

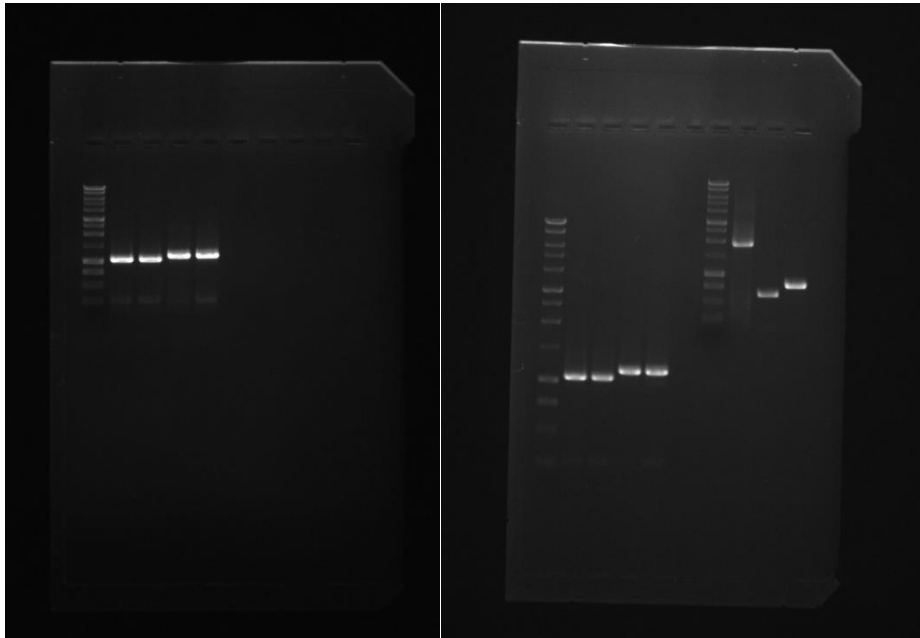
LAB BOOK

05.07 Team meeting

08.07 Electrophoresis

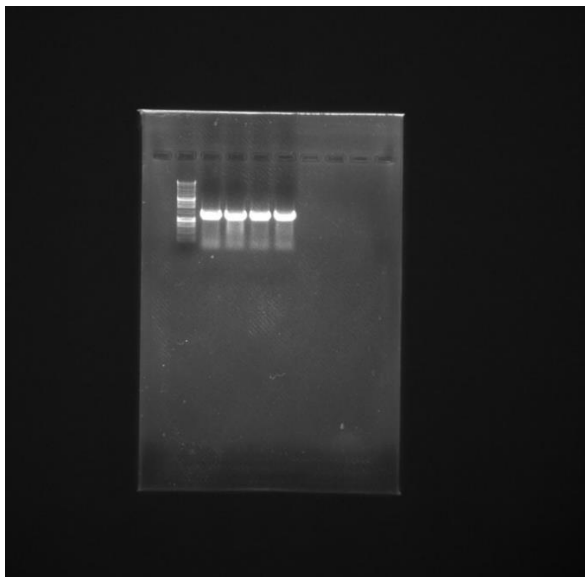
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09.07-21.07 Training the team

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22.07-06.08 Summer break

07.08 competent cells DH5 α , ligase reaction, transformation

08.08 competent cells DH5 α , 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 Break

14.08 miniprep of MP6, preparing competent cells, electrophoresis

15.08 competent cells, miniprep, electrophoresis

16.08 Break

17.08 competent cells, miniprep, electrophoresis

18.08 One of our teammates presented to us a scientific article on topic Clonetegration

19.08 competent cells DH5 α , ligase reaction, transformation

20.08 This day we had a seminar

21.08 For the first time we tried clonetegration. Then ran a PCR and electrophoresis

22.08 We repeated the clonetegration. We made competent 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 Break

27.08 Team meeting

28.08 Wiki planning

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 α

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 Break

04.09 Inoculation of overnight DH5 α 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 α 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 with 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

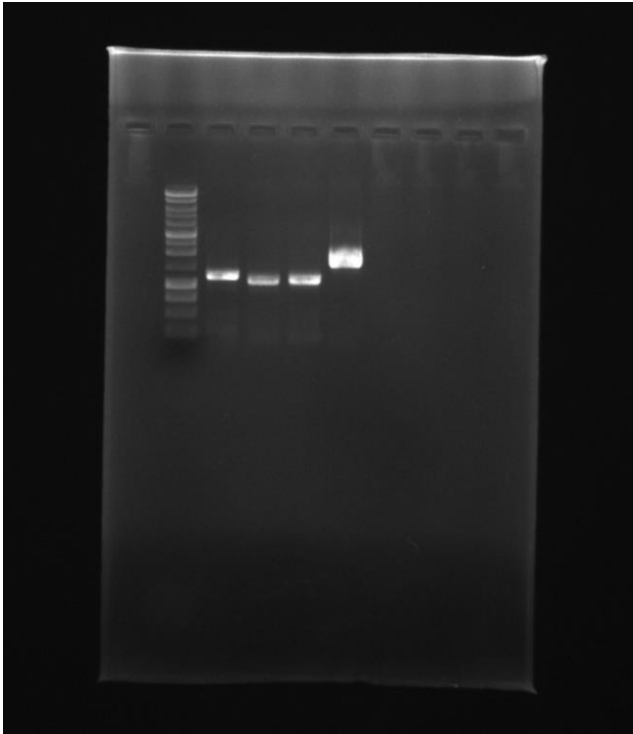
14.09 colony PCR of iGEM constructs with Taq polymerase, electrophoresis

