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Induction of DEL Recombination in the Yeast *Saccharomyces cerevisiae* Using a Microtiter Plate Assay Format

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ABSTRACT

It has been established that a number of mammalian tumors are associated with genomic rearrangements. A system designed to detect genomic rearrangements has been constructed in the yeast *Saccharomyces cerevisiae*. This system consists of a heterozygous duplication of the *bis3* gene in which one copy has been deleted at the 3′ end and the other deleted at the 5′ end (Schiestl et al., 1988). Both copies of the *bis3* gene are nonfunctional. This duplication of the *bis3* gene is separated by the *LEU2* gene and pBR322 plasmid sequences. Homologous intrachromosomal recombination between the *bis3* duplications regenerates an intact *HIS3*$^+$ allele, while deleting the intervening *LEU2* gene and pBR322 DNA. The assay has been accordingly named the yeast DEL (deletion) assay. It has been shown previously that this recombination event is inducible by a variety of chemicals, as well as ultraviolet light and ionizing radiation (Schiestl, 1989; Schiestl et al., 1989). In this work we report the conversion of the yeast DEL assay to a microtiter plate format, which greatly increases its ease of use and quantitative accuracy. Using the microtiter plate format we have found yeast DEL recombination to be inducible by the carcinogens 4-nitroquinoline oxide, epichlorohydrin, methyl methanesulfonate, ethyl methanesulfonate, benzene, and formaldehyde. Chemicals that do not induce DEL recombination include dimethylsulfoxide, acetone, ethanol, L-methionine, and methyl orange. In this format the Yeast DEL Assay is easier to use than the traditional agar plate assay. Results are obtained in 2–3 days, and the labor required is approximately half that required to perform the assay using the agar plate method. The new format, in which microtiter plate wells containing recombinant yeast colonies are scored by color change of the pH indicator dye, methyl orange, is especially amenable to automation.

INTRODUCTION

The association of genome rearrangements and deletions with mammalian cancers, including retinoblastoma, lung cancer, melanoma, neuroblastoma, breast cancer, kidney cancer, colorectal cancer, and leukemia, has been well established (see Cairns, 1981; Hansen and Cavenee, 1988; Klein, 1981; Stanbridge, 1990; Muller and Scott, 1992; Miyao et al., 1993; Reiter et al., 1993; Marchetti et al., 1993; Zalupski et al., 1990; Yang-Feng et al., 1993; Dyer et al.,

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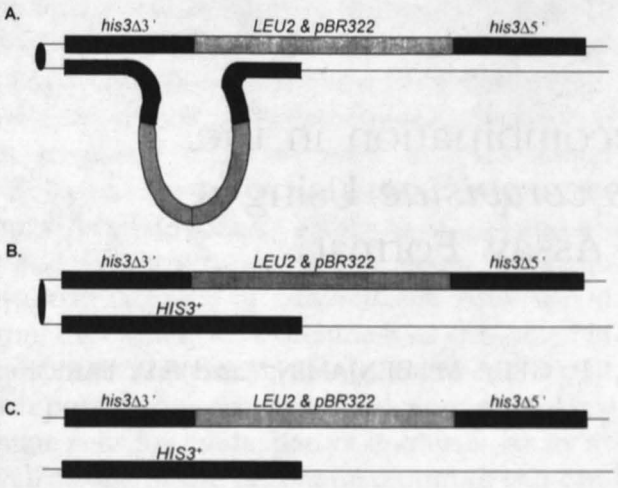


FIG. 1. Del recombination system. A duplication of the *his3* gene (A), in which one copy of this gene is deleted at the 3′ end, and the other is deleted at the 5′ end, is separated by the *LEU2* gene and pBR322 DNA sequence. The yeast, which are *hisLEU*$^+$, undergo recombination (A), and in the process the *LEU2* gene and pBR322 plasmid sequences are deleted (The mechanism of recombination is believed to be gene conversion between sister chromatids [Schiestl et al., 1988]). Deletion of the intervening DNA sequences results in the formation of a HIS3$^+$ allele (B). Cell division results in the segregation of chromosomes and formation of one cell that is *HIS3*$^+$*leu2*$^-$ and one cell that is *his3*$^-$*LEU2*$^+$ (C).

