

Genetic Transformation of Yeast

INTRODUCTION

Transformation is the term used to describe the process whereby a cell takes up foreign DNA that may then be incorporated into its genome and even be expressed. Transformation was first accomplished with bacteria in the early 1900s, but has since been extended to yeast, animal cells, and, with some modifications, even plant cells. In this lab activity, you and others in your lab will attempt to transform several auxotrophic yeast strains (like those studied in a previous lab) using a plasmid that carries a yeast gene (the plasmid was isolated from a bacterial host using the procedure described in a separate lab activity). You will also investigate some factors influencing the efficiency of the transformation process.

The yeast strains you will transform are all auxotrophic for production of the nitrogenous base adenine (which is also considered to be a vitamin, called vitamin B₄). The transformation experiment you will be doing is somewhat like conducting “gene therapy” for yeast cells — you will be trying to provide each mutant strain with a wild-type gene for adenine biosynthesis (enabling it to survive on minimal medium, without added adenine). The wild-type form of the adenine biosynthesis gene is carried on a genetically engineered, bacterial plasmid. Yeast are especially good at taking up and expressing foreign DNA. Even though the plasmid that carries the yeast gene was originally taken from a bacterium, it will be easily taken up and expressed by the yeast used in this activity. However, the efficiency of the transformation process (i.e., the number of cells transformed) will be quite different for different groups of student researchers.

The efficiency of yeast transformation varies significantly from strain to strain, and even somewhat from experiment to experiment when the same strain is used. One possible reason for this variation could be differences in growth rates (between strains and within strains in different experiments). If one strain grows faster than another, or if experimental conditions change, resulting in different growth rates, the number of cells and their physiological condition will also be different. These factors almost certainly affect transformation efficiency. On the other hand, differences besides those related to growth rate may also affect transformation efficiency. For example, different strains may have different kinds of cell surface receptors that may affect their ability to take up foreign DNA.

In this lab activity, different groups of students will attempt to transform different strains of adenine auxotrophs, and each group will attempt the transformation using different volumes of cell suspensions. You will then determine the relative transformation efficiency for each strain, and for each of the volumes used by counting the numbers of colonies produced in each case. To count the numbers of colonies produced, you will need to dilute the various cell samples (i.e., pretransformation cells and each sample of transformed cells) into several concentrations. When the experiment is complete, the entire class will share the data, and you will attempt to determine the relative importance of the differences between strains, as compared to differences in cell numbers.

PROCEDURE

You will conduct the following transformation procedures and prepare cultures to assess transformation efficiency in small student research groups. Different groups will work with different adenine auxotrophic mutants, and each group will attempt three transformations with different volumes of yeast cells.

Step 1: Preparing dilutions to determine the initial concentration of yeast cells — Your instructor will provide you with a sample of an “overnight” yeast culture grown in a solution of complete medium. This solution contains a dense population of auxotrophic yeast cells that will serve as the source of the cells that you will transform. To determine the transformation efficiency for your experiments, you will first need to know the initial concentration of the cells in this overnight culture. You will determine this number by growing three cultures on complete medium at various densities and counting the colonies that result from one of the dilutions (by using three dilutions, you should obtain one that produces an optimum number for counting).

Before preparing the cultures, you will need to dilute your yeast sample so that the concentration of cells will be small enough to allow resulting colonies to be counted. Prepare 1 ml of a 100-fold dilution and 1 ml of a 1,000-fold dilution as follows (using sterile technique as much as possible). Prepare the dilutions in labeled, sterile microcentrifuge tubes using sterile, distilled water.

Make the 100-fold dilution by adding 10 μl of yeast suspension to 990 μl of sterile, distilled water. Make the 1,000-fold dilution by adding 100 μl of the 100-fold dilution to 900 μl of sterile, distilled water. Be sure to fully suspend each of the solutions of cells before taking aliquots for dilution to ensure that a representative density of cells is obtained.

After preparing the two dilutions, you will be ready to prepare your three cultures. First label three culture plates containing complete medium as follows. The labels should include notations to indicate that the medium is complete medium, that the cells are from a specific strain, that they are “nontransformed” cells, and that one of three dilutions, a, b, or c, will be added to the culture medium.

