S. cerevisiae colony PCR (Mark Hickman)

1. Pick up with a toothpick an entire colony (preferably fresh) from YPD plate and place in the bottom of a PCR tube. Repeat for all samples. Then microwave the tubes on high power for 30 sec. (The first time a set of primers are used, include a wild-type purified DNA control, if possible. Add the DNA to reaction mix in step 3Ñdo not autoclave.)

2. Make up enough reaction mixture for all samples. For each 50 ul reaction:

5 ul	10X PCR buffer Ð$MgCl_2$ (Invitrogen)
5 ul	10X dNTPs (stock = 2mM each dNTP)
1.5 ul	50 mM $MgCl_2$ (Invitrogen)
0.5 ul	primer 1
0.5 ul	primer 2
0.25 ul	Taq (Invitrogen)
37.25 ul	dH_2O

3. Resuspend each colony in the reaction mixture by pipeting up and down.

4. PCR conditions:
    a. Annealing temp must be less than the Tm of both oligos (a couple degrees lower than the lowest Tm seems to work better).
    b. Extension time should be greater than 1 min per kb of product (Round up to the next minute; this has worked for products up to 5 kb with an extension time of 5 min).