15.09 miniprep, PCR with Phusion polymerase

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

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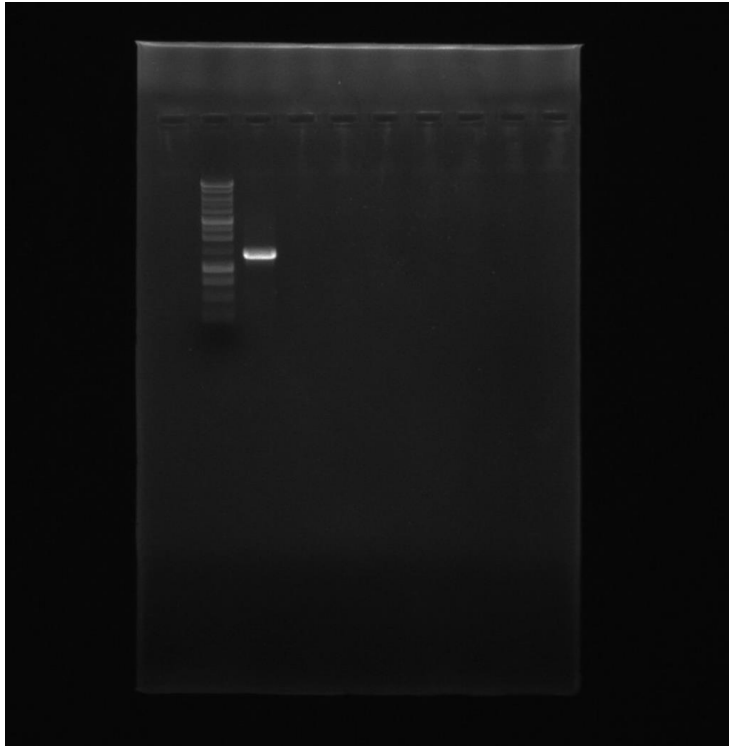
18.09 Team meeting

19.09 Working on poster

20.09 miniprep of RFP and GFP, restriction of RFP and GFP, restriction of fragment tetR generator, electrophoresis, extraction, electrophoresis, writing a project for sponsorship

21.09 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.

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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 α , 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_Suffix-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 DH5 α , 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 α , 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

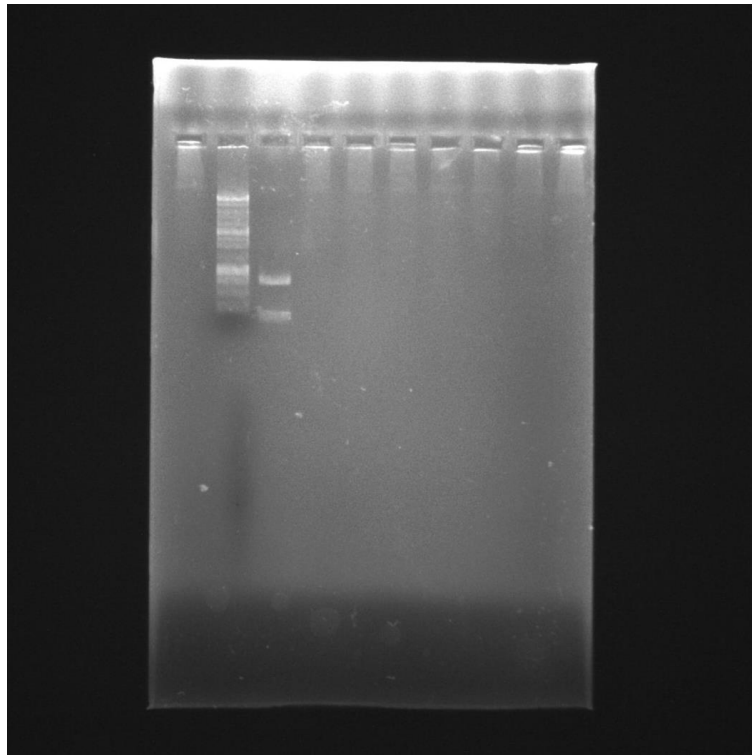
02.10 PCR with Phusion polymerase of previous day's miniprep, electrophoresis, working on wiki

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 α cells, extraction, electrophoresis, quantification, aqua cloning, transformation in DH5 α and inoculation on petri dishes with Chl

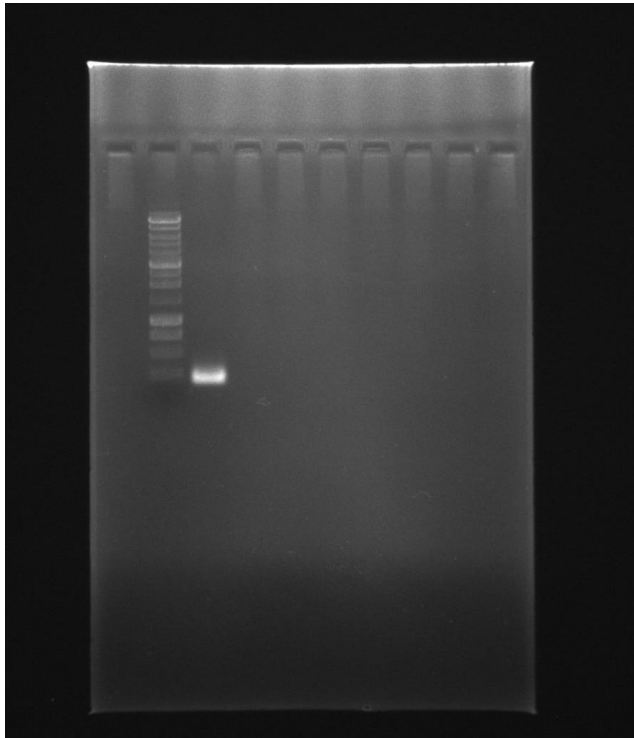
05.10 PCR with supernova fragment, extraction, restriction with Xba1 and Pst1, extraction, electrophoresis, overnight cultures from aqua cloning

