Oct 26, 2020

Introduction to Primer Design (Draft)

Forked from Introduction to Primer Design

1

[1]UCSC

1 Works for me This document is published without a DOI.

UCSC BME 22L

DOCUMENT CITATION

2020. Introduction to Primer Design (Draft). **protocols.io**
https://protocols.io/view/introduction-to-primer-design-draft-bnxkmfkw

FORK FROM

Forked from Introduction to Primer Design , Alyssa Ayala

CREATED

Oct 24, 2020

LAST MODIFIED

Oct 26, 2020

DOCUMENT INTEGER ID

43724

Introduction to Primer Design

Goal

The goal of this lab if for you to design synthetic oligonucleotide primers to amplify a region of DNA on a sequence of interest.

Learning Objectives

- The importance and application of primers
- How to design primers using different software options
- How to use Geneious prime
- How primers are used in amplification methods like LAMP and PCR

Tips and Hazards

When designing primers, there are some key guidelines you should follow to be confident that they will amplify your target correctly.

Here are some key things to keep in mind:

- The **length** of your primers should typically be 18-24 nucleotides
- The **GC content** of these primers should be 40-60%.
- Should start and end with at least 1-2 G-C pairs
- Melting temperature between 50-60°C
- The melting temperature of the forward and reverse primers should be within 5 °C of each other
- The primers shouldn't have complementary regions to avoid primer-primer annealing.

IN SUMMARY: Depending on the amplification protocol performed, there are different criteria to meet when designing a primer. Each amplification process depends on primer design to allow for proper DNA synthesis.

Background

Living organisms depend on primers to initiate DNA synthesis. DNA Polymerase (the enzyme responsible for adding nucleotides to the 3' end of an existing DNA template) relies on a primer to indicate where to latch onto a strand before it synthesizes another strand.

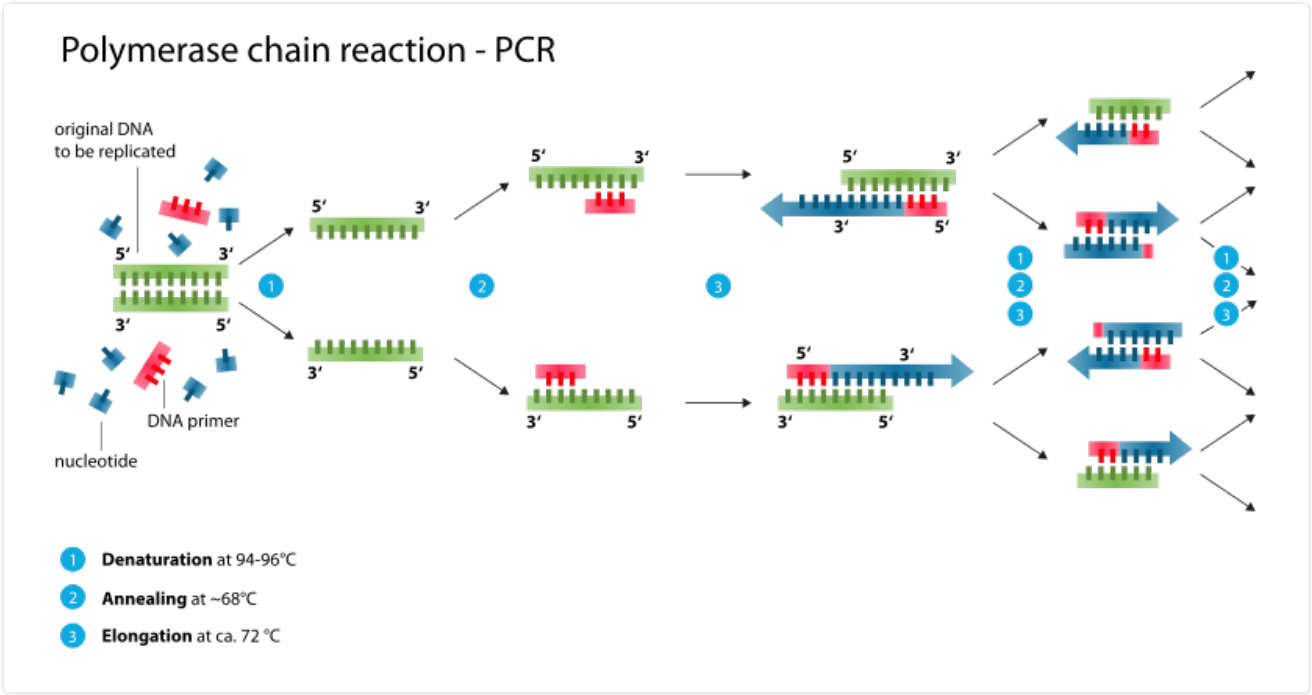
Primers work in pairs, forward and reverse, as one primer moves along the leading strand; the other along the lagging strand to allow proper initiation of DNA synthesis. They bind on the outer regions of the sequence you wish to amplify. In the lab, we design synthetic oligonucleotide primers in order to amplify a region of DNA on a specific sequence. Since these are designed to compliment another sequence, they bind with high specificity and recruit DNA Polymerase to begin replicating DNA.

