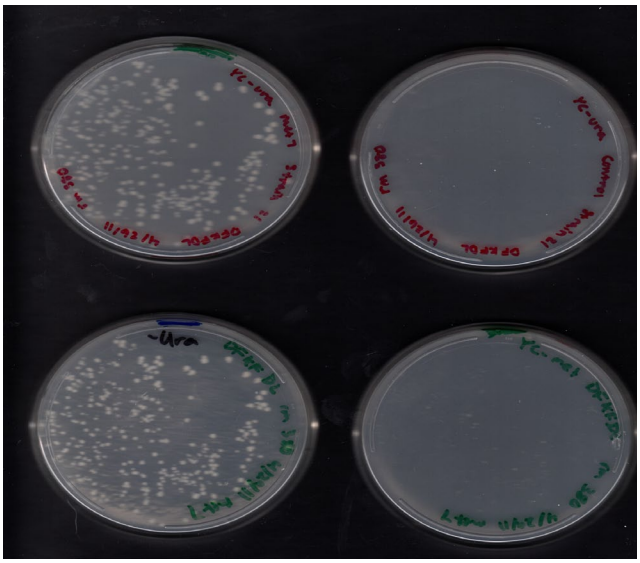
Chapter 12

Yeast Transformation



Techniques for transforming microbial organisms with foreign DNA are essential in modern molecular biology. In this lab, you will transform a *S. cerevisiae met* strain with three different plasmids and use *ura3* complementation to detect transformed cells. You will then use replica plating to determine if *S. pombe Met* genes are functionally equivalent to *S. cerevisiae MET* genes.

Objectives

At the end of this lab, students should be able to:

- explain the processes of transformation and complementation at the molecular level.
- design a selection strategy to isolate transformed strains
- transform *S. cerevisiae* with plasmids and isolate transformants on selective media
- use replica plating to analyze the ability of plasmid-encoded *MET* genes to complement *met* deficiencies

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In this experiment, you may receive a preliminary answer to the semester's research question about the functional conservation of Met proteins in the Ascomycota . During the first part of the semester, your team used selective plating and colony PCR to identify yeast deletion mutants. You then isolated and characterized plasmids that can be used to overexpress Met proteins. These two sets of experiments come together in this lab, when you transform the *S. cerevisiae* deletion strain with the expression plasmids. Through a series of complementation experiments, you will determine if the genes carried on the plasmids are able to compensate for the missing *MET* genes in the mutants. In complementation, the introduced gene restores the normal phenotype to a mutant with a defective gene.

Transformation alters the phenotype of a cell

Transformation refers to the uptake of DNA by a cell, causing a change in its phenotype. Naturally-occurring transformation was first described in 1928 by Frederick Griffith, who described a heat-stable transforming principle from virulent *Streptococcus pneumoniae* that could transform non-virulent *S. pneumoniae* to an encapsulated, virulent form. The transforming principle was subsequently identified as DNA by Avery and colleagues in 1944. Since then, transformation has become an indispensable tool in the molecular biology laboratory. The physical basis for yeast transformation is still incompletely understood, but researchers have empirically developed conditions that give fairly consistent transformation in the lab. Reliable transformation techniques have been developed for bacteria and many eukaryotes, ranging from yeast to mammalian cells.

Transformation conditions have been developed empirically

The challenge in laboratory transformation is to devise conditions under which DNA will pass across the cell wall and plasma membrane of living cells, which are normally impermeable to DNA. Very few cells are naturally competent, or able to take up DNA on their own. Consequently, researchers use a variety of chemical treatments to render cells competent. In general, these chemical treatments have some kind of destabilizing effect on the plasma membrane. The introduction of DNA into these competent cells can be further encouraged by a physical stress, such as a pulse of electric current or temperature elevation. Transformation is not a very efficient process, but because large numbers of microorganisms can be cultured in the laboratory, useful numbers of transformants can be obtained with most microorganisms.