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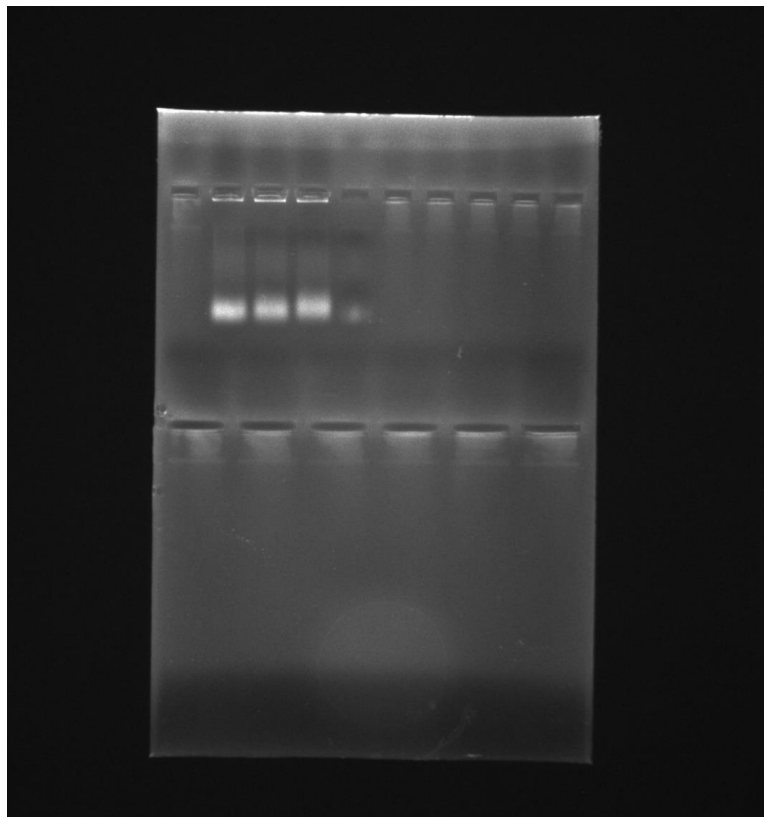
06.10 dilution of Preffix and Sulfix primers with TE buffer, PCR of gRNA with Phusion polymerase, electrophoresis, colony PCR of aqua clones with Taq polymerase, gRNA extraction, restriction of pSB1K3 with EcoR1 and Pst1, electrophoresis, gel extraction, electrophoresis, overnight PCR

061017 gRNA



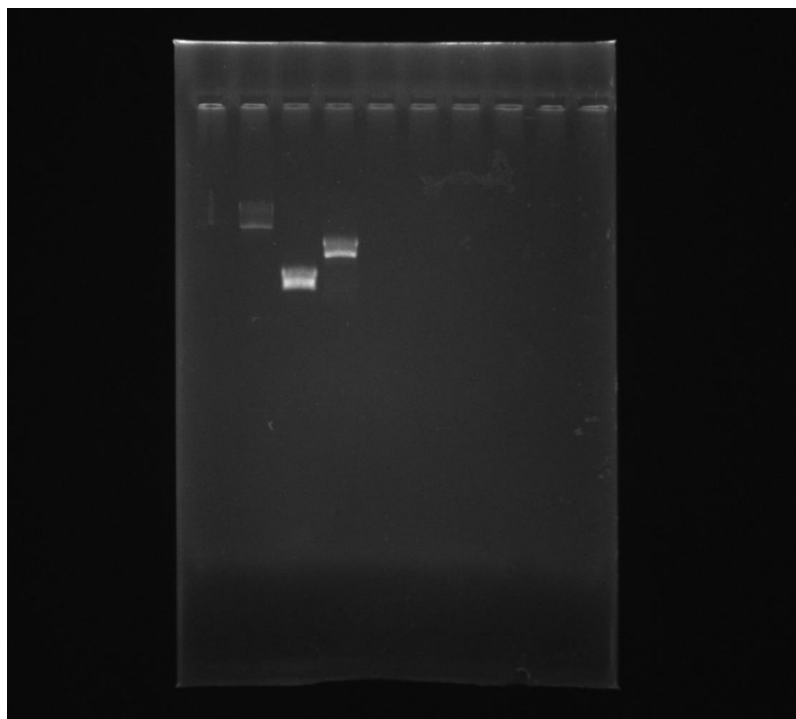
07.10 electrophoresis, PCR with Pfu polymerase, dilution of primers 5-colonysupernova-R and 5-colony-dCas9-C-F, ligase reaction(x2) of pSB1K3 and SuperNova and pSB1K3 and gRNA, electrophoresis, transformation of ligase reaction in DH5 α , overnight PCR

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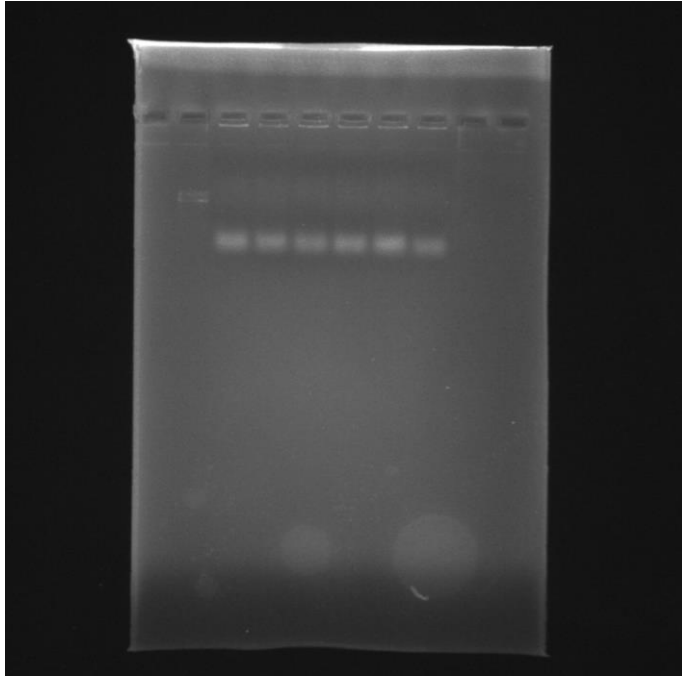
08.10 PCR of Killer Orange, Mini SOC and SuperNova, extraction, electrophoresis, aqua cloning with vector dCas9

