# Rapid Transfer of Low Copy-Number Episomal Plasmids from *Saccharomyces cerevisiae* to *Escherichia coli* by Electroporation

*Laura Gunn and Jac A. Nickoloff**

## Abstract

A simple and reproducible method for transferring low copy-number episomal plasmids from yeast to *Escherichia coli* has been developed. Although slightly more time-consuming than direct transfer methods, which are effective with high copy number plasmids, the method is significantly faster than methods that require purification of yeast DNA. Plasmid DNA is released from yeast cells during brief treatments involving grinding with glass beads and heating. The treated yeast are cooled, electrocompetent *E. coli* is added, the mixture is electroporated, and transformants are selected using standard conditions for *E. coli* electrotransformation. The procedure typically yields sufficient transformants for most applications.

**Index Entries:** Yeast episomes; plasmid rescue; electrotransformation.

## 1. Introduction

Yeast shuttle vectors are often used in molecular and cellular studies of *Saccharomyces cerevisiae,* including those of gene structure/function relationships, DNA repair, mutagenesis, and recombination *(1)*. Shuttle vectors are convenient for such studies because they are easily and rapidly modified in vitro and introduced into *Escherichia coli,* from which large quantities of pure plasmid DNA can be prepared; transferred into yeast for analysis of gene function or for use as substrates of cellular processing enzymes (such as those involved in DNA repair); and then transferred from yeast to *E. coli* for purification and analysis. There are five types of yeast shuttle vectors, all of which have three essential features, including sequences for replication and selection in *E. coli* (an origin of replication and an antibiotic resistance marker), and a yeast selectable marker (usually a gene encoding a enzyme in metabolic pathway, such as *URA3, HIS3, or TRP1*; *2–4*). Plasmids with these minimum features do not replicate freely when introduced into yeast; they must integrate into a yeast chromosome to be maintained, and are therefore called yeast integrating plasmid (YIp) vectors. Integrated YIp vectors must be excised from chromosomal DNA by restriction enzyme digestion and circularized with DNA ligase before they can be returned to *E. coli (5)*. In contrast, shuttle vectors with an autonomously replicating sequence *(ARS)* can exist in yeast as freely replicating circular molecules (episomes), and these can be transferred directly between yeast and *E. coli* hosts. Plasmids with an *ARS* from the yeast 2-μm circle (a naturally occurring plasmid; *6*) are called yeast episomal plasmids (YEp); they exist in yeast at high copy number (30–50/cell) and segregate to daughter cells with high efficiency. Plasmids with a yeast chromosomal *ARS* sequence are called yeast replicating plasmids (YRp). YRp vectors also can occur as episomes with medium to high copy numbers, but they are lost rapidly unless selection pressure is maintained because they are mitotically unstable *(7)*. The addition of a yeast centromere *(CEN)* to an *ARS* plasmid produces a

*Author to whom all correspondence and reprint requests should be addressed. Department of Cancer Biology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

low copy number yeast centromeric plasmid (YCp), which have low copy numbers (1 or 2/cell), and increased mitotic stability (loss rates about 1%/generation). A yeast artificial chromosome (YAC) is an *ARS/CEN* vector with telomere sequences. YAC vectors replicate freely as low copy number linear molecules *(8)*. Only circular DNA transforms *E. coli* efficiently *(9–11)*. Thus, only circular episomal plasmids can be rescued without intermediate enzymatic treatments to release and circularize integrated plasmid DNA.

