Direct transfer of plasmid DNA from yeast to *E.coli* by electroporation

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In molecular biology and genetic studies that utilize the budding yeast *Saccharomyces cerevisiae* as a tool, it is common laboratory practice to transform a yeast strain with a plasmid or a library of plasmids in order to study or identify sequences of interest on the plasmid. Further, it is a common laboratory practice to retrieve these chimeric 'shuttle' plasmids out of yeast and back into *E. coli* for additional analysis or manipulation. This retrieval procedure, however, is much more cumbersome and time-consuming than the initial plasmid transfer into yeast (5).

Observations of several groups have demonstrated that electroporation can facilitate the loss of plasmids from bacterial cells as well as their gain (2), and several more specific experiments have demonstrated that simultaneous electroporation of two different bacteria can cause the transfer of plasmid DNA from one to the other (3, 4, 6). Based on these observations we applied this concept to the direct transfer of plasmid DNA from yeast to *E. coli* by electroporation when the yeast cells harboring the plasmid and the recipient *E. coli* cells were electroporated together in the same cuvette.

The plasmid used is this study was pYES2 (5.6 kb with yeast *URA3* gene and 2 μ origin and the *E. coli* ampicillin-resistance gene and ColE1 origin of replication).

We used *E. coli* strain DH5αF', made electrocompetent by the method of Dower, *et al.* (1) with transformation efficiencies of 2 to 5×10^9 transformants per μg of pUC18 plasmid DNA.

The yeast strains used in these experiments were: GRY549, *MATa*::[trp1-488 his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52, (strain from J. Strathern, NCI-FCRDC, Frederick, MD) and INVSc1, *MAT*α his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52.

The protocol for direct transfer of plasmid DNA from yeast to *E. coli* by electroporation using an Invitrogen Electroporator is as follows:

1. Thaw 40 μl of frozen electrocompetent DH5αF' (or comparable) cells on ice.
2. Using a sterile, plastic pipetteman tip, pick a single colony of *Saccharomyces cerevisiae* from a plate that has been incubated for 2 to 3 days at 30°C and mix with the thawed [ps8x]E. coli cells by pipetting up and down 5 or 6 times.
3. Transfer the yeast/bacteria cell mixture to an ice-cold 1 mm gap electrode electroporation cuvette. Keep on ice.
4. Set and charge the Invitrogen Electroporator device at 750 volts, 50 μF, and 100 Ω. These settings will deliver a pulse with a voltage gradient of 7500 V/cm (reproduction of this voltage gradient on any comparable electroporation device should yield similar results). Place the chilled cuvette with the yeast/bacteria cell mixture into the Electroporator and pulse.
5. Immediately following the pulse, remove the cuvette and place back on ice for 30 to 60 seconds. Meanwhile, change the settings on the Electroporator for optimal conditions for *E. coli*: 1500 volts, 50 μF, and 150 Ω.
6. Put the chilled cuvette back into the Electroporator and apply the second pulse.
7. Remove the cuvette and immediately add 1.0 ml of SOC media, mix well, and transfer to a 15 ml Falcon 2059 tube. Place tube at 37°C with vigorous shaking for 1 hour.
8. Plate 50 to 100 μl of the transformation mixture on LB plates containing 50 μg/ml ampicillin and incubate the plates at 37°C overnight. (There is no growth of the yeast cells on these plates even after several days of incubation at 37°C).

A single Ura$^-$ colony of yeast strain GRY549 or INVSc1 harboring plasmid pYES2 was treated as described above and 1000 and 600 ampicillin-resistant *E.coli* transformants, respectively, were generated.

Plasmid DNA was extracted from several of the ampicillin-resistant bacterial colonies and restriction analysis demonstrated that the transferred plasmids were indeed pYES2.

The plasmid pYES2 is 2μ-based and has a copy number of 30 to 50 copies per yeast cell. While we have not tested the direct transfer of low and medium copy number yeast plasmids such as ARS-based and CEN/ARS-based plasmids, given the high efficiencies obtained with this 2 μ-based plasmid we expect lower copy number plasmids to work, even if at lower efficiencies.

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