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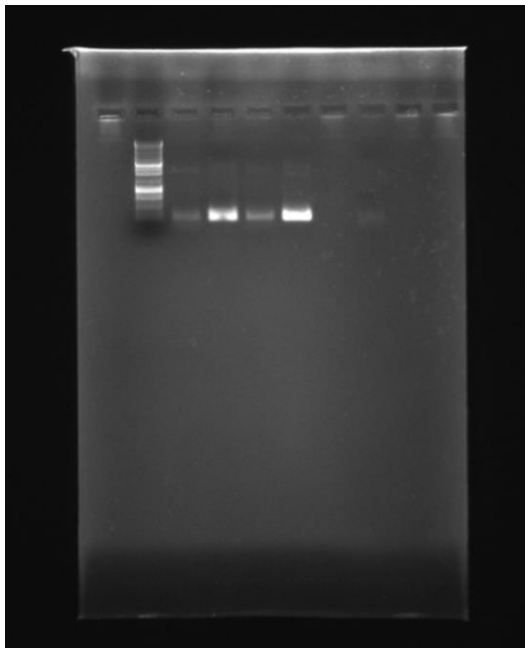
09.10 electrophoresis, miniprep of 1, 2, 3 and 4 pSB1K3 clones + gRNA, PCR with Phusion polymerase, electrophoresis, overnight PCR with Phusion polymerase

91017 gRNAcr



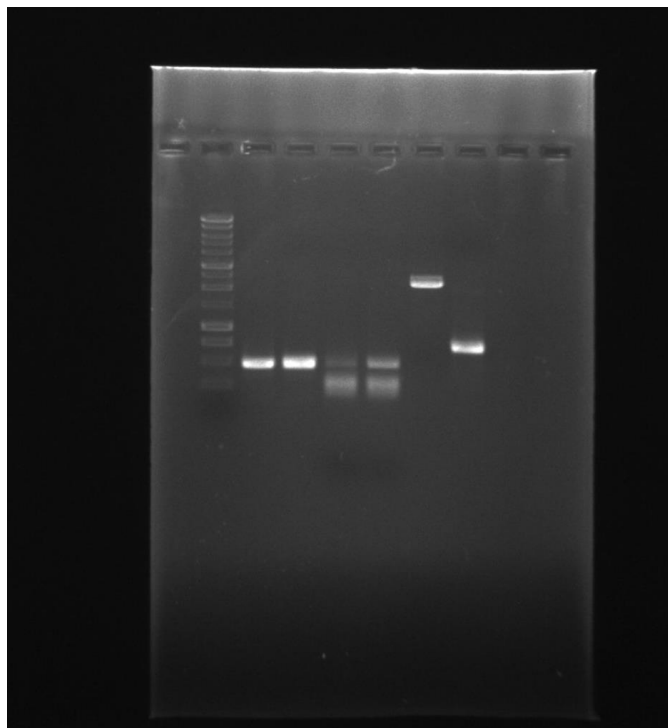
10.10 PCR of 1, 2, 3 and 4 clones of pSB1K3 + gRNA with Phusion polymerase

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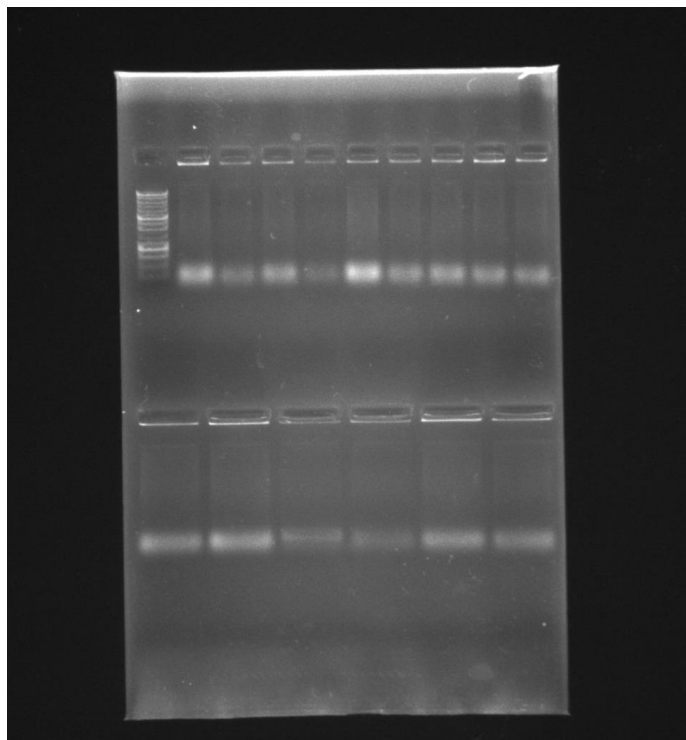
11.10 miniprep of gRNA 1 and 2 and Amp vector, restriction of Amp vector with Spe1 and Pst1, restriction of Tet inverted generator with Xba1 and Pst1 extraction, electrophoresis, overnight cultures of gRNA1 and 2

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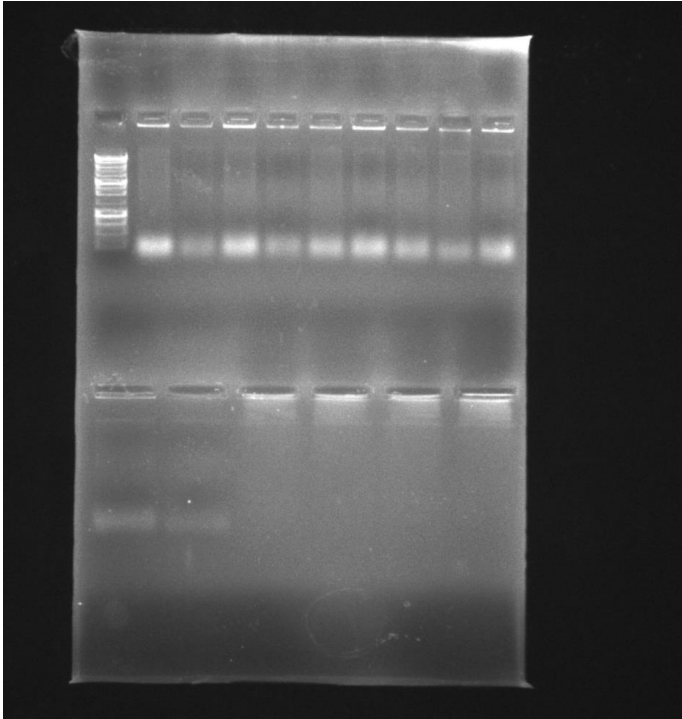


