BIOCHEMISTRY

AGROBACTERIUM COMPETENT CELLS

Day 1

AGROBACTERIUM COMPETENT CELLS

Solutions: * Autoclave solutions 35min

NaCL 0.15M 500ml = 15mL NaCL 5M + 485 ml H2O mq

CaCl2 20mM 200ml = 5.88mg CaCl2 + 200ml H2O

Keep solutions at 4ºC

LB liquid media

Protocol

Day 1: Streak agrobacterium strain GV3101 from a -80ºC aliquot into a LB +Rif (100ug/ml) plate

Day 2 (night): Prepare a 50ml liquid culture (LB+Rif) from the plate and grow it over-night

Day 3 (early morning): 5 ml from the saturated o/n culture into a 100ml LB+Rif media and let it grow to a OD =1.65

Day 3 (evening): ICE ALL THE TIME

- Pellet the cells by using a centrifuge: 5min 4ºC 6000rpm
- Wash pellet with 30ml of sterile NaCl 0.15M
- Pellet the cells by using a centrifuge: 5min 4ºC 6000rpm
- Ressuspend pellet with 2mL CaCl2 20mM
- make 100ul aliquots and freeze with N2