How-To-Do-It

Rapid Transformation of a Color Mutant of Yeast

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One of the most important techniques of modern molecular biology is the ability to introduce a specific gene into an organism and have that genetic information expressed by the organism. This process, called **genetic transformation**, played a critical role in the history of molecular biology and the discovery that DNA was the genetic material. Four scientists, Griffith in the 1920s and Avery, McCarty and MacLeod in the 1940s, showed that the apparent "transformation" of one bacterial type into another required DNA and no other biological molecule.

Today, transformation is not limited to bacteria and, in fact, is used routinely by molecular biologists to manipulate and study the behavior of genes. As an educational tool, it offers the most direct demonstration of the action of a gene. In this paper we describe a transformation technique which is rapid (it can be completed in a single class period), convenient, relatively inexpensive, and uses a safe, easy-to-grow organism—bakers' yeast *(Saccharomyces cerevisiae)*. As a model system yeast has the advantage over bacteria of being a eukaryotic organism with subcellular organization like that of plants and animals, including humans.

Yeast transformation involves four steps. These are:

1. Establishment of **competence**, a state in which the cells become able to take up DNA from their surroundings
2. Uptake of DNA by competent cells
3. A heat shock which by unknown means stimulates the establishment of the added DNA
4. A recovery period in which the cells return to a normal metabolic state and begin to express the genes on the transforming DNA.

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After completion of these four steps, the yeast will express the new characteristics or **phenotype** of the added DNA and will be said to have become transformed.

Our system employs a yeast strain which is defective in a gene, *ADE1*, required in the biosynthesis of the DNA component adenine. (See Manney & Manney 1992 for a full discussion of the adenine biosynthetic pathway.) The metabolic defect in the mutants causes them to require adenine for growth and to accumulate a red pigment that causes the colonies to appear pink or red in color. When these *ade1* mutant yeast are transformed with the normal *ADE1* gene, they become able to grow in the absence of adenine and form colonies that are the normal creamy-white color which is characteristic of bakers' yeast. Thus, this system provides a color indicator of transformation "free of charge," in contrast to the expensive chemical color indicators used in bacterial systems.

The DNA molecules used in this transformation system are plasmids. These are small, circular, independent genetic elements. They behave in many ways like real chromosomes that carry genes in cells, but the plasmids are much more convenient. We have developed two types of plasmids carrying the yeast *ADE1* gene (the cloned *ADE1* gene used in these plasmids was provided by Dr. David Kaback of the University of New Jersey School of Medicine and Dentistry). The YEpADE1 plasmid exists in multiple copies in each transformed yeast cell; however, it is unstable and can be lost from transformed yeast at relatively high frequency. The YCpADE1 plasmid exists in one copy in a haploid yeast cell, just like the normal chromosomes, and, since it carries a yeast centromere, will be stably sorted into the daughter cells each time the yeast divides. Either plasmid can be used effectively to demonstrate the phenomenon of transformation; the differences in behavior of the two plasmids allow the option of further experiments (described below) that illustrate the properties of plasmids and chromosomes.

Background

As part of the GENE (Genetics Education Networking and Enhancement) Project, we collaborated to develop a yeast transformation protocol that would be appropriate in the secondary classroom. (See Manney & Manney 1992 for a description of other components of the GENE project.) Our goals were to develop a simplified protocol that could be accomplished with relatively limited materials and within a normal class period of 50–55 minutes. We have tested this protocol extensively with high school biology students and beginning university students. The protocol has proven to be robust and dependable. The volumes given in the protocol are provided to facilitate quantitation. If quantitation is not desired, then several of the volumes can be approximated. Likewise, the timings for the various incubations can be adjusted to fit various class periods, and there are a number of procedural modifications that can be employed to streamline the experiment if time is very limited.

