Protocol for electrocompetente Agro cells

Glycerol stocks:
for GV3101 (main strain; +Rifampicin)

ON culture + Rifampicin (28°C)

Add ON culture in the main culture (+ Rifampicin) and incubate for 15 – 18 hours (overnight) at 28°C

Divide culture in big centrifuge tubes

All steps on ice

Centrifuge: 3000xg, 10 min, 4°C

Wash 2 times with 10% Glycerol (cool down before using)

After the second wash step and third centrifugation mix the cells together

Centrifuge and resuspend the pellet in 1/800 10%Glycerol

250 µL Glycerol for 200 mL culture
625 µL Glycerol for 500 mL culture
1,25 mL Glycerol for 1 L culture
1,5 mL Glycerol for 1,2 L

aliquot 25 µL/tube, shock frost in liquid nitrogen

store at -80°C

ctal
107
A
B

1.72 pBEN 107 232.5 7.768 ml
1.32 control 303 1.697 ml

Glycerol autoclave always

10 %. - 126 g | 1 L
- 252 g | 2 L
- 378 g | 3 L

30 %. - 378 g | 1 L
- 189 g | 500 mL
- 94,90 g | 250 mL

Trafo electrocomp. Agros
(1 µL) DNA 0,5 µL also ok
+25 µL cells GV3101 (on ice)
add to electroporationcuvette
-> pulse
add 1 mL LB
pipette to tube and shake
at 28°C 3-4 hours
centrifuge and resuspend for plating

Dissolving buffer / Infiltration

100 mL 100 mM MES (pH 5.5 NaOH)
10 mL 1 M $MgSO_4$
1 L

100 mM MES - 1L - 19,52 g pH 5.5 NaOH
1 M $MgSO_4$ - 100 mL - 24,65 g

10^{-1} Verdünnung Agros

$\frac{0,2}{OD_{600}\ Wert}$ • 2000 → x µL + Rest Buffer (total 2 mL)
↳ gesamt Vol.

→ o/n cult. 1:10 mit H_2O verd. für die Rechnung Wert wieder ×10 nehmen

10^{-2} Verdünnung Agros

$\frac{0,02}{OD_{600}\ Wert}$ • 2000 → x µL + Rest Buffer (2 mL)
↳ gesamt Vol.

↳ o/n cult. 1:10 mit H_2O verd. für die Rechnung aber Wert wieder ×10 nehmen

Verdünnungen f. Infiltrationen

1:10	→ 10^{-1}	200 µl / 2 mL
1:100	→ 10^{-2}	20 µl / 2 mL
1:1000	→ 10^{-3}	2 µl / 2 mL
1:10.000	→ 10^{-4}	0.2 µl / 2 mL
1:100.000	→ 10^{-5}	0.02 µl / 2 mL

oder aus Verdünnungen →

Bsp. 10^{-2} Verd. 20 µl / 2 mL
≈ 200 µl 10^{-2} Verd. / 2 mL → 10^{-3}
≈ 200 µl 10^{-3} Verd. / 2 mL → 10^{-4}
≈ 200 µl 10^{-4} Verd. / 2 mL → 10^{-5}
≈ 200 µl 10^{-5} Verd. / 2 mL → 10^{-6} usw.