Colony PCR

Preparing for *E. coli* colony PCR:

1. Resuspend colony or pellet from overnight culture in 10-15ul ddH2O.
2. Set up a PCR reaction:

Water	**to a total of 25ul**
5X Buffer*	**5.0ul**
10mM dNTPs	**0.5ul**
Template	**5.0ul**
5' Primer	**0.5ul**
3' Primer	**0.5ul**
Phusion Polymerase	**0.1 ul**
	= 25ul reaction

   * DMSO can be added for troublesome PCRs (~0.1ul).
3. Use 5ul of the resuspended colony as the template for the PCR.
4. Place tubes in thermocycler and set the times according to this table (3-step protocol):

	2-step protocol		3-step protocol		
Cycle step	Temp.	Time	Temp.	Time	Cycles
Initial denaturation	98°C	30 s	98°C	30 s	1
Denaturation Annealing* Extension	98°C - 72°C	5-10 s - 15-30 s/kb	98°C X°C 72°C	5-10 s 10-30 s 15-30 s/kb	25-35
Final extension	72°C 4°C	5-10 min hold	72°C 4°C	5-10 min hold	1

   ** IMPORTANT: For the **initial denaturation** step, the time should be 3-5 minutes in order to break open the cells. Everything else should be the same. **
5. After PCR has been completed, add dye to samples and load them on a gel to see if the insert is there.