Materials

- *Saccharomyces cerevisiae* culture, red haploid yeast strain with adenine *(ade1)* mutation
- Plasmid DNA/carrier mix, for each student or team: 1 μg YEpADE1 or YCpADE1 plus 20 μg heat-denatured salmon sperm DNA (prepared as described in Schiestl & Gietz 1989 and in Appendix)
- Media: 1 petri plate of YED agar (a non-selective medium: 1% Difco Bacto-Yeast extract; 2% dextrose; 2% agar) per 10–20 students or teams; 1–3 plates of MV agar (selective medium for growth of strains containing plasmids: 0.15% Difco Bacto-Yeast nitrogen base without amino acids and ammonium sulfate; 0.52% ammonium sulfate; 2% dextrose; 2% agar) for each student or team.
- Sterile microfuge tubes, 1.5 mL
- Sterile pipets, micropipets, or microcapillary tubes
- Sterile flat toothpicks
- Sterile water (distilled)
- Glass spreaders*
- Ethanol*
- Flame source*
- Centrifuge to centrifuge microfuge tubes (microfuge or clinical)
- Water bath (42° C) and styrofoam raft
- Sterile solutions:
  - LiAc/TE solution [for 100 mL: 10 mL 1M LiAc stock solution + 1 mL 1M Tris-HC1 stock solution + 1 mL 0.1M EDTA stock solution + 88 mL distilled water; autoclave]
  - TE solution [for 100 mL: 1 mL 1M Tris-HC1 stock solution + 1 mL 0.1M EDTA stock solution + 98 mL distilled water; autoclave]
  - 40% PLT solution [for 200 mL: 160 mL 50% PEG stock solution + 20 mL 1M LiAc stock solution + 2 mL 1M Tris-HC1 stock solution + 2 mL EDTA stock solution + 16 mL distilled water; autoclave]
  - 1M LiAc stock solution: 10.2 g LiAc.2 H_2O + 80 mL distilled water; adjust pH to 7.5 with acetic acid; autoclave.
  - 1M Tris-HCl stock solution: 12.1 g Trizma base + 80 mL distilled water; adjust pH to 8.0 with HCl; adjust volume to 100 mL with distilled water and autoclave.
  - 0.1M EDTA stock solution: 3.72 g ethylenediamine tetraacetic acid, disodium salt + 80 mL distilled water; adjust pH to 7.0 with sodium hydroxide; adjust volume to 100 mL with distilled water and autoclave.
  - 50% PEG stock solution: 100 g polyethylene glycol 4000 plus enough distilled water to adjust volume to 200 mL when dissolved; autoclave.

*See asterisked portion of text below for alternative flameless plating method that does not require use of these items.

Note: The yeast strain, media, and an instructional videotape may be ordered from Dr. Tom Manney, Department of Physics, Cardwell Hall, Kansas State University, Manhattan, KS 66506-2601, (913) 532-6789. The *Handbook for Using Yeast To Teach Genetics* may be purchased by sending a check for $10 or an institutional purchase order for $15 made out to KSU. Chemicals and powders to make the solutions and media may be purchased from a standard supplier such as Sigma (cost of solutions for 40 transformations is approximately $5.50; ingredients for 4 YED plates and 120 MV plates would be about $20). Kits containing pre-mixed solutions, plasmid DNA/carrier mixtures, the yeast strain, and media are available from Bio101, 1060 Joshua Way, Vista, CA 92083, (619) 598-7299. Bio101 also supplies all kit components individually. In the future, bulk discount prices for solutions may be available through the Genetics Society of America; contact the first author for additional information.

Procedure

1. Isolate single colonies of the appropriate yeast strain on a YED plate by streaking for isolation of single colonies with sterile toothpicks. Incubate for about 3 days until colonies are well grown. One plate should provide enough colonies for up to 20 teams (2–4 students per team) of students. Plates can be grown several days in advance and stored in the refrigerator.
2. Using the flat end of a sterile toothpick, scrape about 2–3 colonies from the agar and suspend in 0.5 mL of LiAc/TE solution in a sterile microfuge tube. The suspension should be quite turbid. The LiAc/TE solution assists in making the yeast cells competent. For a discussion of competence and the uptake of DNA in transformation in bacterial systems, see Micklos & Freyer 1990. The basic protocol used in this paper was modified from that of Schiestl & Gietz 1989.
3. To a new sterile microfuge tube, add 21 μL of the plasmid DNA/carrier mix. The YEpADE1 or YCpADE1 plasmid DNA is mixed with a nonspecific, sonicated or fragmented DNA that serves as a carrier. This carrier DNA makes the transformation more efficient and probably helps to protect the plasmid DNA from cellular nucleases that would otherwise destroy the plasmid. If desired, a control tube can be prepared that contains either no DNA or no plasmid (i.e. carrier DNA only).
4. To the DNA tube(s), add 0.2 mL per tube of the yeast/LiAc/TE suspension from Step 2. Be sure that the yeast are well suspended before adding them. Discard the remainder of the yeast/LiAc/TE suspension.
5. To the DNA/yeast suspension(s), add 1.2 mL of 40% PLT solution per tube and mix well by inverting several times. Incubate at room temperature for approximately 20 minutes. The 40% PLT solution helps to force the plasmid DNA into the competent yeast cells. This is followed by a heat shock treatment to complete the process.
6. Heat shock the DNA/yeast/PLT suspension(s) for a minimum of 5 minutes (no longer than 15 minutes) in a 42° C water bath. A suitable water bath can be maintained in most classrooms with careful adjustment of hot and cold faucets. Float the microfuge tubes on the surface of the water with a styrofoam raft cut from the bottom of a styrofoam cup. The plasmids have entered the yeast cells by this stage of the procedure.
7. Centrifuge the suspension to pellet the yeast cells. For a microfuge this will require about 5 seconds. For a standard clinical, bench-top centrifuge, the hinge will need to be removed from the microfuge tube so that it will fit into the centrifuge. Pelleting the yeast will require about 3–5 minutes of centrifuging in a clinical centrifuge.
8. Discard the supernatant (liquid solution) from the microfuge tube(s). Resuspend the pelleted yeast cells by adding 1 mL of TE solution. Tap gently, and use a sterile toothpick or vortex to

mix. The TE solution is used to replace the LiAc and PLT solutions for plating.

