Separation of Spores from Diploid Cells of Yeast by Stable-Flow Free-Boundary Electrophoresis

Abstract. *By the use of stable-flow free-boundary (Staflo) electrophoresis and the electrophoretic mobility difference between ascospores and diploid cells of* Saccharomyces cerevisiae, *a mixture of the two can be separated into spore and diploid cell fractions. The spore fraction that is obtained can then be used for genetic analysis.*

Genetic and physical experiments that utilize ascospores may, as in random ascospore analysis (*1*), be greatly facilitated by the availability of a homogeneous population of spores. Random spore analysis is a useful method for studying such aspects of fungal genetics as recombination and gene conversion. Instead of having to examine spores from individual asci, a laborious procedure involving microdissection, this method is based on random sampling of spores from a large population to study the events of meiosis.

In *Saccharomyces cerevisiae*, a yeast subjected to much genetic analysis in recent years (*2*), pure aqueous spore suspensions have not been obtained, although enrichment has been achieved by oil-water phase separation which leaves the spores in the oil phase (*3*). This report describes a technique—Staflo electrophoresis—that utilizes the electrophoretic properties of spores and diploid cells to obtain an essentially pure (99.04 percent) aqueous suspension of spores.

The Staflo method is a variation of zone electrophoresis in free solution that uses a density gradient for stability (*4, 5*). This method causes solutions of different densities to be layered in a continuous manner at a rate such that the flow is laminar within the Staflo chamber (*5*). The sample is introduced into this chamber at some intermediate density where it forms a layer or zone. An electric field is applied that is normal to both the fluid flow and the sample zone.

In the present experiment the diploid strain of *Saccharomyces cerevisiae*, X801, was used. Its genotype (see *6*) is

$$\frac{\alpha}{a}\ \frac{SU_1}{su}\ \frac{ma}{MA_3}\ \frac{ga_2}{ga_2}\ \frac{Ga_1}{ga_1}\ \frac{mel}{MEL}\ \frac{TR_5LE_1}{tr_5\,le_1}$$

$$\frac{P_1AR_{4\text{-}2}}{p_1\,ar_{4\text{-}2}}\ \frac{HI_2}{hi_2}\ \frac{his\,ad_2}{HI_8AD_2}\ \frac{ur_1}{UR_1}\ \frac{ly_2}{LY_2}$$

After sporulation (*7*) the resultant suspension contained approximately 70 percent nonsporulated cells and 30 percent asci. This suspension was then treated with Glusulase (Endo Laboratories, Garden City, New York), a preparation that digests most of the ascus wall (no separation could be obtained when the ascus wall was not removed). The treated suspension was then sonicated (100-watt Ultrasonic Disintegrator, Measuring & Scientific Equipment Ltd.) for 3 minutes at 21 kc/sec to break up the spore groups, thus leaving a mixture of single spores and diploid cells.

The resultant cell mixture was resuspended in a 2 percent sucrose solution (3×10^6 cells/ml) containing $2.5 \times 10^{-3}M$ tris, $5 \times 10^{-5}M$ ethylenediaminetetraacetate, and $1.9 \times 10^{-4}M$ H_3PO_4 (LKB 3276-GB buffer). It was then introduced into an inlet of the Staflo chamber that corresponded in height to outlet 4 at the rate of 0.2 ml/min. (All of the vertical inlets have sugar solutions of different densities pumped into them at the same rate with the denser solutions entering at respectively lower inlets.) The fluid which exits from the Staflo chamber can be sampled at 48 outlets: three columns across by 16 rows down. Only the center column of outlets was sampled (*8*). Electrophoresis takes place in the latter three-fifths of the chamber. The control sample was obtained by passing the diploid-spore suspension through the Staflo chamber in the absence of an electric field.

The outlet suspensions were plated on nutrient agar (containing yeast extract, peptone, dextrose) and incubated for 3 days. The resultant colonies were tested by replica-plating to minimal medium (a medium that does not contain any of the amino acids or bases associated with the genetic markers in strain X801), and those that grew were classified as diploid. This classification is fairly accurate, since the probability that a spore would exhibit the diploid phenotype (growth on minimal medium) by chance assortment of genes is approximately 0.01. Colonies from outlets 10, 11, 12, and 13 were tested further on various fermentation media. Ability to ferment all three sugars—sucrose, maltose, and melibiose—coupled with growth on minimal medium, indicated with higher certainty that a colony was diploid.