Designing primers manually can often be a tedious task because there are a lot of parameters one needs to follow in order to make sure there is a successful use of these primers. Fortunately, there are a number of software programs out there that will design these primer sequences for you. The only thing that you need to do is download a fasta file of the sequence you wish to amplify and upload it to the site. Depending on the mode of amplification you are doing, there are various sites that design a multitude of primers for whatever task you have at hand. First, we will explain two methods that utilize primers and then you will learn to design your own.

PCR

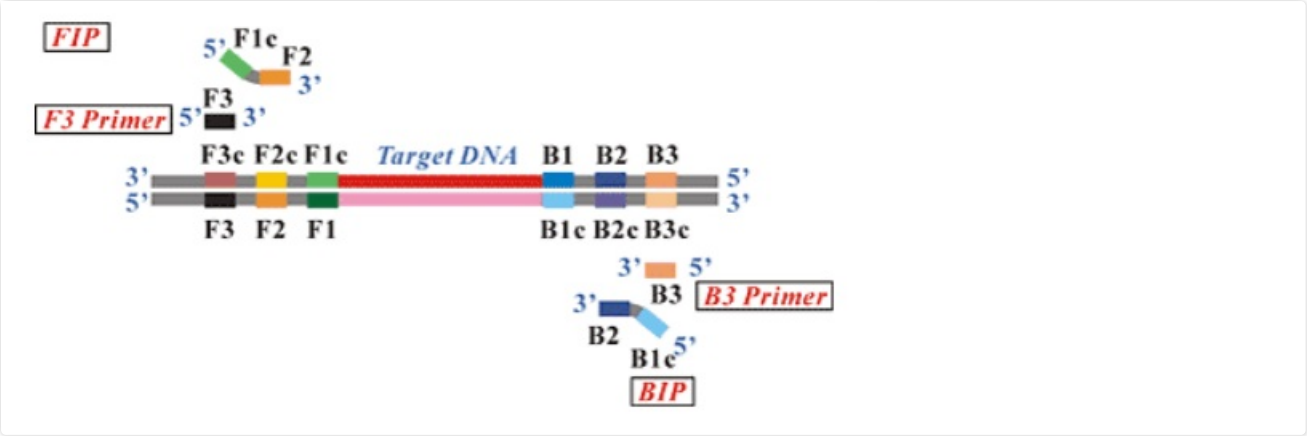
PCR (polymerase chain reaction) is an amplification method that utilizes thermocycling in order to amplify DNA sequences. This technique results in 20-40 cycles of amplification, where temperature changes rapidly and accurately. The first step in PCR amplification is denaturation, where double-stranded DNA (dsDNA) is heated up to 96 C in order to create single-stranded DNA (ssDNA). Denaturation allows primers to access the ssDNA. Primers attach themselves to a complementary sequence on the ssDNA during the Annealing phase. During Annealing, the thermocycler drops in temperature to around 50-60°C, this is an ideal temperature for primer attachment. The next step is DNA elongation, this step requires the thermocycler to increase to about 72°C. This temperature allows for the activity of our Taq polymerase; which is a DNA polymerase that only works at high temperatures. This cycle occurs 20-40 times and allows for the amplification of millions of DNA strands. One can estimate the amount of amplified DNA using this equation 2^n; 2 represents the initial 2 ssDNA while n represents the number of cycles performed. For instance, if there are 30 cycles performed then we can say 2^(30) and that we amplified 1,073,741,824 copies of the original strand of DNA.

When designing primers, we will look at producing two distinct primers. The two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand.



LAMP

Loop-mediated isothermal amplification (LAMP) is an amplification method that utilizes 4 primers that recognize 6 different sequences on a target DNA you wish to amplify. Unlike PCR, it only requires one temperature in order to carry out the reaction, hence the “isothermal” in its title.



Inner Primer- The inner primers recognize 4 distinct sequences on the target DNA. Each contains 3 different sequences that allow it to bind and prime. These primers prime normally in the initial stage and they self-prime in later stages.

- Forward Inner Primer: Contains the F2, F1c, and a small sequence that links them together
- Backward Inner Primer: Contains the B2, B1c, and a small sequence that links the two.

Outer Primer- The outer primer hybridizes outside of the FIP region and initiates strand displacement by releasing a FIP linked strand. This strand then serves as a template for BIP initiated DNA synthesis and Backward outer primer strand displacement.

- Forward Outer Primer: Contains F3
- Backward Outer Primer: Contains B3

LAMP requires that there be two inner primers as well as outer primers. In the initial stage, these primers work the way that they normally would and prime on their respective strands. However, the priming of the forward outer primer and the structure of the forward inner primer causes the primer to loop amongst itself and hybridize forming a self hybridizing loop. The same process happens to the backward inner and outer primers, where the outer primer goes along the strand and causes the inner primer to form a self hybridizing loop. All of these primers working in tandem with each other then causes the target strand to form a dumbbell-shaped structure which then serves as a template for further LAMP cycling.

For LAMP cycling, only the sequences that the inner primers recognize remain in this dumbbell structure. Therefore, the inner primers are the only ones that will continue to work and carry out this form of replication, where the inner prime continues this strand displacement activity, forming various products of various lengths in the process.

Reverse-Transcription as a prelude to PCR: RT-PCR of Sars-CoV 2 as an example

Geneious Prime: Genomic visualization tool used in this class

Disclaimer:

The information provided on this document is intended for the educational purposes of the BME 22L laboratory course. It is worth noting that the information listed on this document is subject to change and is not finalized. Therefore, the information on this document should not be used outside of this course.