

**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 :** prepare E.coli MG1655+ $\Delta$ panD and E.coil MG1655 wild type competent cells and transform pCas plasmid into it

**2018-08-10**

Inoculation culture

Step: Add 5ul chloramphenicol to the test tubes with the 5ml LB liquid medium. Then add E.coil-pSu in LB+cm single colony.

Add E.coil MG1655 wild type and E.coli MG1655+ $\Delta$ panD colony to the test tubes with the 5ml LB liquid medium.

**2018-08-11**

We extract the plasmid pSu and measure the concentration, which is 40.56ng/ul.

We prepare E.coli MG1655+ $\Delta$ panD and E.coil MG1655 wild type competent cells

**2018-08-12**

Conversion and plate coating for bacteria

Step: 2  $\mu$ l pSu plasmid and 50  $\mu$ l MG1655+ $\Delta$ panD and MG1655 WT receptive bacteria solution were mixed into EP tubes. After 30 minutes in the ice box, 90 s in the metal incubator at 42°C, then 2 to 4 minutes in the ice box, 940  $\mu$ l 37°C pre-heated LB liquid medium was added to the EP tubes, then incubated in 37°C shaking bed incubator for 1 hour, then 12000rpm centrifuged for 1 minute. The liquid transfer gun was used to remove 800  $\mu$ l supernatant next to the alcohol lamp, the remaining culture medium was mixed with the bacteria, and then 50  $\mu$ l bacterial solution was added to the LB solid medium (containing cm), pour a certain amount of glass beads and shook constantly to smear the liquid evenly on the surface of the medium, then the glass beads were poured out and labeled on the petri dish and cultured overnight in a constant temperature incubator at 37°C.

**2018-08-13**

Preservation of bacteria