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**The TRAFO Page**

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**HIGH EFFICIENCY TRANSFORMATION**

**Reference: Gietz, R.D. and R.A. Woods. (2002) TRANSFORMATION OF YEAST BY THE Liac/SS CARRIER DNA/PEG METHOD. Methods in Enzymology 350: 87-96.**

**All Solutions for the LiAc/SS-DNA/PEG TRAFO Protocol are listed in the** [**TRAFO Solutions Page.**](http://home.cc.umanitoba.ca/%7Egietz/Solutions.html)

***High Efficiency Transformation Protocol***

This protocol can be used to generate sufficient transformants in a single reaction to screen multiple yeast genome equivalents for plasmids that complement a specific mutation. It can also be used to transform integrating plasmids, DNA fragments and oligonucleotides for yeast genome manipulation. Finally, it is used to optimise the conditions for transformation of a particular yeast strain, for example, the transformation of a plasmid library into a two-hybrid yeast strain transformed with a bait plasmid by the Rapid Transformation Protocol. The High Efficiency Protocol can also be employed to transform a yeast strain simultaneously with two different plasmids, such as the two-hybrid bait and prey plasmids.

**Day 1**

Inoculate the yeast strain into 5 ml of liquid medium (2x YPAD or SC selection medium) and incubate overnight on a rotary shaker at 200 rpm and 30°C. Place a bottle of double strength YPAD broth (2x YPAD) and a 250 ml culture flask in the incubator as well.

**Day 2**

**1.** Determine the titer of the yeast culture by pipetting 10 ml of cells into 1.0 ml of water in a spectrophotometer cuvette and measuring the OD at 600 nm. For many yeast strains a suspension containing 1 x 106 cells/ml will give an OD600 of 0.1. Alternatively, titer the culture using a hemocytometer. see note:

**Note:**

**i)** Dilute overnight YPAD or SC cultures 10-1 or more in water.

**ii)** Carefully place 10 µl of the cell suspension between the cover slip and the baseof haemocytometer. Let the cells settle onto the haemocytometer grid for a few minutes. The grid area is typically 1 square millimeter, divided into 25 equal-sized squares, and the volume measured is 10-4 ml.

**ii)** Count the number of cells in 5 diagonal squares

**iv)** Calculate the cell titer as follows: cells counted x 5 x dilution factor x 1/volume measured by the 25 squares of the haemocytometer. 239 cells x 5 x 10 (dilution factor) x 1/10-4ml = 1.2 x 108 cells/ml.

**v) *Saccharomyces cerevisiae*** divides by budding from a mother cell. Count budded cells as a single cells. Count cells with equal bud sizes as two cells when there is evidence of additional buds forming on eithercell. Some strains form clumps of cells which reduce plating efficiency. A single clump of cells will only give rise to one colony on a plate, which may complicate further analysis.

**2.** Transfer 50 ml of the pre-warmed 2x YPAD to the pre-warmed culture flask and add 2.5 x 108 cells to give 5 x 106 cells/ml.

**3.** Incubate the flask on a rotary or reciprocating shaker at 30°C and 200 rpm.

**Note:**

**i)** It is important to allow the cells to complete at least two divisions.

**ii)**This will take 3 to 5 hours.

**iii)**This culture will give sufficient cells for 10 transformations.  
**iv)** Transformation efficiency (transformants/ µg plasmid/108 cells) remains constant for 3 to 4 celldivisions.

**4.** When the cell titer is at least 2 x 107 cells/ml, which should take about 4 hours, harvest the cells by centrifugation at 3000 *g* for 5 min, wash the cells in 25 ml of sterile water and resuspend in 1 ml of sterile water.

**5.** Boil a 1.0 ml sample of carrier DNA for 5 min and chill in an ice/water bath while harvesting the cells.

**\*\*\*\*** It is not necessary or desirable to boil the carrier DNA every time. Keep a small aliquot in your own freezer box and boil after 3-4 freeze-thaws. But keep on ice when out.**\*\*\*\***

**6.** Transfer the cell suspension to a 1.5 ml microcentrifuge tube, centrifuge for 30 sec and discard the supernatant.

**7.** Add water to a final volume of 1.0 ml and vortex mix vigorously to resuspend the cells.

**Note:**If the cell titer of the culture is greater than 2 x 107 cells/ml the volume then increase the volume to maintain the titer of this suspension at 2 x 109 cells/ml. If the titer of the culture is less than 2 x 107 cells/ml then decrease volume.

