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High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier

Robert H. Schiestl* and R. Daniel Gietz**
Department of Biology, University of Rochester, Rochester, NY 14627, USA

Summary. A method, using LiAc to yield competent cells, is described that increased the efficiency of genetic transformation of intact cells of *Saccharomyces cerevisiae* to more than 1×10^5 transformants per microgram of vector DNA and to 1.5% transformants per viable cell. The use of single stranded, or heat denaturated double stranded, nucleic acids as carrier resulted in about a 100 fold higher frequency of transformation with plasmids containing the 2 μm origin of replication. Single stranded DNA seems to be responsible for the effect since M13 single stranded DNA, as well as RNA, was effective. Boiled carrier DNA did not yield any increased transformation efficiency using spheroplast formation to induce DNA uptake, indicating a difference in the mechanism of transformation with the two methods.

Key words: Yeast – Transformation – ss carrier DNA

Introduction

Transformation of the yeast *Saccharomyces cerevisiae* was initially accomplished by spheroplast formation before addition of the transforming DNA (Hinnen et al. 1978; Beggs 1978). This method gives high transformation frequencies, from 3,000 up to 50,000 transformants per μg of plasmid DNA, with 2 μm vectors (Struhl et al. 1979; Broach et al. 1979; Beggs 1978) and an efficiency of up to one transformant per 10^4 viable spheroplasts (Botstein et al. 1979). Another method, using *E. coli* protoplasts fused to yeast spheroplasts, yielded up to 10% transformed yeast cells (Gyuris and Duda 1986). The disadvantage of both these methods, involving spheroplasts, lies in the fact that transformation is somewhat laborious, is associated with a high frequency of cell fusion (Harashima et al. 1984), and different strains vary considerably in their transformation competence, which seems to be inherited in a polygenic manner (Johnston et al. 1981). A recently developed method, which uses agitation with glass beads (Constanzo and Fox 1988), is convenient but gives a low transformation efficiency of up to 300 transformants per μg of vector DNA.

A simpler method has been developed using intact yeast cells and alkali cations, especially lithium acetate and polyethylene-glycol (Ito et al. 1983), or PEG alone (Klebe et al. 1983), to induce DNA uptake. Currently, the method developed by Ito et al. (1983) seems to be the most commonly used, despite the disadvantage that it gives a lower transformation efficiency than the spheroplast method, yielding about 100 transformants per μg of plasmid containing the 2 μm origin of replication (Ito et al. 1983). Further development of this procedure, using intact yeast cells, yielded a maximum of 7,000 colonies per μg of plasmid DNA (Gietz and Sugino 1988; Brzobohaty and Kovac 1986; Brushi et al. 1987; Keszenman-Pereyra and Hieda 1988) and an efficiency of up to four transformants per 10^4 yeast cells (Brushi et al. 1987).

In the present study we report a procedure for transformation of intact yeast cells which routinely yielded more than 10^5 transformants per μg of DNA and an efficiency of up to 1.5% transformed cells. This procedure has been characterized for amounts and type of carrier DNA, duration of heat shock, and a number of other conditions.

Present addresses:
* Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, USA
** Department of Human Genetics, Faculty of Medicine, University of Manitoba, 250-770 Bannatyne Ave, Winnipeg, Manitoba, Canada R3E 0W3

Offprints requests to: R. H. Schiestl or R. D. Gietz at their present addresses

Table 1. Strains used

E. coli strain	Genotype	Source
HB101	*hsdr⁻, hsdm⁻, recA1*, supE44, *laczE, leuB6, proA2, thi1*	Bethesda Research Laboratories

Yeast strains	Genotype	Source or reference
S35/2-10C	*MATa ura3-52*[a] *leu2-3,112 trp5-27 arg4-3 ade2-40 ilv1-92*	Schiestl et al. 1988
LP2752-4B	*MATa lys1-1 ura3-52 his4-864,1176* pBR313 *his4-260,39*	Schiestl and Prakash 1988
LP2752-4BH⁻	HIS⁻ derivative of strain LP2752-4B	Schiestl and Prakash 1988
DBY747	*Mata ura3-53 leu2-3,112 trp1-289 his3-Δ1*	D. Botstein
RS109-2A	*MATa ura3-52 leu2Δ1*[b] *his3Δ200*[c] *trp1Δ901*[d] *lys2-801 ade2-101*	R. H. Schiestl; Gietz and Sugino 1988

[a] The *ura3-52* allele is described in Rose and Winston (1984)
[b] The *leu2Δ1* allele was constructed by P. Hieter (personal communication)
[c] The *his3Δ200* allele is described in Fasullo and Davies (1987)
[d] The *trp1Δ901* allele was constructed by M. Snyder

Materials and methods

Strains and media. The *E. coli* strain and the yeast strains used are listed in Table 1. Growth and minimal media were prepared as described previously (Schiestl and Wintersberger 1982) with the exception that adenine sulfate (30 mg/l) was added to the YPD medium (YPAD).

