Fungal Genetics Reports

Volume 47 Article 24

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Recommended Citation

Cockram, J. A., and . Sealy-Lewis (2000) "A simple modification of the A. nidulans transformation protocol increases the transformation frequency," *Fungal Genetics Reports*: Vol. 47, Article 24. https://doi.org/10.4148/1941-4765.1220

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Abstract

After transformation of *Aspergillus nidulans* with plasmid DNA the transformants are usually incubated at 37C until transformants appear. We have found that pre-incubation of the transformation plates at room temperature for 24h leads to increased transformation frequencies.

A simple modification of the *Aspergillus nidulans* transformation protocol increases the transformation frequency

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After transformation of *Aspergillus nidulans* with plasmid DNA the transformants are usually incubated at 37°C until transformants appear. We have found that pre-incubation of the transformation plates at room temperature for 24h leads to increased transformation frequencies.

The transformation protocol of Tilburn *et al.* 1983 (Gene 26, 205-221) has been used in our lab virtually without change since it was developed. Recently we discovered that a simple modification increases the transformation frequency.
The results of a typical experiment are shown in Table 1. Details of the protocol not specifically mentioned here are the same as described in Tilburn *et al.* Strain H1326: *biA1; argB2; pyroA4; alcΔ500* was inoculated into *Aspergillus* glucose minimal medium with appropriate supplementation and grown for 16 hours at 25°C. The mycelium was divided into two portions one of which was treated with Novozyme 234 for 1.5 h at 30°C and the other for 1.8h at 25°C. After the protoplast washing procedures the two batches of protoplasts were transformed with pBluearg (constructed by inserting the *Bam*HI/*Pst*I fragment from pILJ16 containing *argB* into Bluescript). After transformation and plating out of the protoplasts on selective media lacking arginine, half the plates from each treatment were immediately incubated at 37°C, and the other half were left at room temperature for 24h before being transferred to the 37°C incubator. As can be seen from Table 1 the plates that were pre-incubated at room temperature result in at least a 2-fold increase in the transformation frequency. This result has been repeated consistently in our laboratory. This is a simple modification of the protocol and with some plasmids/strains that are difficult to transform any modifiation that increases the frequency may be beneficial. Lowering the protoplasting temperature also seemed to lead to increased transformation frequencies in this experiment.

Table 1. Transformation experiment testing protoplasting temperature and regeneration conditions on the transformation frequency

Proplasting Temperature	Regeneration Conditions	Transformants µg/pBluearg
25°C	24h at room temp then at 37°C	65
25°C	37°C from start	20
30°C	24h at room temp then at 37°C	14
30°C	37°C from start	7