12.10 electrophoresis, colony PCR of dCas9 with Pfu polymerase, ligase reaction of yesterdays' vector and insert, transformation in DH5 α , overnight cultures with Amp in the medium

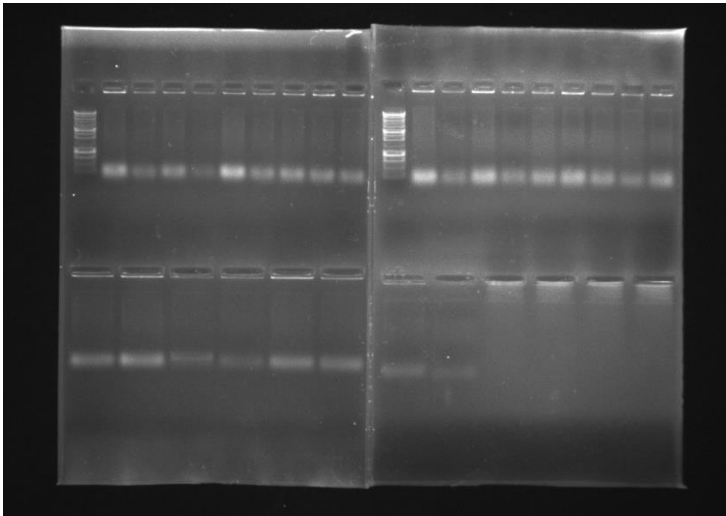
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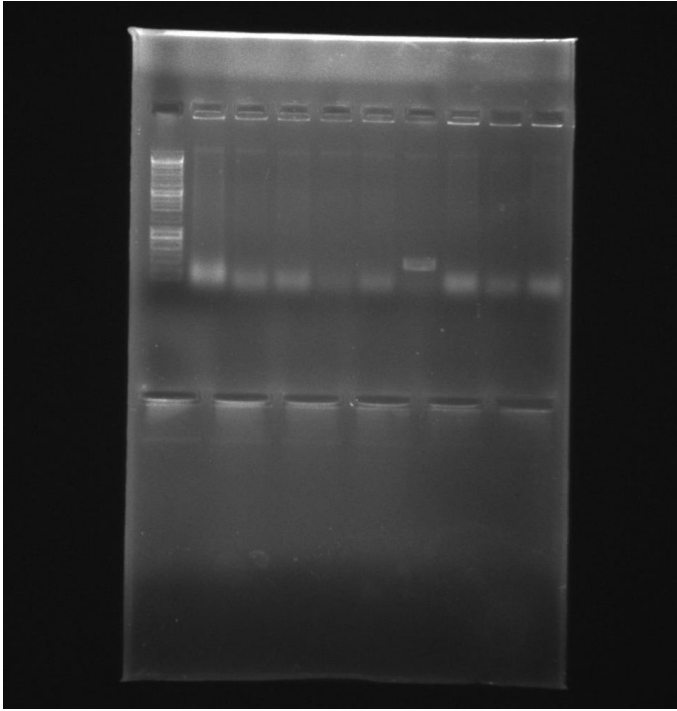


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13.10 PCR of Killer Orange with Pfu polymerase, electrophoresis, restriction of gRNA1 with Eco31, extraction

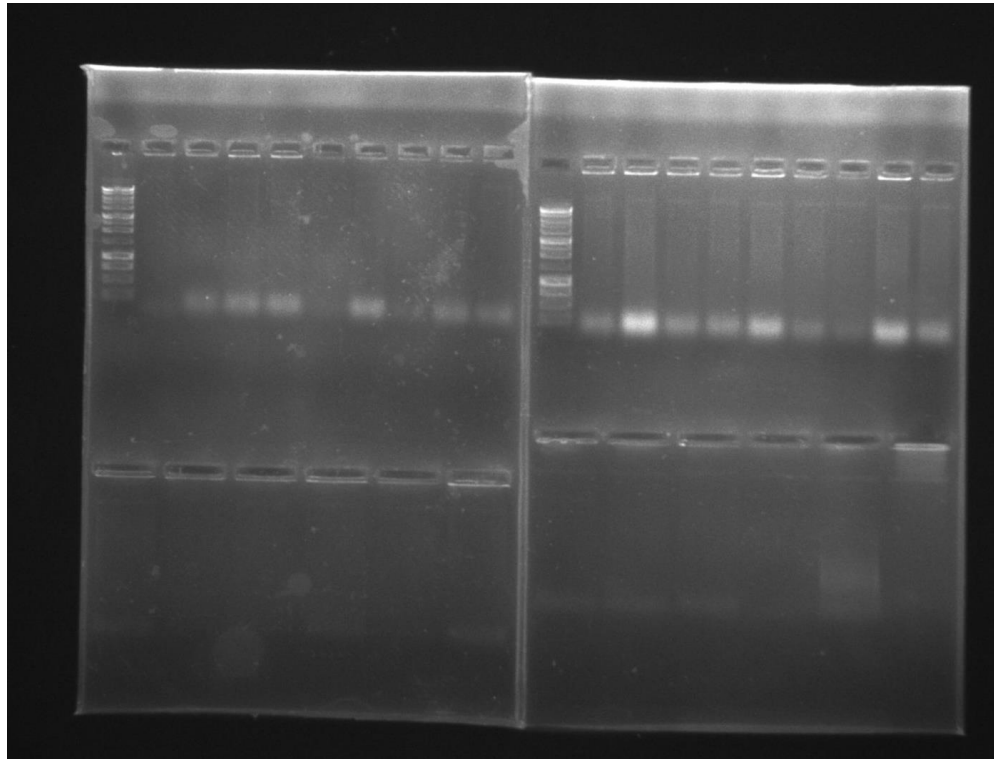
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14.10 working on wiki and presentation