Transformation and other procedures. Large scale plasmid isolation from *E. coli* and electrophoresis of DNA was performed according to Maniatis et al. (1982). *E. coli* transformation was carried out as described in Hanahan (1983). Transformation of yeast is described in Fig. 1; variations are indicated in the individual experiments. For Plasmid DNA isolation from yeast, essentially the method described by Hoffman and Winston (1987) was followed.

Plasmids. Plasmids YEplac195 and YEplac181 (Gietz and Sugino 1988), contain the *URA3* gene and the *LEU2* gene, respectively, as selectable markers and the 2-μm origin of replication for autonomous replication in yeast. YEplac195 and YEplac181 transform yeast at high frequency. In plasmid pDG95 (constructed by R. D. Gietz, unpublished, published in Schiestl and Prakash 1988) the *Hin*dIII fragment (about 150 bp) of the *HIS3* gene has been deleted and replaced by the *URA3* gene. This plasmid was used for selection for gene replacement (Rothstein 1983). A *Bam*HI fragment of plasmid pDG95, containing the *URA3* gene with flanking *HIS3* sequences, was used for transformation and URA⁺ transformants were selected. Plasmid pYAC3 (Burke et al. 1987) contains the yeast *CEN4, ARS1, TRP1, HIS3* and *URA3* sequences as well as telomere sequences. Plasmid pYAC3 was digested with *Bam*H1 to expose the telomeres before transformation.

Preparation of Carrier DNA. DNA (Sigma - D1626 Type III Sodium from Salmon Testes) was dissolved in TE (10 mM Tris/HCl pH 8.0, 1 mM EDTA), 10 mg/ml, by drawing up and down in a 10 ml pipette. It was then incubated overnight at 4°C to give a homogenous viscous solution. Following this it was sonicated twice for 30 s with a large probe at 3/4 power. The resulting DNA had an average size of 7 kb as judged from an ethidium bromide gel and ranged in size from 15–2 kb. Sonication, leading to carrier DNA with smaller average size about 2–1 kb, dramatically reduced the transformation efficiency.

The DNA solution was extracted once with TE saturated phenol, once with phenol (TE sat.):chloroform (50:50) and once with chloroform. The DNA was then precipitated by adding 1/10 vol of 3 M sodium acetate (pH 6.0) and 2.5 vol. of ice cold ethanol (99%). The precipitate was collected by centrifugation at about 12,000 g and washed with 70% ethanol, partially dried under vacuum and then redissolved in TE at 10 mg/ml. Following this, the DNA was denatured in a boiling water bath for 20 min or in a microwave oven by heating until noticeably boiling, and then immediately cooled in an ice water bath. The DNA solution can be stored in aliquots of −20° C and thawed when needed. Repeated freezing and thawing does not noticeable reduce the transformation efficiency.

Sonicated salmon sperm carrier DNA was alkaline denatured by adding 0.5 ml of 2.0 N NaOH to 5 ml of 1 mg/ml DNA in TE and incubated at room temperature for 10 min prior to the addition of 2 ml of 5 M ammonium acetate (pH 7.4) and 2.5 vol. of ethanol. The solution was then incubated on ice for one h. The precipitate was collected by centrifugation and washed with 70% ethanol, partially dried under the vacuum and then redissolved in TE at 10 mg/ml.

Preparation of RNA and single stranded M13 DNA. RNA was extracted from logarithmically growing yeast cells as described in Jensen et al. (1983). Total nucleic acid was applied to an oligo dT column according to the method of Aviv and Leder (1972). The flow through, devoid of poly A⁺ mRNA, was collected and precipitated by the addition of 2.5 vol of ice-cold 95% ethanol. The precipitate was collected by centrifugation, washed with 70% ethanol and dissolved in sterile double distilled water. Single stranded M13 mp19 DNA was isolated according to Kunkel et al. (1987).

