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# Electrophoretic Karyotypes of Fungi: The New Cytology

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**Pulsed-field gel electrophoresis (PFGE) of fungal chromosomes.** The application of PFGE to separate fungal chromosomes in agarose matrices is an exciting and powerful technique for analysis of fungal genomes. Initially employed for separating *Saccharomyces cerevisiae* chromosomes (Schwartz and Cantor 1984; Carle and Olson 1984, 1985), the technique has wide applicability to other fungi. Molecular karyotypes have been described for species representing at least 22 genera of fungi, including nine genera of phytopathogenic fungi (Table 1). The development of molecular karyotypes, physical maps of entire genomes, and fine structure physical and genetic maps of chromosomes are possible when this technique is used in conjunction with other standard molecular techniques, even for fungi that are recalcitrant to classical genetic analysis.

**Problems inherent in classical genetic analysis.** Classical fungal genetics requires a collection of genetic markers that can be followed either by Mendelian genetic analysis or via the parasexual cycle (Pontecorvo 1958). For a variety of reasons, induced mutagenesis has not led to a collection of suitable markers for many fungi. A problem inherent for many species has been finding suitable methods of determining whether presumptive haploid spores contain a uniform base number of chromosomes. Unfortunately, the small chromosomes of many fungi preclude any cytological determination of the karyotype. In other fungi that have chromosomes suitable for cytological karyotyping, the ploidy level has been difficult to ascertain. Cytological karyotypes of field isolates of *Phytophthora megasperma* have a large range in chromosome number, estimated to vary from approximately 11 to 40 (Hansen *et al.* 1986). Whether the range in karyotypes represents different ploidy levels or stages of aneuploidy is unclear, although the technique of molecular karyotyping has provided evidence for levels of polyploidy (Howlett 1989). The selection of induced genetic markers in fungal species that are polyploid or that tolerate aneuploidy could fail simply because mutations arising in one homologue of a pair of disomic chromosomes would be complemented by the wild-type allele of the other homologue.

Other problems unrelated to the isolation of genetic markers could also adversely affect the use of these markers in the analysis of fungal genomes. In *Tilletia* spp., where an elaborate scheme for recovering mutant alleles has been successfully developed (Mills and Churchill 1988), linkage data may be difficult or impossible to obtain simply because strains maintained in culture without weekly transfer either lose their ability to mate or fail to complete the sexual cycle (Trail and Mills 1990). Further complications are inherent in members of the Fungi Imperfecti that lack a sexual cycle, precluding their being subjected to Mendelian genetic analyses. Many of these and other problems can be resolved by the appropriate application of PFGE techniques in combination with now standard molecular and classical genetic approaches.

**Principles and use of PFGE technology.** The intent of this review is to familiarize the reader with approaches and applications of PFGE to the study of fungal genomes without a detailed analysis of PFGE theory and behavior of DNA molecules under various parameters of electrophoresis. Theoretical considerations of the dynamics of DNA molecules in agarose matrices and parameters for resolving DNA molecules by PFGE have been thoroughly discussed in a series of recent studies (Bancroft and Wolk 1988; Birren *et al.* 1988; Cantor *et al.* 1988; Carle and Olson 1987; de la Cruz *et al.* 1990; Deutsch 1987; Hightower and Santi 1989; Holzwarth *et al.* 1987; Mathew *et al.* 1988a, 1988b, 1988c; Olson 1989; Schwartz and Koval 1989; Smith and Cantor 1987; Southern *et al.* 1987). Furthermore, application of PFGE for analysis of chromosomes has been the subject of several recent review articles (Anand 1986; Lai *et al.* 1989; Smith *et al.* 1986). However, a general description of PFGE is necessary as many variations of the technique are used to fractionate fungal chromosomes, and interpretation of the data generated by different techniques can be limited by the particular technique employed.

DNA molecules less than approximately 20 kilobases (kb) long are fractionated in agarose matrices upon conventional electrophoresis because they are sieved. Larger DNA molecules are not sieved and are not resolved by size because their velocity is not proportional to their length. PFGE is a technique that separates DNA molecules in agarose matrices by subjecting them to electric fields that alternate between two directions (Schwartz and Cantor 1984). The time required for a DNA molecule to change directions in response to a switch in the direction of the electric field is size-dependent. Large DNA molecules require more time to reorient than small molecules; hence, resolution of a particular size range of DNA molecules depends on the duration of the switching interval. Pulse times are selected so that DNA molecules of a targeted size spend most of the duration of the pulse reorienting rather than moving through the gel, which accounts for the long periods of time, usually days or weeks, needed to fractionate large DNA molecules.