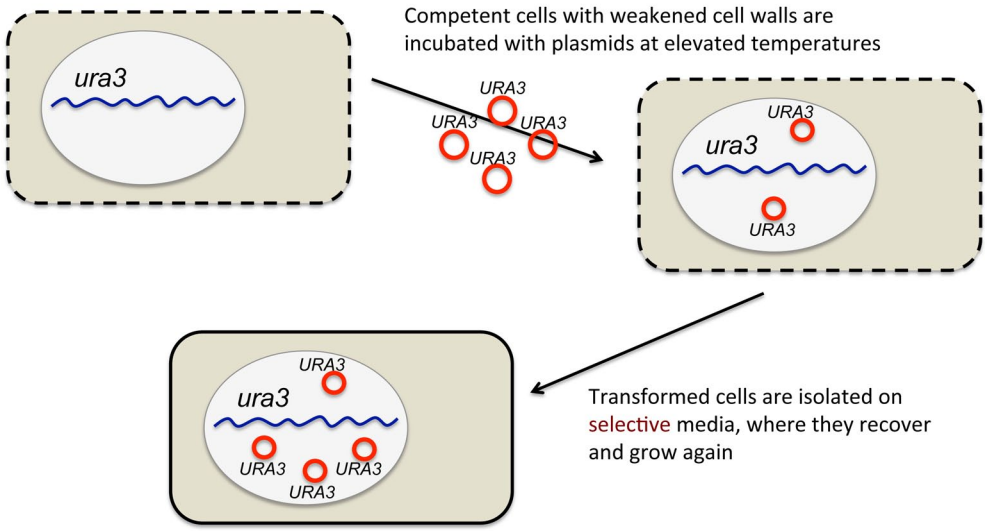
Techniques for yeast transformation are now standard in the laboratory. Depending on the details of the experimental procedure, reactions can yield as many as 10^6 transformants per µg DNA. The structure of the DNA used for transformation greatly affects the transformation efficiency. Transformation efficiencies are considerably higher with supercoiled plasmid DNA than with linear pieces of DNA, possibly because plasmids enter the cell more readily and/or plasmids are less susceptible to endonuclease digestion.

The most commonly used yeast transformation methods use a combination of lithium acetate, single-stranded carrier DNA and polyethylene glycol (PEG). Although no one knows

exactly how these components promote transformation, a number of hypotheses have been advanced. Lithium ions neutralize the negative charges on DNA molecules to be transformed and the phospholipid bilayer of the yeast cell, and they may also generate small holes in the plasma membrane that allow the passage of nucleic acids. Single-stranded DNA acts as a carrier for the plasmid DNA to be transferred into the cell and it may help to protect the latter from endonucleases. The source of the carrier DNA is unimportant. Since the carrier DNA concentration is considerably higher than that of the DNA to be introduced into the cell, the carrier DNA is usually isolated from an inexpensive source, such as salmon sperm. ***It is imperative that the carrier DNA for transformations be single-stranded.*** In our experiments, we will boil the carrier DNA for 5 minutes and then rapidly chill it to prevent reanneling of the DNA helix. PEG may help bring the DNA into closer apposition with the membrane. PEG is often used to promote membrane fusion and is thought to alter water structure around plasma membranes.

Complementation is often used to isolate transformants

The DNA used for transformation must carry a selectable marker whose presence can be detected by screening. Following a transformation, cells are plated on selective media that will allow transformed, but not untransformed, cells to grow. All the pBG1805- (Gelperin *et al.*, 2005) and pYES2.1-derived plasmids that we are using carry a normal copy of the yeast *URA3* gene, as well as the *URA3* promoter, so the gene is regulated much like a normal chromosomal gene. Our yeast deletion strains were derived from strain BY4742, which has the *ura3Δ0* allele (Winzeler *et al.*, 1999) Complementation will occur because the plasmid carries a functional copy of the gene that is defective in the mutant host strain. The Ura3p protein produced from the plasmid-encoded *URA3* gene compensates for the *ura3* deletion in the yeast chromosome, allowing transformed cells to grow in the absence of uracil, as shown below. Because of its reliability, many yeast transformation schemes rely on *URA3* complementation to isolate transformants.



Transformation and plasmid complementation
Competent *ura3* yeast cells are transformed by incubating cells with a plasmid containing the yeast *URA3* gene at an elevated temperature. Transformed cells are selected on media that does not contail uracil.