1993; Ponder, 1988; Pall, 1981; Marx, 1982). A system has been constructed in the yeast *Saccharomyces cerevisiae* (yeast DEL) that detects intrachromosomal recombination events resulting in the deletion of DNA sequences between two direct repeats (Fig. 1; Schiestl et al., 1988). It has been found that DEL recombination can be induced by the treatment of yeast with chemicals or radiation (Schiestl, 1989; Schiestl et al., 1989; Schiestl and Reddy, 1990). Chemicals that induce DEL recombination include a number of proven rodent carcinogens that are not detectable using other short-term genotoxicity assays such as the Ames test. These compounds include benzene, safrole, cadmium sulfate, thiourea, acetamide, thioacetamide, and carbon tetrachloride (Schiestl 1989; Schiestl et al., 1989; Schiestl and Reddy, 1990).

For ease of use as well as greater quantitative accuracy we converted the yeast DEL assay to a microtiter plate format. Both bacterial mutation reversion assays and yeast recombination assays, which use either test tubes or microtiter plates (fluctuation tests), have been described (Green et al., 1976; Parry, 1979; Gatehouse and Delow, 1979; Bridges, 1980; McPherson and Nestmann, 1980). These tests are qualitative in nature because the number of recombinants or revertants per number of viable cells is not determined. In these tests a positive response to a given chemical is simply determined by having a greater number of positive wells in the treated samples than in the untreated controls. Since the yeast DEL assay accounts for the number of viable cells at all stages of the assay, the response to a given chemical is determined as an increase in the recombination frequency relative to that of the untreated control (Schiestl, 1989; Schiestl et al., 1989; Schiestl and Reddy, 1990). This frequency of recombination is determined by a Poisson distribution (Luria and Delbruck, 1943). In this system the recombination frequency is indicative of the recombination rate (Schiestl et al., 1988).

The ability of the yeast DEL assay to detect chemicals that induce genomic rearrangements makes it a valuable short-term genotoxicity assay. Our microtiter plate format reduces the time to perform the assay to 6 h from 24 h, uses common laboratory equipment, and permits the testing of four to five doses of a chemical in three to five replicates per dose. In this paper we describe DEL recombination in yeast exposed to a number of mutagenic and nonmutagenic compounds. The carcinogens ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), 4-nitroquinoline oxide (4NQO), epichlorohydrin, and formaldehyde, which induce DEL recombination in the agar plate assay, also tested positive in the microtiter plate assay. Benzene, which is negative in short-term bacterial assays, also induced yeast DEL recombination. Chemicals such as acetone, dimethylsulfoxide (DMSO), and the amino acid L-methionine, which do not induce DEL recombination in the agar plate format, did not induce DEL recombination using the microtiter plate format.

MATERIALS AND METHODS

Strains

The diploid yeast strain RS112 *(MATa/α ura 3-52/ura3-52 leu2-3,112/leu2-Δ98 trp5-27/TRP5 arg4-3/ARG4 ade2-40/ade2-101 ilv1-*

92/ILV1 HIS3::pRS6/his3-Δ200 LYS2/lys2-801) was used (Schiestl et al., 1988). RS112 contains the DEL system at one *HIS3* chromosomal locus, located on chromosome VIII, while the other chromosome is deleted for the entire *HIS3* locus. Because only one chromosome contains the *his3* duplication, *HIS3*$^+$ recombinants are formed via intrachromosomal recombination between sister chromatids more than 99% of the time (Schiestl et al., 1988).