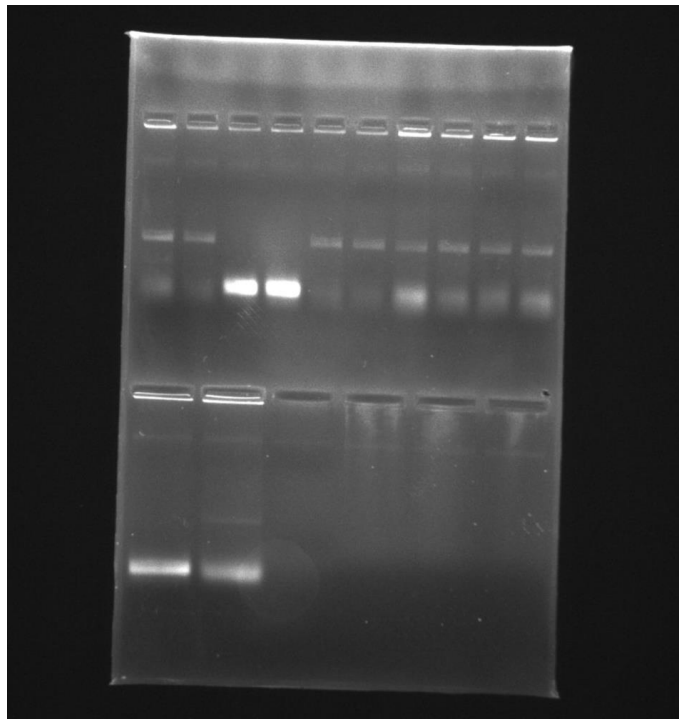
15.10 preparing Visa documents

16.10 working on wiki and presentation



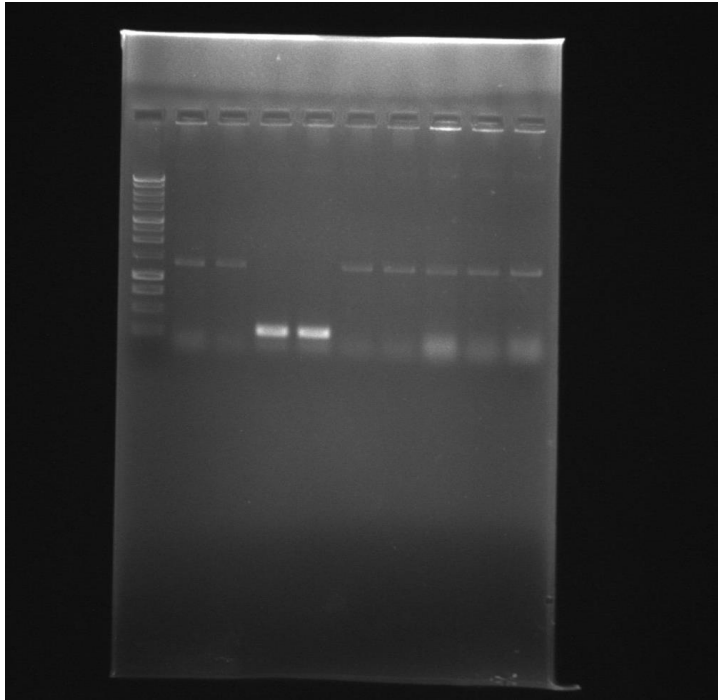
17.10 PCR with Phusion polymerase, electrophoresis

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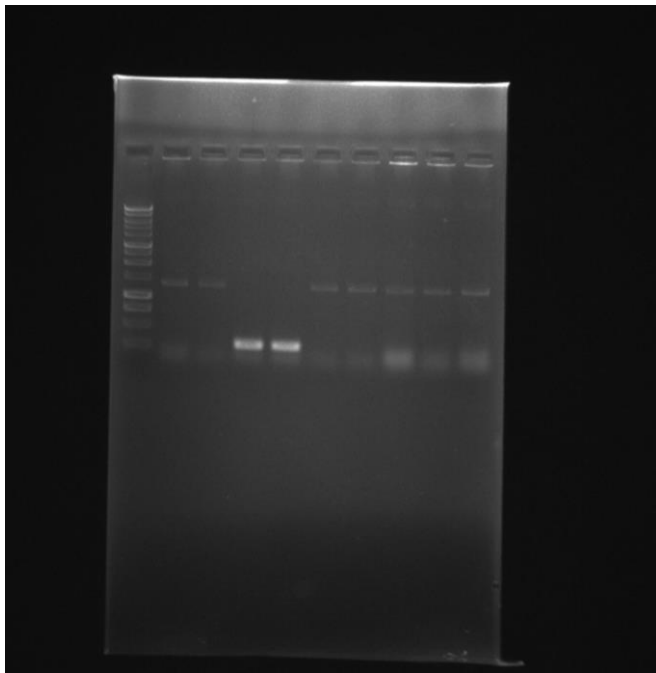


