Yeast Colony PCR protocol

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1. Aliquot 20ul of dH_2O into each well in 96-well plates
2. Use pipette tips to pick up some yeast cells from agar plates (1-2ul will be more than enough) and transfer into above plates
3. Prepare PCR master mixture as following (put the tubes on ice):

		1x rxtn	120x (for 96 rxtns)
a.	10x ExTaq buffer	1.5ul	180ul
b.	dNTP mixture	1.2ul	144ul
c.	20% triton X-100	0.75ul	90ul
d.	Primers (20uM)	0.0375ul each	4.5ul each
e.	Ex Taq	0.0075ul	9ul
f.	dH_2O	6.5ul	780ul

4. Aliquot 10ul of PCR master mix into each well in 96-well PCR plate. Put the PCR plates on ice
5. Use 12-channel pipette to mix the yeast cells and transfer 5ul into the PCR plates. Mix well by pipetting up and down several times.
6. Run the PCR reaction using the following program
   a. 94C 5min
   b. 94C 20sec
   c. 55C 20sec
   d. 72C 45sec
   e. go to b for 35 times
   f. 72C 5min
   g. 4C for ever
7. Add some loading buffer into each well and load all of the reaction mix into 1.5% agarose gel. Run at 140V for 30min.
8. Take pictures and enjoy your perfect knockout!!!!!

Notes:

1. The primer pairs used for yeast deletion project worked very well. I use two pair of primers to check my deletion routinely: Primer A and KanB (positive); Primer A and primer B (negative). Load the pairs next to each other for comparison.
2. Unlike what was described in Dan Gottschling's lab protocol page, it seems that adding more cells helps to generate a much more intense band. Keep the PCR mixture on ice before putting into PCR machine also helps.
3. Same volume can also be used in 384-well PCR plate. Worked well too