Media

Synthetic medium was prepared as previously described (Sherman et al., 1986; Schiestl, 1989). Synthetic complete medium (SC) consists of yeast nitrogen base without amino acids (Difco Laboratories, Detroit, MI), 0.67%; dextrose, 2%; adenine sulfate, 30 mg/L; L-tryptophan, 20 mg/L; L-histidine-HCl, 20 mg/L; L-arginine-HCl, 20 mg/L; L-methionine, 20 mg/L; L-isoleucine 20, mg/L; L-tyrosine, 20 mg/L; L-lysine-HCl, 20 mg/L; uracil 20, mg/L; L-leucine, 30 mg/L; L-homoserine, 118 mg/L; L-threonine, 350 mg/L; L-valine, 30 mg/L; L-phenylalanine, 60 mg/L. Omission media are SC medium lacking one or more required supplements. SC-HIS is SC medium lacking L-histidine-HCl. SC-LEU is SC medium lacking l-leucine. pH indicator dye media contain methyl orange (sodium salt). Amino acids were purchased from Sigma Chemical Co. (St. Louis, MO). Methyl orange was purchased from J.T. Baker (Phillipsburg, NJ).

Chemicals

Methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), epichlorohydrin, 4-nitroquinoline oxide (4NQO), benzene, formaldehyde, acetone, dimethylsulfoxide (DMSO), and L-methionine were purchased from Sigma Chemical Co. (St. Louis, MO). Ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY).

DEL Assay

In more than 99% of yeast DEL recombination events the *LEU2* gene is lost (Schiestl et al., 1988). Therefore the yeast strain was pregrown on medium lacking leucine (SC-LEU) in order to prevent the accumulation of histidine prototrophs in the overnight precultures (Fig. 2). Because histidine prototrophs are selected against during the pregrowth period, the frequency of *HIS3*$^+$ recombination is reflective of the recombination rate (Schiestl et al., 1988).

RS112 cultures were inoculated in 20 ml SC-LEU medium using freeze dried cells and incubated 24 h at 30°C in an environmental shaker. We found that freeze drying does not induce DEL recombination (data not shown). SC-LEU pregrowth medium selects against any *HIS3*$^+$ prototrophs formed during all previous steps. Following the 24 h growth period the cultures were adjusted to an OD_{600} of 0.2 ($1–2 \times 10^6$ cells/ml) in 40 ml SC-LEU medium. The relationship between optical density (600 nm) and

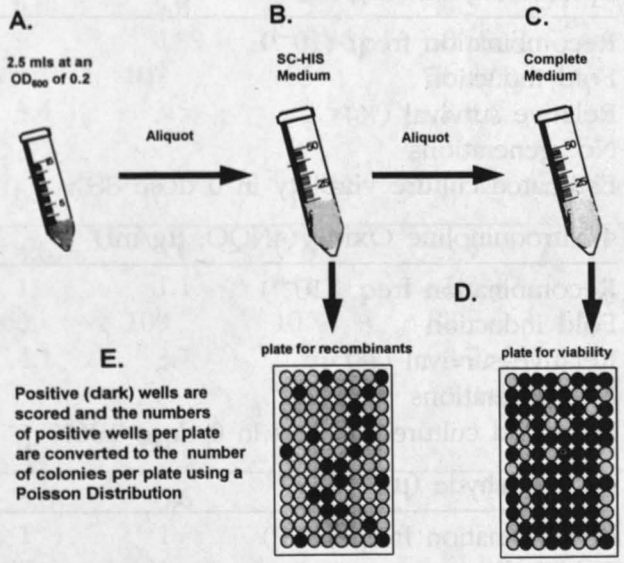


FIG. 2. DEL assay procedure. (A) Each 2.5 ml culture is adjusted to an OD_{600} of 0.2 in 2.5 ml of medium lacking histidine (SC-HIS) after the 17 h incubation. (B) An aliquot of cells is removed from the 2.5 ml of medium lacking histidine (SC-HIS) and transferred to a larger volume of medium without histidine but containing pH indicator dye. (C) An aliquot of culture is removed from the medium from the culture containing pH indicator dye and transferred into synthetic complete medium (SC) containing pH indicator dye. (D) The cultures from B and C are then evenly distributed into sterile 96 well microtiter plates, and the microtiter plates incubated at 30°C for 3 days. (E) After 3 days the plates are scored for the number of positive wells. Using the equation from Materials and Methods, the frequencies of recombination for each chemical dose can be calculated.

