S. c. EasyComp™ Transformation Kit

For the preparation and transformation of competent *S. cerevisiae*

Catalog no. K5050-01

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Important Information

Introduction

The S. c. EasyComp™ Transformation Kit is a simple method to rapidly produce highly competent *Saccharomyces cerevisiae* cells that can be used immediately or frozen and stored for future use. Transformation efficiencies with *Saccharomyces* will vary based on the strain used. In general, transformation efficiencies of $>10^3$ transformants per µg plasmid DNA are obtained.

Kit Components

The *S. c*. EasyComp™ Transformation Kit contains reagents for 6 preparations of competent cells. Each competent cell preparation yields enough cells for 20 transformations.

Component	Purpose	Quantity
Solution I	Wash solution	60 ml
Solution II	Lithium cation solution for making cells competent	6 ml
Solution III	Transformation solution	60 ml

Shipping/Storage

The *S. c*. EasyComp™ Transformation Kit is shipped at room temperature. Store solutions at +4°C.

Product Qualification

The *S. c*. EasyComp™ Transformation Kit is qualified by the preparation and transformation of INVSc-1 cells. Fresh and frozen INVSc-1 cells were made competent and transformed with the kit reagents. Competent cells were transformed with pYES2 vector and selected on Ura^- plates. Fresh and frozen cells must demonstrate $>10^3$ transformants/µg DNA.

Methods

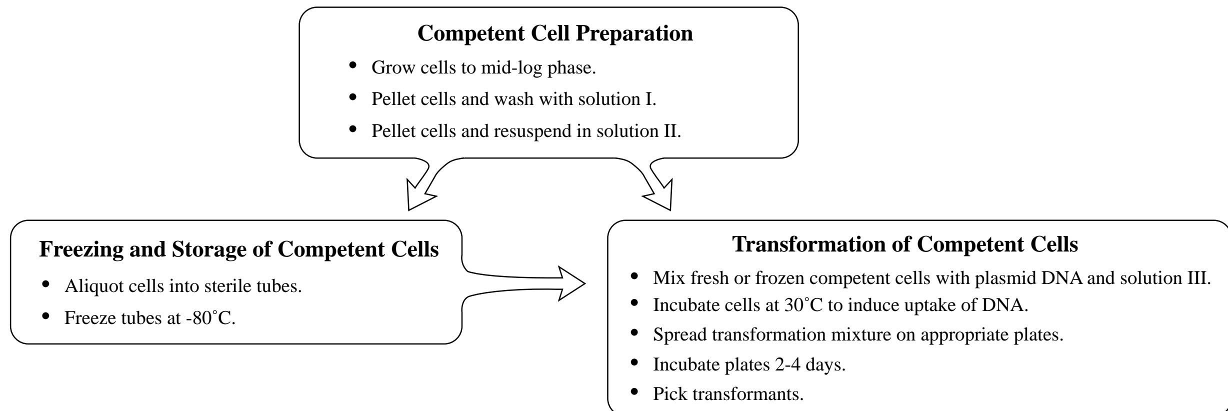
Preparation of Competent Cells

Introduction

The following procedure is for the preparation of competent *S. cerevisiae* cells that can be transformed with plasmid DNA.

Experimental Outline

An outline of the steps required to produce and transform competent *S. cerevisiae* cells is presented below.



Required Reagents and Equipment

- 30°C rotary shaking incubator
- For most yeast strains, use YPD (Yeast Extract Peptone Dextrose) medium (see Media Recipes, page 5). For strains with nutritional requirements, add appropriate supplements (e.g., for Ade^- strains such as L40, add adenine to a final concentration of 0.01%).
- 50 ml, sterile conical tubes
- Centrifuge suitable for 50 ml conical tubes (floor or table-top)
- 1.5 ml sterile screw-cap microcentrifuge tubes
- -80°C freezer
- Styrofoam box or paper towels

Before Beginning

1. Streak a YPD plate with your *S. cerevisiae* strain such that isolated, single colonies will grow. Incubate the plate at 28-30°C for 2 days.
2. Equilibrate Solutions I and II to room temperature.

Continued on next page

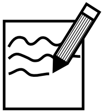
Preparation of Competent Cells, continued

Preparing Competent Cells

1. Inoculate 10 ml of YPD with a single colony of your *S. cerevisiae* strain. Grow overnight at 28-30°C in a shaking incubator (250-300 rpm).
2. Determine the OD_{600} of the overnight culture. It should be between 3.0 and 5.0.
3. Dilute cells from the overnight culture to an OD_{600} of 0.2 to 0.4 in a total volume of 10 ml of YPD.
4. Grow the cells at 28-30°C in a shaking incubator until the OD_{600} reaches 0.6 to 1.0. This will take approximately 3 to 6 hours.
5. Pellet the cells by centrifugation at 500 x g (1500 rpm) for 5 minutes at room temperature. Discard the supernatant.
6. Resuspend the cell pellet in 10 ml of Solution I.
7. Pellet the cells by centrifugation at 500 x g (1500 rpm) for 5 minutes at room temperature. Discard the supernatant.
8. Resuspend the cell pellet in 1 ml of Solution II. Cells are now competent and can be used immediately for transformation (page 3) or stored for future use (page 2).

Freezing and Storage of Competent Cells

1. To freeze cells, aliquot 50 to 200 µl of competent cells into labeled 1.5 ml sterile screw-cap microcentrifuge tubes. **Note:** 50 µl of cells are used for each transformation. Cells can be thawed and refrozen several times without significant loss in transformation efficiency.
2. Place tubes in a Styrofoam box or wrap in several layers of paper towels. Place in a -80°C freezer. **Note:** It is important that the cells freeze down slowly to avoid damage to the cell wall. Do not snap-freeze the cells in liquid nitrogen.