9. Plate 0.2 mL of yeast suspension to each of one or two MV plates (selective media) per DNA tube, either directly, by pipeting and spreading with a bent glass rod sterilized in flaming alcohol, or * by pipeting 0.2 mL into a tube containing 0.8 mL of sterile water and pouring the entire contents onto the surface of the agar plate. Distribute the suspension over the surface by tilting and rotating the plate to spread the cells. If there are places the liquid did not cover, use the blunt end of a sterile toothpick to guide the suspension to cover those areas. MV medium provides only minimal nutrients, an energy source and vitamins. The yeast strain used in this procedure requires adenine and will not grow on MV. The addition of the plasmid with the functional *ADE1* gene allows the growth of the transformants on MV. The control salmon sperm DNA will not support growth on MV and no transformants will be obtained.
10. Incubate for 3–5 days at room temperature or in a 30° C incubator.

Results

Transformants will appear as creamy-white colonies. Numbers will range from 10–200 per plate. The color change and novel ability to grow on minimal medium indicate the action of the introduced *ADE1* gene in the transformed yeast. The plasmid loss extension described below is an easy experiment to show that the transformed characteristics are reversible.

Extensions

Plasmid Loss

Under selective pressure (i.e. the absence of adenine), transformants that lose the unstable YEpADE1 plasmid will not survive. This means that a stable, white phenotype of transformants is maintained. The YEpADE1 plasmid is not stable under nonselective conditions because it is independent of the yeast's chromosomes and is not necessarily evenly distributed during mitosis. Removing the selective pressure allows survival of those cells that have lost the plasmid. Resulting colonies will be red or red-sectored (Manney & Manney 1992). There are two ways to show that the transformants are dependent on growth under selective conditions (MV): Take one of the white transformant colonies and either streak it onto or make dilutions and plate onto nonselective media, YED, that contains adenine. [Directions for streaking for single colonies and serial dilutions and plating are given in Manney & Manney 1990.] The same experiment carried out with transformants containing the YCpADE1 plasmid will show much less loss, since this plasmid is more stable.

Mutagenic Effects of Ultraviolet Light

Transformation can be used to demonstrate the lethal and mutagenic effects of ultraviolet light by irradiating the plasmid DNA before using it for transformation. This will require consistent quantitation of the plasmid DNA used in the transformation technique but would serve as an excellent individual student project. The number of transformants per microgram quantity of plasmid DNA can serve as a measure of the mutagenic effects of various UV doses.

Appendix

Recombinant DNA Safety Considerations

Experiments using yeast plasmids and yeast host strains do not present a significant risk to health or the environment and are exempt from federal *Guidelines for Research Involving Recombinant DNA Molecules* under Section III-D-5.

Preparation of Heat-Denatured Salmon Sperm DNA

Dissolve 100 mg salmon sperm DNA (Sigma Chemicals D1626, Type III Sodium Salt from Salmon Testes) in 10 mL of TE. This may require incubation at 4° C (refrigerator) overnight. Sonicate the suspension twice for 30 seconds each at 0.75 power using a large-bore probe. The shearing should reduce the viscosity. Extract once each with TE-saturated phenol, TE-saturated phenol/chloroform-isoamyl alcohol, and chloroform-isoamyl alcohol (24:1). Precipitate the DNA by adding 0.1 volume of 3M sodium acetate, pH 6.0 and 2.5 volumes of ice-cold 95% ethanol. Vortex well and centrifuge to pellet the white mass of DNA. Dry the pellet under vacuum and resuspend in TE at 10 mg/mL. Denature by heating in a boiling water bath for 20 minutes and then immediately cooling in ice. Store the DNA in aliquots at −20° C (freezer).

Time-Saving Tips

1. For teachers with short class periods or who lack access to many sets of micropipetors, etc., we suggest pre-aliquoting the DNAs and LiAc/TE (1x) solutions in sterile microfuge tubes. This saves a great deal of time in measuring out these reagents during the class period.
2. Plastic bulb-type dropping pipets are available presterilized and are easier for beginners to use than conventional pipets in measurement of the PLT and TE solutions.
3. Save time and flaming by using bent wires or paper clips presterilized in aluminum foil packets for spreading the yeast on plates.

Acknowledgments

This work was supported in part by grants DTE-8954638 and ESI-9252889 from the National Science Foundation. We thank Dr. David Kaback for providing the cloned *ADE1* gene.

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