# [iGEM 2017] PCR

## Introduction

Amplify a specific region of DNA specified by the choice of primers. Generates a high concentration of linear double-stranded DNA fragments.

This protocol contains instructions for:
- one PCR reaction
- creating a master mix for multiple reactions
- colony PCR

The typical cloning pipeline is:
**PCR** -> Gel -> DpnI -> PCR Purification -> Gibson Assembly -> Transformation -> **Colony PCR** -> Inoculation -> Miniprep

*Notes:*
*- IDT recommends that you do not PCR directly off of a gBlock.*
*- For troubleshooting PCR reactions, try* NEB's guide, BioRad's guide, Thermo-Fisher's guide, or many more...
*- An alternate PCR protocol we've tried in the past to troubleshoot is 1.25 uL of each primer and 9 uL NFW, if we are getting off-target banding.*

## Materials

- NEB 2x Q5 Hot Start High Fidelity Master Mix
- Nuclease Free Water (NFW)
- Forward Primer (10 uM)
- Reverse Primer (10 uM)
- Template DNA
- 0.2 mL Tube

## Procedure

### PCR [for one reaction]

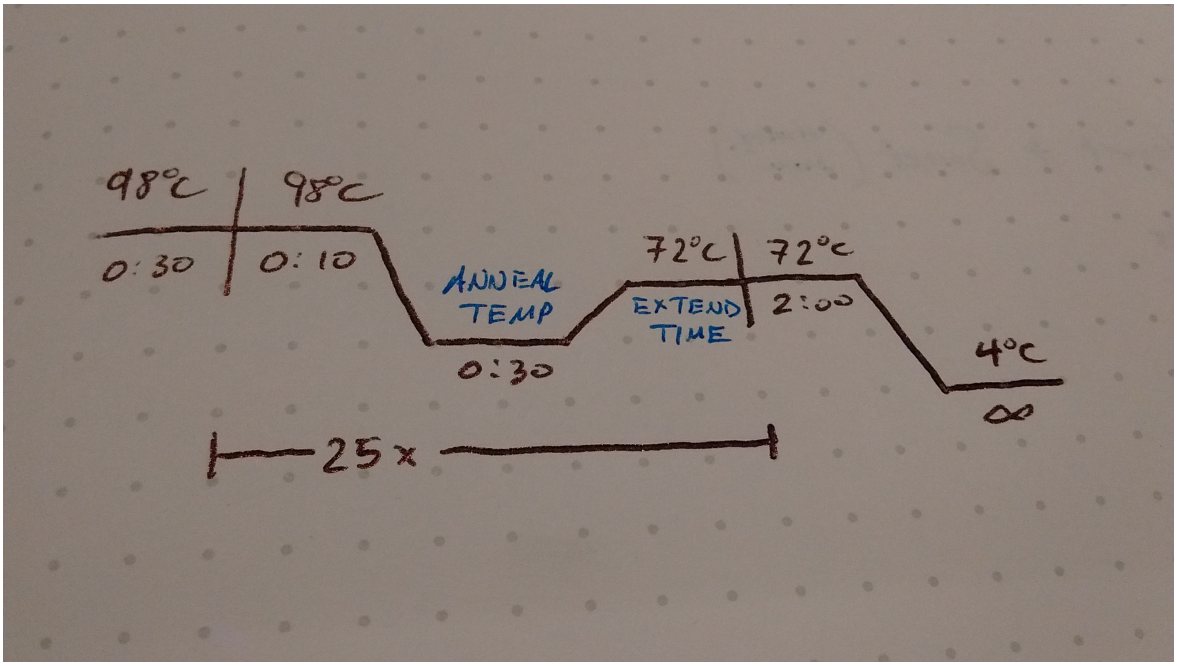
1. In a single 0.2 mL Tube, add (for a total volume of 25 uL):

   9 uL NFW
   1.25 uL Forward Primer
   1.25 uL Reverse Primer
   1 uL DNA sample *(We default to diluting minipreps 1:10 in NFW, but undiluted samples and higher dilution of samples have all worked fine-- it's just a parameter to be modified in troubleshooting.)*
   12.5 uL Q5 2X Hi-Fi Master Mix