TABLE 1. CHEMICALS TESTED USING THE MICROTITER PLATE ASSAY FORMAT

Chemical	*Dosage*				
Methyl methanesulfonate (MMS) (μg/ml)	0	33	65	98	
Recombination freq. (10^{-4})	5.0	26.9	53.1	92.2	
Fold induction	1	5.4	10.6	18.4	
Relative survival (%)	100	76	46	11	
No. generations	3.9	3.7	3.3	2.8	
Estimated culture viability in 0 dose 112%					
Ethyl methanesulfonate (EMS) (mg/ml)	0	0.1	1.0	2.0	
Recombination freq. (10^{-4})	5.2	17.6	37.4	75.9	
Fold induction	1	3.4	7.2	14.6	
Relative survival (%)	100	91	57	20	
No. generations	4.0	4.0	3.4	2.5	
Estimated culture viability in 0 dose 108%					
Epichlorohydrin (μg/ml)	0	50	100	200	
Recombination freq. (10^{-4})	4.0	18.4	40.8	84.1	
Fold induction	1	4.6	10.2	21	
Relative survival (%)	100	108	98	21	
No. generations	3.8	3.8	3.8	2.8	
Estimated culture viability in 0 dose 88%					
4-Nitroquinoline Oxide (4NQO; μg/ml)	0	0.05	0.1	0.2	
Recombination freq. (10^{-4})	4.9	25.0	41.4	32.0	
Fold induction	1	5.2	8.5	6.5	
Relative survival (%)	100	79	63	38	
No. generations	4.4	4.2	4.2	2.5	
Estimated culture viability in 0 dose 106%					
Formaldehyde (μg/ml)	0	50	75	100	
Recombination freq. (10^{-4})	2.7	5.7	7.8	11.7	
Fold induction	1	2.1	2.9	4.3	
Relative survival (%)	100	84	75	46	
No. generations	3.6	3.4	1.9	0	
Estimated culture viability in 0 dose 88%					
Benzene (% volume)	0	0.5	1.0	1.5	2.0
Recombination freq. (10^{-4})	2.2	2.7	4.6	13.2	23.9
Fold induction	1	1.3	2.2	6.1	11.2
Relative survival (%)	100	50	33	24	18
No. generations	3.5	3.4	2.9	2.4	0.9
Estimated culture viability in 0 dose 85%					

(continued)

TABLE 1. (CONTINUED)

Chemical	*Dosage*				
Acetone (% volume)	0	2	3	4	5
Recombination freq. (10^{-4})	5.6	3.5	10.4	9.3	5.3
Fold induction	1	0.63	1.9	1.7	0.95
Relative survival (%)	100	89	30	10	7.5
No. generations	3.5	3.4	2.9	2.4	0.9
Estimated culture viability in 0 dose 102%					
Dimethylsulfoxide (DMSO) (% volume)	0	3	4	5	
Recombination freq. (10^{-4})	3.0	4.7	5.2	4.2	
Fold induction	1	1.6	1.7	1.4	
Relative survival (%)	100	71	72	62	
No. generations	3.4	3.6	3.4	2.8	
Estimated culture viability in 0 dose 68%					
Ethanol (% volume)	0	1	2.5	5	
Recombination freq. (10^{-4})	6.6	5.9	5.7	4.3	
Fold induction	1	0.89	0.86	0.65	
Relative survival (%)	100	107	85	76	
No. generations	3.4	3.5	3.5	2.8	
Estimated culture viability in 0 dose 98%					
L-Methionine (mg/ml)	0	10	15	20	
Recombination freq. (10^{-4})	4.6	5.0	5.6	5.9	
Fold induction	1	1.1	1.2	1.3	
Relative survival (%)	100	109	107	82	
No. generations	4.1	3.7	3.7	3.7	
Estimated culture viability in 0 dose 63%					
Methyl orange (μg/ml)	0	60			
Recombination freq. (10^{-4})	3.8	3.6			
Fold induction	1	1			
Relative survival (%)	100	101			
No. generations	4.1	3.7			
Estimated culture viability in 0 dose 87%					

