

## How to avoid pitfalls and keep out of trouble in the Neurospora lab.

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"Experience is a dear school, but fools can learn from no other." -- Benjamin Franklin, *Poor Richard's Almanac*.

The precautions gathered below, which are based on long experience, are largely repetitive of information scattered under different headings in the descriptions of individual methods.

### ***Culture, incubation, storage, and disposal:***

- Don't incubate cultures in closed containers where humidity reaches 100%. Neurospora can then grow through cotton plugs, past closures, and out of plates, conidiating profusely when it emerges.
- Don't leave grown cultures or crosses for long periods at room temperature or above 5° C, where they are subject to infestation by mites. Culture tubes that have not completely dried down are especially inviting to mites.
- Don't transfer conidiating cultures in drafty conditions, to avoid conidial scatter and airborne cross-contamination. Work close to the flame of a Bunsen burner or alcohol lamp.
- Don't send cultures or contaminated glassware to the dishwasher before they have been autoclaved, lest airborne conidia contaminate the laboratory building.
- Don't discard living material with ordinary garbage or trash. Autoclave before discard, to avoid contaminating the environment and possibly interfering with future studies of ecology and geographical distribution.
- When conidiating strains are obtained from nature or from suspect sources, place the material at minus 20° C for at least 12 hours to kill any contaminating mites and their eggs, or other arthropods. Neurospora can be freed of contaminating bacteria by growing the fungus on chloramphenicol (200 µg/ml, autoclaved in medium). (See *How to sample natural populations*.)

### ***Stock preservation:***

- Don't maintain important stocks by storing vegetative cultures at refrigerator temperatures (~5° C) and transferring them periodically. Under these conditions, mutations accumulate that impair growth and fertility and alter morphology. Except for temporary working stocks, significant strains should be kept in suspended animation by freezing, on silica gel, or by lyophilization. (See *How to preserve stocks*.)
- Always use fresh, optimally grown cultures for preserving stocks in suspended animation. Before being preserved, macroconidiating cultures should have aged sufficiently for the conidia to be dry and powdery.
- Don't expect wet conidia or mycelia to show good survival if they are frozen at minus 20° C. Aco-spores also do not survive freezing at minus 20° C.
- Do not attempt to preserve stocks on silica gel that contains a colored desiccation-indicator.

- For each significant stock, don't fail to record the FGSC number or other accession number of the strain, as well as the allele (isolation) number of each mutant allele it contains.

***Spore and colony counts:***

- Do not suspend ascospores in water for counting or quantitative plating. A viscous medium is needed to avoid sedimentation of the large spores. A 1% solution of polyvinyl pyrrolidone (PVP-360, Sigma) is convenient, nontoxic, and preferable to 0.05% agar, which is commonly used (Metzenberg 1989).
- Do not base estimates of viable-cell numbers on the number of colonies in densely crowded plates. Crowding inhibits a fraction of the viable cells from forming colonies (the "Grigg Effect"). (See Grigg 1958, Davis and de Serres 1970, p. 92.) For reliable numbers of viable cells, use uncrowded plates from higher dilutions.

***Making crosses and recovering progeny:***

- Don't incubate crosses at high temperatures. For *N. crassa*, 25° C is the standard and perithecia fail to form above ~30° C.
- Once ascospores have been exposed to 60° C and then removed to a lower temperature, never return them to 60°. Activated ascospores are hypersensitive to killing by heat.
- Don't attempt to germinate dehydrated ascospores. Rehydrate them prior to heat shock, which can be applied either before or after isolation. Add water to dried-down or partially dried down cross tubes or plates, suspend the ascospores, or place them on a moist surface. Even ascospores that have collapsed and become sickle-shaped as a result of water loss can often be rendered viable.
- For good recovery, don't heatshock ascospores sooner than 7 to 10 days (at 25° or 30° C) after they are ejected from the asci.
- Don't fail to monitor crosses visually for the presence of unpigmented, aborted ascospores that can reveal the presence of unsuspected chromosome rearrangements, ascospore-lethal mutations, or Spore killer elements. Examine ejected ascospores routinely under ~60× magnification using transmitted light. Ninety percent or more of ascospores are expected to be black in normal isosequential crosses. Rearrangements and mutations that affect ascospore development and result in failure of ascospore pigmentation may arise spontaneously and are common following exposure to mutagens.

See *How to make a cross*. See *How to recognize and diagnose chromosome rearrangements*. See *How to distinguish a Spore killer from alternative causes of ascospore abortion*. See *How to enhance perithecial formation and ascospore production of poorly fertile crosses*.

***Use of heterokaryons for allelism tests:***

- Don't fail to ascertain that mutant strains which appear unable to complement one another are fully heterokaryon-compatible. For strains in Oak Ridge background, this is conveniently accomplished by testing for *het*-compatibility with one of the inactive-mating type "helper" strains (See *How to use helper strains*. See *How to test for complementation between mutant strains*.)

***Use of unordered asci to estimate the relation of translocation breakpoints to centromeres:***

- When 4 Black : 4 White unordered asci are infrequent, don't conclude that the observed 4B:4W octads are due to crossing over proximal to a breakpoint. They could result instead from nondisjunction of centromeres, resulting in 3:1 segregation from the translocation quadrivalent at Meiosis I. The two origins can be distinguished by opening perithecia and examining ordered asci to determine whether the four viable, black ascospores are all in the same half-ascus (as expected with 3:1 segregation and nondisjunction), or two of the four are in opposite halves (as expected with crossing over). (See Perkins and Raju 1995.)

## References

Davis, R. H., and F. J. de Serres. 1970. Genetic and microbiological research techniques for *Neurospora crassa*. Meth. Enzymol. 17A: 79-143.

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