Because plasmid DNA is easily purified from *E. coli,* plasmids in yeast are often transferred back to *E. coli* for detailed characterization. For example, molecular cloning may involve the selection of a complementing activity encoded by a DNA fragment from a plasmid library and the subsequent transfer (or "rescue") from yeast to *E. coli* for DNA sequence analysis. Plasmid rescue may also be used to confirm the structure (genotype) of a mutant gene introduced into yeast in order to correlate a genotype with a phenotype. In our studies of homologous recombination, plasmid recombination substrates are introduced into yeast, allowed to recombine with endogenous heteroallelic loci, and total yeast DNA (containing plasmid recombination products) is prepared and electroporated into *E. coli* for detailed structural analysis of the rescued plasmids. In one such study we characterized more than 400 products *(12)*. Although such transfers are highly efficient (often yielding >100 transformants), the purification of yeast DNA from each recombinant was time-consuming and labor-intensive. We were interested in streamlining the plasmid rescue step and began investigating methods to transfer plasmids from yeast to *E. coli* directly, i.e., without requiring preparation of yeast DNA. Procedures have been described for direct transfer of plasmids between bacterial species *(13–15)* and of high copy number shuttle vectors from yeast to *E. coli (16)*. This report describes the development of a rapid procedure for "semidirect" transfer of low copy-number plasmids from yeast to *E. coli* that involves releasing DNA from yeast cells by mechanical means.

## 2. Materials and Methods

### 2.1. Plasmid DNA Preparation and Yeast Culture

Plasmid DNA was prepared and manipulated as described previously *(12)*. The 7.0-kbp low copy-number shuttle plasmid (pUraHAC) contains a *ura*3 allele on a *HIS3/ARS1/CEN4* (HAC) derivative of pUC19, and is similar to HAC plasmids described previously *(17)*. pUraHAC was transformed into yeast strain DY3028 (*12*; *MAT***a***-inc, lys2-Δ1:LYS2-GALHO, ura3-X432, ade2-101, his3-200, leu2-Δ1, trp1-Δ1*) using the method described by Ito et al. *(18)*. Yeast strains were cultured as described previously *(12)*. Yeast carrying the HAC shuttle vector were grown on selective medium (lacking histidine) to maintain selective pressure for the unstable episome.

### 2.2. Electroporation Conditions

Frozen competent *E. coli* strain DH5α were prepared for electroporation with a BioRad (Richmond, CA) Gene Pulser essentially as recommended by the manufacturer, with modifications as described by Miller and Nickoloff *(19)*. All experiments were performed with a single batch of electrocompetent DH5α cells with a transformation efficiency of $6 \times 10^9$ transformants/μg of pUC19. The capacitance and resistance were set at 25 μF and 200 Ω, respectively. Treatments of yeast cell suspensions were performed in 1.5-mL microcentrifuge tubes as follows. "Grinding" involved vortexing with 300 μL of autoclaved, acid washed glass beads (450-μ diameter; Sigma, St. Louis, MO) for 5 min unless noted otherwise. "Heating" involved incubating at 100°C for 1 min. "Freezing/thawing" involved two cycles of incubation for 1 min on powdered dry ice and 2 min in a 65°C water bath. Treated cells were chilled on ice for at least 2 min before being mixed with *E. coli* and subjected to electroporation. Immediately following electroporation, 1 mL of SOC *(20)* was added, cells were incubated for 1 h at 37°C, plated on LB plates containing 100 μg/mL ampicillin *(20)*, and transformant colonies were scored after a 16–24 h incubation at 37°C. Yeast does not grow on LB plates.

Table 1
Permutations Tested in Dual Pulse Procedures

| Set | *E. coli*, µL | Yeast[a] | Cuvet[b] | First pulse, kV | Second pulse, kV | Permutations[c] |
|---|---|---|---|---|---|---|
| 1 | 20 | Colony | 1, 2 | 1.0 | 2.5 | 2[d] |
| 2 | 40 | Colony | 1 | 1.0 | 2.5 | 1[d] |
| 3 | 40 | Colony | 2 | 1.0, 1.5, 2.0 | 2.5 | 3 |
| 4 | 20, 40 | Streak | 1, 2 | 1.0, 1.5, 2.0 | 2.5 | 12 |
| 5 | 20 | Grinding | 1 | 1.0, 1.5, 2.0, 2.5 | 2.0 | 4 |
| 6 | 20 | Grinding | 2 | 1.5, 2.0 | 2.5 | 2 |

[a]"Colony" indicates direct mixing with *E. coli*. "Streak" indicates that cells from 3 × 20 mm streaks were washed by suspending in 0.5 mL of $dH_2O$, centrifuging at 3000*g* for 1 min, and suspending in 20 µL of $dH_2O$, and then added to *E. coli*. "Grinding" indicates that cells from a 3 × 20 mm streak were vortexed with glass beads, as described in Materials and Methods.