The chemicals MMS, EMS, epichlorohydrin, 4NQO, formaldehyde, benzene, and methyl orange were tested a minimum of nine times. The chemicals DMSO, ethanol, acetone, and L-methionine were tested a minimum of three times each.

cell number was determined by performing growth curves in synthetic medium. Cells (2.5 ml) were aliquoted into a series of sterile 15 ml conical tubes and test chemical added to all but one of the 2.5 ml cultures. In general, three to five replicates for each chemical dose were performed in parallel. The tubes were sealed with parafilm and the 2.5 ml cultures incubated for 17 h at 30°C in an environmental shaker. Approximately 85% of the cells in untreated cultures were found to be viable, as determined by counting the cells using a hemacytometer and then plating them on synthetic complete medium agar plates (data not shown). Following incubation with chemical the cells were pelleted at 3600 rpm for 5 min. and the supernatant containing the chemical decanted into the appropriate waste container. The cell pellets were resuspended in 2.5 ml of SC-HIS medium, which allowed only the growth of histidine prototrophs. The optical density of each culture was determined, and the cells in each culture were diluted to an OD_{600} of 0.2 in 2.5 ml SC-HIS medium. Adjustment of the OD_{600} values to a specific value ensured the equality of cell numbers, both viable and inviable, between the 2.5 ml cultures.

Aliquots of cells were taken from the 2.5 ml cultures and transferred to SC-HIS medium that contained the pH indicator dye methyl orange (0.06 mg/ml). Methyl orange itself did not induce recombination of the yeast DEL system, nor did it affect growth or viability of the yeast cultures (Table 1). Aliquots of cells were then removed from the cultures in SC-HIS with dye and added directly to SC medium also containing methyl orange. The synthetic complete medium allows the growth of all viable cells. Following dilution of all cultures into medium with pH indicator dye, the cultures were evenly distributed into individual sterile 96 well microtiter plates. The microtiter plates were then placed in sealed plastic bags and incubated at 30°C for 3–4 days. We found that sealing the plates in bags to prevent evaporative loss of medium from the microtiter plates did not affect assay performance. Growth of yeast clones in the wells of the microtiter plates resulted in formation of distinct yeast colonies, and the color of the pH indicator dye in the well containing clones changed from orange to pink.

Calculations

Recombination frequency is defined as the number of recombinants per number of viable cells. Because the use of microtiter plates restricted cell number to a specific fraction of wells containing yeast colonies, and not total colony number, a Poisson distribution was used to estimate the number of colonies per microtiter plate. The calculation for Poisson distribution was taken from Luria and Delbruck (1943) as follows:

$$-\log_n([\text{total number of wells plated} - \text{number of positive wells}]/\text{total number of wells plated})$$

After the estimated number of cells per recombinant microtiter plate and per viability microtiter plate was obtained, the recombination frequencies could be determined. Therefore, the procedure for determining recombination frequency was as follows:

$$\frac{\text{Number of positive wells (recombinants)} \rightarrow \text{Poisson distribution}}{\text{Number of positive wells (viability plate)} \rightarrow \text{Poisson distribution}} \rightarrow \frac{\text{Number of recombinants}}{\text{Number of viable cells}}$$

Cell survival relative to the negative controls at each compound concentration was determined by dividing the estimated viable cell number of the treated sample by the estimated viable cell number of the untreated sample to obtain the percentage of viable cells. The number of generations the cells grew during the exposure period was determined by (Schiestl 1989; Schiestl et al., 1989):

$$\frac{\log_{10}(OD_{600}\ 17\ h/OD_{600}\ 0\ h)}{0.30103}$$

The OD_{600} at 0 h is the optical density at 600 nm of a culture at the beginning of the 17 h exposure to chemical, and the OD_{600} at 17 h is the optical density at 600 nm of the culture at the end of the 17 h incubation with chemical.

