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erstellt: 15.05.2019 13:54

Eintrag 1/2: copy Marburg Collection 1.0 Part 1

aktualisiert: 08.08.2019 09:10

In Projekt: Toolbox: MC 1.0 kopieren

Keine Tags verwendet

07.05 went to stuki to get the first 59 cultures from the Marburg Collection 1.0 (see excel file). We took all from sheet 1 except for pMC_0_5_Nanoluc as stuki did not freeze that part yet.

15.05 went to stuki to get the next 59 cultures from the Marburg Collection 1.0 (see excel file).

Parts missing:

22.05 Went to get the last parts.

Parts missing:

 [Plan Toolbox kopie \(ausdrucken und mit zu stuki bringen\).xlsx](#)

06.08

strainees noticed that some of the parts in their collection were in the wrong order so ill resequence some parts

since Chuns Lvl 1 assembly didnt work, ill start with these parts:

1_08

2_20

3_07

4_07

5_03

6_02

Kan_ColE1

1_08 and 2_20 as well as Kan_ColE1 yielded no results (N-N-N-N-N), the others were correct.

Autor: Maurice Mager

erstellt: 28.06.2019 17:13

Eintrag 2/2: competent cells

aktualisiert: 11.07.2019 16:01

In Projekt: Toolbox: MC 1.0 kopieren

Keine Tags verwendet

27.06

made new competent cells for the lab

- inoculated 20 ml LB in flask with competent cells from -80 freezer ("Lucy competent cells, 1. colony, DH5alpha) and grow overnight
- 4 ml of ONC in 250 ml LB media grown to OD 0.7
- centrifuge at 5000 rpm 6 °C for 10 minutes
- resuspend in 20 ml 0,1 M CaCl and put on ice for 30 mins
- centrifuge at 5000 rpm 6 °C for 10 minutes
- resuspend in 4 ml 0,1 M CaCl and 1,5 ml 50% glycerol
- make aliquots of 70 µl in eppis, freeze in liquid nitrogen
- store at -80 °C

28.06

- test retrafo with Lvl 0 backbone
- 70 µl of competent cells thawed on ice
- 1 µl (660 ng) of DNA
- 30 min on ice, 90 sec heat shock at 42, 2 min on ice, add 500 µl SOB, 1h regeneration at 37 °C and 550 rpm
- 5000 rpm for 2 mins, remove most of liquid
- vortex and plate out the remaining culture