Then add 2 to 3 drops of sterile, distilled water to the surface of the medium in each plate to facilitate spreading the yeast suspension. Next, add the following volumes of your cell dilutions directly into the sterile drops of water: (a) 1 μl of the 1,000-fold dilution, (b) 10 μl of the 1,000-fold dilution, and (c) 10 μl of the 100-fold dilution. Then use a sterile spreading loop to thoroughly spread the yeast cells over the entire surface of each plate.

Also prepare three additional culture plates containing minimal medium as described earlier, using the same concentrations of yeast suspensions, and label the plates appropriately. These will serve as control cultures for comparison to the cultures you will make after transforming the yeast (Step 8).

Place all six cultures described so far (along with the ones you will prepare in Step 8) into a 30°C incubator, if available, or incubate them at room temperature.

Step 2: Remove three aliquots of the overnight yeast culture to be transformed, and place them into labeled, sterile microcentrifuge tubes. Each aliquot will contain a different volume of cells (and, hence, a different number of cells). Be sure to fully suspend the solution of cells before taking

each aliquot to ensure uniform cell density. These separate aliquots will be treated identically in the subsequent transformation steps, and the results will be used to evaluate the effect of varying the number of cells.

One aliquot should contain 500 μl of cells, a second aliquot should contain 1000 μl , and the third aliquot should contain 1500 μl .

Step 3: Place these tubes into a microcentrifuge with the tubes balanced against tubes from other student groups (balance tubes with the same volumes, and ensure that tubes are labeled so that they can be identified). Run the microcentrifuge for 20 seconds. You will see a dense pellet of yeast cells at the bottom of each tube.

Conduct each of the following steps for all three of your samples in an identical manner.

Step 4: Carefully remove the supernatant leaving an undisturbed pellet of cells at the bottom of the tube. You should use a micropipette with a sterile pipette tip to remove the last portion of the remaining solution. If you notice that some cells are being withdrawn during this process, it is better to leave behind a small amount of solution, rather than disturb the pellet.

Step 5: Add 100 μl of a solution that contains the following: 0.2 M lithium acetate, 40% polyethylene glycol-4000, and 100 mM DTT. Resuspend the pellet with a vortex mixer or by repeatedly aspirating the solution with the micropipette.

The lithium acetate affects cell permeability (to enhance DNA uptake), and the polyethylene glycol promotes interactions between the DNA (added in the next step) and the cell surface (the DTT is a chemical stabilizer).

Step 6: Add 10 μl of plasmid DNA and 5 μl of DNA that is called “carrier” DNA to this solution. Add these volumes very carefully, since changes in volume of the DNA will affect transformation efficiency. Be especially careful when pipetting the carrier DNA since it may be quite viscous.

Single-stranded salmon sperm DNA is often used as carrier DNA. The genes on this DNA will not be expressed by the yeast cells, but the presence of this “extra” DNA somehow facilitates the uptake of the plasmid DNA.

Step 7: Vortex this mixture for several seconds (or mix it thoroughly by repeated aspiration with a micropipette) and then place the tube in a floating rack in a 45°C water bath for 30 minutes. This is the final step in the transformation process. Heating the cells and the DNA apparently creates a dynamic condition that promotes DNA uptake.

Step 8: After the 30-minute incubation period, you will prepare two dilute samples for each tube of transformed yeast and spread aliquots from both dilutions onto one or more Petri plates containing minimal medium (without adenine). It is necessary to prepare dilutions so that the cell density will be low enough that single cells can form discrete colonies. You will prepare two dilutions of the transformed yeast because some samples may produce too many transformed cells to count at the higher dilution, while other samples may produce too few to count accurately at the lower dilution. Prepare the dilutions and spread them on Petri plates with minimal medium, according to the following instructions.

Each tube should contain 115 μl of solution (100 μl of transformation solution with yeast cells, 10 μl of plasmid DNA, and 5 μl of carrier DNA). Add enough sterile, distilled water to each sample to bring the volume up to the original aliquot of cells (i.e., add 385 μl to bring the first sample up to 500 μl , add 885 μl to bring the second sample up to 1,000 μl , and add 1,385 μl to the

third sample to bring it up to 1,500 μl). Prepare a second dilution for each sample that is 10 times less concentrated by mixing 100 μl of the first dilution (make sure it's well suspended) with 900 μl of sterile, distilled water.

Now prepare one culture for each dilution of your three samples of transformed cells on minimal medium, as follows. For each culture, add 100 μl of the cell dilution to the surface of the medium, and spread the solution thoroughly over the entire surface. (*Note:* If you wish to obtain a more accurate estimate of the number of transformed cells, you can prepare multiple cultures in the same fashion and count the resulting colonies from each plate.)

