

Identifying Gene Amplification Using Polymerase Chain Reaction (PCR)

Background Information:

What is Polymerase Chain Reaction (PCR)?

Polymerase Chain Reaction of 'PCR' is a powerful technique used by many scientists to **amplify** (or 'copy') a particular gene or region of DNA in a test tube. Starting with only a few copies of DNA, PCR uses the same basic ingredients as DNA replication in the cell and results in **billions** of copies of your specific gene or region of DNA. **All PCR experiments contain a few vital components:**

- A **DNA template**, which is DNA used as instructions to make more DNA. You will use 'patient' DNA provided to you as a template to amplify the HER2 gene.
- A **DNA polymerase**, which is an enzyme that assembles new DNA molecules. You will use a special kind of DNA polymerase called **Taq** polymerase to copy the HER2 gene.
- Forward and reverse **primers**, or small single-stranded pieces of DNA used to start or 'prime' DNA synthesis, which bind specifically to the 5' and 3' sequences on either side of the DNA of the HER2 gene. These primers tell the DNA polymerase where to start copying the gene.
- **Deoxynucleotide triphosphates (dNTPs)**, which are the building blocks of DNA, and are used to build the new DNA molecules during the PCR reaction.
- **Buffer** (usually in a 10X concentrate, which you dilute with pure water) that helps to stabilize the pH of the solution and contains the right type of salts to reproduce the conditions in a cell so that the *Taq* DNA polymerase can function.

Today you will be using **2X OneTaq Master Mix (Fig 1)**, which contains all the ingredients for your PCR **except** the DNA template, primers and nuclease-free water. The 2X OneTaq Master Mix shown in Fig. 1 is green because it has a special dye added to it. The 2X OneTaq MasterMix you will be using is not green.

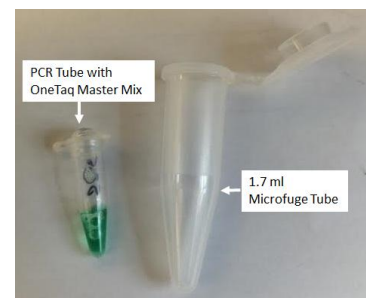


Figure 1: 0.2 ml PCR Tube with OneTaq Master Mix Compared to a 1.7 ml Microfuge Tube

How do you know which primers to use?

Scientific experiments build on what is already known. In order to make the Taq DNA polymerase specifically copy **your** gene of interest and not just any piece of DNA, you need to use primers that will bind only to the DNA on either side of the region you want to copy. You will be using a mixture containing a Forward and a Reverse primer, called a **primer pool**. Each primer in the primer pool was

originally derived from the specific DNA sequence on either side of the HER2 gene. A primer pool is a collection or mixture of primers, usually used in PCR.

Procedure:

Note: Work in groups of 2.

1. Obtain ice in your ice container. PCR primers should be kept cold. The 2X OneTaq Master Mix, which contains the Taq polymerase, must be kept cold.
2. PCR tubes are very small and do not permit complicated labels. Use the table below (**Table 1**) to list each patient sample that will be tested by you and your lab partner, and the number that you will use to label your PCR tubes. **Your PCR tubes should also include your initials**, such as 'JT' in the example below.

Table 1: DNA Samples and PCR Test Tube Labels

PCR Tube Label Species (or sample number) from which DNA was purified	
Example: Joe Ting's Samples are labeled 'JT' plus other sample information	
PCR Tube Label	Sample Description
JT0	<u>Negative Control</u>
JT+2	<u>Positive Control</u>
JTA, JTB, JTC	<u>Patient Sample A, Patient Sample B, Patient Sample C (3 separate tubes)</u>

3. The PCR tubes are the small **0.2 mL clear tubes provided by the instructor**. Obtain 5 tubes for your group: one for each of your three patient samples, one for a 2 copy HER2 positive control, and one for a negative control. Put the tubes on ice.
4. Label each PCR tube as you described in **Table 1**, for example 'JTA'.
5. Label one PCR tube with one of the lab partner's initials and a zero for your group's negative control, for example: 'JT0'.
6. Label one tube with one lab partner's initials and a '+2' and a number for the two copies of the HER2 gene in the positive control DNA, example: 'JT+2'.
7. To set up the PCR reaction with your patient sample, add the following components to the PCR tube labeled with initials and patient ID (letter), example 'JTA', 'JTB', or 'JTC' in the order listed below. Try to keep the components on ice as much as possible.
 - a. 12.5 uL of the 2X OneTaq MM
 - b. 10.0 uL of your patient DNA sample (labeled with a letter A-L)
 - c. 2.5 uL of the primer pool (labeled 'Primer')
8. To set up your positive control reactions, add the following components to the PCR tubes labeled with initials and positive control designations (example: 'JT+2'), in the order listed below. Try to keep the components on ice as much as possible.

- a. 12.5 uL of the 2X OneTaq MM
 - b. 10.0 uL of your positive control DNA sample (labeled '+2')
 - c. 2.5 uL of the primer pool (labeled 'Primer')
9. To set up your negative control, add the following components to the PCR tube labeled with initials and the number zero (example: 'JT0'), in the order listed below. Try to keep components on ice as much as possible.
 - a. 12.5 uL of the 2X OneTaq MM
 - b. 10.0 uL of nuclease-free water (labeled 'H₂O')
 - c. 2.5 uL of the primer pool (labeled 'Primer')
10. Refer to Table 2 for a summary of how to mix each PCR reaction.

Table 2: PCR Reaction Set Up

	Sample Names				
Reagent	Negative Control Ex: "JT0"	Positive control Ex: "JT+2"	Patient A Ex: "JTA"	Patient B Ex: "JTB"	Patient C Ex: "JTC"
2X One Taq MM	12.5 uL	12.5 uL	12.5 uL	12.5 uL	12.5 uL
DNA Sample	0	10 uL	10 uL	10 uL	10 uL
Primer pool	2.5 uL	2.5 uL	2.5 uL	2.5 uL	2.5uL
Sterile water	10 uL	0	0	0	0

11. Mix your PCR tubes containing the PCR mixtures by closing the lid and flicking the bottom of the tube with your finger.
12. Spin down the components of your PCR tubes in the small table-top centrifuge.
13. Working with your instructor and the rest of your class/camp, put all 5 of your group's tubes into the **thermocycler**. A thermocycler is a type of machine used to automate polymerase chain reaction. It can be programmed to cycle through all of the temperatures required to complete the PCR.

The PCR reaction conditions are as follows:

1 cycle	95°C	2 min	[initial denaturation]
30 cycles	95°C	30 sec	[denaturation]
	58°C	30 sec	[low-temp annealing]
	72°C	1 min	[elongation]
1 cycle	72°C	5 min	[final elongation]
1 cycle	4°C	overnight	