Pichia EasyComp™ Kit

For the preparation and transformation of competent *Pichia* cells

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Important Information

Introduction

The *Pichia* EasyComp™ Kit uses a simple method to rapidly produce competent *Pichia* cells that can be used immediately or frozen and stored for future use. Transformation efficiencies with *Pichia* will vary based on the strain used and the efficiency of plasmid integration into the host chromosome. In general, transformation of 50 µl of competent cells with 3 µg of linearized plasmid DNA will yield approximately 50 colonies.

Kit Components

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

Component	Description	Quantity
Solution I	Sorbitol solution containing ethylene glycol and DMSO for the preparation of competent cells	75 ml
Solution II	PEG solution for the transformation of competent cells	150 ml (2 x 75 ml)
Solution III	Salt solution for washing and plating transformed cells	150 ml (2 x 75 ml)

Shipping/Storage

The *Pichia* EasyComp™ Kit is shipped at room temperature. Store Solutions I and III at +4°C. Store Solution II at room temperature.

Product Qualification

The *Pichia* EasyComp™ Kit is qualified by the preparation and transformation of GS115 with the Pichia expression vector pPICZα A. Transformation of 50 µl of competent GS115 cells with 3 µg of linearized pPICZα A vector must yield at least 30 colonies following growth on YPD+Zeocin plates.

Preparation of Competent Cells

Introduction

This procedure is for the preparation of competent *Pichia pastoris* cells. The cells produced with the *Pichia* EasyComp™ Kit can be transformed immediately or frozen and stored for future use.

Required Reagents and Equipment

- 30°C rotary shaking incubator
- YPD (Yeast Extract Peptone Dextrose) medium (see **Media Recipes**, page 4)
- 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

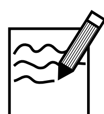
Streak a YPD plate with your *Pichia pastoris* strain such that isolated, single colonies will grow. Incubate the plate at 28-30°C for 2 days.

Equilibrate Solution I to room temperature.

Protocol

1. Inoculate 10 ml of YPD with a single colony of your *Pichia* strain. Grow overnight at 28-30°C in a shaking incubator (250-300 rpm).
2. Dilute cells from the overnight culture to an OD_{600} of 0.1-0.2 in 10 ml of YPD. Grow the cells at 28-30°C in a shaking incubator until the OD_{600} reached 0.6-1.0. This will take approximately 4 to 6 hours.
3. Pellet the cells by centrifugation at 500 x g for 5 minutes at room temperature. Discard the supernatant.
4. Resuspend the cell pellet in 10 ml of Solution I. No incubation time is required.
5. Pellet the cells by centrifugation at 500 x g for 5 minutes at room temperature. Discard the supernatant.
6. Resuspend the cell pellet in 1 ml of Solution I. The cells are now competent.
7. 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.
8. At this point, the cells may be kept at room temperature and used directly for transformation or frozen for future use. To freeze cells, place tubes in a Styrofoam box or wrap in several layers of paper towels and place in an -80°C freezer. It is important that the cells freeze down slowly. **Do not snap-freeze the cells in liquid nitrogen.**
9. Proceed to the transformation procedure.



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 *Pichia* cells. Transformation efficiency may vary with each strain and vector used.

Required Reagents and Equipment

- 30°C incubator
- Water baths or heat blocks at 30°C and 42°C
- Microcentrifuge at room temperature
- RDB plates [for vectors containing the *HIS4* gene (i.e. pPIC9), see **Media Recipes**, page 4]
- YPDS with 100 µg/ml Zeocin™ plates [for vectors containing the Zeocin™ resistance gene (i.e. pPICZ), see **Media Recipes**, page 4]

Before Beginning

The PEG in Solution II may precipitate at temperatures below 27°C. If you see a precipitate, warm the solution at 37°C, swirling occasionally, until the precipitate dissolves. To prevent formation of a precipitate, store Solution II at room temperature.

Equilibrate Solution III to room temperature.

Equilibrate the appropriate number and type of plates to room temperature. You will need one plate for each transformation.

You may want to include controls to check for contamination. We recommend a no DNA and a plasmid only control.

Transformation Protocol

1. For each transformation, thaw one tube of competent cells at room temperature and aliquot 50 µl into a sterile microcentrifuge tube. If transforming fresh cells use 50 µl of cells from **Preparation of Competent Cells,** page 1, step 7.
2. Add 3 µg of linearized *Pichia* expression vector DNA to the competent cells. See the *Pichia* Expression Kit manual or the appropriate *Pichia* Expression Vector manual for information regarding sites and protocols for linearization.

   Note: Using greater than 3 µg of DNA may increase transformation efficiencies in some cases. The volume of DNA should not exceed 5 µl. Linearized DNA can be used directly from a restriction digest reaction without affecting transformation efficiency. Phenol chloroform extraction and ethanol precipitation are not necessary.
3. Add 1 ml of Solution II to the DNA/cell mixture and mix by vortexing or flicking the tube.
4. Incubate the transformation reactions for 1 hour at 30°C in a water bath or incubator. Mix the transformation reaction every 15 minutes by vortexing or flicking the tube. Failure to mix the transformation reaction every 15 minutes will result in decreased transformation efficiency.
5. Heat shock the cells in a 42°C heat block or water bath for 10 minutes.
6. If transforming with a Zeocin-resistant plasmid, split the cells into 2 microcentrifuge tubes (approximately 525 µl per tube) and add 1 ml of YPD medium to each tube. Incubate the cells at 30°C for 1 hour to allow expression of Zeocin™ resistance. For all other vectors, proceed directly to step 7.
7. Pellet the cells by centrifugation at 3000 x g for 5 minutes at room temperature. Discard the supernatant.