Results

Two common methods have been used to promote DNA uptake in yeast cells; lithium acetate treatment of intact yeast cells (LiAc transformation; Ito et al. 1983) and digestion of cell walls to produce spheroplasts (spheroplast transformation; Beggs 1978; Hinnen et al. 1978). We have developed a method for high efficiency transformation of intact yeast cells using LiAc transformation. Fig. 1 outlines the procedure which in our hands maximized the transformation efficiency. A

1. Inoculate cells from an O/N culture in 300 ml liquid YPAD
2. Grow to 5–10 × 10^7 cells/ml (2 to 3 × higher efficiency is obtained by diluting to 2 × 10^6 cells/ml in fresh YPAD and growth for another two generations)
3. Spin at 5,000 rpm for 5 min in a GSA rotor, Sorvall centrifuge, at room temperature
4. Resuspended in 10 ml glass-distilled sterile water, transfer to 40 ml tubes
5. Spin at 7,000 rpm for 5 min in a SS34 rotor, Sorvall centrifuge, at room temperature
6. Resuspended in 1.5 ml sterile 1 × TE, LiAc [made fresh from 10 × filter sterile stocks; 10 × TE (0.1 M Tris-HCl, 0.01 M EDTA, pH 7.5); 10 × LiAc (1 M LiAc pH 7.5, adjusted with diluted acetic acid)]
7. Sonicate cells for 3 min in an ultrasonic (Branson 2200) water bath (Only for highest transformation efficiency of strains showing clumping)
8. Incubate for 1 h at 30°C with constant agitation
9. Prepare DNA in microfuge (Brinkman) tubes, up to 5 µg of transforming DNA and 200 µg of salmon sperm carrier DNA in a maximum of 20 µl; add appropriate volume of 10 × TE, LiAc if more than 20 µl DNA solution is used (carrier DNA was prepared as described in the Methods section, this is the most important step for highest transformation efficiency)
10. Add 200 µl of yeast suspension to each microfuge tube
11. Incubate 0.5 h at 30°C with agitation
12. Add 1.2 ml sterile 40% PEG 4000 solution (40% PEG 4000, 1 × TE, 1 × LiAc, made fresh from sterile 50% PEG stock, and sterile 10 × TE and 10 × LiAc)
13. Incubate 30 min at 30°C with agitation
14. Heat shock for 15 min in a 42°C waterbath (Important)
15. Centrifuge tubes in microfuge for 5 s
16. Wash cells twice with 0.5 ml sterile 1 × TE, throroughly resuspend cells each time with the help of a sterile toothpick
17. Resuspend in 1 ml of 1 × TE and plate 200 µl onto one selection plate. (we found that the agar is important for the transformation efficiency, Difco Agar gives the best results)
18. Incubate at 30°C until transformants appear

This protocol is based on the method initially developed by Ito et al. (1983). The above method routinely yields up to 1 × 10^5 transformants per µg of DNA and up to 1.5% transformed yeast cells

Fig. 1. Protocol for high efficiency transformation of intact yeast cells using single stranded nucleic acids as carrier

number of different conditions have been evaluated for their effect on transformation.

Medium and cultivation of cells

Addition of adenine in YPAD medium for the preculture phase gave a slightly better transformation efficiency, especially with *ade*$^-$ mutants. The highest transformation efficiency was obtained when cells were harvested at 1 × 10^6 cells/ml, or when an overnight culture, grown to about 1 × 10^7 cells/ml, was diluted to 2 × 10^6 cells/ml and allowed to grow for another 1–2 generations. In this case the transformation efficiency was 3-fold higher than without recultivation.

Carrier DNA denaturation gives high transformation efficiency

The addition of various types of carrier DNA to LiAc transformation of intact yeast cells (Ito et al. 1983) with the 2 µm plasmid YEPlac195 (selection for URA3$^-$, Gietz and Sugino, 1988) dramatically increases the transformation efficiency with strain LP2752-4B (Table 1) for both types of carrier DNA used. Native salmon sperm DNA gave a 20-fold increase and native calf thymus DNA gave a 200-fold increase over transformation levels without carrier (Table 2). However, in each case, when boiled and quickly chilled or alkaline denatured carrier DNA was used, the transformation efficiency increased again. Denatured salmon sperm DNA increased the transformation efficiency another 100-fold while denatured calf thymus DNA gave a smaller 5-fold increase (Table 2).

