26/09/2015 15:31

1. Aliquot of 100 uL of DH5 α Ca2+ competent cells was mixed with 10 uL of ligation 27.07.15 (from yesterday) and incubated on ice for 30 min.

1: 30 uL of ligation mixture2: 10 uL of ligation mixture

make prewarmed 37 C medium

- 2. Uptake of DNA was induced by heat shock 5 min at 37 C. (or <u>I min at 42 C</u>)

 maybe put some water for better heat transfer
- 3. After the heat shock the cells were diluted in 500 uL of prewarmed LB medium (37C) and incubated for 1 h by shacking at 250 rpm
- 4. 350 uL aliquotes of cell suspensions were plated onto LB agar with Amp 100, Tet 10, Km 20 antibiotics.
- 5. Plates were incubated overnight at 37 C