18.10 Visa interview and running 2 electrophoresis

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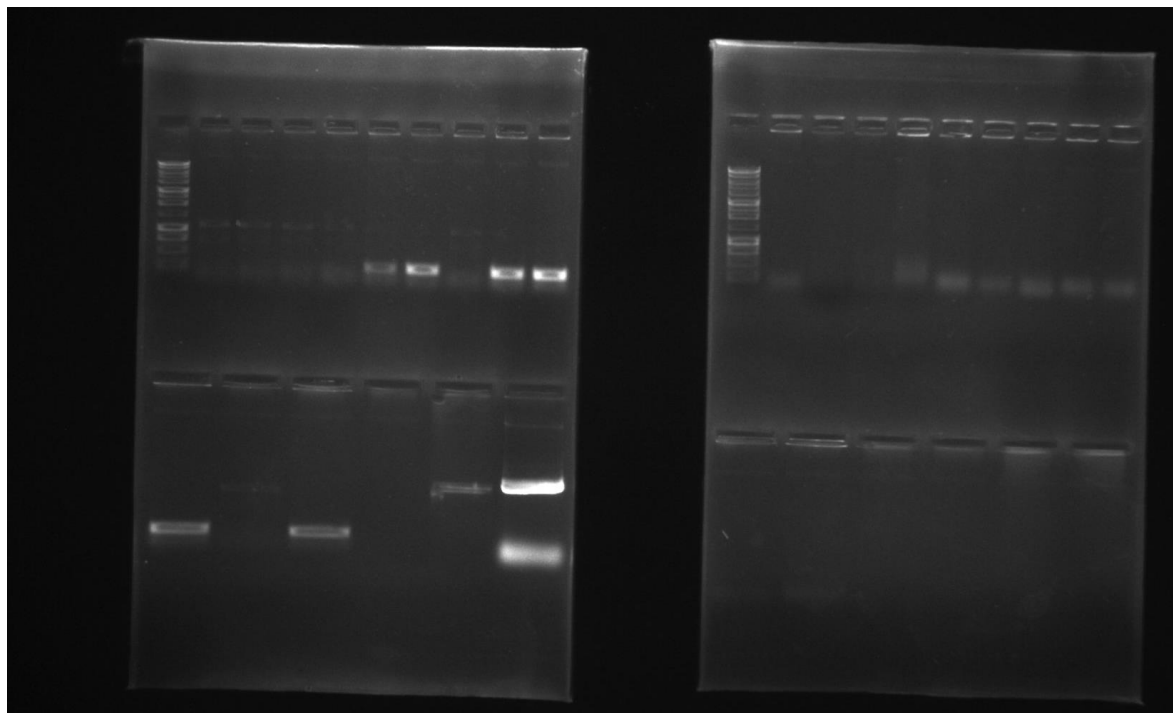


181017 no saturation



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

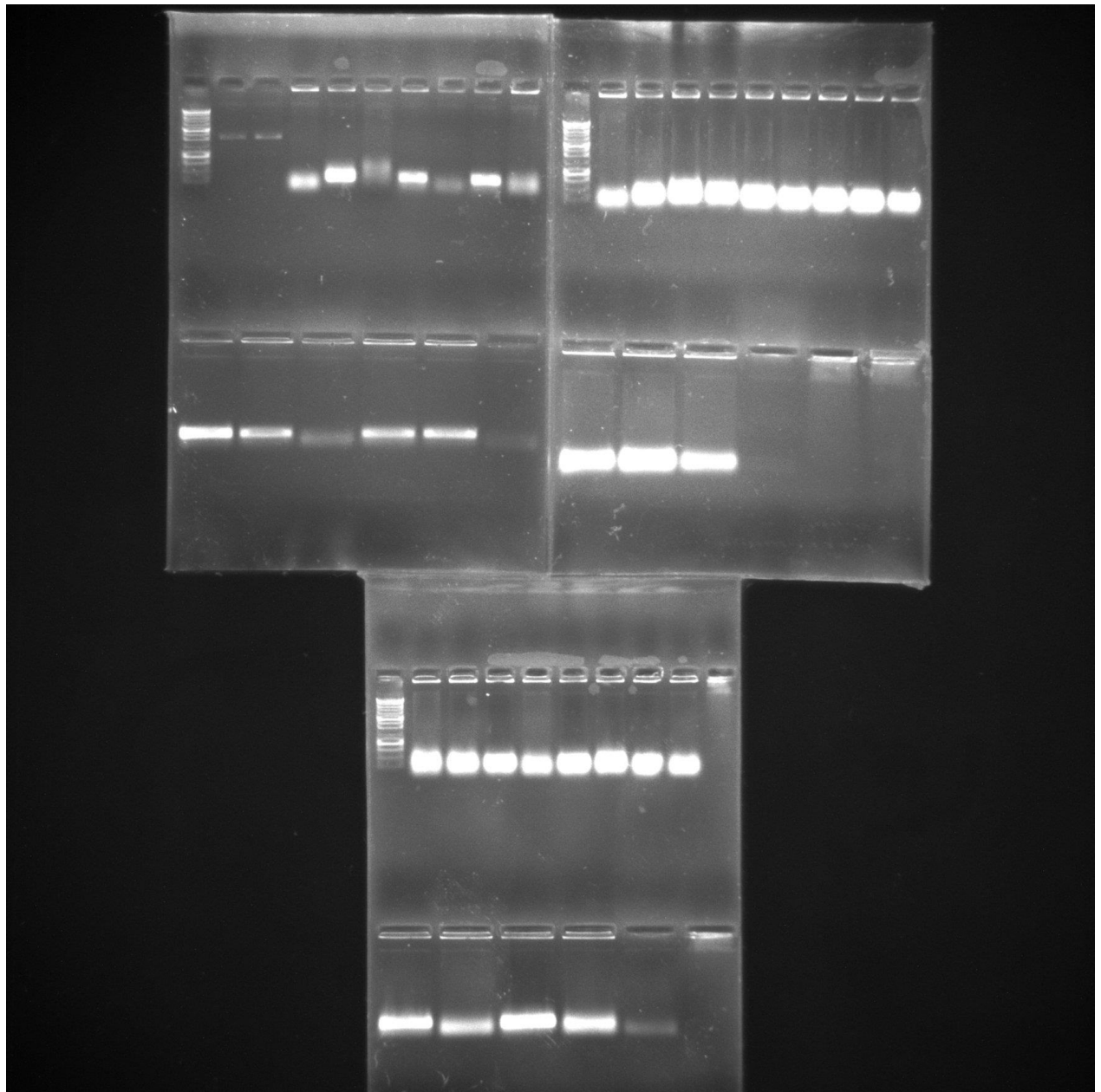
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20.10 PCR, electrophoresis, extraction of Killer Orange and Mini SOC, overnight PCR

