Ustilago maydis

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.536 – 01/2002

Microorganism	*Ustilago maydis* fbd11, protoplasts
Cell type	Phytopathogenic fungus, yeast-like growing sporidia (diploid)
Molecules injected	Plasmid DNA (pNEBUH, pSMUT)
Growth medium	YEPS (1% (w/v) yeast extract, 2% (w/v) bacto-tryptone, 2% (w/v) saccharose)
Washing solution	SCS (1 M sorbitol, 20 mM sodium citrate (pH 5.8)); 1 M sorbitol
Electroporation solution	1 M sorbitol
Selection plates	1.5% agar in YEPS with 1 M sorbitol in 94 mm diameter plastic petri dishes - 10 ml bottom layer containing 300 µl hygromycin B/ml agar - 10 ml top layer of agar (without hygromycin B) poured off 10 to 20 min. prior to use
Cuvette	2 mm gap width

Reference Robert Fischer ♦ Department of Biology ♦ Genetics ♦ Philipps University of Marburg
Karl-von-Frisch-Str. 1 ♦ D-35032 Marburg
Phone +49 6421 282 7080 ♦ Fax +49 6421 282 8971 ♦ e-mail: rfischer@mailer.uni-marburg.de

Preparation of protoplasts:

1. Inoculate 50 ml of YEPS with a primary culture of *Ustilago maydis* and grow cells overnight at 30 °C to a cell density of $O.D._{600}$ of 0.5 to 0.7.
2. Harvest by centrifugation at 3,000 rpm for 5 min at room temperature.
3. Wash one time with 25 ml SCS.
4. Digest the cell walls by resuspending cells in 2 ml filter sterilized SCS containing 3 mg/ml Novozyme-234, incubate until 20 to 50% of protoplasts have been formed (observe by microscope), then add 25 ml 1 M ice-cold sorbitol.
5. Centrifuge for 7 min at 2,300 rpm at 4 °C.
6. Wash three times with 25 ml ice-cold 1 M sorbitol. Centrifuge for 7 min at 2,300 rpm at 4 °C.
7. Resuspend in 1 ml ice-cold 1 M sorbitol (density: 10^7 to 10^8 protoplasts/ml). Keep on ice.

Electroporation of protoplasts:

1. Add 1-2 µl pNEBUH DNA or 10-20 µl pSMUT DNA (1 µg/µl) to 400 µl of protoplasts. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	480 V
Time constant (τ)	5 ms

4. Incubation for 10 to 20 min. on ice is possible but not required.
5. Plate on selective plates (freshly poured top layer).

Ustilago maydis

Multiporator/Eppendorf Eporator®

Expected results:

The protoplast viability was not determined because of incomplete treatment with Novozyme-234: death of protoplasts may occur to certain percentage, but probably not of remaining cells with intact cell wall.

Transformation efficiency (transient) approx. 400 colonies/µg autonomously replicating vector pNEBUH.

Transformation efficiency (stable) approx. 1 colony/µg integration vector pSMUT.

Note:

The preparation of protoplasts is very critical to obtain a high transformation efficiency. Cell well degradation by Novozyme-234 continues during washing steps in 1 M sorbitol causing burst of protoplasts.

eppendorf

Eppendorf AG · 22331 Hamburg · Germany · Phone +49 40-5 38 01-0 · Fax +49 40-5 38 01-556

e-mail: eppendorf@eppendorf.com · Internet: www.eppendorf.com