Continued on next page

Transformation of Competent Cells, continued

Transformation Protocol, continued

8. If transforming with pPICZ or pPICZα, resuspend each tube of cells in 500 µl of Solution III and combine the cells into one tube. For all other vectors, resuspend the cells in 1 ml of Solution III.
9. Pellet the cells by centrifugation at 3000 x g for 5 minutes at room temperature. Discard the supernatant.
10. Resuspend the cell pellet in 100 to 150 µl of Solution III.
11. Plate the entire transformation on appropriate selection plates using a sterile spreader. Incubate the plates for 2 to 4 days at 30°C. Each transformation should yield approximately 50 colonies.

Troubleshooting

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

Problem	Probable Cause	Possible Solution
Low efficiency of transformation	The pH of Solution I or Solution III may have drifted. The pH of both solutions should be 8.0	Check the pH of Solutions I and III. If the pH is low, increase it by adding NaOH. If the pH is high, decrease it by adding HCl. Store solutions at +4°C in order to minimize drift in pH.
	Transformation reaction not mixed during incubation	Be sure to mix the transformation reaction every 15 minutes throughout the 1 hour incubation at 30°C. Vortexing works best.
	Incubation time is too short or temperature is too low.	*Pichia pastoris* 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 **Preparation of Competent Cells**, step 6, page 1, in a smaller volume (i.e. 500 µl)

Media Recipes

Stock Solutions

10X YNB (13.4% Yeast Nitrogen Base w/Ammonium Sulfate w/o amino acids)

Dissolve 134 g of yeast nitrogen base (YNB) with ammonium sulfate and without amino acids in 1000 ml of water and filter sterilize. It may be necessary to heat in order to dissolve YNB. Store at +4°C. The shelf life of this solution is approximately one year.

500X B (0.02% Biotin)

Dissolve 20 mg biotin in 100 ml of water and filter sterilize. Store at +4°C. The shelf life of this solution is approximately one year.

10X D (20% Dextrose)

Dissolve 200 g of dextrose (D-glucose) in 1000 ml of water. Autoclave for 15 minutes or filter sterilize. The shelf life of this solution is approximately one year.

100X AA (0.5% of each Amino Acid)

Dissolve 500 mg each of L-glutamic acid, L-methionine, L-lysine, L-leucine, and L-isoleucine in 100 ml of water. Filter sterilize and store at +4°C. The shelf life of this solution is approximately one year.

Zeocin™

Zeocin™ is available from Invitrogen. Please refer to the table below for ordering information.

Item	Amount	Catalog no.
Zeocin™	1 g	R250-01
	5 g	R250-05

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Media Recipes, continued

YPD

Yeast Extract Peptone Dextrose Medium (1 liter)

1% yeast extract
2% peptone
2% dextrose (D-glucose)

1. Dissolve the following in 900 ml of water:
   10 g yeast extract
   20 g of peptone
2. Autoclave for 20 minutes on liquid cycle.
3. Add 100 ml of 10X D.

Store the medium at room temperature. The shelf life is several months.

YPDS/Zeocin™ Agar

Yeast Extract Peptone Dextrose Sorbitol Medium with Zeocin™ (1 liter)

1% yeast extract
2% peptone
2% dextrose (D-glucose)
1 M sorbitol
100 µg/ml Zeocin™

1. Dissolve 10 g yeast extract, 20 g of peptone, 182.2 g of sorbitol in 900 ml of water.
2. Add 20 g of agar.
3. Autoclave for 20 minutes on liquid cycle.
4. Add 100 ml of 10X D.
5. Cool the solution to ~60°C and add 1.0 ml of 100 mg/ml Zeocin™. Pour plates.

Store YPDS plates with Zeocin™ at +4°C in the dark. The shelf life is one to two weeks.

RDB Agar

Regeneration Dextrose Medium (1 liter)

1 M sorbitol
1% dextrose
1.34% YNB
4 x 10^{-5}% biotin
0.005% amino acids

1. Dissolve 186 g of sorbitol in 700 ml of water and add 20 g of agar.
2. Autoclave 20 minutes on liquid cycle.
3. Cool and maintain the liquid medium in a 60°C water bath.
4. Prepare a pre-warmed (45°C) mixture of the following stock solutions:
   100 ml of 10X D
   100 ml of 10X YNB
   2 ml of 500X B
   10 ml of 100X AA
   88 ml of sterile water
5. Add the mixture from Step 4 to the sorbitol solution. Pour plates immediately. Store RDB plates at +4°C. The shelf life is several months.

Technical Service

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http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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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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3E Company
Voice: 1-760-602-8700

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