The results of the Staflo electrophoresis of the diploid-spore mixture are presented in Table 1 and Fig. 1. The total collection time was 200 minutes. The steady-state time of electrophoresis was 146 seconds (*p*H 8.5, 25°C), during which the cells traveled horizontally 18 cm. (Sedimentation effects are negligible during this period.) Average mobilities of cells from each outlet are calculated by using the vertical distance traveled (from the height of outlet 4 to the height of the given outlet; the distance between outlets is 1.25 mm). The resistivity values used for calculating the average mobilities were based on averages of the measured inlet and outlet resistivities; cur-

Table 1. Separation of spores from vegetative cells.

Outlet	Total clones examined	Frequency of spore colonies	Millions of cells* exiting through outlet	Average mobility of cells exiting through outlet [(cm/sec)/(volt/cm)] $\times 10^{-4}$
(i)	(a)	(b)	(c)	(d)
4	474	0.3301	0.48	0.1
5	388	.1521	1.26	.3
6	431	.0302	1.08	.6
7	421	.0261	2.02	.9
8	506	.0296	2.16	1.1
9	572	.2937	3.16	1.5
10	2061	.8321	6.35	1.9
11	2480	.9641	4.05	2.3
12	720	.9833	0.91	2.8
13	2080	.9904	.09	3.5
Control	1044	.5661		

Average spore electrophoretic mobility

$$\sum_{i=4}^{i=13} (b_i\, c_i\, d_i) \Big/ \sum_{i=4}^{i=13} (b_i\, c_i) = 2.0 \times 10^{-4} \left(\frac{\text{cm}}{\text{sec}}\right) \Big/ \left(\frac{\text{volt}}{\text{cm}}\right)$$

Average vegetative cell electrophoretic mobility

$$\sum_{i=4}^{i=13} (1 - b_i)\, c_i\, d_i \Big/ \sum_{i=4}^{i=13} (1 - b_i)\, c_i = 1.1 \times 10^{-4} \left(\frac{\text{cm}}{\text{sec}}\right) \Big/ \left(\frac{\text{volt}}{\text{cm}}\right)$$

* Based on hemacytometer counts.

Table 2. Segregation frequencies in spore samples.

Gene	Controls	Outlet 11	Outlet 13
TR_5	0.631	0.567	0.552
LE_1	.656	.567*	.533*
P_1	.458	.557*	.486
AR_4	.542	.567	.500
HI_2HI_8	.321	.295	.248*
LY_2	.524	.548	.495
UR_1	.536	.552	.467
AD_2	.540	.533	.448*
	Number of spores assayed		
	504	210	210

* Indicates that the ratio differs from the corresponding control ratio at the 5 percent level of significance.

rent density was 8.3×10^{-4} amp/cm^2. The data presented in columns (a) and (b) of Table 1 demonstrate that separation of spores from diploid cells can be attained, the maximum separation being 99.04 percent in outlet 13. Separation is possible because the diploid cells have a distribution of electrophoretic mobilities lower than that of the spores; the weighted averages of the two distributions are 1.1×10^{-4} and 2.0×10^{-4} (cm/sec)/(volt/cm), respectively, for this experiment.

The spore colonies were examined genetically, by omission media tests, to determine if the cell populations exiting from each outlet were different from the controls. Omission tests involve replica plating to a series of synthetic media, each member of which lacks one of the different nutrients associated with the genetic blocks in strain X801. Therefore, the presence or absence of functional genes can be determined. With regard to the segregation frequencies of different genes (spores growing on a given omission medium divided by the total spores tested) outlets 10 and 12 did not differ from the controls. However, the segregation frequencies in samples from outlets 11 and 13 were significantly different from the controls for certain genes (Table 2). These results could indicate that the absence or presence of various biochemical functions can affect the electrophoretic properties of a cell. The deviations from .50, or .25 for histidine, observed in the controls may have been the result of colonies arising from aggregations of spores rather than from individual spores. Also, presence of a nonrandom sample of spores in two- and three-spore asci, or selective germination and growth of spores of particular genotypes, may have caused these deviations.