RESULTS

Spontaneous Recombination Frequencies

Because the yeast cultures in each assay were pregrown in medium that prevented the accumulation of *HIS*$^+$ recombinants, the numbers of recombinants per 96 well microtiter plates in the untreated control samples were extremely consistent from experiment to experiment. By plating 30,000–40,000 cells per microtiter plate (300–400 cells per well) in SC-HIS medium, it was possible to obtain, on average, 10 positive wells (wells containing growing *HIS*$^+$ yeast colonies) per microtiter plate in the untreated controls. These *HIS3*$^+$ colonies represented the spontaneous recombination events. By having an average of 10 positive wells per recombinant microtiter plate in the untreated cultures it was possible to detect increases in the numbers of positive wells (recombinants) in the treated cultures without quickly saturating the numbers of positive wells per microtiter plate. It also allowed the detection of decreasing numbers of positive wells per microtiter plate in the treated cultures when cell viability decreased. For example, a decrease in the average number of positive wells per plate to five would represent a 50% decrease in the number of recombinants due to reduced cell viability.

The number of viable cells per well per recombinant plate was determined by taking an aliquot of cells suspended in SC-HIS medium and immediately diluting the aliquot in SC medium. The number of viable cells per well was then determined by taking the number of positive wells in the synthetic complete medium microtiter plate, using a Poisson distribution, and multiplying by the dilution factor used. The recombination frequencies in the untreated control cultures ranged from 1×10^{-4} to 5×10^{-4}. This was found to be consistent with recombination frequencies previously reported for DEL recombination in *S. cerevisiae* (Bailly et al., 1992; Schiestl and Prakash, 1990; Schiestl and Reddy, 1990; Schiestl, 1989; Schiestl et al., 1989; Schiestl and Prakash, 1988). Inductions in recombination greater than twofold over those of the untreated controls were considered to be positive in the microtiter plate format, because results obtained at this level of induction had an experimental coefficient of variation of ±20%.

Effect of Methyl Orange

The pH indicator dye methyl orange, which is orange at a pH of 4.5 and pink at a pH of 3.0, and turns color from orange to pink upon growth of a yeast colony in a microtiter plate well, was used to facilitate scoring of the microtiter plates at the end of the experiment. The pH of low-density yeast cultures in synthetic media was found to be 4.5–5.0, while the pH of stationary phase yeast cultures and media from microtiter plate wells containing yeast colonies was found to be 2.5–3.0. It was important to determine whether growth in the presence of dye altered either the recombination frequencies or cell viability. Ten independent cultures, containing no test chemical, were incubated for 17 h as described in the Materials and Methods section. The cultures grew to optical densities at 600 nm of 3.0–4.0 after incubation for 17 h at 30°C, which is equivalent to growing three to four cell generations. This growth is consistent with that obtained using the agar plate method (Schiestl, 1989; Schiestl et al., 1989). Following the exposure period, the untreated yeast cultures were grown in microtiter plates containing media with or without 60 μg/ml methyl orange. No significant difference was seen in the spontaneous recombination frequency in the presence or absence of methyl orange (Table 1). These spontaneous recombination frequencies were found to be consistent with those previously reported for the yeast DEL system (Bailly et al., 1992; Schiestl and Prakash, 1990; Schiestl and Reddy, 1990; Schiestl, 1989; Schiestl et al., 1989; Schiestl and Prakash, 1988). No difference in the size of the colonies was observed when those growing in the presence or absence of methyl orange were compared. No new pink wells were observed in the microtiter plates after incubation in excess of the initial 3 days at 30°C. Wells did not change from orange to pink when no yeast colonies were present.

