this page should go to: http://2017.igem.org/Team:Edinburgh_OG/Notebook

# Yuri's lab work
# Engineering the P1 phage with CRISPR SaCas9

## <Designing of *E.coli* testing platform>

May

12th: Decide target regions to be taregeted by the CRISPR Cs9 system
and be inserted into the *E. coli* testing platform
17th: Continue designing
25th: Make competent cells from *E. coli* TOP10
30th: Design primers
Decide the strategy to construct the phagemid (J72110-J72114) with SaCas9 and spacers

## < Amplification of plasmids>

June

1st: Order IDT to synthesise the CRISPR and spacer cassettes
6th: Amplify the phagemid (Transformation, overnight culture)
7th: Make competent cells from *E. coli* C600 (Lysogen of P1 phage)
8th: Mini-prep phagemid (J72110-J72114)
Digestion to check the Mini-prep product
14th:Maxi-prep phagemid
29th: PCR to amplify the DNA parts for the KPC spacers

## < Inserting SaCas9 Part2 into the phagemid-SaCas9 Part1>

July

4th: Digestion of phagemid-SaCas9 Part1
Gel extraction
5th: Ligation of Phagemid-SaCas9 Part1 and SaCas9 Part2
Transformation
7th: Mini-prep phagemid with complete SaCas9

Digestion to check the construct
Completion of Phagemid - SaCas9

## <Inserting KPC spacers>

10th: Digestion of phagemid-SaCas9, KPC spacer PartA , B
Ligation (Fail)
11th: Digestion of phagemid-SaCas9, KPC spacer Part A
PCR: amplify the KPC spacer Part A and B
12th: Mini-prep phagemid-SaCas9
Digestion of phagemid-SaCas9, KPC spacer Part A and B, SaCas9 Part2
Gel extraction: phagemid-SaCas9
13th: Ligation
KPC Part A+ B (Fail)
phagemid-SaCas9+ KPC PartA >> Transformation
SaCas9 Part 2 + KPC Part A(Fail)

14th: Colony PCR1(Pick 4 colonies for KPC, pick 4 for VanA)
17th: Colony PCR2
Gel running: Check the result of colony PCR1 (Fail)
18th: Gel running: Check the result of colony PCR1, 2 (Fail)
Mini-prep from colony cultures (4 samples)
Digestion to check the construct
(None of them was desired construct)
19th: Pick another 20 colonies and culture them overnight
2nd Ligation of phagemid-SaCas9 and KPC spacer Part 1
Transformation
20th: Pick 9 colonies and culture them overnight
21st: Colony PCR (with a new primer) (Fail)
Mini-prep 29 colonies
24th: Digestion to check the construct
Successfully got several samples of phagemid-SaCas9 with spacers

## <Producing P1 phage>

24th: Transformation of *E. coli* C600 (Lysogen of P1 phage) using phagemid-SaCas9 with spacers
25th: Harvest colonies and culture them over night
26th: Add arabinose to the culture, to turn the phages into lytic, and harvest engineered phage

## <Infection of the *E. coli* with P1 phage>

26th: Mix phages and *E.coli* testing platform, and plate them onto LB plates (with no antibiotics/Chloramphenicol /Kanamycin)
27th:Observation of GFP from *E. coli* to check the cleavage of target
28th:Check the efficiency of phage infection and the CRISPR system

## <Making Biobricks>

September
12th:Extract the SaCas9 gene from the phagemid-SaCas9 by PCR, with primers which can attach prefix and suffix to the gene.
13th: Digestion of the PCR products SaCas9 and backbone (pSB1C3)
14th: Ligation and transformation
15th: Colony PCR
16th: Do overnight culture and Maxi-prep
20th: Digestion to check the construct