

Fungal Genetics Reports

Volume 41

Article 10

Tergitol enables the rapid and inexpensive scoring of nutritional and drug-resistance markers in the progeny of *Neurospora crassa* genetic crosses

W . Michael Gray
Bob Jones University

Matthew S. Sachs
Oregon Graduate Institute of Science and Technology

Follow this and additional works at: <https://newprairiepress.org/fgr>



This work is licensed under a [Creative Commons Attribution-Share Alike 4.0 License](https://creativecommons.org/licenses/by-sa/4.0/).

Recommended Citation

Gray, W. M., and M.S. Sachs (1994) "Tergitol enables the rapid and inexpensive scoring of nutritional and drug-resistance markers in the progeny of *Neurospora crassa* genetic crosses," *Fungal Genetics Reports*: Vol. 41, Article 10. <https://doi.org/10.4148/1941-4765.1372>

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.

Tergitol enables the rapid and inexpensive scoring of nutritional and drug-resistance markers in the progeny of *Neurospora crassa* genetic crosses

Abstract

After cultures from the progeny ascospores of a genetic cross containing nutritional markers are obtained, it is often necessary to test them on a variety of media. Testing using individual culture tubes may be ideal, but is time consuming. In contrast, inoculation of plates with conidial suspensions is relatively quick.

Tergitol enables the rapid and inexpensive scoring of nutritional and drug-resistance markers in the progeny of *Neurospora crassa* genetic crosses

W. Michael Gray and Matthew S. Sachs** - *Biology Department, Bob Jones University, PO Box 34467, 1700 Wade Hampton Boulevard, Greenville, SC 29614 **Department of Chemical and Biological Sciences, Oregon Graduate Institute of Science and Technology, 20000 NW Walker Road, PO Box 91000, Portland, OR*

After cultures from the progeny ascospores of a genetic cross containing nutritional markers are obtained, it is often necessary to test them on a variety of media. Testing using individual culture tubes may be ideal, but is time consuming. In contrast, inoculation of plates with conidial suspensions is relatively quick.

Tatum originally reported that medium containing tergitol results in an initial phase of colonial growth (Tatum et al. 1949. *Science* 109:509-511), and Springer reported the use of tergitol as a substitute for sorbose in *N. crassa* transformation procedures (Springer, *Fungal Genet. Newsl.* 1991 38:92). The limitations of sorbose are that it adds expense and that it takes longer to score phenotypes on sorbose-containing media due to reductions in cell growth rate.

In crosses testing the segregation of *arg-12s*, *pyr-3*, and an *arg-2-hph* fusion gene, we found that the sensitivity of *arg-12s pyr-3* progeny to arginine in the absence of uridine, the uridine requirement of *pyr-3* progeny, and the hygromycin-resistant phenotype of progeny containing an *arg-2-hph* fusion gene could be examined using plates containing tergitol. Tergitol containing media may be useful to other investigators for large scale nutritional screening.

Our procedure was as follows: sucrose-containing plating media (containing Vogel's Medium N (Vogel, 1956. *Microbiol. Genetics. Bull.* 13:42-43)) and appropriate supplements were prepared and autoclaved in 100 ml aliquots in milk dilution bottles for 20 minutes. After cooling media to 50°C following autoclaving, Tergitol NP-10 (Sigma) was added to a final concentration of 0.005% by volume. Tergitol was not sterilized prior to use. Tergitol clings to the inside of the pipet tip and flushing of the tip with the sterile medium was essential in dispersing the entire volume of tergitol. Bottles of media were then gently mixed to avoid foaming and poured into standard 100 mm diameter plastic petri dishes at approximately 20 ml of medium per plate. Plates were dried prior to inoculation.

Grids were printed on acetate transparency film and taped to the bottom of the petri dishes. The grids consisted of four rows of five columns (20 squares); individual squares in the grid were approximately 13 mm x 13 mm.

Conidia were collected with a wet inoculating loop, transferred to 1 ml of sterile water, and briefly mixed with a vortex mixer. Six microliters of each suspension were applied as a spot to a

square in the same grid position on each of the media involved in the screening process. Once all positions were inoculated and all liquid absorbed by the media, plates were incubated at 32oC. Growth was checked after 12, 15, 18, 24 and 48 hours of incubation. Scoring of a few progeny could be done after 15 hours, but reliable screening required 24 hours. Growth remained in the form of colonies even on extended incubation, but the aerial spreading along the lid of the petri dish which occurred was seen as potentially compromising the integrity of data collected after 48 hours.

Brian Christman, Daniel Wong, S. Randal Olsen, Jennifer McNew, Scott Jackson, Deborah Lindner, Alyssa Ward and Rachel Weiler participated in this work, which was supported by the American Society for Microbiology/National Science Foundation's Scientist-Educator Team Program (W.M.G. and M.S.S.) and by the National Institutes of Health (M.S.S.)