Chemicals Tested in the Microtiter Plate Format

The established carcinogens MMS, EMS, epichlorohydrin, and 4NQO (McCann et al., 1975; IARC, 1973–1987) were tested for their ability to induce DEL recombination in the microtiter format. Inductions in recombination rate twofold

over those of the untreated controls were considered positive responses (Schiestl, 1989; Schiestl et al., 1989). Each of these compounds has been shown to be positive in the Ames assay and SCE assays, and all gave strong positive responses in the microtiter-based DEL assay, as they had in the agar plate DEL assay (Schiestl, 1989; Schiestl et al., 1989). MMS, EMS, and epichlorohydrin, which alkylate DNA at the O^4 position of thymine, the O^6 position of guanine, and induce an adaptive response in yeast (Chen et al., 1989) induced DEL recombination 14–21-fold at the highest concentration of chemical used (Table 1). 4NQO, in which the quinilone ring of the compound causes DNA adducts at the N^2 position and C^8 position of guanine (Daubersies et al., 1992), induced DEL recombination approximately eightfold. As is characteristic of DEL recombination, many positive responses were observed when concentrations of the compounds entered the cytotoxic ranges. That is, recombination frequencies increased as cell viability decreased. The carcinogen formaldehyde, which has been shown to cause increased incidence of nasal cancers in rats (IARC, 1973–1987; Milman and Weisberger, 1985; Butterworth and Slaga, 1987) was positive in both the agar plate DEL assay (Schiestl, 1989; Schiestl et al., 1989) and the microtiter plate DEL Assay (Table 1). The carcinogen benzene, which is negative in the Ames assay (Ashby and Tennant, 1991) but induces SCE in human lymphocytes (Erexson et al., 1985), induced DEL recombination approximately 11-fold (Table 1).

The solvents acetone, DMSO, and ethanol are frequently used in short-term tests to dissolve chemicals that are insoluble or poorly soluble in water. Yeast cells were treated with these solvents in concentrations up to 5% final volume. The recombination frequencies in the untreated controls for all three solvents were as expected. Acetone caused significant cell death when used in volumes above 2% (Table 1). At 5% acetone, cell viability dropped to 7.5% relative to the negative control. Yeast DEL recombination was not induced at these cytotoxic concentrations, which indicates that cell death alone does not induce recombination. The solvents DMSO and ethanol, which reduced viability to approximately 60% when used at concentrations of 5% total volume, did not induce yeast DEL recombination (Table 1). We concluded that the concentrations of DMSO and ethanol tested did not induce yeast DEL, and that these solvents could therefore be used with confidence in concentrations up to 3% during the exposure period. The amino acid L-methionine, which sometimes gives a false-positive response in other short term tests (DeSerres and Ashby, 1981), did not induce DEL recombination (Table 1). Results obtained for acetone, DMSO, ethanol, and L-methionine were consistent with those obtained by Schiestl (1989; Schiestl et al., 1989).

DISCUSSION

The prescreening of chemicals using short-term microbial test systems costs between $2,500 and $10,000 per compound and produces results within 1 week (Lave and Omenn, 1986; Sivak et al., 1987). Short-term tests that can predict carcinogenicity in mammalian systems with a high degree of accuracy are desirable for the prescreening of chemicals prior to their introduction into long and costly animal studies. The yeast DEL assay has been reported to have a high degree of specificity, with no false positive results being obtained with the 100 compounds tested so far (Schiestl, 1989; Schiestl et al., 1989; Schiestl and Reddy, 1990; Sommers et al., unpublished data). Whether the sensitivity of the yeast DEL assay is due to the quantitative nature of the assay, the ability of the yeast DEL assay to detect agents that cause chromosomal rearrangements, or both, is not known. Yeast DEL recombination has been shown to be induced by chemicals that are both positive and negative in bacterial mutation reversion assays but induce tumor formation in rodents (Schiestl, 1989; Schiestl et al., 1989; Schiestl and Reddy, 1990). The carcinogen benzene, which is negative in short-term bacterial tests, is positive in the yeast DEL assay.

