

Zymolyase Treatment

- Transfer a small portion of a well-isolated colony (~1 mm) into 50 µl solution containing 60 U/ml of Zymolyase

(This solution is generated by resuspending 0.6 mg of 100T Zymolyase in 1 mls of water. Ordering information: Seikagaku Corp., #120493-1).

- Sterile yellow tips work great for this task.

- Repeat this step for each of the 3 isolates and the wild-type control.

- Incubate the Zymolyase solutions at 37 ° C for 30 minutes followed by 10 minutes at 95 ° C.

- It is not necessary to remove the Zymolyase solution by pelleting the cells before the PCR.
- The Zymolyase treated cells can be stored at 4 ° C for several days before being used as template for the colony PCR. However, fresher is probably better.
- The amount of cells that gets picked into the Zymolyase does not seem to be too critical - similar PCR results were obtained when 1 µl, 5 µl or 10 µl of the Zymo treated cells were used as template.