Colony PCR using Zymolyase

03-07-11, modified by Sigurd Braun (original from Suzanne Komili)

Use:
Diagnostics of yeast (*S. pombe, S. cerevisiae*) genomic DNA after chromosomal integration

Requirements:
Zymolyase (e.g. zymolyase 100T, 500 mg, Cat. #120493-1, $400, **Seikagaki America, Inc.**)
sodium phosphate buffer (0.1 M, pH 7.5), PCR machine

Protocol:
Zymolyase solution:

- Make 2.5 mg/ml zymo solution in 0.1 M sodium phosphate buffer pH 7.5
- Mix by inverting, not all will go into solution
- Spin down and aliquot supernatant (e.g. 100-200 ul) for storage -20º C (alternative: filter sterilize)

note:
in most protocols, the concentration of zymolyase refers to 20T zymolyase; when using 100T zymo, however, less enzyme can be used

Lysis:

- Aliquot 15 ul of zymo solution into PCR tube (can be done in 96well format)
- Scrape small amount of yeast colony (pipette tip size) into PCR tube and swish to to resuspend

- Heat to 37º C for 20 min and then 95º C for 5 min in PCR machine
- Mix by inverting, not all will go into solution

PCR:

- Dilute lysis solution 1:10 by adding 150 ul ddH2O
  (can now be stored at 4ºC or -20ºC)
- Use 2.5 ul as template for 25 ul (or 5 in 50 ul) PCR reaction