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Genetic Transformation of *Candida glabrata* by Electroporation

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[Abstract] Here, we report a method for the transformation by electroporation of the human fungal pathogen *Candida glabrata* (*C. glabrata*). The protocol can be used for transformations in single well or in 96-well microtiter plates. It has been extensively used to generate a genome-scale gene deletion library using the *C. glabrata* background recipient strain ATCC2001 (Schwarzmüller *et al.*, 2014).

Materials and Reagents

1. Recipient strain [ATCC2001, HTL or clinical isolates (Schwarzmuller *et al.*, 2014)]
2. DNA deletion construct / transforming DNA (highly purified)
3. Electroporation cuvette (Life Technologies, Invitrogen™, catalog number: P51050)
   This product has been discontinued.
4. Deep well plate (96-well) (Nunc®, catalog number: 7322662)
5. Reservoir (autoclavable) (VWR International, catalog number: 6130466)
6. Multichannel pipette (200 µl) (BrandTech Scientific)
7. Sterile water (double-distilled)
8. Bacto™ peptone (BD Biosciences, catalog number: 211820)
9. Bacto™ yeast extract (BD Biosciences, catalog number: 212720)
10. Bacto™ agar (BD Biosciences, catalog number: 214030)
11. Glucose (Merck KGaA, catalog number: 108337)
12. Tris base (Sigma-Aldrich, catalog number: T1503)
13. EDTA (Sigma-Aldrich, catalog number: E6758)
14. Lithium acetate dihydrate (Sigma-Aldrich, catalog number: L6883)
15. DL-Dithiothreitol (DTT) (Sigma-Aldrich, catalog number: D0632)
16. Nourseothricin-dihydrogen sulfate (Werner BioAgents, catalog number: 5.0)
17. TE buffer (see Recipes)
18. DTT solution (see Recipes)
19. YPD media (see Recipes)
20. Solid selective media (see Recipes)

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Equipment

1. 96-wel electroporation plate (2 mm gap) (Harvard Apparatus, catalog number: HTP962450450)
2. Electroporation cuvettes (1 mm gap) (Life Technologies, Invitrogen™, catalog number: P51050)
   Note: This product (catalog P51050) has been discontinued. Please contact vendor for possible alternatives
3. Culture flasks with baffles
4. Centrifuge for tubes (50 ml) (Eppendorf, catalog number: 5702R)
5. Centrifuge for tubes (1.5 ml) (Eppendorf, catalog number: 5417R)
6. Rotary shaker for culture flasks (New Brunswick Scientific, catalog number: innova44)
7. Stratagene Stratalinker 2400 UV Crosslinker (Artisan Technology Group®, catalog number: 532741)
8. Single-well scaleTransfection apparatus consisting of capacitance extender, pulse controller and gene pulser (Bio-Rad Laboratories, AbD Serotec®, catalog numbers: 165-2087; 165-2098; 165-2077)
9. 96-well microtiter plates
   a. 96-well electroporation device (BTX The Electroporation Experts, catalog number: ECM63 or Harvard Apparatus, catalog number: 450422)
   b. Plate handler (BTX The Electroporation Experts, catalog number: HT-100 or Harvard Apparatus, catalog number: 450400)

Procedure

1. Culture preparation
   a. Grow the recipient strain overnight in YPD media at 30 °C with shaking at 160 rpm.
   b. Dilute the culture in fresh YPD media to an OD_{600} of about 0.3, and continue culture until an OD_{600} of 1.5 is reached (50 ml of culture are sufficient for 12 transformations).
   c. Harvest the culture in 50 ml tubes by centrifugation at 1,000 *x g* for 5 min. Wash the cell pellet(s) once with 25 ml of sterile water at room temperature.
2. Treatment of cells
   a. Each cell pellet derived from 50 ml culture is very gently resuspended by slow pipetting in 8 ml sterile water, 1 ml 10x TE buffer and 1 ml 1 M LiAc.
   b. Incubate the cell suspension(s) for 30 min at 30 °C and 130 rpm.
   c. Add 250 µl of DTT and incubate the suspension(s) for 60 min at 30 °C and 130 rpm.