[b]Width of electrode gap in mm.

[c]All permutations were performed once except those in set 6, which were each performed twice.

[d]Parameters that resulted in arcing.

## 3. Results and Discussion

Marcil and Higgins *(16)* described the direct transfer of high copy-number YEp vectors from yeast to *E. coli* using a two-pulse electroporation procedure. The reported transfer efficiencies were high enough to suggest that this procedure would also be effective for direct transfer of low copy-number YCp vectors *(16)*. However, in four attempts, this procedure yielded no *E. coli* DH5α transformants when DH5α cells were mixed with yeast strain DY3028 containing a 7.0-kbp YCp vector (pUraHAC), despite the fact we used electrocompetent *E. coli* with a 1.2–3-fold higher transformation efficiency (as measured using supercoiled pUC19) than used by Marcil and Higgins *(16)*. We reasoned that the observed low transfer efficiency may be owing to the inefficient electrotransfer of plasmid DNA from yeast cells into the yeast/*E. coli* cell suspension or low viability of recipient *E. coli* after two electric pulses. We explored several different approaches for improving plasmid release from yeast cells, including various procedures involving one or two electric shocks, and procedures in which yeast cells were mechanically disrupted prior to mixing with *E. coli* and electroporation. Because transferring low copy number plasmids from yeast to *E. coli* is very efficient if transfer is effected using purified yeast DNA *(12)*, we focused our efforts on procedures that were easier to perform than this existing method. The starting material for all of the experiments described below was strain DY3028 carrying pUraHAC grown for 2–3 d on plates lacking histidine, to insure that most cells carried the unstable plasmid element.

### 3.1. Tests of Dual Electric Pulse Procedures

Transferring plasmid DNA from yeast to *E. coli* using two pulses presumably involves an initial electric field-mediated transfer of plasmid DNA out of yeast cells and a second transfer of liberated DNA into *E. coli*. We performed six sets of experiments with 24 permutations of dual pulse procedures, consisting of 26 tests (Table 1). Yeast from single 2-d-old colonies or streaks (approx 3 × 20 mm) were mixed with 20 or 40 µL of electrocompetent *E. coli* and shocked first with 1.0, 1.5, or 2.0 kV in cuvets with 1- or 2-mm electrode gaps, returned to an ice bath for 1 min, and then shocked a second time with 2.5 kV. Initial pulses had time constants averaging 4.6 ms (range: 4.2–4.8 ms) and second pulses had time constants averaging 4.3 ms (range: 3.8–4.8 ms). Only two of these tests yielded any *E. coli* transformants (Table 1; set 6): one and three transformants were obtained with initial pulses of 1.5 and 2.0 kV, respectively. These results are in sharp contrast to those reported previously using comparable electrical parameters to transfer a 5.6-kbp high copy-number plasmid from yeast to *E. coli (16)*. Because almost none of the tested conditions yielded transformants, these experiments

Table 2
Plasmid Transfer and *E. coli* Viability in Single and Dual Pulse Procedures