**The apparatus.** Schwartz and Cantor (1984) first demonstrated that the chromosomes of *S. cerevisiae* could be fractionated by PFGE. A modification of PFGE, the orthogonal-field-alternation gel electrophoresis (OFAGE) technique that employed two nonhomogeneous electric fields, was used by Carle and Olson (1985) to resolve 15 of the 16 yeast chromosomes in a single gel. However, the bands were distorted and nonlinear, making analysis difficult.

Carle *et al.* (1986) subsequently demonstrated that large DNA molecules can be fractionated by field-inversion gel electrophoresis (FIGE) rather than by two orthogonally positioned pairs of electrodes. By simply reversing the current briefly, the DNA molecules reorient and travel backward. With FIGE, the forward pulse is of longer duration than the reverse, and the fractionation of DNA molecules of any size range can be maximized by selecting the appropriate pulse interval. FIGE has some technical limitations, and caution is warranted in directly comparing FIGE-derived data to data derived by means of other PFGE modalities (Ellis *et al.* 1987; Zimm 1988).

The transverse-alternating-field electrophoresis (TAFE) system described by Gardiner *et al.* (1986) orients homogeneous electric fields transversely to the gel. This modification eliminates the curved bands produced by FIGE and OFAGE. However, because the angle between the electric fields at the top and bottom of the gel varies from 115° to 165°, respectively, the DNA molecules do not move at a constant velocity throughout the gel. Consequently, mobility of DNA bands is not directly related to size, and assignment of sizes to unknown bands is potentially inaccurate. With the use of 24 electrodes arranged in a closed hexagonal contour with orientation angles of 120° or 60°, Chu *et al.* (1986) produced a major advance in technology for fractionating large DNA molecules. This system, designated CHEF (contour-clamped homogeneous electric field), separates large DNA molecules in straight bands in pulsed fields. Furthermore,

**Table 1.** Electrophoretic karyotypes of phytopathogenic and other fungi

| Species | Estimated no. chromosomes | Estimated length range (kb) | Reference or source |
|---|---|---|---|
| Phytopathogenic fungi | | | |
| *Cochliobolus heterostrophus* | | | |
| A Chromosomes | 15 | 1,300–3,700 | Tzeng 1990; C. Bronson[a], personal communication |
| B Chromosomes | 1 | 1,300 | |
| *Fusarium oxysporum* | | | |
| f. sp. *conglutinans* | 8 | 2,200–6,200 | E. Momol and H. C. Kistler[b], personal communication |
| f. sp. *raphani* | 11 | 630–6,400 | |
| *Magnaporthe grisea* | | | |
| A Chromosomes | 5–6 | 3,000–12,000 | M. Orbach, B. Valent, and F. Chumley[c], personal communication; D. Z. Skinner, H. Leung, and S. Leong[d], personal communication |
| B Chromosomes | 1–4 | 500–2,000 | |
| *Nectria haematococca* | 10–20 | 400–>4,000 | V. Miao, D. E. Matthews, and H. D. VanEtten[e] personal communication |
| *Phoma tracheiphila* | 12 | 700–1,600 | Rollo *et al.* 1989 |
| *Phytophthora megasperma* | 9–14 | 1,400–4,000 | Howlett 1989 |
| *Septoria tritici* | 17–18 | 330–3,500 | B. McDonald[f], personal communication |
| *Tilletia caries* | 12–15 | 880–4,000 | McCluskey *et al.* in press; B. W. Russell and D. Mills, unpublished data |
| *T. controversa* | 12–16 | 880–4,000 | |
| *Ustilago hordei* | 16–21 | 170–3,150 | McCluskey and Mills 1990 |
| *U. maydis* | 20 | 300–>2,000 | Kinscherf and Leong 1988 |
| *U. tritici* | 13 | 250–3,000 | K. McCluskey, B. W. Russell, and D. Mills, unpublished data |
| Nonphytopathogenic fungi | | | |
| *Aspergillus nidulans* | 8 | 2,900–5,000 | Brody and Carbon 1989 |
| *Candida albicans* | 7 | 1,100–3,000 | Magee *et al.* 1988; Snell and Wilkins 1986 |
| *Cephalosporium acremonium* | 8 | 1,700–4,000 | Skatrud and Queener 1989 |
| *Filobasidiella neoformans* | 11 | 400–1,000 | De Jonge *et al.* 1986 |
| *Hansenula* spp. | 4 | 700–1,000 | De Jonge *et al.* 1986 |
| *Histoplasma capsulatum* | 7 | 2,000–5,700 | Steele *et al.* 1989 |
| *Kluyveromyces marxianus* | 5–7 | 1,000–4,000 | Steensma *et al.* 1988; Sor and Fukuhara 1989 |
| *Neurospora crassa* | 7 | 4,000–12,000 | Orbach *et al.* 1988 |
| *Rhodosporidium toruloides* | 10 | 400–4,000 | De Jonge *et al.* 1986 |
| *Rhodotorula mucilaginosa* | 9 | 200–1,000 | De Jonge *et al.* 1986 |
| *Saccharomyces cerevisiae* | 16 | 170–2,200 | Carle and Olson 1985 |
| *Schizosaccharomyces pombe* | 3 | 3,500–5,700 | Smith *et al.* 1987 |