An understanding of why the yeast DEL assay can detect carcinogens that are both positive and negative in bacterial assays lies in the coupling of DNA repair and recombination in yeast. It has previously been determined that the genetic regulation of yeast DEL recombination involves genes required for the excision repair and double-strand break repair of DNA, since

mutations in the excision repair genes RAD1 and RAD10, in addition to the RAD52 gene, reduce the frequency of DEL recombination (Schiestl and Prakash, 1988; Schiestl and Prakash, 1990). In the same studies it was also shown that the RAD1 and RAD10 gene products affect intrachromosomal (DEL) recombination but not interchromosomal recombination in yeast. In addition, DEL recombination is reduced synergistically in *rad1 rad52* double deletion strains, again indicating that DEL recombination is regulated by multiple genetic pathways (Schiestl and Prakash, 1988; Schiestl and Prakash, 1990). Therefore, the assay bears resemblance to both bacterial reversion assays and chromosome aberration assays.

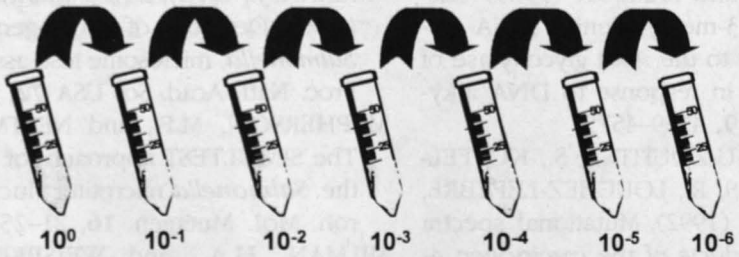
Results presented here indicate that chemicals that induce yeast DEL recombination in the agar plate format also induce yeast DEL recombination in the microtiter plate format. Those chemicals that do not induce yeast DEL recombination in the agar plate assay do not induce yeast DEL recombination in the microtiter plate assay. The conversion of the assay to a microtiter plate format decreases the time required to perform the assay. Equalization of cell number by optical density (600 nm) removes the need to perform and score multiple platings from serial dilutions for both cell viability and recombinants, as is required for the agar plate assay (Figs. 2, 3). The elimination of cell counts from the microtiter plate assay format removes this time-consuming task (Figs. 2, 3). Microsoft Windows-based software has been written to perform all of the calculations described in this text and to summarize the data in an easy-to-read report. Because the microtiter plate format involves primarily liquid handling, and recombination events are detected as color changes, the assay is amenable to automation by existing robotics systems.

To increase the ability of the yeast DEL assay to detect mutagens, we are currently testing derivatives of the yeast strain RS112 in which the DNA excision repair gene *RAD2* has been deleted. The *rad2* mutation is comparable to the excision repair mutations *uvrA* and *uvrB,* which are currently used in *Salmonella* and *E. coli trpE65* mutagenicity tests. Construction of mutant yeast strains that increase the porosity of

A. Set up cultures for exposure to test compound. Determine the starting cell number (average of three counts) for each sample to be tested by counting cells using a phase contrast microscope and a hemocytometer.

B. Determine cell number after the overnight exposure to test compound in each sample tested using a phase contrast microscope and hemocytometer (average of three counts).

C. Wash out test compound and perform serial dilutions.



D. Plate cells for both recombinants and viability. 2 plates for recombinants and 2 plates for viability for each serial dilution.

E. Incubate the agar plates for 2 -3 days.

F. Score all plates and calculate the results by hand.

FIG. 3. Yeast DEL assay using the agar plate format. The agar plate format requires multiple cell counts using a hemacytometer for each sample tested, serial dilutions for each sample tested, and multiple platings for recombinants and viability for each sample tested.

the yeast cell wall, which would be comparable to the *rfa* mutations in *Salmonella,* are also in progress. S9 fraction, which has been used to activate cyclophosphamide in the yeast DEL assay by Schiestl and Reddy (1990), is currently being used to test other compounds that require metabolic activation.

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