| Expt | *E. coli*, μL | Cuvet[a] | V1, T1[b] | V2, T2[c] | Viability[d] | Number of transformants |
|---|---|---|---|---|---|---|
| 1 | 20 | 2 | 2.0 (4.3) | 2.5 (4.3) | 168 | 0 |
| 2 | 40 | 2 | 2.0 (4.2) | 2.5 (4.2) | 132 | 1 |
| 3 | 20 | 2 | 2.0 (arc) | — | — | — |
| 4 | 40 | 2 | 2.0 (3.1) | 2.5 (2.9) | 163 | 0 |
| 5 | 20 | 1 | 1.0 (3.4) | 2.0 (2.9) | 39 | 0 |
| 6 | 20 | 1 | 1.5 (3.8) | 2.0 (3.7) | 23 | 0 |
| 7 | 20 | 1 | 2.0 (3.5) | 2.0 (2.9) | 24 | 0 |
| 8 | 20 | 1 | 2.5 (arc) | — | — | — |
| 9 | 20 | 1 | 1.0 (3.6) | — | 1160 | 2 |
| 10 | 20 | 1 | 1.5 (3.8) | — | 168 | 0 |
| 11 | 20 | 1 | 2.0 (3.5) | — | 63 | 0 |
| 12 | 20 | 1 | 2.2 (3.8) | — | 24 | 0 |

[a]Width of electrode gap in mm.
[b]Voltage in kV (time constant) for first pulse.
[c]Voltage in kV (time constant) for second pulse.
[d]Number of *E. coli* colonies formed on LB plates lacking antibiotics from 100 μL of a $10^4$-fold dilution of transformed cells after 1 h incubation in 1 mL of SOC.

were inconclusive regarding the relative importance of *E. coli* cell volume, or electric field strength (controlled by voltage and electrode gap distance). Some experimental conditions caused samples to arc, especially those with a smaller volume of *E. coli* cells and/or a narrower electrode gap. Samples that arced were not processed further in this or subsequent experiments. All experiments in set 4 failed. Interestingly, the (slightly) successful experiments in set 6 used identical conditions, except that yeast cells were treated by grinding with glass beads before electroporation, indicating that mechanical disruption of yeast might facilitate plasmid transfer. Because transformants were never obtained with yeast colonies, all subsequent experiments were performed using cells from streaks.

To determine whether the low transfer efficiency was a consequence of low *E. coli* viability after two electric pulses, we compared plasmid transfer efficiency and *E. coli* viability using dual and single pulse procedures. In these tests, yeast cells were treated by grinding with glass beads as described earlier. Only two of the experimental conditions yielded transformants (Table 2), indicating that grinding yeast prior to electroporation, though helpful, does not yield transformants reproducibly. Again, varying *E. coli* cell volume and field strengths had no demonstrable effect. These experiments confirmed that *E. coli* cell viability is strongly reduced by dual pulses, and inversely proportional to field strength. Interestingly, the experiment that yielded two transformants involved only a single shock and had the greatest *E. coli* viability (Table 2; experiment 9).

### 3.2. Tests of Single Electric Pulse Procedures

The results described above suggested that plasmid release from yeast cells by mechanical disruption, rather than by an initial electric pulse, might improve transfer efficiency by increasing *E. coli* viability. Sixteen experiments were performed using a single pulse of 2.5 kV, which is presumably optimal for transforming *E. coli*. Three parameters were varied, including the field strength (by using 1- and 2-mm electrode gaps), the yeast cell treatment, and the volume of *E. coli* used. In eight experiments, yeast cells were treated by grinding for 2.5 or 5 min continuously, or for a total of 5 min at 1-min intervals alternating with 1-min incubations on ice. The time con-

Table 3
Effects of Various Yeast Cell Treatments on Single Pulse Plasmid Transfer

| Expt | Yeast treatment[a] | $T^b$ | Viability[c] | Number of transformants |
|---|---|---|---|---|
| 1 | Grinding/heating | 4.3 | 120 | 20 |
| 2 | Grinding/heating | 4.5 | 157 | 6 |
| 3 | Grinding/heating | 4.3 | 193 | 4 |
| 4 | Grinding/heating | 4.3 | 245 | 7 |
| 5 | Heating/grinding | 4.2 | 19 | 0 |
| 6 | Heating | Arc | — | — |
| 7 | Freezing/thawing | Arc | — | — |
| 8 | Freezing/thawing/heating | Arc | — | — |
| 9 | Heating/freezing/thawing | 4.1 | 235 | 0 |
| 10 | Grinding/freezing/thawing | 3.7 | 127 | 6 |
| 11 | Grinding/freezing/thawing/heating | 3.5 | 163 | 1 |
| 12 | Triton X-100/grinding | 4.1 | 30 | 0 |
| 13 | Triton X-100/grinding/heating | 4.0 | 85 | 1 |
| 14 | Triton X-100/heating/grinding | 4.2 | 90 | 0 |

