BIO 101 Protocol

WHOLE CELL YEAST PCR KIT

2016-200 **200 preps**

- **1 hour enzymatic reaction in PCR tubes or 96 well microplate followed by PCR**
- **Release plasmid, YAC, and genomic DNA from yeast cells for PCR analysis**
- **No phenol/chloroform extractions**
- **No alcohol precipitation**
- **No centrifugation**
- **Positive control (primers/control yeast to amplify genomic actin DNA**

Shipping & Storage:

The kit is shipped at room temperature. However, upon receipt the ENZYME MIXX, CONTROL ACTIN PCR PRIMER SET (both lyophilized powder) should be stored at -20ºC. The slant containing control yeast BIO 96 should be stored at 4ºC. After the ENZYME MIXX is mixed with the ENZYME BUFFER the resulting WHOLE CELL LYSIS SOLUTION is stored at -70ºC insingle use aliquots. The reconstituted control actin primer is stored at -20ºC.

"Check with BIO 101... We have you time in mind!"

BIO 101

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Whole Cell Yeast PCR Kit Protocol

Kit Components

ENZYME MIXX*[1]		2016-201
ENZYME BUFFER, Sterile	1.1 ml	2016-202
CONTROL YEAST BIO 962	slant	2016-205
CONTROL ACTIN PCR PRIMER SET*	125 reactions	2016-206

*Store at -20ºC. To reconstitute see protocol.
[1] Lyophilized Powder
[2] Store at 4ºC.

Introduction

The WHOLE CELL YEAST PCR KIT is designed to release DNA (plasmid, YAC, and genomic DNA) from whole cells (colonies or liquid cultures) for PCR analysis. Using a proprietary Enzyme Mixx, the tough yeast cell wall is disrupted in a single one-hour incubation and the resulting cell suspension is used directly in the PCR reaction. Such a treatment is essential to give reproducible PCR results without purifying the DNA first.

Optimal performance is obtained when Molecular Biology CertifiedTM yeast media from BIO 101 is used. This is particularly important if yeast liquid cultures are directly used without pelleting first.

Preparation of reagents before first use

Before first use, prepare the WHOLE CELL LYSIS SOLUTION, CONTROL ACTIN PCR PRIMER SET and a frozen stock of CONTROL YEAST.

I. Preparation of WHOLE CELL LYSIS SOLUTION:

a. Gently tap ENZYME MIXX vial on the bench top to collect enzyme powder at the bottom of the vial.
b. Resuspend ENZYME MIX with the contents of ENZYME BUFFER tube (1.1 ml). Gently mix to dissolve at room temperature for 10 minutes or less. Do not heat, even though the solution may appear grainy at this point.
c. Aliquot in quantities that will be convenient for your needs. For example, if you expect to PCR 10 samples at once, freeze aliquots of 50 µl (5 µl/sample).
d. Freeze aliquots at -70ºC. To ensure maximum efficiency, thaw aliquots once and use entire contents. Do not refreeze. Enzyme is stable for 6 months after reconstitution.

II. Preparation of Control Actin PCR Primer Set.

a. Spin tube containing primer set for 5 minutes at room temperature to collect the lyophilized oligos at the bottom of the tube.
b. Add 140 µl of autoclaved H2O to get a 10 µM stock solution.
c. Spin for 10 minutes at room temperature.
d. Store at -20ºC.

III. Preparation of a frozen stock for control yeast BIO 96.

a. Inoculate a 5 ml YPD culture and grow overnight at 30ºC with shaking.
b. Transfer 800 µl to a sterile microfuge tube, add 200 µl sterile 80% glycerol and
invert to mix.
c. Store at -70ºC.

To revive cells, scoop some off the frozen surface, streak onto a YPD plate and incubate at 30ºC until growth is observed. Do not allow the frozen cells in the storage vial to thaw.

Protocol

Note: To use Positive PCR Control, streak new YPD plate with control yeast each time. A grid is included on page 6 that can fit up to 100 yeast colonies on the same plate for easier processing of multiple samples. Optimal results are obtained with fresh colonies / cultures. If using liquid cultures, it is essential to use high-quality ingredients (Molecular Biology CertifiedTM yeast media is available from BIO 101).

1. Yeast colonies or pelleted cells:
   Lightly touch a fresh yeast colony or a cell pellet from a liquid culture with a sterile, disposable pipette tip and transfer to a microfuge tube or a PCR tube (or a 96 well microplate if processing multiple samples) containing 5 µl WHOLE CELL LYSIS SOLUTION. Suspend the cells by gently swirling with pipette tip while avoiding the formation of bubbles. (Transfer ~ 1/4 of a 2 mm yeast colony. Cells must be a homogenous suspension for optimal results.)

   Yeast liquid cultures:
   Transfer 1 µl cells from an overnight culture to a microfuge tube or a PCR tube (or a 96 well microplate if processing multiple samples) containing 5 µl of WHOLE CELL LYSIS SOLUTION. Pipette up and down or gently swirl to ensure a homogeneous cell suspension.

2. Incubate at 37ºC for 1 hour and use the resulting cell suspension (NOT the clear supernatant) for a 100 µl PCR reaction. To lyse cells, include a 10-15 minute incubation step at 95ºC as the first PCR step. Spin the completed PCR reaction for 2 minutes before to loading the gel in order to collect the cell debris at the bottom of the tube.

PCR cycle conditions to amplify genomic actin DNA from control yeast.
5 µl CONTROL YEAST BIO 96 (processed as above)
5 µl CONTROL ACTIN PCR PRIMER SET
dNTP to 200 µM
10 µl 10x Buffer with MgCl2
H2O to 100 µl
Mix and incubate 10-15 minutes at 95ºC. Add 5 units of Taq Polymerase and start a 40 cycle PCR reaction.
Reaction Conditions:
[95ºC 1 minute, 50ºC 1 minute, 72ºC 1 minute]40 cycles [72ºC 4 minutes]
Store at 4ºC.
Expected product size ~700 bases

Trouble shooting guide

No PCR signal

1. Insufficient amount of yeast cells used. Upon resuspension of the transferred cells in whole cell lysis solution the resulting suspension should be somewhat cloudy and not clear.

2. Cells with damaged cell walls may have settled to the bottom of the tube; resuspend and use cell suspension in the PCR reaction (do not use the clear supernate which contains few cells).

3. Inefficient cell wall damage, thus no lysis at 95ºC: increase the incubation time at 37ºC in step 2 and use a fresh colony/culture.

Notes

Product Use Limitation & Warranty

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General Information

BIO 101 is a pioneer in developing kits for molecular biology research. We introduced the **GENECLEAN®** Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

Technical Support and Ordering Information

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