

Editors note: The National Institutes of Health has recently approved the use of Neurospora crassa and of Saccharomyces cerevisiae for use as HVI systems for the cloning of recombinant DNA. The following represents the pertinent sections as approved and published in the Federal Register (Fed. Reg. 44, No 71: 21730-21736, April 11, 1979).

Definition of a eukaryote microorganism which is on HVI system (moderate level of containment). Such an organism is (1) nonpathogenic (2) not adapted to normal escape routes (air, people, sewage) or is specially modified to minimize such escape and (3) shows little or no genetic exchange with other organisms encountered in escape route. Laboratory strains of Neurospora crassa when modified as specified in this report, and laboratory strains of Saccharomyces cerevisiae, both of which do not have the biological association with man characteristic of the enteric bacteria, clearly fulfill these criteria of HVI systems.

Saccharomyces cerevisiae as an HVI system. This yeast is nonpathogenic to man, animals and plants^{1,2,3,4,5,6} is not normally found in sewage^{7,8} or in the intestinal tract^{2,3} and has no aerial dispersal mechanism. Of all strains of Group 1 Saccharomyces (the group to which the laboratory strain of S. cerevisiae belongs) encountered at random in nature, it is estimated that less than 5% are capable of mating with any given haploid strain of S. cerevisiae (H.J. Phoff, personal communication) and under natural conditions no other species of yeast will mate with the laboratory strain. 9, 10 Furthermore, no natural vectors are known that might effect interspecific transfer of genetic material. Because of these properties, laboratory strains of S. cerevisiae require no special modification to serve as HVI systems.

Neurospora crassa as an HVI system. N. crassa is nonpathogenic to man, animals and plants.^{11,12,13,14} It is an obligate aerobe and is not found in sewers^{15,16} or in the intestinal tract.¹¹ It is only found sporadically in temperate regions.¹³ N. crassa is only capable of hybridization and gene exchange to a very limited extent with the three most closely related species N. tetrasperma, N. sitophila and N. intermedia.^{17,18} Crosses with other Neurospora species or with fungi of other genera have been unsuccessful.^{13,19} Numerous vegetative incompatibility genes in N. crassa strains found in nature block transfer of genetic material from strain to strain by vegetative fusion.^{14,20} No vectors are known that might effect interspecific or intergeneric transfer of genetic material.

Since wild type conidia can be dispersed aerially, the HVI designation shall apply only to the following strains in which either survival away from a special substrate, or aerial dispersal, are greatly compromised.

- (i) inl (inositolless) strains 37102, 37401, 46316, 46802, 64001 and 89601. These strains require concentrations of the order of 5 micrograms/ml inositol for maximum growth.²¹ At 0.5 microgram/ml growth and sporulation are poor. On substrates without inositol the mutants rapidly die (inositolless death).²² Mutant 46802 is non-reverting, the other mutants revert at frequencies of less than 10^{-7} .²¹
- (ii) csp-1 (UCLA37) and csp-2 (FS590, UCLA101) (conidial reporation). These mutants form adherent macroconidia that cannot fall free, even with vigorous tapping.^{23,24} No reversions have been reported since the discovery of the mutants in 1974.
- (iii) ear (UCLA191) (easily wettable). Macroconidia become wet and go into water suspension instantly (wild type is hydrophobic). Macroconidia appear sticky, do not fall free of mycelium even with vigorous tapping.²⁵ No reversions have been reported since the discovery of the mutants in 1976.

Wild type Neurospora crassa as an HVI system. Because of the fact that Neurospora has never been implicated in adverse effects for man, animals or plants and has no close association with man, animals or plants in nature, and because N. crassa in interspecific combination is incapable of forming heterokaryons,^{20,26} wild type N. crassa may be permitted to be used in experiments that require HVI provided these are carried out at physical containment one level higher than required for HVI. However, if P3 physical containment is specified for HVI, this level is considered adequate for unmodified N. crassa. For P2 physical containment special care must be exercised to prevent aerial dispersal of macroconidia.

Disabled Saccharomyces cerevisiae as an HV2 system. Since laboratory strains of S. cerevisiae intrinsically more than satisfy HVI criteria, and, since the ability of laboratory strains to establish themselves in the wild is low, the main route of escape of a cloned segment to the environment would be through mating of the host to a more robust wild type yeast. Therefore, a sufficient basis for construction of an HV2 S. cerevisiae would be a drastic reduction in the frequency of mating and, therefore, transmission of a cloned segment over the HVI level by the introduction of sterility mutations. Even without such mutations, on exceedingly low frequency of mating would be expected under dilute natural conditions where mating pheromones would not be in concentrations necessary for the initiation of mating.

Equivalence of lower eukaryote HV systems with those of E. coli. Except for the cloning of complete genomes of eukaryote viruses, the S. cerevisiae and N. crassa HVI systems and S. cerevisiae HV2 systems are to be equivalent to EK1 and EK2, respectively; no special action by the RAC is needed for experiments requiring these levels of containment. Experiments involving com-

plete genomes of class I eukaryote viruses will require P3-HV1 or P2-HV2 containment. Other eukaryote viruses are to be handled on a case-by-case basis.

Editors note: A description of six criteria which must be satisfied in order that a yeast strain can be certified as a HV2 host is not reproduced here. (The primary consideration is the use of sterility mutations which essentially preclude mating of the proposed HV2 strain with all other yeast strains.) It is of interest that the Recombinant DNA Advisory Committee (RAC) is now considering whether to approve four mutant strains of *Saccharomyces cerevisiae* as HV2 hosts; also under consideration are a number of potential vectors, all derivatives of the plasmid pBR322 (some also contain sequences derived from the yeast 2-micron plasmid or from yeast chromosomal DNA). These vectors are designed to permit cloning in both suitable *E. coli* K12 and yeast hosts. For additional information please consult the Office of Recombinant DNA Activities, the National Institutes of Health. The article by Perkins and Björkman in this issue of the Newsletter describes the *Neurospora* mutants which have been approved for cloning.

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