, dilution of primers 5-colonysupernova-R and 5-colony-dCas9-C-F, ligase reaction(x2) of pSB1K3 and SuperNova and pSB1K3 and gRNA, electrophoresis, transformation of ligase reaction in DH5 $\alpha$ , overnight PCR





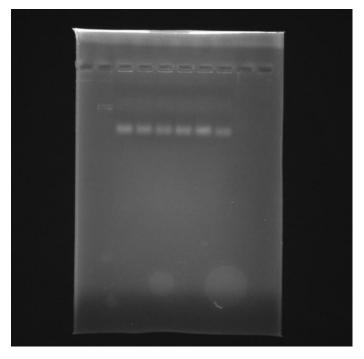
08.10 PCR of Killer Orange, Mini SOC and SuperNova, extraction, electrophoresis, aqua cloning with vector dCas9



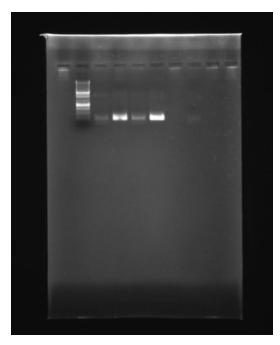


09.10 electrophoresis, miniprep of 1, 2, 3 and 4 pSB1K3 clones + gRNA, PCR with Phusion polymerase, electrophoresis, overnight PCR with Phusion polymerase

### 91017 gRNA cr

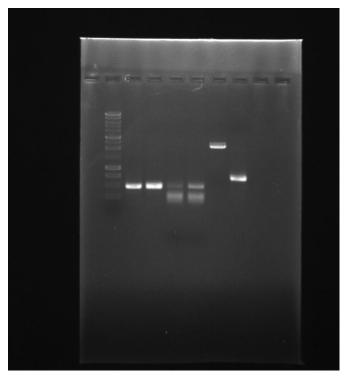


10.10 PCR of 1, 2, 3 and 4 clones of pSB1K3 + gRNA with Phusion polymerase **101017** 

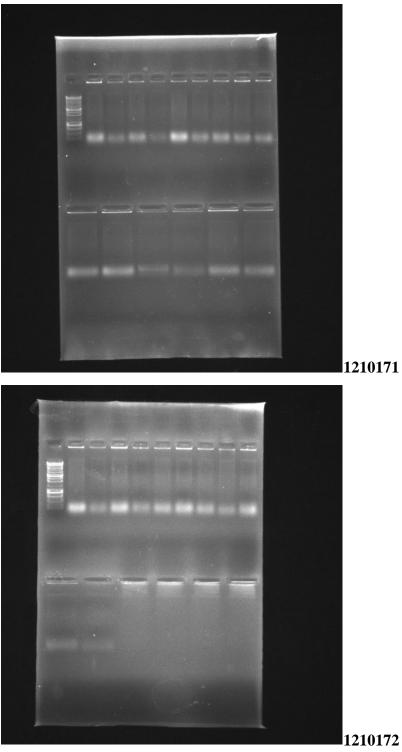


11.10 miniprep of gRNA 1 and 2 and Amp vector, restriction of Amp vector with Spe1 and Pst1, restriction of Tet inverted generator with Xba1 and Pst1 extraction, electrophoresis, overnight cultures of gRNA1 and 2

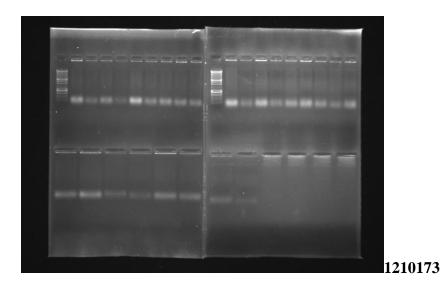
## 111017



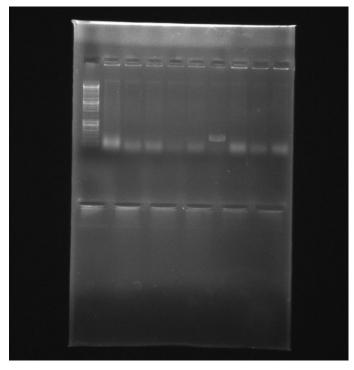
12.10 electrophoresis, colony PCR of dCas9 with Pfu polymerase, ligase reaction of yesterdays' vector and insert, transformation in DH5 $\alpha$ , overnight cultures with Amp in the medium





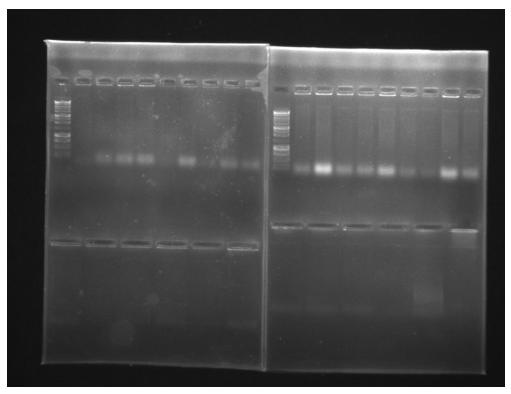


13.10 PCR of Killer Orange with Pfu polymerase, electrophoresis, restriction of gRNA1 with Eco31, extraction

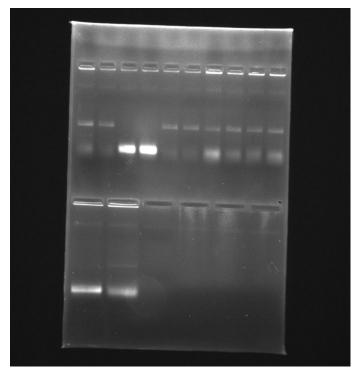


- 14.10 working on wiki and presentation
- 15.10 preparing Visa documents
- 16.10 working on wiki and presentation