[a]Grinding, heating, freezing, and thawing procedures are described in Materials and Methods; Triton X-100 treatments performed as described in the text. Samples not subjected to grinding were suspended in 20 µL of $dH_2O$.
[b]Time constant; all pulses were at 2.5 kV, 25 µF, and 200 Ω using a 2-mm electrode gap.
[c]Viability assayed as described in Table 2.

stants for these experiments were similar to previous values. Four of these experiments yielded one or two transformants. There was no apparent advantage of one grinding treatment over another, nor was there any effect of varying the two other parameters. The remaining eight experiments involved treating cells with zymolyase, using conditions similar to those used to digest the yeast cell wall during genomic DNA preparation *(21)*. Cells from a streak were washed in 100 µL of 0.9*M* sorbitol, suspended in 10 µL of 0.9*M* sorbitol, 0.1 mg/mL zymolyase 100T (Kirin, Japan), incubated at 37°C for 1 h, and added to *E. coli* and pulsed as described. No transformants resulted when yeast were treated with zymolyase alone, or with zymolyase followed by grinding.

We next examined the effects of 11 different yeast cell treatments on transfer efficiency, involving grinding, heating, and freezing/thawing. We also tested whether cell membrane disruption could be effected by performing some of these physical treatments in the presence of 5% Triton X-100. Triton X-100 was chosen because it is a nonionic detergent and should not affect electrical conductivity or increase arcing. Fourteen experiments were performed with 20 µL of *E. coli*, 2-mm electrode gaps, and a 2.5-kV pulse. Included were four repetitions of a grinding/heating protocol, which was effective and reproducible (Table 3; experiments 1–4). We mapped 12 plasmids rescued by this protocol and all 12 had the expected structure (data not shown). Interestingly, plasmid transfer was inefficient when these steps were reversed, i.e., when heating preceded grinding. Only one other treatment yielded more than one transformant (grinding/freezing/thawing; experiment 10), but no further tests of this protocol were performed. Triton X-100 inhibited plasmid transfer. Triplicate determinations using the grinding/heating protocol indicated that the amount of yeast, in the range of $1–3 \times 10^7$ cells, does not significantly affect transfer efficiency (data not shown).

In a final set of experiments, we tested the efficiency of an initial 1.5-kV pulse of yeast cells in 20 µL of $dH_2O$ without *E. coli*, followed by addition of *E. coli* and a second 2.5-kV pulse. Triplicate experiments using 20 µL of *E. coli* produced no transformants. This "split pulse" protocol was also unsuccessful when yeast cells were treated before the initial pulse by grinding, or by heating, and it was less effective than the single pulse procedure

when yeast cells were treated by grinding/heating, yielding only two transformants in three attempts.

In summary, the steps used for "semidirect" transfer of low copy-number shuttle plasmids to *E. coli* are: grind 1–3 × $10^7$ yeast cells for 5 min, heat to 100°C for 1 min, chill on ice for 2 min, add 20 µL of electrocompetent *E. coli* (competence >5 × $10^9$ transformants/µg of pUC19), transfer the mixed cell suspension to a cuvet with a 2-mm electrode gap, pulse with 2.5 kV, 25 µF, 200 Ω, immediately add 1 mL of SOC, incubate for 1 h at 37°C, and plate using an appropriate antibiotic. This procedure is nearly as fast as direct transfer, and though not as efficient as procedures that use pure yeast DNA, it requires 2–3 d less time, and it is well suited to applications that require rescue of few transformants.

## Acknowledgments

The authors thank Doug Sweetser and Jennifer Whelden for technical assistance. This research was supported by grant CA 55302 to JN from the NIH.