Step 9: Place these cultures (along with the ones prepared in Step 1) in a 30°C incubator for 3 to 4 days, or allow them to grow at room temperature for somewhat longer.

Step 10: The following describes the rationale for determining the transformation efficiency for each sample of your yeast strain. Obviously, this analysis must be performed after the incubation step described in Step 9. To determine transformation efficiency, you will need to determine two values: (1) the number of cells per milliliter in the original culture (pretransformation) and (2) the number of transformed cells in each sample. You will only need to determine these values for one dilution in each case. Some dilutions will produce too many colonies to count accurately, and others will produce too few. If possible, select a dilution that yields more than a few colonies per plate, but not one in which the colonies overlap one another. To count the colonies, you should draw a grid on the bottom of the culture plate (using a permanent marker or a wax pencil) and count the colonies in each square of the grid to avoid overlooking any.

The following describes each type of culture that you will generate in this experiment and gives you instructions for collecting and evaluating data from each. In all, you will have at least 12 different cultures (more, if you prepared multiple plates from your transformed cells).

Three cultures will represent pre-transformation cells at three different densities (a, b, and c) on complete medium. You should count the colonies produced by cells at one of these dilutions, and determine *the average number of cells per milliliter* in the original overnight culture (remember to take into account the necessary dilution factors).

Three cultures will represent pre-transformation cells at three different densities (a, b, and c) on minimal medium. You should count the colonies produced (if any) by cells at one of these dilutions, and determine *the average number of cells per milliliter* from the original overnight culture that were able to survive without adenine (remember to take into account the necessary dilution factors).

At least six cultures will be prepared from transformed cells (more, if you prepared multiple plates from these cells). For each volume of cells (500 μl , 1000 μl , and 1500 μl), you will have two cultures prepared from different dilutions. You should count the colonies produced by cells at one of these dilutions, and estimate *the number of cells that were transformed* for each 1 ml (ie., 1000 μl) of the original aliquot. Remember that you only tested a portion of each sample, and you will have to estimate the total number of transformed cells based on the amount you tested. Also, remember to account for the dilution factor if you counted colonies from the diluted sample.

- Q1** What was the average number of cells per milliliter in your overnight sample? Show your calculations, indicating with notes what each number in your calculation represents.
- Q2** A. Did any colonies appear on the minimal medium containing nontransformed cells? If so, how many such cells per milliliter were present in the original overnight culture?
- B. It is reasonable to expect that you may have observed a very small number of nontransformed cells growing on minimal medium. Propose a reasonable hypothesis explaining why this kind of result might be expected to occur from time to time.
- Q3** For the transformed cells, compare the number of cells per ml that survived on minimal medium for the various strains tested by different groups in your class. If any colonies grew on minimal medium for a given strain, subtract the number of colonies per ml that grew on minimal medium from the apparent number of transformed cells per ml. Also compare the number of transformed cells per ml for the different volumes (i.e., cell numbers) for these strains. Prepare a table that shows this information. You will be instructed to evaluate these data in following questions.
- Q4** Is there an apparent difference in the transformation efficiency for the various adenine auxotrophic strains tested by your class? Describe the rationale for your answer to this question, citing specific examples.

- Q5** Is there an apparent difference in the transformation efficiency when different numbers of cells are used (i.e., the three different volumes should have proportionally different numbers of cells)? Respond to the following considerations as you answer this question.

One might expect to see some increase in the number of transformed cells as the initial number of cells used for the transformation increases. Did this trend occur for most (all?, some?, any?) strains tested by your class, or was a different general trend observed? If an increase occurred, was there generally a linear relationship between increases in the initial number of cells and increases in the number of transformed cells? Illustrate the relationship (linear or otherwise) between these factors for several strains by graphing the data (volumes of cell suspensions vs. numbers of cells transformed). Describe what factors (cell density, DNA concentration, etc.) may contribute to the trend you observed and discuss specifically how these factors may be related to the trend (i.e., formulate a hypothesis).

- Q6** If differences in transformation efficiency existed between the different strains studied by your class, do the differences seem to be related primarily to differences in growth rates (as indicated by different cell densities in overnight cultures) or to some other factor(s)? Explain your answer with reference to specific data from the table you prepared in response to Question 3.