[a] Iowa State University, Ames.
[b] University of Florida, Gainesville.
[c] Du Pont, Wilmington, DE.
[d] D. Z. Skinner, Kansas State University, Manhattan; H. Leung, Washington State University, Pullman; and S. Leong, University of Wisconsin, Madison.
[e] V. Miao, University of Oregon, Eugene; D. E. Matthews, Cornell University, Ithaca, NY; and H. D. VanEtten, University of Arizona, Tucson.
[f] Texas A. & M. University, College Station.

up to 40 samples may be electrophoresed in a single gel, providing the possibility of direct comparisons of molecular karyotypes of a large number of individuals (Chu 1989; Vollrath and Davis 1987). A new system, now available, uses programmable, autonomously controlled electrodes (Clark *et al.* 1988) and promises to enhance the applicability of PFGE.

Other systems have been devised for separating large DNA molecules (see Birren *et al.* 1988; Clarke *et al.* 1988; Serwer 1987). For the investigator willing to assemble an apparatus for PFGE at a considerable savings in cost, an electrophoresis device has been described in detail and shop-ready plans are being made available (Chu *et al.* 1986; Schwartz *et al.* 1989).

**Preparation of fungal material for molecular karyotyping.** The ability to visualize fungal chromosome-sized DNA molecules by PFGE has been dependent on the identification of reliable and reproducible conditions for producing protoplasts. A variety of fungal cell-wall-degrading enzymes are commercially available for protoplast isolation, although the specificity and effectiveness of individual or combinations of enzymes, concentration of the isotonic medium, and reaction conditions have to be established for each species (Bellis *et al.* 1987; Hamlyn *et al.* 1981). Once produced, the protoplasts are then gently mixed with molten low-melting temperature agarose and cast into plugs with a plug-casting device. The plugs are incubated in a solution of proteinase, a detergent such as sodium dodecyl sulfate, and EDTA to degrade DNA-binding and other proteins, destroy the integrity of the nuclear and cytoplasmic membranes, and inhibit nuclease activity, respectively. Plugs stored in 0.5 M EDTA at 5° C for at least a year give reproducible results with PFGE (McCluskey and Mills 1990). Gels of 0.6–1.5% agarose are prepared as for conventional electrophoresis of DNA, and the entire plug or a sliver, depending on the concentration of DNA, may be inserted into a well and sealed in with agarose. The duration of the pulse, field strength, and length of time samples are electrophoresed are determined by the sizes of the DNA molecules.