Note

We have observed that higher transformation efficiencies are often obtained with frozen versus freshly prepared cells. You may choose to use some of the cells immediately following preparation and freeze the remaining cells in small aliquots.

Transformation of Competent Cells

Introduction

The following protocol can be used to transform either freshly prepared or frozen competent *S. cerevisiae* cells. Transformation efficiencies may vary with each strain and vector used. Special instructions are included if you are using Zeocin™-resistant vectors and yeast strains from Invitrogen's Hybrid Hunter™ Two Hybrid Kit (Catalog no. K5000-01).

Required Reagents and Equipment

- 30°C incubator or water bath
- Microcentrifuge at room temperature
- Appropriate selective plates for your strain and plasmid. For example, if you are using Zeocin™ resistant yeast plasmids from Invitrogen, please see page 5 for recipes for the appropriate selective plates.

Before Beginning

1. Equilibrate Solution III to room temperature.
2. Equilibrate the appropriate number and type of plates to room temperature. You will need one plate for each transformation.
3. You may want to include controls to check for contamination. We recommend "minus DNA" and "plasmid-only" controls.

Transformation Protocol

1. For each transformation, thaw one tube of competent cells at room temperature and aliquot 50 µl into a sterile microcentrifuge tube, or use 50 µl of fresh competent cells.
2. Add 1 µg of vector DNA to the competent cells. **Note**: Using up to 5 µg of DNA may increase transformation efficiencies in some cases. The volume of DNA should not exceed 5 µl.
3. Add 500 µl of Solution III to the DNA/cell mixture and mix by vortexing vigorously or by flicking the tube.
4. Incubate the transformation reactions for 1 hour in a 30°C water bath or incubator. Mix the transformation reaction every 15 minutes by vortexing vigorously or by flicking the tube. Failure to mix the transformation reaction every 15 minutes will result in decreased transformation efficiency.

   If transforming with vectors containing the Zeocin™ resistance gene, proceed to Step 5. For all other vectors, proceed to Step 9.
5. Add 1 ml of YPD (or YPAD if using the yeast strain L40) to each tube. Incubate the cells in a 30°C shaker for 1 hour to allow expression of Zeocin™ resistance.

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Transformation of Competent Cells, continued

Transformation Protocol, continued

6. After the 1 hour recovery time, pellet the cells by centrifugation at 3000 x g for 5 minutes at room temperature. Discard the supernatant.
7. Resuspend the cell pellet in 100 to 150 µl of appropriate medium, TE, or Solution III.
8. Using a sterile spreader, plate the entire transformation on appropriate plates containing Zeocin™. Proceed to Step 10.
9. For all vectors except those containing the Zeocin™ resistance gene, plate 100 µl of the transformation reaction from Step 4 on appropriate selection plates using a sterile spreader.
10. Incubate the plates for 2 to 4 days at 30°C. Each transformation should yield approximately 100 colonies depending on the strain you are using.

Troubleshooting

The table below provides solutions to possible problems you may encounter when preparing and transforming competent *S. cerevisiae* cells.

Problem	Probable Cause	Possible Solution
Low efficiency of transformation	Transformation reaction not mixed during incubation	Be sure to vortex the transformation reaction every 15 minutes throughout the 1 hour incubation.
	Incubation time is too short or temperature is too low.	Transformations may be incubated for longer periods of time (up to 3 hours) and at higher temperature (35-37°C). This may, in some instances, result in higher transformation efficiencies.
	Cell density is too low (OD_{600} <0.6)	Resuspend cells from Preparing Competent Cells, page 2, Step 8, in a smaller volume (i.e. 500 µl)

Appendix

Media Recipes

Stock Solutions

10X D (20% Dextrose)

Dissolve 200 g of dextrose (D-glucose) in 800 ml of water. Bring the volume up to 1000 ml. Autoclave for 15 minutes or filter sterilize. Store at +4˚C. The shelf life of this solution is approximately one year.

YPD (YPAD) ± Zeocin™

Yeast Extract Peptone Dextrose Medium (± Adenine, ± Zeocin™) (1 liter)

1% yeast extract
2% peptone
2% dextrose (D-glucose)
± 0.1 g adenine
± 300 µg/ml Zeocin™

1. Dissolve the following in 900 ml of water:
   10 g yeast extract
   20 g of peptone
   (0.1 g adenine, if using L40 or other Ade$^-$ strain)
2. Autoclave for 20 minutes on liquid cycle.
3. Add 100 ml of 10X D.
4. If desired, cool the solution to <60°C and add 3.0 ml of 100 mg/ml Zeocin™ just prior to use.

Store medium at room temperature. The shelf life is approximately two months.

YPD (YPAD) Agar ± Zeocin™

1% yeast extract
2% peptone
2% dextrose (D-glucose)
2% agar
± 0.1 g adenine
± 300 µg/ml Zeocin™

1. Dissolve the following in 900 ml of water:
   10 g yeast extract
   20 g of peptone
   (0.1 g adenine, if desired)
2. Add 20 g of agar.
3. Autoclave for 20 minutes on liquid cycle.
4. Add 100 ml of 10X D.
5. If desired, cool the solution to <60°C and add 3.0 ml of 100 mg/ml Zeocin™.

Store YPD plates at +4˚C. If Zeocin™ has been added, store plates in the dark. The shelf life is one month.

Technical Service

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3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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Technical Service, continued

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References

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