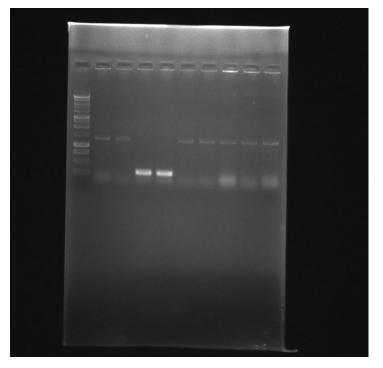


17.10 PCR with Phusion polymerase, electrophoresis171017

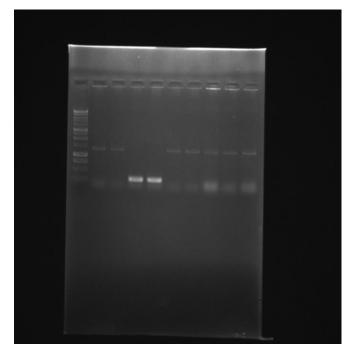


18.10 We had a Visa interview ima 2 gela?!?!?!

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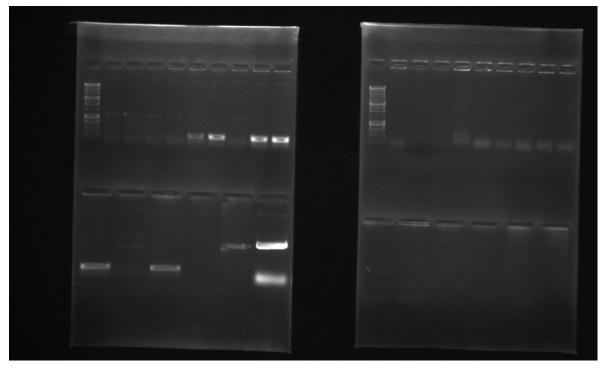


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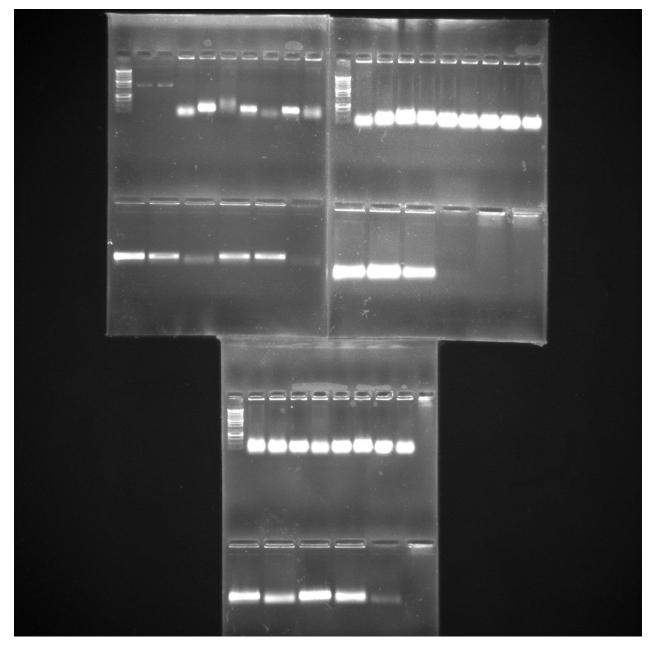
19.10 miniprep of pSB1C3, dCas9, Killer Orange, FtsZ gRna1 and FtsZ gRna2, PCR, electrophoresis, sponsorship project presentation





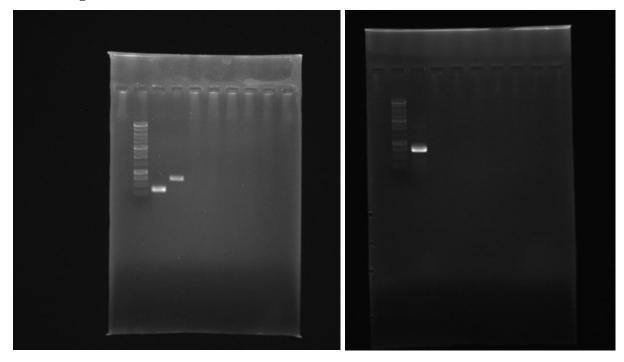
20.10 PCR, electrophoresis, extraction of Killer Orange and Mini SOC, overnight PCR 21.10 PCR of gRNA with Taq polymerase, electrophoresis, ligase reaction, miniprep of pSB1C3, restriction Mini SOC, Killer Orange and PSB1C3 with Pst1 and EcoR1, electrophoresis, gel extraction and quantification, overnight cultures, overnight PCR of pSB1K3 + gRNA1 with Phusion polymerase

# 211017 final vectors

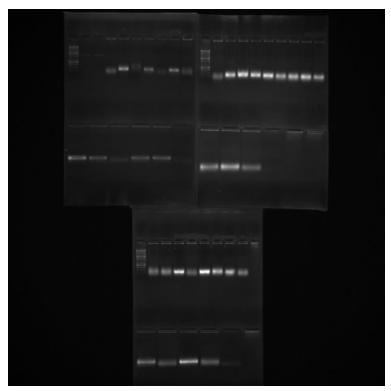


# 211017 3-gel

211017



# 211017 final



#### 211017 after band excision



22.10 Ligase reaction of Killer Orange and pSB1C3, ligase reaction of Mini SOC and pSB1C3, miniprep of GFP and RFP with Tet, transformation in DH5 $\alpha$ , inoculate in petri dishes with Chl, inoculation of GFP and RFP on LB + Tet medium, working on wiki

23.10 Half of our team worked on our wiki while the other half did laboratory work