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Experimental considerations

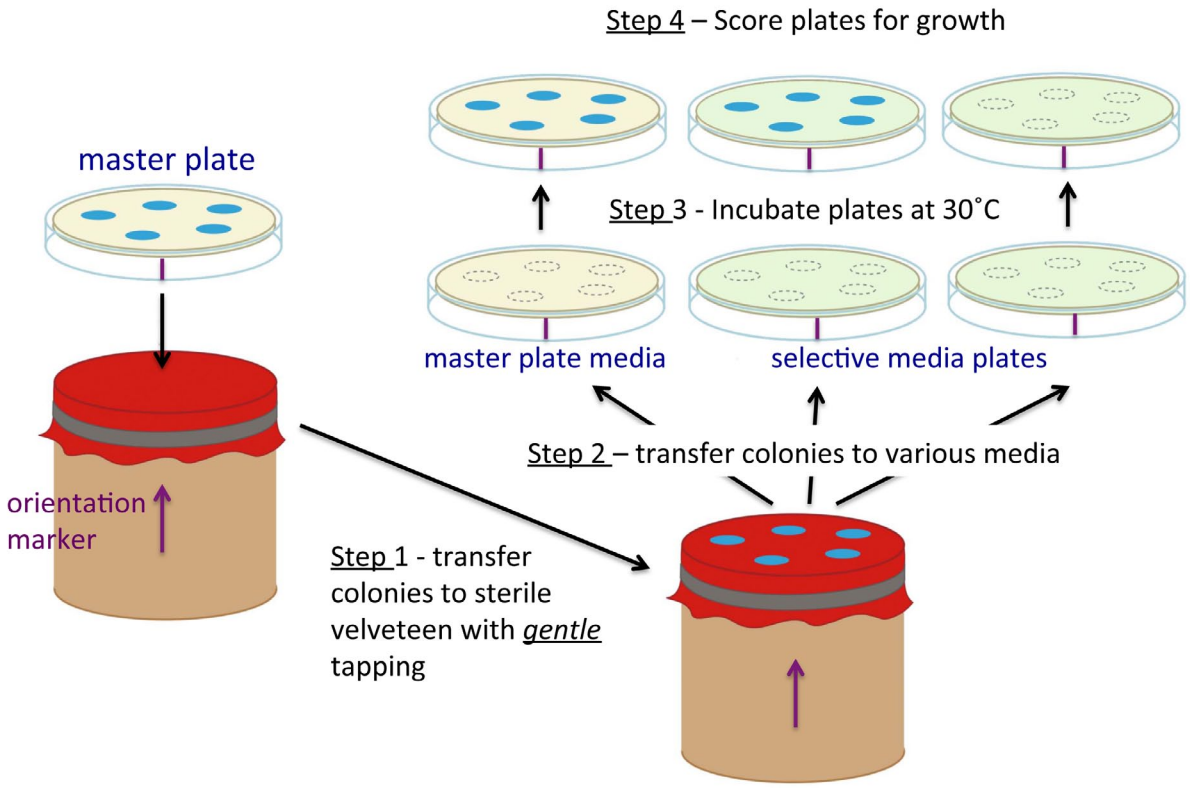
You may be wondering why we are not using *MET* gene complementation to isolate transformants, since this is the goal of our semester's project. There are several reasons why we are using YC-Ura plates, rather than YC-Met plates, to isolate tranformants. First, we need to ensure that the overexpression plasmids have successfully transformed the deletion strains and it is possible that the Met fusion proteins encoded by the plasmids will be unable to complement the *met* deficiencies in transformants. *URA3* gene complementation offers a well-tested and reliable means to assess successful transformation that is independent of methionine metabolism.

A second issue relates to uncertainties associated with regulation of the plasmid ORFs by the *GAL1* promoter (Johnston, 1987). The *GAL1* promoter is an inducible promoter that is normally repressed when cells are grown in glucose and induced when galactose replaces glucose as the carbon source. In its normal chromosomal location, the *GAL1* promoter responds to a variety of positive and negative transcription regulators (Chapter 13). Although a large number of studies have established that the *GAL1* promoter functions well in ectopic locations, such as plasmids, the promoter is not as tightly regulated in plasmids as in the yeast chromosome. Some of this difference may relate to copy number. Both pBG1805 and pYES2.1 are multi-copy plasmids.

