Colony PCR Using Yeast Spheroplasted Cells

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[Abstract] The first part of this protocol involves the removal of yeast cell walls using the Zymolase enzyme. The resulting spheroplast cells can then be used as template for PCR. This quick and easy to implement protocol describes how to prepare spheroplasted yeast cells for colony PCR.

Materials and Reagents

1. Spheroplasted yeast cells
2. Zymolase 20 T [AMS Biotechnology (Europe)]
3. Sorbitol
4. Sodium phosphate
5. Zymolyase solution (see Recipes)

Equipment

1. Sterile pipette tips
2. Standard laboratory PCR machine
3. Wooden toothpicks

Procedure

1. Touch an average-size yeast colony (0.5-2 mm) or a cell pellet from a liquid culture with a sterile pipette tip.
   Note: Intact cells, a colony on a plate or liquid cultures which are stored at 4 °C for up to 3 months could still be used for this method. Wooden toothpicks should be avoided because they may interfere with either the release of DNA from yeast cells or the PCR reaction itself.
2. Rinse the cells off the tip with 10 µl Zymolase solution by pipetting up and down, this spheroplasts them.

3. Incubate for 10 min at 37 °C.
4. Use 2 µl spheroplasted yeast cells for 50 µl PCR reaction.
   Note: Spheroplasted cells should be made fresh; if not, the PCR will not be as efficient.

Recipes

1. Zymolyase solution
   2.5 mg/ml Zymolyase (20 T)
   1.2 M sorbitol
   0.1 M Na phosphate (pH 7.4)
   Aliquots of Zymolyase solution can be stored at -20 °C for at least 6 months.

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