**8.** Pipette 100 µl samples (ca. 108 cells) into 1.5 ml microfuge tubes, one for each transformation, centrifuge at top speed for 30 sec and remove the supernatant.

**9.** Make up sufficient Transformation Mix for the planned number of transformations plus one extra. Keep the Transformation Mix in ice/water.

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| --- | --- | --- | --- |
| **Number of Transformations** | | | |
| **Reagents** | **1** | **5 (6X)** | **10 (11X)** |
| **PEG 3500 50% w/v** | **240 µl** | **1440 µl** | **2640 µl** |
| **LiAc 1.0 M** | **36 µl** | **216 µl** | **396 µl** |
| **Boiled SS-carrier DNA** | **50 µl** | **300 µl** | **550 µl** |
| **Plasmid DNA plus Water** | **34 µl** | **204 µl** | **374 µl** |
| **Total** | **360 µl** | **2160 µl** | **3960 µl** |

10. Add 360 µl of Transformation Mix to each transformation tube and resuspend the cells by vortex mixing vigorously.

11. Incubate the tubes in a 42°C water bath for 40 min.

**Note:**The optimum time can vary for different yeast strains. Please test this if you need high efficiency from your transformations.

12. Microcentrifuge at top speed for 30 sec and remove the Transformation Mix with a micropipettor.

13. Pipette 1.0 ml of sterile water into each tube; stir the pellet by with a micropipette tip and vortex .

**Note:**We like to be a gentle as possible at this step if high efficiency is important. Excessive washing washes away transformants!!!!

14. Plate appropriate dilutions of the cell suspension onto SC selection medium. For transformation with an integrating plasmid (YIp), linear construct or oligonucleotide, plate 200 µl onto each of 5 plates; for a YEp, YRp or YCp library plasmid dilute 10 µl of the suspension into 1.0 ml of water and plate 10 and 100 µl samples onto two plates each. The 10 µl samples should be pipetted directly into 100 µl puddles of sterile water on the SC selection medium.

**Note:**When spreading yeast inoculum onto the plate gently distribute the fluid completely with a sterile glass rod with a minimum of strokes. Allow the fluid to be taken up by the plate prior to incubation.

15. Incubate the plates at 30°C for 3 to 4 days and count the number of transformants.  
The transformation efficiency (transformants/1 µg plasmid/108 cells) can be determined by calculating the number of transformants in 1.0 ml of resuspended cells per 1.0 microgram plasmid per 108 cells. For example, if the transformation of 1.0 x 108 cells with 100 nanogram plasmid resulted in 500 colonies on a plate of SC dropout medium spread with 1 µl of suspension (usually dispensed into a 100µl puddle of sterile water on the plate).  
Transformation Efficiency = 500 x 1000 (plating factor) x 10 (plasmid factor) x 1 (cells/transformation x 108).  
Transformation Efficiency = 5 x 106 transformants/1.0 µg plasmid/108 cells.  
Transformation efficiency declines as plasmid concentration is increased (Gietz et al. 1995) but the actual yield of transformants per transformation increases. For example, 100 nanogram of plasmid in a transformation might give a Transformation Efficiency of 5 x 106 and a yield of 5 x 105 transformants whereas with 1 µg of plasmid the Transformation Efficiency might be 2 x 106 and the yield 2 x 106 per transformation. In order to obtain 5 x 106 transformants it is simpler to set up two or three transformations with 1 µg of plasmid DNA, or a single 3 fold scaled up transformation, than to carry out 10 reactions with 100 ng of plasmid in each.

**Note:** Two plasmids, such as an expression plasmid and a library plasmid pool, can be co-transformed into a single cell by including both plasmids in the same transformation reaction. The efficiency is reduced, however, because only about 30-40 % of all transformed cells take up more than one plasmid molecule. An alternative approach, which has a higher efficiency is to transform in the expression plasmid first, using this or the [**Quick and Easy protocol**](http://home.cc.umanitoba.ca/%7Egietz/Quick.html), and then use the protocol found on the [**2 Hybrid System TRAFO Page**](http://home.cc.umanitoba.ca/%7Egietz/2HS.html) to transform in the library plasmid pool.

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