Following the isolation of transformants of YC-Ura plates, you will analyze *MET* gene complementation on YC-Met plates containing either D-glucose and D-galacatose. Keep in mind that galactose and glucose may not function as simple "ON" and "OFF" switches because the regulatory balance is altered in transformed cells. It is possible, for example, that "leaky" gene transcription could occur in the presence of the normal repressor, D-glucose. In this case, *MET* genes would complement *met* mutants grown in D-glucose. It is also possible that transformed cells could produce excessive quantities of Met and proteins that are detrimental, or even fatal, to transformed cells.

Replica plates accelerate the screening process

As noted above, transformation is an inefficient process, so researchers want to make the most of every cell that has been transformed. In our experiments, we will be isolating transformed cells for their ability to grow in the absence of uracil, but we are really interested in their ability to grow in the absence of Met. Replica plating offers a means to quickly screen a plate of cells for their ability to grow in a wide range of media, while retaining information about individual colonies. As shown on the opposite page, the original plate of transformants becomes the "master plate." An imprint of the master plate is made by *GENTLY* tapping the inverted plate on a piece of sterile cotton velveteen immobilized on a block. This imprint can then be transferred to plates with different kinds of selective media, establishing the genotype of the transformants. In our experiments, we will make transfer replicas of the transformation reactions (isolated on YC-Ura plates) to YC-Ura plates that are also lacking Met, with either glucose or galactose as a carbon source.



Replica plating provides a rapid screening method for analyzing phenotypes.
Colonies on a master plate are transferred to a sterile piece of velveteen. Copies of the mater plate are transferred to additional selective or indicator media to monitor phenotypes under additional conditions.

Exercise 1 - Yeast transformation

The following protocol is a slight modification of the "Quick and Dirty" transformation protocol described by Amberg *et al.* (2005). With careful attention to detail and cooperative strains, this procedure can yield thousands of transformants per µg plasmid DNA. Modifications to this method can increase its efficiency by several orders of magnitude (Gietz and Schiestl, 2007), which would be required if linear pieces of DNA were being used to tranform yeast.

Prepare a transformation master mix

1. Prepare a transformation master mix. The following ingredients provide enough reagents for five transformation reactions. Combine and mix in a microcentrifuge tube:

    100 µL sterile 2 M lithium acetate (freshly prepared)
    400 µL sterile 50% PEG-3350
    4 µL 2-mercaptoethanol (STINKY!! add this in the fume hood!)

Set up individual transformation reactions - <u>for each transformation</u>:

2. Add 15 µL of the denatured salmon sperm DNA (2 mg/mL) to a new microcentrifuge tube ***labeled*** with the name (or code) of the plasmid.

Note: It is important for the salmon sperm DNA to be single-stranded for this procedure to work well. Boil the DNA for 5 minutes to denature the DNA. Quick chill the DNA by placing it immediately on ice. Keep the DNA on ice until you are ready to use it.

3. Add 5 µL of miniprep plasmid DNA to the appropriately labeled microcentrifuge tube.
4. Add 100 µL of transformation mix from step 1 to each microcentrifuge tube. Vortex for 10-15 seconds to mix the contents.
5. Using a sterile toothpick or micropipette tip, scrape a large yeast colony (or the equivalent of a "match head" of yeast) from a YPD plate. Transfer the yeast to the microcentrifuge tube containing the transformation/DNA solution (step 4) by twirling the toothpick several times. Be sure that the cells are uniformly suspended before proceeding.

Repeat steps 2-5 for each of your transformation reactions. ***Be sure to include a control that contains no plasmid DNA.***

6. Incubate the transformation mixtures at <u>37˚C</u> with shaking for <u>30-45</u> minutes.

Plate the transformed cells on selective media lacking uracil.

7. Remove 10 µL of the resuspended cells and add them to 90 µL of sterile water in a microcentrifuge tube. This sample will be serially diluted for a spot plate (step 9) that you will use to calculate the transformation efficiency.
8. Spread the remainder of the mixture on a selective media plate lacking uracil. Transfer the transformation reaction to the plate, and then shake out ~4 sterile glass beads that will spread the cells. Cover the plates and spend 0.5-1 minutes agitating the plates so that the beads spread the transformation mixture evenly over the surface of the plate. Discard the glass beads into the appropriate waste containers, so they can be sterilized and used again. Incubate the plates at 30˚C until colonies can be detected. The earliest that colonies will be visible is usually 2 days. If the colonies are small, allow them to grow an additional day(s) at 30˚C. Count the number of colonies on the plate.

Determine the number of viable cells in the transformation mixture.