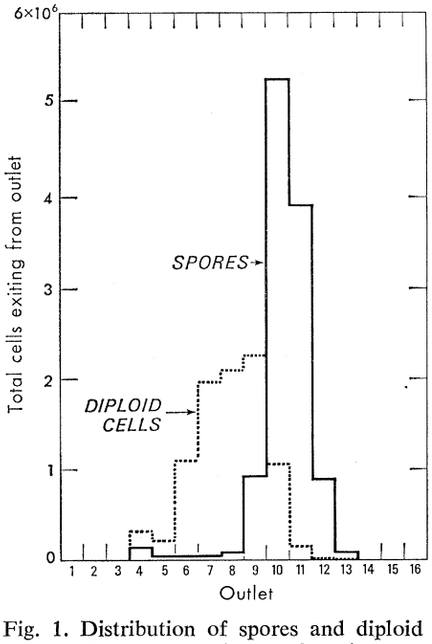

Fig. 1. Distribution of spores and diploid cells in the center column of outlets.

Although the segregation frequencies in some outlet populations differed from the controls with regard to individual genes, gene linkages were not appreciably affected. In the controls and outlet populations all unlinked gene pairs assorted randomly. The linkage of the gene pair tr_5-le_1 was observed to differ somewhat from the controls: the percent recombinants for the controls was 18.7; corresponding values of 12.8, 13.6, 11.7, and 9.6 were obtained for spores sampled from outlets 10, 11, 12, and 13 respectively. These outlet recombination frequencies are comparable to the value 13.3 reported by Mortimer and Hawthorne (*9*).

These experiments demonstrate the following: (i) spores and diploid cells have different electrophoretic mobilities which presumably reflect differences in cell wall electrical charge density; (ii) utilizing these differences in mobilities, Staflo electrophoresis enables the separation of spores from diploid cells; and (iii) the spore sample so obtained can be used to determine genetic linkages.

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6. The symbols used to define the genotype are the following: *a/α*, mating type; carbohydrate utilization: ***SU***, sucrose; ***MA***, maltose; ***GA***, galactose; ***MEL***, melibiose; nutritional requirements: ***TR***, tryptophan; ***LE***, **leucine;** ***AR***, **arginine;** ***HI***, histidine; ***AD***, adenine; ***UR***, uracil; ***LY***, lysine; genetic petite: ***P***. Upper and lower case refer, respectively, to ability and inability to ferment the particular sugar, to grow in the absence of a particular nutrient, and, for petite, to grow in the absence of a fermentable carbon source. The subscripts refer to particular loci.
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Minimum Condition for Stereopsis and Anomalous Contour

Abstract. *Horizontal borders were deleted progressively from square and circular dot patterns containing disparate forms. Stereoscopic depth and anomalous contour occur in the absence of horizontal borders and the form in depth does not always assume the shape of the dot patterns. The stereoscopic effects depend on a laterally disparate form in the stereogram.*

The recent use of dot and letter matrix stereograms has raised again the issue of the necessary conditions for stereoscopic vision (*1–3*). When the half-images in Fig. 1a are viewed stereoscopically an inner white square appears in the dashed plane in front of two squares of dots, whereas viewing Fig. 1b produces a white surface in depth (dashed plane) with curved vertical and *straight* horizontal contours. The perceived contours are anomalous because they occur in the absence of any corresponding brightness gradient in the visual displays. Furthermore, the depth effects contradict traditional theories of stereopsis, inasmuch as all of the dots in the half-images are equidistant, thus prohibiting any contour or edge disparity (*4*).

It has been suggested that the dot patterns contain lateral form disparity which accounts for the depth effects (*3*). In the square matrices (Fig. 1a), the enclosed white forms are located differently within each half-image due to the selective omission of dots. Notice that the right inner column is absent for the left half-image whereas the left