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Protocol

Zymolyase-Treatment and Polymerase Chain Reaction Amplification from Genomic and Plasmid Templates from Yeast

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Here, we present a protocol for amplifying DNA fragments from the genome of, or plasmids transformed into, yeast strains that require the use of the lytic enzyme zymolyase to break open the yeast cells by digesting the cell wall. Yeast strains requiring such treatment include YM4271 and Y1HaS2, whereas other yeast strains (e.g., MaV103) might not require treatment with Zymolyase.

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MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Agarose gel (1%, w/v, in 0.5× TBE buffer)
DNA molecular mass markers
dNTP solution (PCR grade)
Primers (various, specific for desired template)
Taq DNA polymerase (thermostable) and associated 10× PCR buffer

We have found that Taq *DNA polymerase from Invitrogen (10342-053) works robustly.*

TBE buffer <R>
Water (sterile, PCR grade)
YAPD plates (150-mm) <R> or selective medium

YAPD medium is yeast extract peptone dextrose (YEPD) medium with extra adenine, which is added because the yeast strain used has mutations in the adenine synthesis pathway. Growing on YAPD, rather than on selective medium, increases the efficiency for amplifying the reporter constructs from the yeast genome of integrated yeast one-hybrid (Y1H) DNA-bait strains, but colonies grown on selective medium are suitable for amplifying from plasmid templates.

Zymolyase suspension <R>

Equipment

Incubator (set at 30°C)
PCR plates (96-well) or PCR tubes (0.2-mL)
Thermal cycler

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Toothpicks and/or pipette tips (sterile)
Tubes (sterile; 1.5-mL)

METHOD

1. Grow yeast on appropriate plates (e.g., solid YAPD or selective medium) overnight at 30°C in an incubator.
2. For each yeast colony, aliquot 15 µL of zymolyase suspension into a sterile polymerase chain reaction (PCR) tube (0.2 mL) or the wells of a 96-well PCR plate.

   Because Zymolyase has low solubility in water, it is important to mix the suspension thoroughly before and periodically (every 30 sec) during distribution into the tubes or wells.
3. Using a sterile toothpick or pipette tip, transfer a small amount (approximately one-eighth of a match head) of each yeast colony grown in Step 1 to each Zymolyase aliquot from Step 2.

   Too much yeast will inhibit the PCR reaction.
4. Transfer the PCR tubes or plate to a thermal cycler and incubate the yeast–enzyme mix for 30 min at 37°C. Then heat-inactivate the enzyme for 10 min at 95°C.
5. Remove the PCR tubes or plate from the thermal cycler and dilute the lysate by adding 85 µL of sterile PCR-grade water.

   The lysate can be stored at −20°C to await the subsequent PCR reactions.
6. For each amplification, in a sterile PCR tube (or well of a PCR plate) prepare the following PCR reaction mix:

Diluted lysate (from Step 5)	5 µL
Forward primer (20 µM)	1 µL
Reverse primer (20 µM)	1 µL
dNTPs (1 mM)	5 µL
10× PCR buffer	5 µL
Taq (or other thermostable) DNA polymerase	2 units
Water	to 50 µL

   Remember to include a negative-control PCR reaction that lacks template.
7. Place the tubes in a thermal cycler and run the following PCR program, as tabulated below.

Cycle number	Denaturation	Annealing	Polymerization
1	2 min at 94°C		
35 cycles	1 min at 94°C	1 min at 56°C	1 min per kb of sequence to be amplified at 72°C
Last cycle			7 min at 72°C

   The conditions of the PCR reaction might need to be optimized.
8. Run 5–10 µL of the PCR reaction from Step 7 on a 1% (w/v) agarose gel in 0.5× TBE buffer (pH 8.3) alongside DNA molecular mass markers.

   When analyzing the PCR products, remember that amplification using vector primers will generate PCR products larger than the insert.

   See Troubleshooting.

TROUBLESHOOTING

Problem (Step 8): Yeast colony PCR fails to generate a product.
Solution: Aside from the generic problems that can affect PCR (e.g., forgotten, degraded, or incorrectly prepared reagents) there are factors specific to yeast colony PCR that require consideration. The

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first is that the yeast need to be effectively lysed by the zymolyase to release the template for the PCR. Zymolyase is an enzyme and needs to be treated somewhat carefully by ensuring that melted suspensions are always kept on ice and do not freeze-thaw the same aliquot of suspension more than three times. Also remember that the zymolyase is a suspension that needs to be regularly mixed (every 30 sec) while aliquoting or some samples will receive less enzyme than others. The second factor to consider is that adding too much yeast to the lysis solution will inhibit the PCR. An effective method for determining what is the correct amount of yeast to lyse is to setup a series of Zymolyase treatments with different amounts of yeast and then test which lysate is successful in the PCR.

RECIPES

TBE Buffer

Prepare a 5× stock solution in 1 L of H_2O:
- 54 g of Tris base
- 27.5 g of boric acid
- 20 mL of 0.5 M EDTA (pH 8.0)

The 0.5× working solution is 45 mM Tris-borate/1 mM EDTA. TBE is usually made and stored as a 5× or 10× stock solution. The pH of the concentrated stock buffer should be ~8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10× as opposed to 5×). However, 5× stock solution is more stable because the solutes do not precipitate during storage. Passing the 5× or 10× buffer stocks through a 0.22-µm filter can prevent or delay formation of precipitates.

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YAPD Plates

Reagent	Quantity (for 2 L)
Peptone	40 g
Yeast extract	20 g
Adenine hemisulfate dehydrate	0.32 g
Glucose (40%, w/v) in water, sterile	100 mL
Agar	35 g

Dissolve the first three powders in 950 mL of water in a 2-L flask and add a stir bar. In a second 2-L flask, add the agar to 950 mL of water (do not add a stir bar as it will cause the agar to boil over in the autoclave). Autoclave for 40 min at 15 psi on liquid cycle. Immediately pour the contents of the first flask, including the stir bar, into the agar-containing flask. Add the glucose, mix well on a stir plate, and cool to 55°C. Pour into 150-mm sterile Petri dishes (~80 mL per dish), dry for 3–5 d at room temperature, wrap in plastic bags, and store for up to 6 mo at room temperature.

Zymolyase Suspension

Mix 200 mg of Zymolase-100T powder (Associates of Cape Cod 120493-1) in 100 mL of sterile 0.1 M sodium phosphate buffer (pH 7.5). (The powder will not dissolve totally; some precipitate will be visible even after 30 min of mixing.) Divide into aliquots of 1 mL and store for up to 12 mo at −20°C.

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