How to transform *Neurospora crassa* by electroporation

Laura Navarro-Sampedro, María Olmedo, Luis M. Corrochano
University of Seville, Spain

Background

Transformation of *Neurospora crassa* conidia by electroporation provides an easy alternative to the chemical transformation of protoplasts. Whole ungerminated conidia can be easily transformed with DNA without the need for protoplast preparation and storage. The major drawback of this technique is the need for a cell electroporator. However, it is possible to share the electroporator between different labs since the time required for each electroporation experiment is short. Different protocols for the transformation of *Neurospora* by electroporation have been published (Chakraborty and Kapoor 1990, Kothe and Free 1996, Turner et al. 1997). The protocol described here is derived from that of Margolin et al. (1997, 2000) and is routinely performed in our laboratory.

Method

Obtaining a fresh conidial stock

1. Obtain a fresh stock of conidia from *Neurospora crassa* by the inoculation of a 250 ml flask with 50 ml of Vogel's agar with supplements as required.
2. Collect conidia after 2-3 weeks of growth by adding 50 ml of 1M sorbitol. The suspension of conidia is filtered through an sterile gauze or cheesecloth.
3. Wash the conidial suspension three times with 1 M sorbitol. The suspension is centrifuged in a tabletop centrifuge (3000 rpm, 3 min) and the conidia are resuspended using 50 ml 1 M sorbitol. After the final wash, transfer the conidial pellet into an eppendorf tube to obtain a high-density stock of conidia.
4. Dilute and count the conidial stock, and adjust the final conidial suspension to 2.5×10^9 conidia/ml in 1 M sorbitol.

Electroporation procedure

5. Add 40 µl of conidia (1×10^8 conidia) and 300 ng of DNA to a sterile eppendorf tube. Gently mix the conidia/DNA suspension. Keep the eppendorf tube with the mixed conidia/DNA on ice for 5 min. Prepare one conidia/DNA mix for every electroporation experiment.
6. Transfer the conidia/DNA mix to an ice-cold 0.2 cm electroporation cuvette (electroporation cuvettes Plus, 2 mm gap cuvette. BTX, San Diego, USA).
7. Electroporate with the following parameters: voltage gradient: 7.5 kV/cm (1.5 kV in a 0.2 cm cuvette), capacitance: 25 µF, resistance: 600 ohms. We use the BTX Electro Cell Manipulator 600, but other electroporators should work as well.
8. After electroporation add 1 ml of 1 M sorbitol to each cuvette. The suspension is mixed by gently pipetting up and down.
9. Plate 100 µl from the conidial suspension onto sorbose medium containing the appropriate supplements and incubate at 34ºC. Sorbose agar is used to promote colonial growth.
10. After 3-5 days of growth pick the trasformants and transfer them to small tubes for vegetative growth and homokaryon purification.
11. Verify the nature of the transformants by DNA hybridization with the appropriate DNA probes.

A convenient alternative: freezing conidia for electroporation (Selker lab)

12. The stock of conidia prepared in step 4 (2.5 x 10^9 conidia/ml in 1 M sorbitol) is divided in aliquots, transferred to eppendorf tubes and stored at -80ºC (no quick freezing required).

13. For electroporation, thaw the conidial stock at room temperature and wash conidia once with 1M sorbitol: Conidia are centrifuged in a microcentrifuge (15 seconds) and resuspended in 1 ml 1M sorbitol. Then conidia are centrifuged again and resuspended in the initial volume of 1M sorbitol. The resulting conidial suspension (2.5 x 10^9 conidia/ml in 1 M sorbitol) is now ready for electroporation following step 5.

NOTES:

The freeze/thaw cycle causes bursting of conidia and build up of salts in the tube so washing the conidia with 1M sorbitol is essential.

The possible reduction in transformation efficiency compared to freshly harvested conidia has not been measured. If transformation with high efficiency is required we recommend using freshly harvested conidia but most transformation experiments should work using frozen conidial stocks.

Frozen conidial stocks should not be stored at -80ºC for more than three months, but it is possible to freeze/thaw the same tube several times and obtain transformants or transform conidia stored in the freezer for over two years. In these cases, we expect that the transformation efficiency should be low but sufficient for many applications.

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References

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