## References

1. Sherman, F. (1991) Getting started with yeast. *Methods Enzymol.* **194,** 3–21.
2. Rose, M., Grisafi, P., and Botstein, D. (1984) Structure and function of the yeast *URA3* gene: expression in *Escherichia coli. Gene* **29,** 113–124.
3. Struhl, K. and Davis, R. W. (1977) Production of a functional eukaryotic enzyme in *Escherichia coli*: Cloning and expression of the yeast structural gene for imidazole-glycerolphosphate dehydratase (his3). *Proc. Natl. Acad. Sci. USA* **74,** 5255–5259.
4. Tschumper, G. and Carbon, J. (1980) Sequence of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. *Gene* **10,** 157–166.
5. Gunn, L., Whelden, J., and Nickoloff, J. A. (1995) Transfer of episomal and integrated plasmids from *Saccharomyces cerevisiae* to *Escherichia coli* by electroporation, in *Electroporation Protocols for Microorganisms* (Nickoloff, J. A., ed.), Humana, Totowa, NJ, in press.
6. Broach, J. R. and Volkert, F. C. (1991) Circular DNA plasmids of yeasts, in *The Molecular and Cellular Biology of the Yeast Saccharomyces cerevisiae* (Broach, J. R., Pringle, J. R., and Jones, E. W., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 297–331.
7. Fitzgerald-Hayes, M., Clarke, L., and Carbon, J. (1982) Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. *Cell* **29,** 235–244.
8. Burke, D. T. and Olson, M. V. (1991) Preparation of clone libraries in yeast artificial chromosome vectors. *Methods Enzymol.* **194,** 251–270.
9. Conley, E. C. and Saunders, J. R. (1984) Recombination-dependent recircularization of linearized pBR322 plasmid DNA following transformation of *Escherichia coli. Mol. Gen. Genet.* **194,** 211–218.
10. Deng, W. P. and Nickoloff, J. A. (1992) Site-directed mutagenesis of virtually any plasmid by eliminating a unique site. *Anal. Biochem.* **200,** 81–88.
11. Ray, F. A. and Nickoloff, J. A. (1992) Site-specific mutagenesis of almost any plasmid using a PCR-based version of unique-site elimination. *Biotechniques* **13,** 342–346.
12. Sweetser, D. B., Hough, H., Whelden, J. F., Arbuckle, M. A., and Nickoloff, J. A. (1994) Fine-resolution mapping of spontaneous and double-strand break-induced gene conversion tracts in *Saccharomyces cerevisiae* reveals reversible mitotic conversion polarity. *Mol. Cell. Biol.* **14,** 3863–3875.
13. Summers, D. K. and Withers, H. L. (1990) Electrotransfer: direct transfer of bacterial plasmid DNA by electroporation. *Nucleic Acids Res.* **18,** 2192.
14. Pfau, J. and Youderian, P. (1990) Transferring plasmid DNA between different bacterial species with electroporation. *Nucleic Acids Res.* **18,** 6165.
15. Kilbane, J. J. and Bielaga, B. A. (1991) Instantaneous gene transfer from donor to recipient microorganisms via electroporation. *Biotechniques* **10,** 354–365.
16. Marcil, R. and Higgins, D. R. (1992) Direct transfer of plasmid DNA from yeast to *E. coli* by electroporation. *Nucleic Acids Res.* **20,** 917.
17. Nickoloff, J. A., Singer, J. D., Hoekstra, M. F., and Heffron, F. (1989) Double-strand breaks stimulate alternative mechanisms of recombination repair. *J. Mol. Biol.* **207,** 527–541.
18. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153,** 163–168.
19. Miller, E. M. and Nickoloff, J. A. (1995) *Escherichia coli* electrotransformation, in *Electroporation Protocols for Microorganisms* (Nickoloff, J. A., ed.), Humana, Totowa, NJ, in press.
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
21. Philippsen, P., Stotz, A., and Scherf, C. (1991) DNA of *Saccharomyces cerevisiae. Methods Enzymol.* **194,** 169–182.

Received September 16, 1994; Accepted October 17, 1994