The limiting step in the development of molecular karyotypes for some species, or even among strains within a species, has been to arrive at reproducible conditions for protoplast formation. However, McCluskey *et al.* (in press, 1990) recently discovered that extremely reproducible molecular karyotypes can be obtained for some fungi, including filamentous forms, without the formation of protoplasts. With this simplified procedure, yeasts, yeastlike cells, sporidia, or conidia may be suspended directly in molten agarose; filamentous forms are first suspended in an isotonic medium and briefly macerated in a glass tissue grinder to obtain small, unclumped fragments. The plugs that contain either intact cells or fragments are then digested with proteinase in the presence of sodium dodecyl sulfate and EDTA at 50° C for 24 hr, as before. Mycelia and sporidia of *Tilletia controversa* and *T. caries* prepared by this simplified technique yield extremely reproducible banding patterns devoid of the smear of background fluorescing material typically observed in the lanes of samples prepared from protoplasts (McCluskey *et al.* 1990). Furthermore, molecular karyotypes have been obtained for individual colonies of *S. cerevisiae, Ustilago hordei, U. tritici*, and *U. maydis* that were removed from petri plates and suspended directly into molten agarose. This procedure should provide an efficient method for rapidly screening the karyotypes of large numbers of individuals such as colonies derived from field isolates, asexual and sexual spores, and transformants.

**Fungal molecular karyotypes.** The successful separation of *S. cerevisiae* chromosomes by PFGE (Schwartz and Cantor 1984; Carle and Olson 1985) paved the way for similar studies of other fungi (Table 1). The electrophoretic karyotype of a phytopathogenic fungus by OFAGE was first published for *U. maydis* (Kinscherf and Leong 1988). At least 20 chromosome-sized DNAs ranging in length from approximately 300 to longer than 2,000 kb (perhaps approaching 4,000 kb) were detected among field isolates and laboratory strains. Each of the strains was determined to have a different karyotype resulting from length polymorphisms between homologous chromosomes. With the use of homologous and conserved, heterologous gene probes of fungal and mammalian origin, seven genes were mapped by hybridization to Southern-blotted bands or clusters of chromosomes. Hybridization of multiple bands to an internal fragment of the *U. maydis hsp70* (heat shock protein) gene probe provided evidence for an unlinked, multigene family that has been characteristic of *hsp70* genes in a variety of organisms. Furthermore, the hybridization of the heat shock sequence to a novel band in strain F200 of *U. maydis* is suggestive of a translocation. The smallest chromosome of F200 also appeared to be novel and was surmised to have arisen by interchromosomal recombination. When this smallest band was excised and used to probe Southern-blotted chromosomes of reference strain 518, it hybridized to a doublet band more than twice its size. These preliminary results provide physical evidence for a chromosomal rearrangement, such as a translocation, which can be pursued further by classical genetics and restriction fragment length polymorphism (RFLP) analysis in conjunction with PFGE.

**Chromosome length polymorphisms and aneuploidy among field isolates.** In a recent study (McCluskey and Mills 1989, 1990), collections of teliospores representing the 14 known races of *U. hordei* (Tapke 1945; Pedersen and Kiesling 1979) were germinated to obtain presumptive haploid meiotic products (basidiospores) to culture for molecular karyotyping by CHEF PFGE. Surprisingly, each strain selected to represent one of the 14 collections was determined to have a unique karyotype that differed from all other strains either with respect to the number of chromosome-sized bands, a chromosome length polymorphism, or both. However, when the analysis was expanded to include 10 meiotic tetrads representing teliospores from three of the collections, a conserved karyotype was observed among all progeny derived from a particular collection, with one exception. The exception was noted among progeny of an unordered tetrad that showed 2:0 segregation for a chromosome-sized band approximately 1,000 kb long.

Variability in the number of bands seen among strains of any fungus could arise because of aneuploidy for chromosomes that also have length polymorphisms. An

analysis of the chromosomal constitution of widely used homothallic wine strains of *S. cerevisiae* has produced convincing evidence for the occurrence of aneuploidy among most strains (Bakalinsky and Snow 1990). By means of classical genetic analyses and CHEF PFGE to visualize the karyotypes, the strains were determined to be disomic, trisomic, and tetrasomic for some of their chromosomes. The variability in numbers of chromosomes already seen in related strains of many phytopathogenic fungi (Table 1) may indicate a high tolerance for this condition as observed in *S. cerevisiae* (Parry and Cox 1970).

Chromosome length polymorphisms could initially arise through gross chromosomal rearrangements such as insertions (for example, by transposable elements), deletions, duplications, and translocations. PFGE of fungal chromosomes has revealed translocations in *U. maydis* (Kinscherf and Leong 1988) and *Magnaporthe grisea* (D. Z. Skinner, H. Leung, and S. Leong, personal communication) and deletions in *Septoria tritici* (B. McDonald, personal communication).