2. Program the Thermal Cycler using the preprogrammed 'PCR' protocol:

   Calculate ANNEALING_TEMP using the NEB online calculator. (*Be sure to set 'Product Group' to 'Q5 Hot Start' and 'Polymerase/Kit' to 'Q5 Hot Start Hi-Fidelity 2X Master Mix'!)*
   Calculate EXTENSION_TIME by the rule-of-thumb '30 sec. per 1kb of final fragment length'. *(People say anywhere betwen 20-45 sec/kb is okay, so this value is flexible. It's okay to fudge the extension time so that many reactions can fit in one thermal cycler run, if thermal cyclers are limiting.)*

Edit the 'PCR' protocol to include the calculated values, such that the final protocol is:




3. Proceed to run a gel (Gel Electrophoresis) of the PCR product to confirm it is the right size. After that (or simultaneously, if you're strapped for time), proceed to DpnI Digestion.

## Creating a Master Mix for multiple reactions

4. Sometimes we are using the same primer set on a number of different DNA templates. Or sometimes we are doing the same PCR multiple times, but with different thermal cycler parameters. In such situations it is efficient to create a single Master Mix of PCR solution. To do this, follow the table below:

Table1

| | A | B | C |
|---|---|---|---|
| 1 | **Number of Reactions** | | **1** |
| 2 | Upscale Factor | | 1.1 |
| 3 | | Single PCR Volumes: | **Final MasterMix Volumes:** |
| 4 | 10 uM Forward | 1.25 | **1.375** |
| 5 | 10 uM Reverse | 1.25 | **1.375** |
| 6 | Nuclease Free Water | 9 | **9.9** |
| 7 | Q5 2x-HiFI MM | 12.5 | **13.75** |

Here, change the **Number of Reactions** and add to an appropriate-volume tube the **Final MasterMix Volumes**. If necessary, alter the Single PCR Volumes. The upscale factor is currently set to 10% arbitrarily to account for volume errors from pipetting.

5. Add 24 uL PCR Master Mix solution to 1 uL Template DNA to complete the PCR solution for one reaction.

## Colony PCR [one reaction]

6. In order to validate the success of a transformation, we PCR directly off of the colonies on a plate. This protocol describes the process for a single reaction, but typically we will PCR 4-8 colonies per plate (depending on our level of confidence for the success of the gibson assembly and transformation). For a single Colony PCR:

7. We first set up the Colony Solution. In a 0.2 mL tube, add the following and mix gently to homogenize:

   10 uL NFW
   1 colony *(see Inoculation protocol for good colony-picking principles)*

8. Then in another 0.2 mL tube, set up the PCR solution by adding:

   3 uL NFW
   0.5 uL Forward Primer
   0.5 uL Reverse Primer
   1 uL Colony Solution
   5 uL Q5 2X Hi-Fi Master Mix

9. Proceed with standard PCR / PCR Master Mix protocols. Master mix calculators for colony PCRs:

10. Traditionally colony PCRs are done in 10µls, but we can do 5µl or even 8µl colony PCRs. (In both cases 1µl of template is used for ease of use with multichannel pipette). However, often times low copy bands will be less visible in 5µl PCRs. For non gibson transformations it is recommended to save colonies in NFW, but to not colony PCR them unless something interesting in experiments comes up. For gibsons PCRs, it may be advisable to do 8µl Colony PCRs when working with low copy backbones.

Table2

| | A | B | C |
|---|---|---|---|
| 1 | Number of Reactions | | 8 |
| 2 | Percent Upscale | | 1.1 |
| 3 | | uL / Tube | uL - > MM |
| 4 | 10 uM Forward | 0.5 | 4.4 |
| 5 | 10 uM Reverse | 0.5 | 4.4 |
| 6 | Nuclease Free Water | 3 | 26.4 |
| 7 | Q5 2x MM | 5 | 44 |

For a diagnostic colony PCR, we proceed to the gel-running stage but do not go on to DpnI or PCR purification. However, it is theoretically possible to try and do a gibson assembly off Colony PCR product--but the efficiency will be lower.