2. Grow up a fresh culture of the strain to be preserved on an appropriate medium with 4% agar. One 13 x 100 mm slant usually gives enough material for one silica gel tube. For conidiating stocks, 5 or 6 days at 25°C is about right. Slow growing and nonconidiating strains should not be allowed to age beyond 6 or 8 days — a larger amount of material should be obtained by using smaller tubes or several tubes.

3. Suspending the material in sterile water to give a densely turbid suspension. The procedure differs for conidiating and for nonconidiating strains. With conidiating strains, suspensions can be made in the original culture slant if 4% agar medium is used. About 0.5 ml sterile water is gently introduced, using a thin-walled disposable Pasteur pipette. The cotton plug is replaced and conidia are suspended by shaking, using a vortex-type mixer. About 0.3 ml nontax milk is then pipetted in and stirred gently to avoid breaking the agar surface, and the entire suspension is pipetted onto silica gel.

(Both water and nonfat milk are conveniently dispensed in 10 x 75 mm tubes, and autoclaved 10 minutes. Brewery store powdered nonfat milk is dissolved in water to give a concentration at least full strength.)

Nonconidiating strains, such as fluffy or multicent, or scanty growers, are not suspended in the growth slant. Instead, a sterile blade (platinum-iridium, nichrome, or stainless steel) is used to slice or peel the mycelial mat off the agar surface and transfer it to a 10 x 75 mm tube containing 0.3 ml water. The more mycelium the better, and the unavaluable small amounts of agar do not affect preservation. Do not use just aseptic hyphae, but include mycelium from the agar surface.

In the 75 mm tube, use a pipet or glass rod to grind the mycelium against the tube wall (stirring motion) until a smooth homogenate result. (Be patient! It may take several minutes.) Many revolutions are more effective than heavy pressure. When the homogenate is creamy and has been vibrated, add 0.5 ml milk and proceed as with conidiants.

4. The 1 ml suspension is pipetted dropwise over the silica gel in a prelabelled tube. (Before removing the plug and introducing the pipet, hold the silica gel tube horizontally and shake it so that the particles lie evenly along the tube, providing clearance for the pipet tip to reach the butt. The pipet is then gradually moved up the tube as the suspension flows out onto the layer of particles.

5. Replace plug and recheck correspondence of labels.

6. Vibrate briefly with the mechanical mixer, so as to distribute inoculum over as many grains as possible throughout the silica gel tube, and to prevent lumping.

7. Place tube in an ice-water bath for 15 minutes.

8. After a few hours at room temperature the particles should appear dry. If they remain soggy because of over-saturation, add more sterile silica gel and mix. (Silica Gel, Chromatographic Grade, type 1, 60-200 mesh is convenient for adding, being a fine powder.) In a humid climate, storage of the unsealed tubes in a desiccator may be desirable.

9. One day after the tube appears dry, it is sealed against moisture by covering the plug and mouth of tube with a 20 mm square of Paraffilm. At this time we etch a stock number on the glass with an engraving tool.

10.

11.

*A method of staining Neurospora nuclei.

**A procedure is described for the cytological preparation of vegetative and meiotic nuclei of Neurospora crassa with Schiff's reagent. The main objective behind developing this technique was to determine microspectrophotometrically the relative DNA content at various stages of meiosis in order to follow the timing of DNA synthesis pertaining to recombination. The details of the microspectrophotometric analysis will be published elsewhere (Iyengar et al., 1977 Genet. Res. Comb. (In press)).

Stains of both mating types of N. crassa CBS 285-62 were obtained from Central Bureau voor Schimmelcultures, Baarn, The Netherlands. The technique for crossing these stains was essentially the same as that of Barry (1966 Neurospora NewsL, 10: 12).

Fixation: a) Perithecia: The fixative, containing 9 parts of n-butyl alcohol, 6 parts of glacial acetic acid, and 2 parts of 10% aqueous chromic acid (Lu 1962 Con. J. Bot. 40: 843), was directly poured into cultures containing perithecia. The cultures were then placed in a vacuum desiccator to ensure complete infiltration with the fixative. After 24 hours, strips of agar bearing perithecia were cut out of the cultures and washed in running water for an hour. The strips were then immersed in small vials containing Newman's fluid (Newcomer 1953 Science 118: 161) consisting of 6 parts isopropyl alcohol, 3 parts propionic acid, 1 part acetic, 1 part petroleum ether, and 1 part dioxane. After 24 hours, the strips were transferred to the vials containing 70% ethanol and stored at 4°C. The fixed perithecia can be stored for 3-4 months without any deterioration in the quality of preparations.

b) Hyphae and conidia: Hyphae and conidia were scraped from growing cultures and directly fixed in 3:1 ethanol-acetic acid for 24 hours, transferred to 70% ethanol, and stored at 4°C.

Staining: a) Meiotic nuclei: Perithecia were scraped from agar strips, hydrolysed in 1 N HCl for 8 minutes at 60°C, washed in distilled water, and treated with methanol for 2-3 minutes. The perithecia were then immersed in the conventional Schiff's reagent prepared according to Groenman (1953 Z. wiss. Micr. 61: 225) with the modification that 0.1% paraphenylindan (Merck, Darmstadt) was used instead of 0.5%.

c) Vegetative nuclei: Hyphae and conidia were hydrolysed in 1 N HCl at 60°C for 12 minutes and stained with Schiff's reagent according to the method as described for perithecia.

Preparation of Slides: Perithecia were placed on a clean slide in a drop of 45% acetic acid and mycelial fragments and remnants of agar were removed with a needle and pair of forceps under a dissecting microscope. The asci were expressed through the ostiole by carefully pressing the perithecium with a flattened needle. Perithecium wall and other debris were removed and the clumps of asci were teased apart and then carefully covered with a glass coverslip. The slide was exposed to steam for a few minutes and then the asci were flattened by pressing the coverslip with a thumb. This helps to increase the contrast between the cytoplasm and the chromosomes. For good results, exposure to steam should not be too long but should last until the asci took the stain.

Also, pressure should be applied to the coverslip in a single steady motion, otherwise the asci may get distorted or overlap. The excess acetic acid was then blotted out with a filter paper and the coverslip sealed with transparent nail polish. The slides thus prepared can be stored for 3-4 days at 4°C. The preparation of slides with hyphae and conidia was the same as that described for ascis. - -- Botany Department, Bose Institute, Calcutta 700009, India. 17