Baking Bright Ideas: How I Genetically Modified Yeast To Produce GFP

By Nayra Cadavid

Have you ever found yourself caught in the grip of intense cravings for green bread? Just imagine the sensation that only this bread can deliver. How it feels almost like a whisper from your taste buds—a subtle yet vibrant fusion of flavors.

Well, I can't relate.

Honestly, it doesn't look that appetizing. But that doesn't matter because you don't need to have precise goals to explore with science. I don't want to bake green bread, I wouldn't eat it, yet, my curiosity leads me to the fascinating world of gene editing, where I find my deepest enthusiasm. And since it's only through hands-on experiences that genuine learning occurs, I immersed myself in creating a simulation using Benchling. I directed baker's yeast to produce a green fluorescent protein, so that's the process and science I'll delve into shortly.

So let's commence by gaining insight into the tool utilized for this simulation

Benchling

For the past few months, I've been delving into the world of gene editing, and it was time to get my hands dirty. However, lacking extensive lab equipment at home, I discovered a valuable tool to expand my knowledge in this field—Benchling, a popular program for individuals interested in biology. This program simplifies the process of copying information from various sources, such as enzymes and plasmids, and seamlessly pasting them without any difficulty, that way, one can conduct endless simulations and analysis.

Using Benchling, you can learn a lot of useful biology techniques and knowledge. What makes it even better is that when you're working on a project, it feels like you're actually in a lab, getting hands-on experience with your learning.

With that being said, let's explore the simulation done: what I did, how I did it, and what I learned from it.

Process:

As this is one of my first times utilizing Benchling, and since I was just learning and had limited knowledge and expertise in the field of gene editing, I followed a tutorial by Stanford Biome, from where I learned how to edit a yeast plasmid to incorporate green fluorescent protein (GFP).

Let's start from starch:

What is a plasmid?

A plasmid is a crucial component in molecular biology that assumes the form of a long, circular DNA sequence. It is primarily found in bacteria and certain other microscopic organisms; and what sets plasmids apart is their physical separation

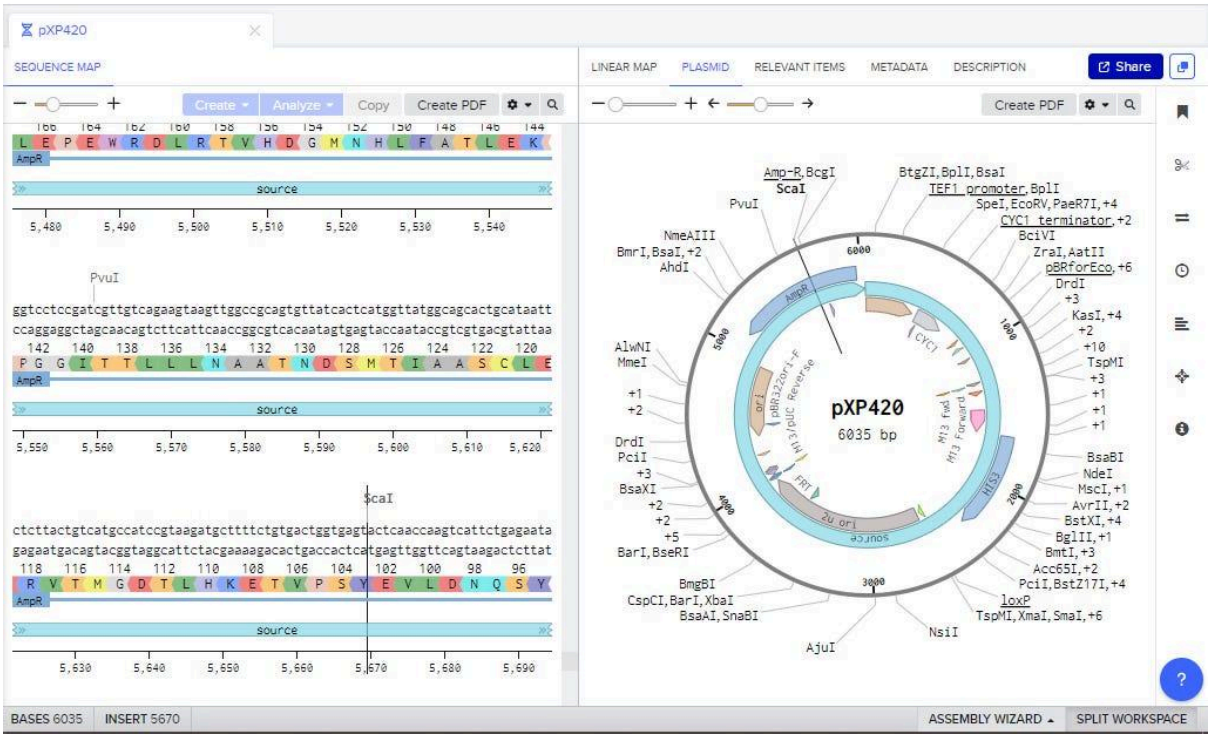
from chromosomal DNA within a cell, existing independently. These self-replicating entities are particularly important because they carry genes that facilitate their DNA replication and enable them to transfer from one host cell to another. Essentially, plasmids act as genetic messengers, aiding in the processes of DNA transfer and replication within microbial life.

