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Preparation of electrocompetent cells

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Josef Hoff[1]

[1]Phillips University

Josef Hoff

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Protocol status: Working
We use this protocol and it's working

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Abstract

Preparation of electrocompetent cells

Weinstock paper:

Matthew T Weinstock, **Eric D Hesek**,**Christopher M Wilson**, **Daniel G Gibson**

Vibrio natriegens as a fast-growing host for molecular biology

Nature Methods volume 13, pages 849–851 (2016)

To prepare the day before:

-LB I media +V2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl2)
-electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7) (sterile filtrated)
-over night culture of V.n from the cryo-stock in LBI + v2 salts (37 °C ; at 200 r.p.m)

Preparing culture :

Depending on how many aliquds you want to have, incubate media with your overnight culture for a starting OD of 0.05.
The culture is grown at 37 °C in a baffled flask, shaking at 200 r.p.m. until an OD600 between 0.5 to 0.8 is reached.
*be careful when they reach an OD near 0.1, *V. natriegens*is very fast growing, so start measuring in shorter time periods.
Prechill the electroporation buffer

Washing:

From here on try always to keep your culture on ice.

-The culture is then put on ice for 15 min.
(the original Protocol sais to directly fill them into your prechilled centrifugatin containments)
-The cells are pelleted at 3000x g. for 20 min at 4 °C.
-The supernatant is carefully decanted and the cell pellets are gently suspended in 10mL of **chilled** electroporation buffer.
-The suspensions are transferred to a **chilled**50mL falcon tube and the tube is filled top with additional **chilled** electroporation buffer (50mL) and inverted several times.
-The cells are centrifuged down at 3000x g for 15 min at 4 °C.

-The wash is repeated two times for a total of three washes.

Aliquotation:

After the final wash, the supernant is carefully decanted the cells are gently resuspended in residual electroporation buffer.
Measure the OD in a 1/20 dilution against electroporation buffer.

The volume is adjusted with additional electroporation buffer to bring the final OD600 to 16.

The Cells are aliquoted (80µL) into chilled 1.5µL centrifugation tubes, directly frozen in liquid nitrogen and stored at −80 °C until use.

Attachments

PDF

Preparation of elect...

50KB

Troubleshooting