

25/5 overnight liquid culture TOP 10
26/5 Made E. coli Top10 competent cells
6/6 Made E. coli C600 (P1 lysogen) competent cells
7/6 Amplified phagemid/phagemid-pCAS by transformation
12/6 Digestion check of the J72114-J72100 plasmid
13/6 Maxi-prep of the J72114-J72100 plasmid
14/6 Digestion check for J72114-J72100-pCas9
15/6 Maxi-prep J72114-J72100-pCas9
16/6 Resuspension of gBlocks Cpf1(p1) fragment; Digestion of phagemid by AvRII&BsiWI; Digestion of Cpf1(P1) by AvRII&BsiWI;
19/6 Gel extraction of Cpf1(p1)-digested and Phagemid-digested; Ligation of phagemid-digested (AvRII&BsiWI) and Cpf1(P1)-digested (AvRII&BsiWI)
20/6 Transformation of the product of ligation phagemid-digested (AvRII&BsiWI) and Cpf1(P1)-digested (AvRII&BsiWI)
21/6 Cpf1(p1) work: Blue-white screening& colony picking and liquid culture
21/6 Digestion of Cpf1(P2) by HindIII&AvRII
22/6 Glycerol stock of bacteria pellet and resuspend VanA/KPC spacer AND primers
26/6 Resuspension of gBlocks VanA/KPC spacer1 fragment
27/6 PCR for spacer1-CPF1 with primer FOR-universal&REV-Bsal
29/6 Gel extraction: Kpc-spacer1 & VanA spacer1 PCR product; Digestion of Kpc-spacer1 & VanA spacer1 by Bsal
3/7 Mini-prep: phagemid-Cpf1-p1, phagemid-saCas-p1; Digestion check mini-prep product; Digestion of phagemid-Cpf1-p1 by HindIII and AvRII; Gel running of digestion product.
4/7 Gel extraction of phagemid-Cpf1-p1-digest; Ligation of phagemid-cpf1-p1&cpf1-p2; Transformation&plate
5/7 Liquid culture for colony of phagemid-Cpf1(p1-p2)
6/7 Glycerol stock for liquid culture; Mini-prep of phagemid-cpf1-p1-p2; Digestion check of phagemid cpf1(p1-p2); Digestion of phagemid-cpf1-p1-p2 by Bsal.
7/7 Gel extraction of phagemid-cpf1-p1-p2
10/7 Ligation of phagemid-cpf1&kpc-spacer1 and phagemid-cpf1&VanA-spacer1; Transformation & plate with ligation product at room temperature
11/7 Transformation & plate with ligation product at 4°C ; Ligation of phagemid-cpf1&kpc-spacer1 and phagemid-cpf1&VanA-spacer1 again; Liquid culture of colony on 10/7
12/7 Miniprep (*8) of phagemid-cpf1 and phagemid-cpf1-kpc/vanA; Digestion check of phagemid-cpf1 and phagemid-cpf1-kpc/vanA by AvRII&HindIII and BsiWI separately (DID NOT WORK); Gel running and liquid culture of colony on 11/7
13/7 Colony PCR check for phagemid-cpf1-kpc/vanA by primer FOR-cpf1-8&REV-aCas
14/7 Gel running of PCR product on 12/7 (DID NOT WORK)
15/7 Phagemid PCR check of phagemid-cpf1-kpc/vanA by another PCR program
18/7: Gel running of PCR product on 15/7 (DID NOT WORK); Digestion check plasmid Phagemid-cpf1-kpc/vanA by BsiWI&Bsal (SUCCESSFULLY)
19/7: Transformation & plate of Final construct phagemid-Cpf1-Kpc/VanA to competent cell C600

20/7 Mini-prep of phagemid-CPF1, Phagemid-Cpf1-VanA and Phagemid-Cpf1-KPC; liquid culture of C600 colony.

21/7 Produce phage with phagemid-Cpf1-VanA/KPC; liquid culture of phage with Moclo-VanA/KPC and phage without spacer with Moclo-VanA/KPC; First round plate on LB, Chl, Kan

24/7 Transformation & plate of Final construct phagemid-Cpf1-Kpc/VanA to competent cell C600

25/7 Second round plate on LB, Chl, Kan and colonies were picked from first round plate which aims to show the CRISPR work or not; produce phage again from 24/7 C600 cells.

26/7 Observation of second round plate result, CRISPR DID WORK ON TWO COLONIES; First round plate again.

27/7 Re-plated ones which CRISPR worked for double check