In a systematic study the generality of this observation was extended to other 2 µm plasmids, other types

Table 2. Influence of the treatment of carrier DNA on transformation efficiency

Carrier	Treatment of carrier		Transformants/µg of					
		Denaturation	YEplac195	Fold incr.	pDG95	Fold incr.	YAC3	Fold incr.
None	–	–	37	1×	1.74	1×	210	1×
Salm. sp.	Sonicated	–	739	20×	525	301×	211	1×
Salm. sp.	Sonicated	Boiled	67,000	1,810×	1,940	1,115×	2,550	12×
Salm. sp.	Sonicated	Alkaline treated	64,320	1,738×	–	–	–	–
Calf thymus	Sonicated	–	7,900	213×	–	–	–	–
Calf thymus	Sonicated	Boiled	37,000	1,000×	–	–	–	–
Salm. sp.	*Hae*III digested	–	768	20×	–	–	–	–
Salm. sp.	*Hae*III digested	Boiled	32,190	870×	–	–	–	–

100 µg of carrier DNA and a 10 min heat shock was used for each transformation. The DNA was boiled by incubation for 30 min in a boiling water bath. *Hae*III digestion was carried out with 0.5 units per µg DNA for 3 h

of transformation events, other sources of carrier DNA and other yeast strains. When another 2 μm plasmid, YEPlac181 (selection for LEU2$^+$, Gietz and Sugino 1988), was used the same increase in transformation efficiency was observed with denatured DNA, and a slightly higher transformation efficiency was obtained when compared to plasmid YEPlac195 (data not shown). The frequency of gene replacement and transformation with a linear yeast artificial chromosome was also increased 4- and 10-fold respectively, when heat denatured, instead of native salmon sperm carrier, DNA was used (Table 2). Plasmid pDG95 (constructed by R. D. Gietz, unpublished, published in Schiestl and Prakash 1988) was used for selection of gene replacement events (Rothstein 1983). The yeast artificial chromosome, YAC3 (Burke et al. 1987 was also used). The relatively lower increase, of only 4-fold, in transformation frequency by heat denatured DNA, may be due to the fact that an additional step, namely recombination, is required to yield transformants. A large number of abortive transformants was always present.

Salmon sperm DNA was sheared for different time periods with a sonicater and an aliquot was examined on an agarose gel for size distribution; other aliquots were used for transformation after boiling. Carrier DNA, not sonicated, resulted in a transformation efficiency of 4.6×10^4 per μg of vector DNA. Peak transformation efficiency was reached with sonicated carrier DNA of mean size 7 kb, ranging from 15 to 2 kb, giving 5.5×10^4 transformants per μg of DNA. Decreasing the size of carrier DNA to about 500 bp decreased the transformation efficiency to 1.9×10^4 transformants per μg of vector DNA. Because reducing the average M.W. of carrier DNA to 7 kb gives the best transformation efficiency, and because less viscous DNA solutions are easier to handle, we recommend reducing the size by sonication or by other means. We found that shearing by passage through a 20 gauge needle was unsatisfactory in reducing the average M.W. enough to circumvent the formation of a gel upon chilling after boiling the sample. After digestion with a 1/2 unit per microgram for 3 h of the restriction endonuclease *Hae*III, salmon sperm DNA was found to enhance the transformation efficiency after boiling (Table 2). Presumably other restriction enzymes would be similarly effective if no sonicater were to be available.

For most of this study, strain LP2752-4B (Table 1) was used. The study was extended to other strains by using RS35/2-10C, DBY747 and RS109-2A (Table 1). The transformation efficiency for 2 μm plasmids for all of these strains increased at least 20-fold after boiling of salmon sperm carrier DNA (data not shown). In fact we use this procedure routinely (Schiestl and Prakash 1988; Schiestl et al 1989; Gietz and Prakash 1988; and papers in preparation) and so far we have not found any strain which did not give a satisfactory high transformation efficiency.

Proof of transformation

The putative transformants isolated with the procedure shown in Fig. 1 satisfied all genetic and molecular requirements for genetic transformation in yeast. Southern blots verified that the transformants in fact contained the correct plasmids. Furthermore, plasmids could be isolated from yeast cells which were transformed with 2 μm and *CEN ARS* plasmids. These plasmids were transformed into *E. coli*. Also, integrated plasmids could be rescued from yeast after digestion of yeast DNA with appropriate restriction enzymes and ligation. Restriction enzyme digests verified that the correct plasmids were rescued. 2 μm and *CEN ARS* plasmids conferred a mitotically and meiotically unstable phenotype to the yeast cells transformed with this procedure and they were lost at frequencies characteristic for the respective plasmids (Struhl 1983). This procedure was also used for the cloning of different DNA repair and CDC genes in yeast. With DNA fragments designed to replace or disrupt genes, such as pDG95 which replaces the *HIS3* gene with the *URA3* gene, 95% of the URA$^+$ transformants showed the desired his$^-$ phenotype (Schiestl and Prakash 1988). Different DNA repair genes were deleted with a similar frequency (Schiestl and Prakash 1988; Schiestl et al. 1989; Gietz and Prakash 1988; and papers in preparation). Integration and deletion events obtained by this transformation method segregated as a single genetic marker in a multitude of crosses. Thus we conclude that the isolates obtained with this improved method (Fig. 1) are due to genetic transformation.

