Fungal Genetics Reports

Volume 42 Article 25

A rapid method of heterologous gene cloning using cotransformation of lambda genomic DNA banks in Aspergillus nidulans

M. S. Stanley
University of Wolverhampton

P. Hooley
University of Wolverhampton

N. J.W. Clipson
University of Wolverhampton

Follow this and additional works at: http://newprairiepress.org/fgr

Recommended Citation
Stanley, M. S., P. Hooley, and N.J. Clipson (1995) "A rapid method of heterologous gene cloning using cotransformation of lambda genomic DNA banks in Aspergillus nidulans," *Fungal Genetics Reports*: Vol. 42, Article 25. https://doi.org/10.4148/1941-4765.1355

This Regular Paper is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.

A rapid method of heterologous gene cloning using cotransformation of lambda genomic DNA banks in Aspergillus nidulans

Abstract

Relatively few fungal genera possess the extensive collections of mutants or reliable transformation systems that are found in favoured species such as *Aspergillus nidulans*, *Saccharomyces cerevisiae* or *Neurospora crassa*. Consequently, heterologous gene cloning protocols that circumvent the requirement for mutant construction or the development of transformation vectors in a previously uncharacterized species, are attractive. Lambda vectors probably provide the most versatile group of cloning vehicles but, with some exceptions (Pall and Brunelli 1994 Fungal Genet. Newsl. **41**:63-65), they do not carry selectable markers for fungal transformation. Cooley et al (1990 Mycol. Res. **94**:145-151) nevertheless demonstrated that lambda molecules could be cotransformed effectively into *Septoria nodorum* along with a conventional vector carrying a selectable marker. Here, we describe an extension of that protocol demonstrating that such cotransformed molecules can be rescued efficiently from *Aspergillus nidulans* as a host. Lambda genomic DNA libraries of other fungi may thus be screened by complementation of *A. nidulans* mutants. Additionally, the protocol offers a second method of gene bank screening via conventional plaque hybridization, so exploiting the large cloning capacity of lambda replacement vectors. Recently, we have cloned DNA capable of complementing the *A. nidulans* salt sensitivity mutation sltA1, (Spathas 1978 Aspergillus Newslett. **14**:28), from the marine hyphomycete *Dendryphiella salina*, using this cotransformation approach.

Creative Commons License

This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

A rapid method of heterologous gene cloning using cotransformation of lambda genomic DNA banks in *Aspergillus nidulans*

M.S. Stanley, P. Hooley and N.J.W. Clipson - School of Applied Sciences, University of Wolverhampton , West Midlands , WV1 1SB , U.K.

Relatively few fungal genera possess the extensive collections of mutants or reliable transformation systems that are found in favoured species such as *Aspergillus nidulans*, *Saccharomyces cerevisiae* or *Neurospora crassa*. Consequently, heterologous gene cloning protocols that circumvent the requirement for mutant construction or the development of transformation vectors in a previously uncharacterized species, are attractive. Lambda vectors probably provide the most versatile group of cloning vehicles but, with some exceptions (Pall and Brunelli 1994 Fungal Genet. Newsl. **41**:63-65), they do not carry selectable markers for fungal transformation. Cooley et al (1990 Mycol. Res. **94**:145-151) nevertheless demonstrated that lambda molecules could be cotransformed effectively into *Septoria nodorum* along with a conventional vector carrying a selectable marker. Here, we describe an extension of that protocol demonstrating that such cotransformed molecules can be rescued efficiently from *Aspergillus nidulans* as a host. Lambda genomic DNA libraries of other fungi may thus be screened by complementation of *A. nidulans* mutants. Additionally, the protocol offers a second method of gene bank screening via conventional plaque hybridization, so exploiting the large cloning capacity of lambda replacement vectors. Recently, we have cloned DNA capable of complementing the *A. nidulans* salt sensitivity mutation *sltA1*, (Spathas 1978 Aspergillus Newslett. **14**:28), from the marine hyphomycete *Dendryphiella salina*, using this cotransformation approach.

