|  |  |
| --- | --- |
| Time: 2h+8h+2h+8h  Transformation 30m  Plating 30m  Colony plate 8h  Colony culture 8h | 5. Transformation of ligated plasmids  1. Prepare LB plates  (See preparations)  2. Brief spins the ligation reactions. Add 2µL (or 5µL) of each ligation reaction to a sterile 1.5 tube  3. Thaw competent cells in an ice bath (just thawed, ~5min), gentle flicking the tube to mix the cells.  4. Transfer 50µL of cells to the ligation reaction tube. Gentle flicking tube to mix.  5. Incubate on ice for 20~30min.  6. Heat shock the cells in water bath for 2 min at 37°C.  Immediately return the tubes to ice for 2 min.  7. Add 900µL LB and incubate for 45min in shaker at 37°C (~150rpm)  8. Centrifuge 16000g for 2min, discard 800µL of supernatant and resuspend cell pellet in remaining supernatant. Plate out 200µL of culture onto a plate.  9. Incubate plates overnight at 37°C, then store plate at 4°C.  10. Pick single colony and put in 2mL LB media + Ampicillin (100µg/mL) using a 5mL tube lose cap. Incubate in shaker over night at 37°C.  ----  Notes:  1. Do dry plate before use. A wet plate can be a nightmare to plating and subsequent colony formation. It takes forever for it to complete dry and if it is not dry well, colonies grow altogether.  2., Do not exceed maximum colony formation culture golden 8 hours threshold, make life easier. Satellite colony may grow if exceed the time limit.  3. Do miniPrep within 2days if do it after the culture is not possible, put bacterial in fridge for longer period, there maynot be much plasmid left. |