Single stranded M13 DNA and RNA as carrier give high transformation efficiency

Theoretically, there could be two possible explanations for the high transformation efficiency obtained after boiling or alkaline treatment of carrier DNA. First, it may be that an inhibitor of transformation is destroyed by the boiling process or the alkaline treatment. In fact when DNA from yeast is isolated, an inhibitor of *E. coli* transformation copurifies with yeast DNA (Devenish and Newlon 1982; Holm et al. 1986). Alternatively, and more likely, the denaturation of DNA, and the presence of single stranded DNA, after boiling and alkaline treatment is responsible for the increase in transformation efficiency.

Table 3. Single stranded M13 DNA and RNA as carrier for highly efficient yeast transformation

Carrier	Treatment of carrier		Transformants/μg of	
	Sonicated	Heat denaturation	YEplac195	Fold increase
Salm. Sp.	–	–	249	1×
Salm. Sp.	–	Boiled	22,600	91×
M13	–	–	21,020	84×
M13	–	Boiled	48,140	193×
M13	+	–	5,650	23×
M13	+	Boiled	6,330	25×
rRNA	–	–	18,760	75×
rRNA	–	Boiled	16,050	65×
tRNA	–	–	339	1.4×

100 μg of carrier nucleic acids and a 10 min heat shock were used for each transformation. DNA was boiled by incubation for 30 min in a boiling water bath

Table 4. Influence of different amounts of boiled carrier DNA on transformation efficiency

Amount of carrier DNA	Transformants/μg of vector DNA	Fold increase
0	37	1×
10 μg	720	20×
50 μg	29,700	803×
100 μg	37,000	1,000×
200 μg	42,500	1,149×
500 μg	28,000	757×

Sonicated boiled salmon sperm DNA was used as carrier, a 10 min heat shock was applied and YEPlac195 was used as transforming plasmid. Carrier DNA was boiled by incubation for 30 min in a water bath

The issue of whether an inhibitor of transformation was inactivated, or wheter denatured DNA was active, was approached by different means. First, we tried to determine whether the DNA can be purified from such a putative inhibitor of transformation using a CsCl/EtBr gradient. Commercially available salmon sperm, as well as calf thymus DNA, was first prepared as indicated in the Materials and methods section and further purified by a CsCl/EtBr gradient according to Maniatis et al. (1982). Boiling the DNA again increased the frequency of transformation with CsCl-purified DNA about 100-fold when salmon sperm DNA was used and 5-fold when calf thymus DNA was used (data not shown). Thus we were not able to purify the DNA from a putative inhibitor of transformation.

On the other hand if denaturation of DNA, and thus single stranded DNA regions, are required for the increase in transformation efficiency, single stranded M13 DNA or even RNA may be as effective as boiled double stranded DNA. The experiments in Table 3 show that, in fact, single stranded M13 DNA or RNA is as effective as denatured salmon sperm carrier DNA. Boiling increases the efficacy of single stranded M13 DNA only 2-fold and does not increase the efficiency of RNA. It is also noticeable that sonication of single stranded M13 DNA, which in this case produced fragments of less than 500 basepairs, decreased the efficiency 10-fold which is also in agreement with the data from boiled salmon sperm DNA. Furthermore, when tRNA was used no increase in transformation efficiency was observed (Table 3) presumably because of the small size of tRNA. Thus, the increase in transformation efficiency by single stranded DNA may require a size of at least two kilobases. We conclude that, most likely, the single strandedness in denatured carrier DNA is responsible for the high transformation efficiency and that single stranded M13 DNA and rRNA are equally efficient.

Additional factors affecting transformation efficiency

The transformation procedure was optimized in terms of the amount of carrier DNA, duration of the heat shock and the amount of transforming vector DNA. In Table 4 the effect of different amounts of boiled carrier DNA on the frequency of transformation is shown. A peak in transformation efficiency can be seen at 200 μg of boiled carrier DNA per transformation batch. In the rest of this study, utilizing 100 μg of carrier DNA, a slightly suboptimal concentration employed.