The occurrence of deletions at RFLP loci among field isolates of *S. tritici* is reported to be unexpectedly high (McDonald and Martinez 1990). Approximately 10% of randomly selected fragments screened for use as probes in RFLP analysis failed to hybridize with total genomic DNA in at least one of six strains. These probes also failed to hybridize to the isolated chromosomes of different strains of a single population, and the chromosome that incurred the deletion could be inferred by the linkage of these fragments to other single-copy probes which do hybridize to a specific chromosome in each strain (B. McDonald, personal communication). Hybridization of single-copy probes to Southern-blotted chromosomes also revealed obvious examples of chromosome length polymorphisms, some of which varied by 20%. Length polymorphisms longer than 100 kb have been observed for the large chromosomes of *S. cerevisiae* (Ono and Ishino-Arao 1988) and for some chromosomes of *U. hordei* (McCluskey and Mills 1990). Chromosome length polymorphisms in the protozoan *Plasmodium falciparum* also have been shown by PFGE and Southern hybridization to result from deletions (Corcoran *et al.* 1986).

The inheritance of chromosome length polymorphisms has been investigated in *S. cerevisiae* (Ono and Ishino-Arao 1988). Examples of 2:2 segregation for several chromosomes in meiotic tetrads were taken as evidence for the occurrence of a length polymorphism for a pair of homologous chromosomes. Furthermore, a length polymorphism associated with chromosome I was mapped by classical genetic analysis and OFAGE to the right arm at a position distal to *ade1*, and was attributed to a single structural alteration. These approaches to the study of the evolution of chromosomes may provide insight into the mechanisms by which plant pathogenic fungi overcome multiple plant resistance genes in a single event.

Cytological analysis of chromosomes from two isolates of *P. megasperma* revealed karyotypes of approximately 12 and 24 chromosomes (Howlett 1989), suggesting the occurrence of diploid and tetraploid strains. OFAGE revealed evidence of at least nine chromosome-sized bands in each strain, corroborating the interpretation from cytological studies. Furthermore, this type of analysis is currently being used in phylogenetic studies of *Phytophthora* species (Howlett 1990).

**Fungal supernumerary B chromosomes.** PFGE of the chromosomes of *Nectria haematococca* (Miao and VanEtten 1989) and *Magnaporthe grisea* (M. J. Orbach, B. Valent, and F. Chumley, personal communication) suggests the presence of B chromosomes characteristic of certain species of higher plants and orthopteran insects (Jones and Rees 1982). The phytoalexin pisatin, produced in the pea plant, provides protection against certain strains of *N. haematococca*, whereas other strains are highly virulent on pea (VanEtten *et al.* 1989). In these virulent strains a pisatin demethylation activity is encoded by a gene designated *Pda1*, which negates the biological activity of pisatin. When PFGE-separated chromosomes of virulent and avirulent strains of *N. haematococca* were blotted and probed with the *Pda1* gene, the probe hybridized to a small chromosome that was present only in the virulent strains. This chromosome is not faithfully transmitted to progeny in crosses in which both parents are virulent, a property characteristic of B chromosomes.

Cytological investigation of *M. grisea* suggested that the haploid number of chromosomes is six (Leung and Williams 1987), and either four, five, or six bands ranging in length from approximately 3,000 to 12,000 kb have been detected by PFGE (D. Z. Skinner, H. Leung, and S. Leong, personal communication; M. Orbach, B. Valent, and F. Chumley, personal communication). Additionally, in most strains pathogenic to rice, one to four smaller bands (500–2,000 kb) that segregate abnormally in crosses have been detected, although currently no genes have been shown to be linked to these chromosomes. Their instability and absence from some strains suggest that they too are B chromosomes and dispensable. Certain relevant structural features of these chromosomes, for example, whether they are acentric, will be more important factors in determining instability than merely their relatively small size. Among the 15–19 chromosome-sized DNA bands of *U. hordei*, as many as 14 in some strains that are approximately 1,200 kb or shorter appear to be faithfully transmitted to progeny (McCluskey and Mills 1990). Furthermore, a probe made of the actin gene from *Aspergillus nidulans* hybridizes to a single, small chromosome that varies in length from 570 to 650 kb in each of the 14 representative strains.

