

# Transformation of *Aspergillus fumigatus* by electroporation

## Introduction

This procedure allows transformation of *A. fumigatus* with efficiencies averaging 200 transformants/ $\mu\text{g}$  DNA. Efficiencies up to 1000 transformants/ $\mu\text{g}$  have been observed. It is adapted from the protocol of O. Sanchez and J. Aguirre developed for *A. nidulans*.

## Materials

- YG medium : 0.5% Yeast Extract, 2% D-glucose supplemented with 5 mM uridine and 5mM uracil when a *pyrG* strain is used
- ice-cold sterile water
- YED pH 8.0 : 1% yeast extract, 1% glucose, 20 mM HEPES, adjust pH to 8.0 with 1 M NaOH)
- EB (ice-cold) : 10 mM Tris-HCl pH 7.5, 270 mM sucrose, 1 mM Li-acetate
- Selective medium : *Aspergillus* minimal medium (0.52 g/l KCl, 0.52 g/l  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1,52 g/l  $\text{KH}_2\text{PO}_4$ , trace elements 1X, 1% D-glucose, 5 mM Na-glutamate, adjust to pH 6.8 with 1 M NaOH, 1.5% Oxoid agar) when a *pyrG* strain is used

## Equipment

- Rotary shaker at 37°C
- Rotary shaker at 30°C
- Table-top centrifuge
- BioRad electroporation device (settings 1 kV, 400 W, 25  $\mu\text{F}$ )
- 0.2 cm BioRad electroporation cuvettes
- 37°C incubator

## Procedure

1. Inoculate 125 ml YG medium at  $10^7$  spores/ml with washed conidia. Conidia are collected from *A. fumigatus* lawns grown on complete medium agar plates or slants (3-4 days at 37°C) and resuspended in PBS. Tween 20 0.1%, filtered and washed 5 times in sterile distilled water.
2. Grow for 4 hr at 37°C at 300 rpm on rotary shaker
3. Collect swollen spores by centrifugation 5 min 4000 x g at 4°C
4. Resuspend spores in 200 ml ice-cold sterile water, centrifuge 5 min at 4000 x g at 4°C
5. Resuspend spores in 12.5 ml YED pH 8.0 and incubate 60 min at 30C at 100 rpm on rotary shaker

6. Centrifuge 5 min at 4000 x g
7. Resuspend spores in 1 ml ice-cold EB at  $10^9$  conidia/ml and keep on ice
8. Mix 50  $\mu$ l conidial suspension with 1 to 2  $\mu$ g DNA in a total volume of 60 $\mu$ l in Epp. tubes and keep on ice for 15 min
9. Transfer suspension to 0.2 cm electroporation cuvette and electroporate with BioRad instrument
10. Add 1 ml ice-cold YED and transfer to pre-cooled sterile 15 ml tube, keep on ice for 15 min
11. Incubate at 30°C for 90 min at 100 rpm on rotary shaker, tubes in horizontal position
12. Spread 100, 200, and 700  $\mu$ l on minimal medium agar plates and incubate at 37°C
13. Transformants are observed after 36-48 hours at 37°C

## Timetable

Fungal culture (1) 4 h

Transformation (2-12) 4-5 h

## Tips and general comments

1. Freshly harvested conidia should be used to ensure highest transformation efficiencies.
2. As is usual with transformation of filamentous fungi, highest transformation efficiencies are obtained with a linearized vector. Targeted integration appears to occur at a lower frequency than that observed following protoplast transformation when this protocol is used.
3. This protocol has been used with different markers including *pyrG* and *hyg*<sup>R</sup>. With the latter, selection of transformants can be obtained by plating the transformation mixture directly on hygromycin-containing plates or by plating the cells on non-selective medium, incubating ON at 37°C and then overlaying the plates with hygromycin-containing soft agar (0.7%) medium. The final hygromycin concentration in the plate should be 200  $\mu$ g/ml.
4. A *pyrG* strain can be obtained upon written request from Christophe d'Enfert, Laboratoire des *Aspergillus*, Institut Pasteur, 75724 Paris Cedex 15, France.

## References

Sanchez, O. and J. Aguirre. **(1996)**. Efficient transformation of *Aspergillus nidulans* by electroporation of germinated conidia. Fungal Genetics Newsletter 43: 48-51.

Weidner, G., d'Enfert, C., Koch, A., Mol, P., and Brakhage, A.A. **(1998)** Development of a homologous transformation system for the human pathogenic fungus *Aspergillus fumigatus* based on the *pyrG*

gene encoding orotidine monophosphate decarboxylase. Current Genet. 33: 378-385.

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