Yeast colony PCR

Yeast Cell Lysis

1. Aliquot 3 μL of 0.02 M NaOH into PCR tubes.
2. Using a sterile pipette tip, pick a small colony and resuspend in NaOH.
    - If the solution is cloudy, you've added enough cells.
3. Boil the samples on a PCR machine by incubating the tubes at 95C for 10 minutes.
    - The boiled samples are stable at room temp for some time.

PCR

1. Prepare the reaction cocktail solution containing:
    - 12.5 μL 2x iProof HF master mix (BioRad)
    - 0.5 μL foward primer (10 μM)
    - 0.5 μL reverse primer (10 μM)
    - 8.5 μL ddH2O
2. Aliquot 22 μL of the rxn cocktail to each boiled sample (25 μL total reaction volume).
3. Run the following PCR cycle:
    1. 5 min at 94°C
    2. 30 cycles of:
        1. 30 sec at 94°C
        2. 30 sec at 55°C (or appropriate annealing temperature)
        3. 30 sec - 1 min/kb at 72°C
    3. 10 min at 72°C
    4. 4°C hold

Notes

- Note that the 0.5 μL of 10 μM primer is about 2x more than standard PCR protocols.
- In lieu of the iProof master mix, you could try making your own with your favorite polymerase and buffer, 200 μM final concentration dNTPs, and 1 μL of 100% DMSO/rxn.
- The expected PCR product should be as short as possible. Anything less than ~5 kb can be easily amplified.