**Use of molecular karyotypes for species identification.** Although for some species of fungi, for example, *Tilletia* spp., *U. hordei*, and *Phytophthora* spp. (Table 1), the base number of chromosomes is variable, presumably because of aneuploidy, the relative numbers and sizes of these chromosomes produce a distinctive pattern when visualized by PFGE. This feature of electrophoretic karyotypes may be extremely useful for distinguishing between closely related species. *Kluyveromyces marxianus* var. *marxianus* and *K. m.* var. *lactis* of the yeast *K. marxianus* were investigated by OFAGE to determine the validity of classifying them as the same species (Steensma *et al.* 1988). Hybrids readily form in crosses of these variants, suggesting that they could be one species. However, recombination of genetic markers was not observed in the progeny, and electrophoretic karyotypes of the progeny were the sum

of the patterns of the parents, whose karyotypes were distinctly different from each other. Although these variants are currently classified as a single species, the authors argue that, in support of genetic and biochemical data, the electrophoretic karyotypes of these variants and their hybrid progeny provide compelling evidence for reclassifying them as different species.

Analogous studies are under way to examine the validity of classifying *T. caries* and *T. controversa* as separate species (McCluskey *et al.*, in press; B. W. Russell and D. Mills, unpublished data). However, unlike *K. marxianus*, genetic markers segregate according to Mendelian laws of inheritance in $F_1$ hybrid progeny (Trail and Mills 1990). The karyotypes of *T. caries* and *T. controversa* (Table 1) share similarities with respect to length (approximately 900–4,000 kb) and number of chromosomes (12–15). Moreover, the number and range of size of chromosomes in the $F_1$ hybrid progeny are also similar to the parents rather than additive. There is also evidence for extensive recombination between homologous chromosomes that apparently differ in size resulting in the formation of intermediate-sized chromosomes and a unique karyotype for each of the progeny (B. W. Russell and D. Mills, unpublished data). The origin of recombinant chromosomes is being determined by probing the Southern-blotted chromosomes with single-copy genomic fragments (B. W. Russell and D. Mills, unpublished data). Although these results are preliminary, the electrophoretic karyotypes provide strong evidence for the reclassification of *T. caries* and *T. controversa* as variants of a single species. A similar conclusion was drawn from studies of a comparison of polypeptides among three species of *Tilletia* (Kawchuk *et al.* 1988).

It is apparent that the use of electrophoretic karyotypes will become another taxonomic tool for classifying some fungi. When coupled with other molecular techniques (for example, RFLP and Southern blot analyses), the relatedness and origin of specific chromosomes can be determined. Other commonly used molecular genetic techniques for analyses of DNA (Maniatis *et al.* 1982) will enable fine-structure physical maps of isolated chromosomes to be constructed. In this regard, the three chromosomes of *Schizosaccharomyces pombe* have been isolated, and the first complete restriction map of a eukaryotic genome has been produced (Fan *et al.* 1989). Moreover, individual chromosomes have been excised from gels and used to make chromosome-specific DNA clone libraries (K. McCluskey and D. Mills, unpublished data), facilitating mapping of linkage groups among strains with dissimilar karyotypes.

**Summary.** The discovery that PFGE can be efficiently used to separate very high molecular weight DNA in agarose matrices has had a rapid and profound effect on approaches used in the analysis of fungal karyotypes, the study of genome organization and linkage, and the construction of genomic libraries. Modifications to PFGE have quickly led to the resolution of fungal chromosomes estimated to be at least 12,000 kb long, and a new generation of systems promises to allow larger chromosomes to be resolved. This new technology, when used in conjunction with standard procedures for analysis of DNA, will provide an efficient method of genetic analysis. Moreover, the discovery that chromosome electrophoresis can be performed directly on yeastlike cells, spores, or mycelia will hasten the advancement of both PFGE and fungal molecular genetics. The ability to remove colonies from the surface of agar-solidified medium and to prepare high-quality chromosome samples will allow the screening of large numbers of isolates or strains, making feasible the application of this technique to population level studies in mycology and plant pathology.

## ADDENDUM IN PROOF

Variable molecular karyotypes also have been observed for strains of *Colletotrichum gloeosporioides* (A. Masel, K. Braithwaite, J. Irwin, and J. Manners 1990. Highly variable molecular karyotypes in the plant pathogen *Colletotrichum gloeosporioides*. Curr. Genet. 18:81-86).

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