The experiment shown in Table 5 demonstrates that the length of incubation at 42°C before plating the cells onto selective medium influences the number of transformants. The application of a heat shock increased the transformation efficiency about 8-fold with its peak at 15 min duration. A heat shock of 10 min was used in the

Table 5. Influence of different incubation times at 42 °C (heat shock) on transformation efficiency

Time of incub. at 42 °C	Transformants µg of DNA	Fold increase
0	12,900	1×
2.5 min	31,600	2.5×
5 min	56,900	4.4×
10 min	94,400	7.3×
15 min	107,000	8.3×
20 min	100,000	7.8×
10 min 10 min ice	78,000	6.1×

After incubation at 42 °C in a water bath, the cells were cooled for 15 s or 10 min as indicated in an ice water bath. 100 µg of sonicated boiled salmon sperm DNA was used as carrier for each transformation and YEPlac195 was used as transforming plasmid. Carrier DNA was boiled by incubation for 30 min in a wather bath

Table 6. Influence of the treatment of carrier DNA on transformation efficiency using spheroplasting to promote DNA uptake

Strain	Heat denaturation of carrier DNA	Transformants per µg of plasmid	Relative percent
LP2752-4B	None	820	120
LP2752-4B	Boiled	680	100
S35/2-10C	None	720	258
S35/2-10C	Boiled	278	100

100 mg of sonicated salmon sperm DNA was used as carrier for each transformation and YEPlac195 was used as transforming plasmid. Carrier DNA was boiled by incubation for 30 min in a water bath

other experiments. It has been previously described that a 10 min incubation of cells on ice after the heat shock increased the transformation efficiency (Brushi et al. 1987). This step was not effective in our procedure and led to a slight decrease in transformation frequency (Table 5).

Other factors tested for their influence on transformation are described below. Usually Difco Agar was used in the selection plates, when Gibco Agar was used only 36% of transformants were obtained from the same dilution plates onto different plates, but no difference in cell survival was detectable between the two media. On selection medium made with purified agarose cells grew poorly and only 39% of transformants were obtained as compared to Difco Agar. Addition of 1 molar sorbitol to the selection medium decreased the transformation efficiency to 50%. Ommission of EDTA from all transformation solutions had no detectable effect on the transformation efficiency with 2 µm vectors; ommission of Tris/HCl reduced the transformation efficiency 10-fold. In transformation of spheroplasts, regeneration of cells in 30% YPD for 20 min, which was then added to the plating mix (Hinnen et al. 1978), is beneficial to the cells before plating onto selective medium. When we used the same incubation in YPD in our transformation method the efficiency decreased slightly. Whenever possible, disposables should be used to handle cells and media; if dishes are to be reused extreme care should be taken in cleaning. Traces of soap seem to greatly reduce the transformation efficiency.

Transformation of spheroplasts does not increase in efficiency with boiled carrier DNA

The transformation protocol using spheroplast formation to facilitate DNA uptake gives a high transformation efficiency in the absence of boiled carrier DNA (Beggs 1978; Broach et al. 1979). Therefore we examined whether the efficiency can be further increased when boiled carrier DNA was used. For both strains tested with this protocol, boiling of the carrier DNA did not increase the transformation efficiency (Table 6). This indicates that the two transformation procedures, using spheroplast formation of LiAc treatment, may be based on different mechanisms of DNA uptake.

Discussion

We have developed an improved yeast transformation method (Fig. 1), using LiAc to induce DNA uptake (Ito et al. 1983), which reproducibly yields more than 10^5 transformants per µg of vector DNA and up to 1.5% transformed cells. To our knowledge this is the highest transformation efficiency reported in yeast with a method using LiAc to induce DNA uptake. We found that heat denatured DNA, or single stranded nucleic acids as carrier, are far more effective in our transformation protocol than is native double stranded DNA. The addition of native carrier DNA to heat denatured carrier DNA specifically decreased the transformation efficiency. An almost linear increase of the number of transformants with increasing amounts of transforming plasmid DNA has been obtained in the range of one nanogram up to 5 microgram of plasmid DNA per transformation batch. Heat denatured carrier DNA did not increase the transformation efficiency using spheroplast formation to induce DNA uptake.

Previously reported frequencies of transformation using LiAc to promote DNA uptake ranged between 400 and 7,000 colonies per µg of plasmid DNA (Ito et al. 1983; Gietz and Sugino 1988; Brzobohaty and

Kovac 1986; Brushi et al. 1987; Keszenman-Pereyra and Hieda 1988), and an efficiency of up to four transformants per 10^4 yeast cells (Brushi et al. 1987). Another method, using spheroplast formation to induce DNA uptake, gave higher transformation frequencies of 3,000 to 50,000 transformants per µg of plasmid DNA with 2 µm vectors (Struhl et al. 1979; Broach et al. 1979; Beggs 1978) and a yield of up to one transformant per 10^4 viable spheroplasts (Botstein et al. 1979). The main reason for researchers continuing to use the method involving spheroplast formation is probably the higher transformation efficiency in spite of the disadvantages that this method is somewhat more laborious and more complex to control in its conditions and that it is associated with a high frequency of cell fusion (Harashima et al. 1984). Since our method gives an even higher efficiency than the spheroplast method it will be especially useful in approaches requiring high transformation competence. Thus, our method has already successfully been used to clone different genes in yeast and four different strains gave about the same high transformation efficiency. Furthermore, we have transformed an extensive number of strains and have not found any strain which did not give a satisfactorily high transformation efficiency.

