iGEM BIELEFELD 2021

P.L.A.N.T.

Plant-based **L**igand **A**ctivated **N**oxious agent **T**racker

Transformation of competent *Agrobacteria* by electro purification 1/4

Preparation of competent bacteria:

- From the glycerol stock, spread a culture on a LB + antibiotics petri dish (28°C)
- pick a colony and make a liquid preculture in 3 mL of YEB + antibiotics for 24 hour (saturation)
- the day before (around 4.30 p.m.): inoculate 2x 100 mL of YEB + antibiotics with 2x 50 µL of preculture (keep 1 mL of virgin medium to make the blank with spectro):
- Antibiotics used: GV3101> Rifampicin 50 µg/mL + Gentamycin 20 µg/mL
- We can also start the culture in the morning: in this case, put the necessary quantity to have an initial OD = 0.1 (approximately 1-5 mL / 100 mL of culture) and take the OD after 4-5 hours
- in the morning: measure the OD around 9 a.m. or 10 a.m. until you have an OD between 0.6 and 0.9
- cool the culture in ice and centrifuge at 4500 rpm for 10 min, 4°C (4 Falcon tubes 50 mL)
- still in ice: Wash the pellets 3x with 25 mL of sterile water + 10% glycerol
    - aliquot with 50 µL, immerse the tubes in liquid nitrogen, then store at -80°C

from: A. Lucchin

Transformation of competent *Agrobacteria* by electro purification 2/4

Transformation by electroporation:

- place the 0.1 cm electroporation tank in ice for at least 10 min
- defrost the agro in ice
- add 1 µL of DNA (5-50 ng) to 50 µL of Agrobacteria
- transfer the mixture to the electroporation tank still placed in ice
- wipe the outside of the tank and tap to drop the mixture
- (On the machine), select the „Agr“ program then place the tank in the support
- press the button to trigger the flow of current
- from there, work in a sterile manner
- add 800 µL of sterile LB/YEB
- incubate 2 h at 28°C at 180 rpm
- spread over LB + antibiotics (Rifampicin 50 µg/mL, Gentamicin 20 µg/mL for the strain & carbenicilin 50 µg/mL)
- incubate 48 to 72 hours at 28°C

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Transformation of competent *Agrobacteria* by electro purification 3/4

Washing of the electroporation tanks: Alternate 3-4 times washing with 70% ethanol followed by washing with UHQ water. Finish with a 70% ethanol wash. Place the vats upside down on a wipe paper and let dry.

Strains:

- C58GV3101: Rif^R $Genta^R$ and naturally resistant to Ampicilline

Media:

- YEB (Yeast Extract Broth)

YEB	Final concentration	1 L
Bacto-tryptone	0.5%	5 g
Yeast Extract	0.1%	1 g
Beef extract	0.5%	5 g
Bacto-pectone	0.1%	1 g

- complete to 1 L with H_2O UHQ
- Adjust pH to 7.4 under agitation
- autoclave

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Transformation of competent *Agrobacteria* by electro purification 4/4

Add on a sterile manner:

YEB	Final concentration	1 L
$MgSO_4$ 1 M – sterile	2 mM	2 mL
Sucrose*	0.5%	5 g

*Disolve first the sucrose in 20-20 mL of sterile water and sterilse the solution with a syringe and a 0.2 µm filter under sterile conditions
*or for 1 L, prepare 900 mL of autoclaved YEB and add 100 mL of a solution containing 5 g of autoclaved sucrose

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