Colony PCR Protocol

We use this protocol to confirm modifications to the yeast genome. It often (but not always) works when our expected product is less than 1000 bp. We often give it a try when we expect longer products as well. When these reactions fail, we usually prepare a genomic DNA prep and use this as our template.

1. Use a pipet tip to scoop up a tiny bit of a colony and suspend in 20 µl 0.25% SDS in a microfiuge tube.
2. Heat tube at least 3 min @ 95°C, vortex, then spin in microfuge for 30 seconds.
3. Use 1 µl of the supernatant as the template in a PCR reaction
4. To the DNA (on ice) add the reaction components below. If you are doing multiple reactions, make a master mix and add 24 µl to each tube

	1 Rxn	42 Rxns
10x colony PCR buffer	2.5 µl	105 µl
50mM $MgCl_2$	0.75 µl	31.5 µl
10mM dNTPs	0.5 µl	21 µl
Primer F (50 µM)	0.5 µl	21 µl
Primer R 50 µM)	0.5 µl	21 µl
20% Triton X-100	1.25 µl	52.5 µl
Taq pol (5 units/ µl)	0.25 µl	10.5 µl
H_2O	17.75 µl	745.5 µl
DNA	1 µl	(1 µl /tube)
	25 µl	1008 µl aliquot 24 µl/tube

5. The program below is our starting place for reactions in which the PCR product is less than 500 bp. For longer templates increase the 72°C step.

COLONY PCR

Step 1	95°C 2min
Step 2	95°C 1min
Step 3	55°C 30 sec
Step 4	72°C 30 sec
Step 5	Go to step 2 x 35
Step 6	72°C 10 min
Step 7	4°C forever

10 x Colony PCR buffer

- 0.125 M Tris-HCl pH 8.5
- 0.56 M KCl