Very little is known about the molecular mechanism of yeast transformation and which specific events are involved. Using LiAc to induce DNA uptake we have shown in this paper that single stranded nucleic acids as carrier DNA substantially increase the transformation efficiency as compared to double stranded carrier DNA. A 10-fold difference in transformation efficiency between native calf thymus and salmon sperm carrier DNA has been observed in Table 2. Calf thymus is a metabolically active tissue and DNA is expressed, whereas during spermatogenesis in eucaryotes the composition of proteins changes and structural rearrangements occur in the chromatin which lead to condensation and repression of the chromatin (Bloch 1976). The source and method of isolation may be responsible for producing more single stranded regions in the calf thymus than in the salmon sperm DNA used. This may explain the fact that for calf thymus DNA a higher level of transformation was observed with native DNA but a similar level was observed with heat denatured DNA.

The mechanisms responsible for transformation using spheroplast formation versus LiAc treatment to induce DNA uptake seem quite different in several respects. Spheroplast formation is accompanied by a low plating efficiency, presumably due to cell lysis, and it is true to a certain extent that a lower plating efficiency gives a higher transformation efficiency (Orr-Weaver et al. 1983). For this transformation method an isotonic medium, usually one molar sorbitol, is absolutely required to give transformants. With our procedure we found that the plating efficiency after treatment is higher than 50% and moreover one molar sorbitol reduces the transformation efficiency. In the spheroplast transformation method, cell lysis probably leads to a release of nucleases from lysed cells and double stranded carrier DNA increases the transformation efficiency (Orr-Weaver et al. 1983), probably by diluting the effect of the nucleases on the transforming vector DNA. Thus the slight decrease in spheroplast transformation efficiency with heat denatured carrier DNA versus native double stranded DNA (Table 6) could be explained if heat denatured carrier DNA could not protect the transforming vector DNA as well as double stranded DNA from nucleases released by cell lysis. LiAc treatment does not seem to be associated with cell lysis and the specific requirement for single stranded nucleic acids as carrier may reflect a specific requirement of the mechanism of uptake of DNA by intact cells in this procedure rather than simply dilution of nucleases.

The increase in the transformation frequency by heat denatured DNA is likely the result of its single strandedness, rather than a destruction of an inhibitor of transformation, since the same effect has been obtained with alkaline denatured DNA and with DNA purified by CsCl/EtBr gradient centrifugation. Furthermore single stranded M13 DNA and rRNA are just as effective.

Since spheroplasts can be transformed with high efficiency (Struhl et al. 1979; Broach et al. 1979; Beggs 1978), it may be assumed that the cell wall acts as a barrier in transformation as has also been suggested by other studies (Brushi and Howe 1985). The cell wall of cells treated with Li^+, and a variety of other agents which enhance genetic transformation in yeast, becomes porous and leaky for nucleic acids, because RNA was found in the supernatant of cells treated with LiAc and SDS, but not in the supernatant of cells treated with SDS alone (Brzobohaty and Kovac 1986). Thus, pores may be present in Li^+-treated cells through which vector DNA can enter the cell. In addition, Brushi et al. (1987) have shown that up to the point of removal of the PEG and DNA solution, transformation is still completely DNAse I-sensitive. This suggests that the transforming DNA is still accessible to the enzyme, and the cells have not yet taken up the vector DNA. Thus, the cell wall may play a part in accessibility of the transforming DNA to the yeast plasma membrane. Single stranded nucleic acids may enter the cell and carry the vector DNA along. It may also be possible that DNA uptake is an active process, and this mechanism may be induced by single stranded DNA.

The described transformation protocol will be useful for approaches requiring high efficient transformation, such as cloning of genes or to study recombination with in vivo constructed substrates. It is also hoped that this method will be useful for strains in which other methods work poorly. In fact we have never encountered any problem with an extensive list of strains. Our protocol may contribute to the understanding of DNA uptake by intact cells and will be useful in studies aimed at further characterization of this process. Lastly, since single stranded DNA may be a specific requirement for intact yeast cells to take up DNA, this may also be true for other organisms and it may be worth while to try in systems where currently only poor transformation efficiencies can be obtained.

