Fungal Genetics Reports

Volume 43 Article 29

Quick method for producing template for PCR from Neurospora cultures

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Recommended Citation

Yeadon, P. J., and D.E. Catcheside (1996) "Quick method for producing template for PCR from Neurospora cultures," *Fungal Genetics Reports*: Vol. 43, Article 29. https://doi.org/10.4148/1941-4765.1325

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Abstract

We have used the following method routinely for the past year and found it reliable even in undergraduate classes.

Quick method for producing template for PCR from Neurospora cultures

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We have used the following method routinely for the past year and found it reliable even in undergraduate classes. We grow our cultures in 13 x 100 mm tubes on 1.5 ml slopes of Vogel's medium containing the appropriate supplements, sucrose as the carbon source and Difco agar. The following volumes are appropriate for this tube size. New tubes are recommended. To a fresh, well- grown culture, add 1.5 to 2 ml DNA-free 1 x TE (pH 8.0) and vortex. Transfer the conidial suspension into a new sterile 10 ml polypropylene disposable tube and place in a boiling water bath for 10 min. Move to ice for 10 min, transfer to Eppendorfs and centrifuge at 13,000 rpm for 5 min at room temperature. Store the supernatant liquid at - 20 C and use 2 ul as template in a 50 ul PCR reaction. Products appear as usual, can be digested with restriction enzymes and are suitable for sequencing after our standard clean-up (Promega "Wizard PCR preps" DNA purification system). The template can be thawed and refrozen several times without loss. The substitution of water for TE results in unstable template and less reliable results.