

Yeast colony PCR-Phusion

- 1) Add 25 μ l of zymolase to a tube.
- 2) Pick a blob of yeast into zymolase (half size of match head).
- 3) Incubate at 37°C for 10 min. Vortex vigorously. Incubate for 20 min at 37°C. Vortex vigorously.

- 4) Make Phusion master mix

10 μ l commercial 5x Phusion buffer

5 μ l dNTPs

5 μ l betaine

1 μ l DMSO

2 μ l Phusion

24 μ l dH₂O

1 μ l 50 μ M F oligo (#1)

1 μ l 50 μ M R oligo (#2)

Mix and aliquot 50 μ l per well

- 5) Add 1 μ l lysate.

- 6) Run on PCR

5min 94°C

35 cycles of

94°C-30 sec

55°C 30 sec

72°C 2 min

72°C 5 min to finish.