How to do replica plating.
David Perkins

Background

Because of the rapid spreading growth habit of wild type Neurospora, replica plating has required using modified strains and special techniques. Replica plating, invented by Lederberg and Lederberg (1952) has therefore not been used routinely for genetic analysis of Neurospora, as it has for bacteria, yeast, Chlamydomonas, and Aspergillus. Effective replication procedures have been devised for special purposes such as selecting for mutants with novel requirements, altered substrate utilization, or increased sensitivity to irradiation or chemical agents (Reissig 1959, 1960; Schroeder 1970; Littlewood and Munkres 1975; Nilheden *et al.* 1975; DeLange and Mishra 1981). Replica plating of conidia from heterokaryons has been used in an elegant method for determining the frequency of recessive lethal mutations over the entire genome (Stadler and Crane 1979, Stadler and Macleod 1984. See '*How to detect and recover recessive lethal mutations*'.).

Procedures

Several mutant combinations have proved suitable for replication. Variously marked strains for replication are listed in the FGSC Catalog.

Replication using cr-1 rg: This double mutant forms small, profusely conidiating colonies on standard media. A master plate is prepared by inoculating conidia of *cr-1 rg-1* onto the surface of appropriately supplemented agar medium to give 50-100 colonies per plate. When colonies have grown up and conidiated, they are replicated to plates containing test mediua, using velveteen (Stadler and Crane 1979) or moistened filter paper (Maling 1960, Schroeder 1970). (See Maling 1960 for photograph).

Replication using sn cr-1: The procedure is identical to that for *cr-1 rg* (Chalmers and St. Lawrence 1979, DeLange *et al*. 1981, Stadler and Macleod 1984). The two double mutant strains are phenotypically similar, but *sn cr-1* is generally preferred because it is fertile as female (allowing homozygous crosses to be made if desired for testing allelism) whereas *cr-1 rg* is female-sterile (Perkins 1971).

Replication using cr-1: Colonies of the surface-conidiating *crisp* single mutant are less restricted than those of the double mutants with *rg-1* or *sn*. Maling (1960) isolated progeny from crosses homozygous for *cr-1* and scored segregating markers by inoculating segregants to fixed points on a master plate with a needle replicator such as that used routinely in Aspergillus (photograph in Maling 1960). Although successful for scoring, needle replication has not been generally used with Neurospora because of the need to introduce markers into *cr-1* strains and because of the reduced speed and productivity of crosses that are homozygous for *cr-1*.

Replication using cot-1: The *cot-1* strain is phenotypically indistinguishable from wild type at permissive temperatures but grows as small, aconidiate colonies above 32°C. Replication is accomplished by inoculating master plates with *cot-1* conidia or with ascospores from crosses homozygous for *cot-1*, overlaying the inoculum with a disk of filter paper (Littlewood and Munkres 1972) or fabric (Nilheden *et al*. 1975) and incubating at restrictive temperature until colonies have grown into the overlying material. The disk is then peeled off and placed on the surface of a test plate or plates long enough for inoculum to grow into the agar, all at restrictive temperature. Concentration of supplements in the master plate must be kept low, to avoid carry-over to the test plates. An earlier variant of this (Reissig 1959, 1960) placed a cigarette-paper disc on the surface of an uninoculated master plate and spread ascospores on top of the paper, which was peeled off and transferred to test plates after colonies had grown into the underlying medium on the master plate..

General comments: Agar should be stiff enough to avoid fracture (~4%) and the surface should be dry enough to avoid running of conidia in a surface film. It is wise to include a fully supplemented terminal test plate to show that inoculation has been effective. Orientation on the master and raplica plates must be clearly indicated. For practical details see the original publications.

References

Chalmers, J. H., and P. St. Lawrence. 1979. *cpl.-1*: A Neurospora mutant sensitive to chloramphenicol. Neurospora Newslett. 26: 8.

DeLange, A. M., and N. C. Mishra. 1981. The isolation of MMS- and histidine-sensitive mutants in *Neurospora crassa*. Genetics 97: 247-259.

Lederberg, J., and E. M. Lederberg. 1952. Replica plating and indirect selection of bacterial mutants. J. Bacteriol. 63: 399-406.

Littlewood, R. K., and K. D. Munkres. 1972. Simple and reliable method for replica plating *Neurospora crassa*. J. Bacteriol. 110: 1017-1021.

Maling, B. 1960. Replica plating and rapid ascus collection of Neurospora. J. Gen. Microbiol. 23: 257-260.

Nilheden, E., K. Holm, and H. G. Kolmark. 1975. Glycerol nonutilizing mutants in *Neurospora crassa*. Isolation by net replication. Hereditas 79: 239-250.

Perkins, D. D. 1971. Conidiating colonial strains that are homozygous fertile and suitable for replication. Neurospora Newslett. 18: 12.

Reissig, J. L. 1959. Replica plating with *Neurospora crassa*. Microbial Genet. Bull. 14: 31-32.

Reissig, J. L. 1960. Forward and back mutation in the *pyr-3* region of Neurospora. 1. Mutations from arginine dependence to prototrophy. Genet. Res. 1: 356-374.

Schroeder, A. L. 1970. Ultraviolet-sensitive mutants of Neurospora. 1. Genetic basis and effect on recombination. Mol. Gen. Genet. 107: 291-304.

Stadler, D., and E. Crane. 1979. Analysis of lethal events induced by ultraviolet in a heterokaryon of Neurospora. Mol. Gen. Genet. 171: 59- 68.

Stadler, D., and H. Macleod. 1984. A dose-rate effect in UV metagenesis in Neurospora. Mutat. Res. 127: 39-47.

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