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References

Aviv H, Leder P (1972) Proc Natl Acad Sci USA 69:1408–1412
Bloch D (1976) In: King RC (eds) Handbook of genetics, vol 5. Plenum Press, New York, pp 139–167
Burke DT, Carle GF, Olson MV (1987) Science 236:806–812
Beggs JD (1978) Nature 275:104–109
Botstein D, Falco SC, Stewart SE, Brennan M, Scherer S, Stinchcomb DT, Struhl K, Davis RW (1979) Gene 8:17–24
Broach JR, Strathern JN, Hicks JB (1979) Gene 8:121–133
Bruschi CV, Comer AR, Howe GA (1987) Yest 3:131–137
Bruschi CV, Howe GA (1985) J Cell Biochem 1519:150
Brzobohaty B, Kovac L (1986) J Gen Microbiol 132:3089–3093
Constanzo MC, Fox TD (1988) Genetics 120:667–670
Devenish RJ, Newlon CS (1982) Gene 18:277–288
Fasullo MT, Davis RW (1987) Proc Natl Acad Sci USA 84:6215–6219
Gietz RD, Prakash S (1988) Gene 74:535–541
Gietz RD, Sugino A (1988) Gene 74:527–534
Gyuris J, Duda EG (1986) Mol Cell Biol 6:3295–3297
Hanahan D (1983) J Mol Biol 166:557–580
Harashima S, Takagi A, Oshima Y (1984) Mol Cell Biol 4:771–778
Hinnen A, Hicks JB, Fink GR (1978) Proc Natl Acad Sci USA 75:1929–1933
Hoffman CS, Winston F (1987) Gene 57:267–272
Holm C, Meeks-Wagner DG, Fangman WL, Botstein D (1986) Gene 42:169–173
Ito H, Fukuda Y, Murata K, Kimura A (1983) J Bacteriol 153:163–168
Jensen R, Sprague GF, Herskowitz I (1983) Proc Natl Acad Sci USA 80:3035–3039
Johnston J, Hilger F, Mortimer R (1981) Gene 16:325–329
Keszenman-Pereya D, Hieda K (1988) Curr Genet 13:21–23
Klebe RJ, Harriss JV, Sharp D, Douglas MG (1983) Gene 25:333–341
Kunkel TA, Roberts JD, Zakour RA (1987) Methods Enzymol 154:367–382
Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
Orr-Weaver TL, Szostak JW, Rothstein RJ (1983) Methods Enzymol 101:228–244
Rose M, Winston F (1984) Mol Gen Genet 193:557–560
Rothstein RJ (1983) Methods Enzymol 101:202–211
Schiestl RH, Igarashi S, Hastings PJ (1988) Genetics 119:237–247
Schiestl RH, Prakash S (1988) Mol Cell Biol 8:3619–3626
Schiestl RH, Reynolds P, Prakash S, Prakash L (1989) Mol Cell Biol 9:1882–1896
Schiestl RH, Wintersberger U (1982) Mol Gen Genet 186:512–517
Struhl K (1983) Nature 305:391–397
Struhl K, Stinchcomb DT, Scherer S, Davis RW (1979) Proc Natl Acad Sci USA 76:1035–1039

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- grow 2ml o/n
- dilute the o/n to $OD_{600} = 0.2$ and grow to $OD_{600} = 0.6 - 0.8$

COMPETENT YEAST CELLS

- Spin down, wash with H_2O and resuspend in 1/100 volume of LiAc buff
- 2×10^8 cells/ml in hemacytometer (dilution 1/10, count)

put in 2 x 50 ml blue top tubes
enough for 10 transformations
Spin in International (IEC) for 4 min 30 rpm
Rinse with sterile dI H_2O (40ml vortex spin
Resuspend in 1/100 ~~total~~ original vol ~~(2x25)~~ LiOAc buffer, pull

Split it in 10 tubes eppendorf (100µl each)
Add plasmid (~1µg) + carrier 6µl at 10mg/ml Salmon Sperm DNA
Do one plate with no plasmid DNA
Add 700µl PEG_{3400} buffer vortex briefly
Incubate @ 30°C 1/2 hr no agitation
Heat shock 10 min @ 42°C
Spin 10 sec in eppendorf Remove
sup with pipet, Spin again remove sup
Resuspend in 150 µl H_2O, plate

PEG buffer

40% PEG in
LiOAc buffer

Autoclave

~~50ml~~ 0.5M ~~10 ml~~

LiOAc buffer

0.1M LiOAc	10 ml
10mM Tris pH8	1 ml
1mM EDTA	0.2 ml

Autoclave