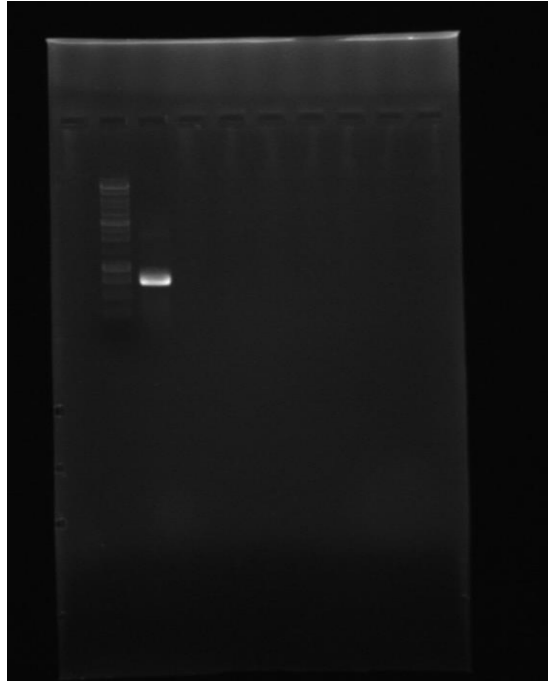
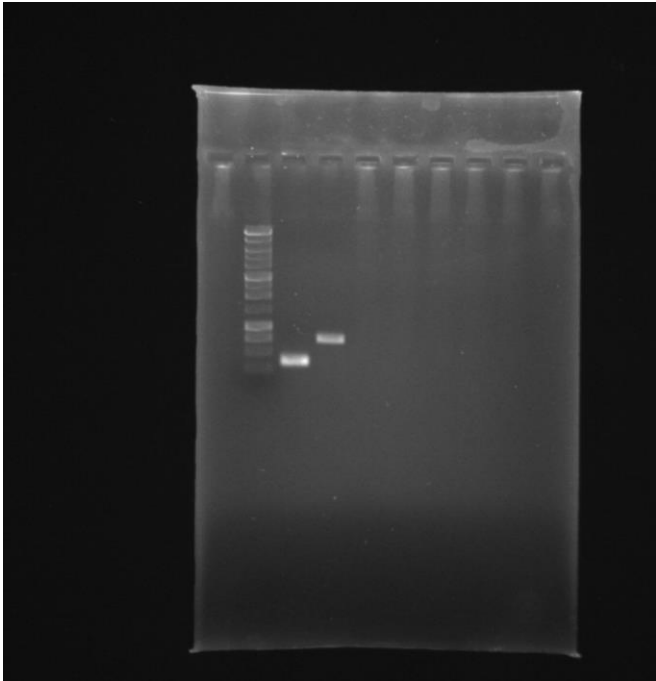
21.10 PCR of gRNA with Taq polymerase, electrophoresis, ligase reaction, miniprep of pSB1C3, restriction Mini SOC, Killer Orange and PSB1C3 with Pst1 and EcoR1, electrophoresis, gel extraction and quantification, overnight cultures, overnight PCR of pSB1K3 + gRNA1 with Phusion polymerase

211017 final vectors

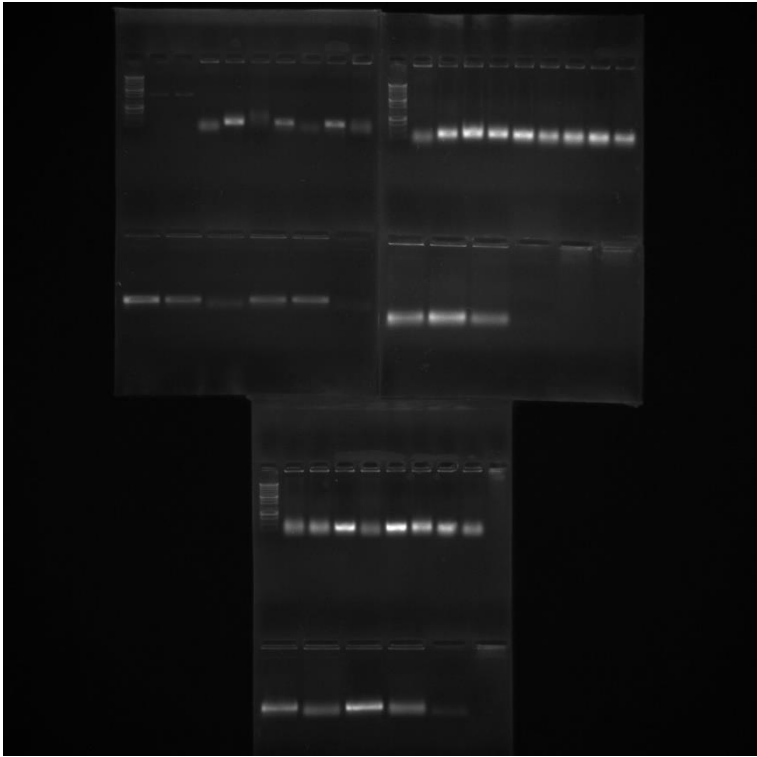


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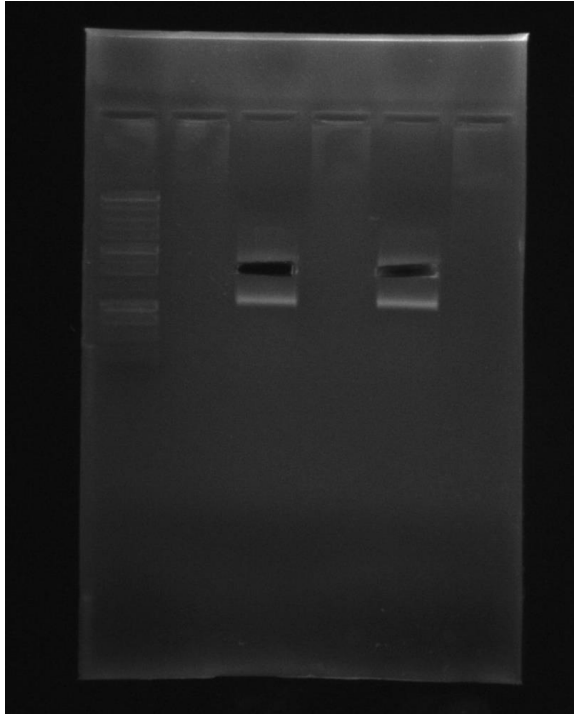
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211017 final



211017 after restriction



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 α , 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

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