This protocol is based on a protocol by Knight.
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∅∅∅∅∅ Colony PCR

Colony PCR is a fast and efficient method of screening ligated colonies for properly ligated inserts. As many ligations rely on the random chance of a part's proper ligation, it becomes important to screen colonies bearing ligated plasmids for these proper clones. Colony PCR suspends raw bacterial colonies in water or LB, then adds the solution itself to the PCR mixture; the high temperature of the PCR reaction will lyse the cells and free the DNA.

While it is possible to screen by growing overnight, digesting, and running a subsequent gel, this method takes far more time and resource; by the same token, however, colony PCR can be highly unreliable due the wildly uncontrollable nature of what is essentially contaminating a PCR mixture with the complete contents of a bacterial cell.

Before starting, decide how many colonies you intend to pick and screen.

Solutions
5Prime master mix
diH2O or ddH2O
Bacterial colonies (on plate)
Forward, reverse primer solution

Materials
10, 50µL pipette
PCR tubes
Colony picker

You will also need access to a
PCR machine

Procedure

1. Add **20µL H2O** to tubes in a PCR tube strip. One tube should exist for every colony screen desired.

2. Using a **sterile colony picker**, pick a colony and rubt the picker around inside the PCR tube until a cloudy-ish solution forms. This should be done for all colonies you intend to screen in the 20µL. Sterilize the implement in between picking.This will be your **colony template solution.**

3. Generate a PCR master mix.
   ⇒ *Multiply this mixture by the number of samples you intend to screen and run a subsequent PCR reaction on. This is necessary, as it can be very difficult to accurately measure 0.2µL of polymerase in glycerol. It also can be helpful to add 3-5µL extra water to the master mix (or make 10-20µL extra), in case you run out of mix for your last sample due to pipetting error.*

   4µL **diH2O** or **ddH2O**
   0.25µL **Forward primer solution**
   0.25µL **Reverse primer solution**
   5µL **5Prime master mix**

4. Add **9.5µL PCR master mix** to a **second** clean **PCR tube strip** according to your sample.

5. Add **0.5µL colony template solution** to each tube containing PCR mix.

6. Design a program for your PCR reaction. If using VF2 and VR primers, run the samples on the following PCR program:
   ⇒ *Alternatively, use the PCR or similar program already entered into the PCR machine. The current standard program is "*`MSBTVFVR`*."*

   A **95°C** for **15 mins**
   B **94°C** for **30 seconds**
   C **56°C** for **30 seconds**
   D **68°C** for **1 minute** *per kb expected fragment size*

   Repeat B-D 35 or so times.

   E **68°C** for **20 minutes**
   F **4°C indefinitely**

7. View the results of the reaction using the **gel electrophoresis** protocol.