

# 4. PCR simulation

MIÉRCOLES, 21/9/2022

## Overview

This doc will show how to use primers already designed and saved on Benchling for applications such as PCR. This can be combined with writing out PCR protocols and data collection in a electronic lab notebook to gain confidence in experimental design and generate a reproducible lab project.

A basic understanding of the concept of Polymerase Chain Reaction and familiarity with common terms such as DNA template, primers is need it to understand the tasks and concepts on this document.

To review what a PCR is please see this [video](#) (in English) or access with your cell phone the augmented reality video (in Spanish) located on the file below.

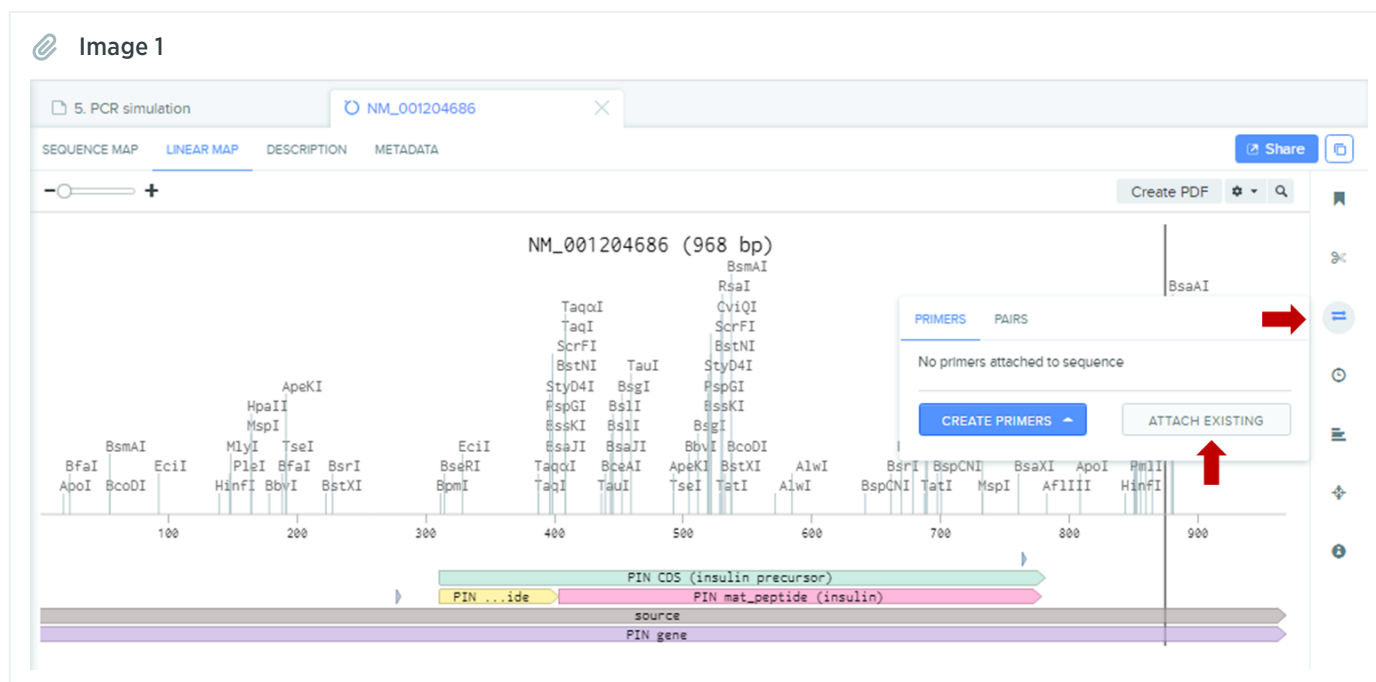
 PCR Zappar



1. Download the [zappar](#) application on your mobile phone.
2. Scan the Image
3. Watch the video. You can move your phone to see details on the video while on front of the picture.
4. Answer the quiz questions.

## Getting the target sequence and linking the primers

1. The first step to simulate a PCR in silico on Benchling is to open or import a sequence to work with. We will import the [Aplysia californica](#) insulin precursor (PIN), mRNA (NM\_001204686).  
To do so, click on the plus sign (+) on the projects column and select DNA/RNA -> Import DNA/RNA sequences
2. A new window will pop. Select **Import from data base**. Type, or copy and paste the sequence ID (NM\_001204686) and click on search.
3. The sequence info and data will appear and now we can import to our working folder on Benchling.
4. Once the sequence is open, we can create primers [manually](#) or using [Benchling Primer Wizard](#). We can use these primers for our example [Ins\\_FW](#) and [Ins\\_Rv](#)
5. To simulate the PCR we need to attach the primers to the target sequence if they are imported or created individually.  
To attach the primers click on the primers icon and attach primers (**Image 1**).



6. A new window will pop up where you can select for the folder containing your primers and click on **find binding sites** (**Image 2**)

**Image 2**

5. PCR simulation NM\_001204686

SEQUENCE MAP LINEAR MAP **FIND BINDING SITES** DESCRIPTION METADATA

☒ Highlight matching bases Attach Selected Primers

Find binding sites for all primers in specified folders

Use primers in Primers x Add locations

with a nucleotide type of DNA

with at least 15 matching bases

separated by  $\leq 0$  mismatches

and no more than 10 total mismatches

with  $T_m$  above 40 °C and under 100 °C.

☐ where entire primer matches

☐ where primer matches exactly once on the sequence

with 3' position in 1 - 968 Use Selection

**Find Binding Sites** [T<sub>m</sub> parameters](#)

7. The software will look for primers able to attach to the target sequences and when done, you can select them and click on **attach primers**.

To know more about [clinical microbiology and identification of pathogens](#)