**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 3-Do not autoclave.)

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

   5 ul 10X PCR buffer DMgCl$_2$ (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$_2$O

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).