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

PCR is a rapid method of amplifying low concentrations of DNA into high concentrations capable of being viewed or used.

This protocol will utilize NEB's *Phusion* polymerase, a high power and high accuracy polymerase capable of amplifying large DNA fragments at very high rates.

******Phusion*** should only be used when for ***cloning or producing stocks*** when the DNA of interest is known to work with PCR! Do not use ***Phusion*** for colony PCR or routine assays unless absolutely necessary, as it is five times as expensive as standard ***taq*** polymerase!

Solutions
HF or GC buffer (depending on DNA %GC content)
dNTP solution
Forward primer solution, concentrated
Reverse primer solution, concentrated
Phusion polymerase solution
ddH2O
Template solution

Materials
10, 50µL pipette
PCR tubes
1.5mL centrifuge tubes.

You will also need access to a
PCR machine
Tabletop quick centrifuge

Procedure

1. Generate a PCR master mix by adding these reagents to a **1.5mL centrifuge tube**.
   ⇒ *This reaction will produce a 100µL mix; 50µL reactions (or less) can be easily achieved by dividing these volumes proportionally.*

   62µL **diH2O** or **ddH2O**
   2.0µL **dNTP solution**
   5.0µL **Forward primer solution**
   5.0µL **Reverse primer solution**
   1.0µL ***Phusion*** **polymerase solution**

2. Properly divide the solution among your samples and add it to each tube in a **clean PCR tube strip.**

3. Add **5/(*# of samples*)µL template solution** to each tube. Mix by uptaking and expelling liquid with your pipette.

4. Centrifuge the tubes in a **tabletop quick centrifuge** for **20 seconds.**

5. Design a program for your PCR reaction. If using VF2 and VR primers, run the samples on the following PCR program:

   A **95°C** for **15 mins**
   B **94°C** for **15 seconds**
   C **56°C** for **20 seconds**
   D **68°C** for **30 seconds** *per kb expected fragment size*

   Repeat B-D 35 or so times.

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

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