1. A conventional genomic DNA bank of the donor fungus was made into lambda EMBL3 using a Stratagene kit. Usually, the bank was amplified by flooding *E. coli* (DH1) lawns on agarose plates with SM buffer (50 mM Tris-HCl, pH 7.5; 100 mM NaCl; 8 mM MgSO4; 0.01% gelatin) and DNA isolated following standard PEG precipitation, phenol extraction and ethanol precipitation, (Maniatis et al. 1982 Molecular Cloning. A Laboratory Manual, Cold Spring Harbor).

2. DNA amplified in this way was mixed at an approximately equimolar ratio with pDJB3 and cotransformed into protoplasts of *A. nidulans* using the techniques of Ballance et al. (1985 Gene **36**:321-331). The recipient strain carried the selectable marker for transformation (*pyrG89*) along with the target mutation for complementation (*sltA1*), which confers salt sensitivity (poor growth on plates containing 0.5 M NaCl).

3. Transformed colonies, regenerating upon pyrimidine-free media containing 0.6 M KCl, were replica plated onto media with 2.0 M NaCl, selecting for *sltA1*+. However, as the regeneration medium itself exerted a strong selection pressure for salt tolerant clones, the precise frequency of *sltA1*+ transformants could not be estimated.

4. Total DNA was isolated from putative clones (*sltA1*+) using the method of Raeder and Broda (1985 Lett. App. Micro. **1**:17-20). The recovery ("rescue") of cotransformed lambda molecules was attempted by exposing around 5 ug of this total DNA to Gigapack Gold II packaging

extracts (Stratagene: efficiency 1 x 10(8) pfu/ug). Normally, 100 ul of packaged extract (500 ul total) were mixed with 200 ul of *E. coli* (DH5a) cells grown to mid-logarithmic phase in Luria broth containing 0.8% maltose. Routinely, cells were resuspended in 10 mM MgSO4 and kept at 4 C prior to exposure to packaged DNA.

5. Recovered lambda molecules ("rescues") were selected in two ways: (i) by their ability to produce plaques in bacterial lawns; or (ii) by selection for the ampicillin resistance marker which allowed bacterial colony growth on Luria Broth agar plates with 100 ug/ml ampicillin.

6. "Rescues" appeared at a relatively low frequency with around 10-15 ampicillin resistant *E.coli* colonies or around 5-6 plaques resulting from infection of 200 ul of cells with 100 ul of packaged extract. In each case, DNA from an individual, putative fungal clone yielded ampicillin resistant colonies or plaques but not both. Some ampicillin resistant colonies grew very slowly in liquid culture - DNA could be isolated from such rescues by standard plasmid mini-preparations or from cell-free supernatants, using a lambda DNA preparation technique. Supernatants of these slow-growers could also prove infective, yielding plaques on a bacterial lawn. Such rescues then appeared "phagemid" in nature. This suggested that recombination had occurred between the lambda molecule and the cotransforming plasmid at some point in the transformation or rescue procedure. Southern blot analysis confirmed the rescue of *D. salina* DNA from the alternative host.

7. The successful cloning and rescue of *D. salina* sequences that complemented *sltA1* was confirmed by retransformation (by cotransformation) back into the original salt sensitive *A. nidulans* host (Stanley et al., in preparation). Retransformants could be placed into at least three different groups based upon their salt sensitivity. Odd retransformants appeared even more sensitive than the original *A. nidulans* host, others were more tolerant and grew well up to 1.0 M NaCl whilst the majority could grow when 2.0 M NaCl was added to the agar medium. Control transformations with pDJB3 alone yielded occasional salt tolerant colonies but at a much lower frequency than the retransformants.

The protocol described here may be applicable to heterologous cloning strategies for other ascomycetes and deuteromycetes to avoid the lengthy development of specialized transformation vectors and techniques. However, we have experienced difficulties in isolating reasonable quantities of DNA from "rescues", in particular from those phagemid molecules that appear to have a limited infective ability and carry ampicillin resistance determinants. Often, when the cotransformation of rescued molecules was attempted back into *A. nidulans* with pDJB3, selection of stable pyr+ expression was difficult. Adoption of this protocol must consider the possibility of the scrambling of DNA inserts produced by recombination events.

Acknoledgements: This work was supported by the Natural Environment Research Council and we thank A.J.Clutterbuck for providing a *sltA1* mutant.

DOI: 10.4148/1941-4765.1355