9. Prepare a series of 4 additional dilutions of the cells set aside in step 7. Use these dilutions for a spot plate on YPD media. Each row on the plate should contain cells from a different transformation reaction. Incubate the cells at 30˚C or room temperature until individual colonies can be detected. ***Do not allow the plate to overgrow, because you need to distinguish individual colonies.***

Calculate the transformation efficiency. The efficiency of transformation is influenced by both the quality of the DNA used and the precise details of the transformation procedure.

10. ***Calculate the fraction of cells that were transformed as shown below.*** The total volume of transformation mixture was ~120 µL, including yeast cells. Ten µL was used for spot plating and the remaining 100 µL was used for the transformation.

$$\text{Fraction cells transformed} = \frac{\text{Transformed cells (cell on YC-Ura plate)}}{\text{Number of cells in the transformation mixture}}$$

Calculate the number of cells in the transformation mixture (N) from the spot plate:

$$N = \text{\# colonies in most dilute spot (5 µL)} \times \text{Dilution factor} \times (22)$$

correct for spot plate dilutions

110 µL of transformation mixture plated on YC-Ura

11. Transformation efficiencies are usually expressed by the number of cells transformed per µg DNA. In the last lab (Chapter 11), you analyzed your plasmid preparations on agarose gels and obtained a rough estimate of the DNA concentrations of your plasmid preparations. Note that you analyzed 7 µL of plasmid prep on those gels. In this transformation lab, you used 5 µL of your plasmid preps.

Calculate the transformation efficiency:

A. Multiply that number of transformed cells on the YC-plate by 1.1
(Only 100 out of 110 µL in the transformation reaction were plated.)

B. Convert the ng of plasmid in your transformation reaction to µg.

C. Divide the calculated value in A by that in B.

Exercise 2 - Replica plating and complementation

This exercise will be performed at the next lab session after transformants will have had a chance to grow.

Your initial selection of transformants was done on plates that lacked uracil, but contained methionine. Next, you will test the ability of your transformed strains to grow on media lacking methionine using replica plating. We will use methionine-free media containing either glucose or galactose for replicas, and you will also prepare a fresh master plate. Predict which transformants will grow on each of the plates.

It is important to have a light touch during replica plating!! The goal is to transfer a small portion of cells from each colony on the master plate (the plates carrying your transformants) to a number of plates containing different media.

1. Place an orientation mark with a Sharpie on the perimeter of your master plate as well as the plates that will be used for replicas.

2. Remove the lid from your master plate and invert the plate on the block, aligning the orientation marker on the plate with the marker on the block. *GENTLY* and *EVENLY* tap on the bottom of the plate to transfer cells to the velveteen. Remove the master plate and replace the lid. You should see faint white colonies on the velveteen.

3. Repeat step 3 with plates containing the following media:
   - Medium without uracil or methionine, containing glucose
   - Medium without uracil or methionine, containing galactose
   - Medium without uracil, containing glucose and methionine

Test yourself

An investigator transforms strain BY4742 (*MATα his3-Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met6::KAN*^{R}) with the plasmids listed in the table below. The transformants are selected on appropriate media and then replica plated to the media listed in the table below. Complete the table below by indicating when cells will grow (+ or Y) or not grow (- or N).

Plasmid	**YC**	**YC-His**	**YC-Leu**	**YC-Lys**	**YC-Ura**	**YC-Met**
pYES2.1-*MET6 LYS2*						
pYES2.1-*met6Δ2 HIS3*						
pBG1805-*MET3 LEU1*						
pBG1805-*HIS3 LEU2*						
pYES2.1-*HIS2 LEU2 LYS2*						
pBG1805-*LEU2 LYS3*						

References

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Gelperin DM, White MA, Wilkinson ML *et al.* (2005) Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. *Genes Develop* **19:** 2816-2826.

Gietz RD & Schiestl RH (2007) High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nat Protoc* **2**: 31-34.

Johnston, M (1987) A model fungal regulatory mechanism: the *GAL1* genes of *Saccharomyces cerevisiae*. *Microbiol Rev* **51:** 458-476.

Winzeler, EA, Shoemaker, DD, Astromoff, A *et al.* (1999) Functional characterization of the *Saccharomyces cerevisiae* genome by gene deletion and parallel analysis. *Science* **285:** 901-906.