

As one part of the entire project, I aim to construct and test the a plasmid expressing sgRNA which target chloramphenicol resistance gene.

Purpose of the experiment : test pTargetF plasmid expressing sgRNA which target chloramphenicol resistance gene and crisper/cas9 system

2018-09-04

Inoculation culture

Step: Add 5ul kan and 5ul cm to the test tubes with the 5ml LB liquid medium. Then add MG1655 WT-pSu-pCas and MG1655+ΔpanD-pSu-pCas single colony.

2018-09-05

We prepare MG1655 WT-pSu-pCas and MG1655+ΔpanD-pSu-pCas wild type competent cells

2018-09-06

Conversion and plate coating for bacteria

According to the following form, added the corresponding substance to EP tube.

	MG1655 WT-pSu-pCas	MG1655+ΔpanD-pSu-pCas
pTargetF-panD		
pTargetF-cm		

Add bacterial solution to the LB solid medium (containing kan and spec)

2018-09-07

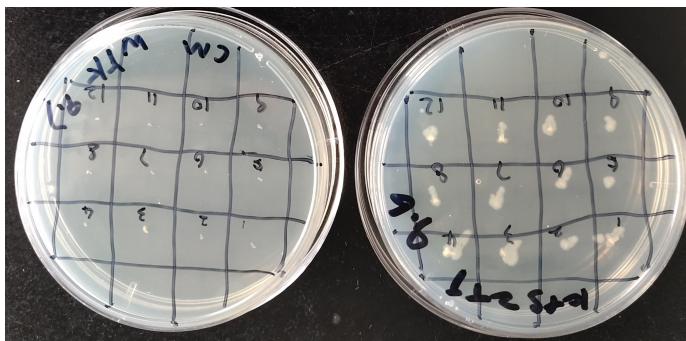
Result

	MG1655 WT-pSu-pCas	MG1655+ΔpanD-pSu-pCas
pTargetF-panD	-	+
pTargetF-cm	+	+

We select MG1655 WT-pSu-pCas-pTarget-cm and culture it both in LB+kan+spec and LB+cm.

2018-09-08

	LB+kan+spec	LB+cm
E.coli MG1655-ppp	+	-



Successful!

2018-10-01

Construct part into standard carrier