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d. Add 40 ml of sterile pre-cooled water and centrifuge the suspension(s) at 1,000 *x g* for 5 min and 4 °C.
e. Carefully resuspend cell pellets by slow pipetting in 25 ml of sterile water (4 °C) and repeat the centrifugation step.
f. Carefully resuspend cell pellets in 5 ml pre-cooled 1 M sorbitol and repeat the centrifuging step. Finally, resuspend the pellets of electrocompetent cells in 550 µl of pre-cooled 1 M sorbitol.

3. Electroporation

Single-well scale

a. Transfer 45 µl aliquots of electrocompetent cell suspensions into sterile precooled tubes.
b. Add 5 to 10 µl of highly purified transforming DNA (1 to 3 µg), mix gently and thoroughly and incubate tubes on ice for 10 min.
c. Apply electro pulses (200 Ω, 1.5 kV, 25 µF), the time constant should be at about 4.6 Ω. µF.
d. Add 950 µl of YPD media to each cuvette and transfer the suspension to a tube.

96-well scale

a. Transfer 5 to 10 µl aliquots of the highly purified transforming DNA (1 to 3 µg) into each well of the electroporation plate.
b. Add 45 µl of electrocompetent cell suspensions and incubate the plate for 10 min on ice. Make sure that the DNA samples and the cell suspension are at the bottom of the wells.
c. Cover the plate with a sterile lid or a seal it with a foil for the incubation.
d. After the incubation place the plate in the plate handler and pulse each column individually (200 Ω, 1.5 kV, 25 µF).
e. Transfer the cell suspension into a 96-well plate with 950 µl of YPD media.
f. The media in the plate can be used to flush the wells of the electroporation plate.

4. Regeneration of cells

a. Incubate cell suspensions in tubes or in a plate for 1 to 4 h (depending on the marker) at 30 °C without shaking.
b. Afterwards, centrifuge the tubes or the plate at 1,000 *x g* for 5 min.
c. Discard supernatants and resuspend cell pellets in 100 µl of sterile water.
d. Plate cell suspensions on selective media and incubate plates for 1 to 2 days at 30 °C until colonies are visible.

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Notes

1. The protocol was optimized for *C. glabrata* ATCC2001 and all derivative strains.
2. Speed of rotary shaker depends on the type of culture flasks used. Flasks without baffles require higher shaking speeds about 220 rpm.
3. Handle cells as gently as possible after adding LiAc (no vortexing!) and keep them on ice. Add LiAc AFTER adding sterile water and the TE buffer (this automatically dilutes the LiAc to the appropriate concentration).
4. In section “Procedure” part 2 we mention that cell pellets should be resuspended carefully by pipetting. For this step we recommend a manual 1,000 µl pipette. By slow aspiration and release the cell pellet can be gently resuspended.
5. Regeneration time depends on the selective marker. We experienced that transformants with a *HIS3* marker can be plated after 1 h, while those with a *NAT1* marker may require up to 4 h of regeneration.
6. The electroporation cuvettes and plates can be reused. They should be cleaned after use with a mild detergent and then thoroughly rinsed with distilled water. After drying, they can be sterilized using a Crosslinker (3 times “Auto-Crosslink” - equals an exposure of 3 times 120 mJoules).

Recipes

1. TE buffer
   10 mM Tris base (pH 8)
   1 mM EDTA
2. DTT solution
   Dissolve 1 M dithiothreitol in 0.01 M NaAc (pH 5.2)
3. YPD media (yeast extract peptone dextrose)
   25 g/L Bacto™ peptone
   12.5 g/L Bacto™ yeast extract
   2% glucose
4. Solid selective media (nourseothricin)
   25 g/L Bacto™ peptone
   12.5 g/L Bacto™ yeast extract
   2% glucose
   2% agarose
   0.2 g/ml nourseothricin

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