Now, let's delve into the process.

Step 1

The first step was to find an empty backbone yeast plasmid. One can copy them from add gene.

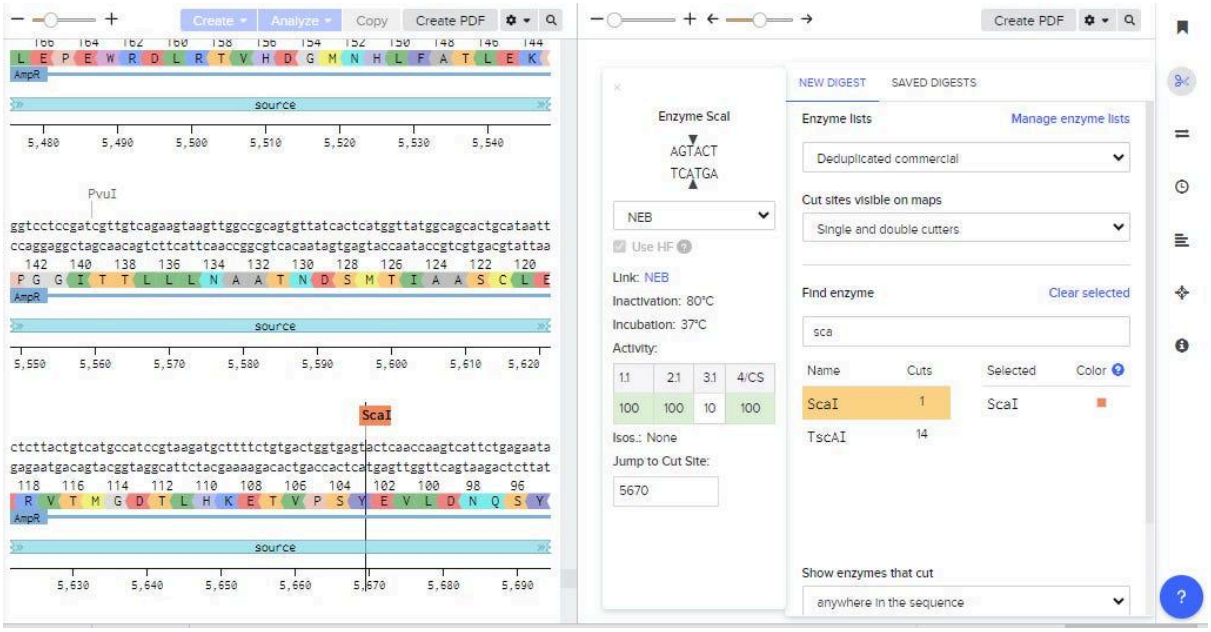
For this simulation, it utilized the plasmid called pXP420.



Step 2

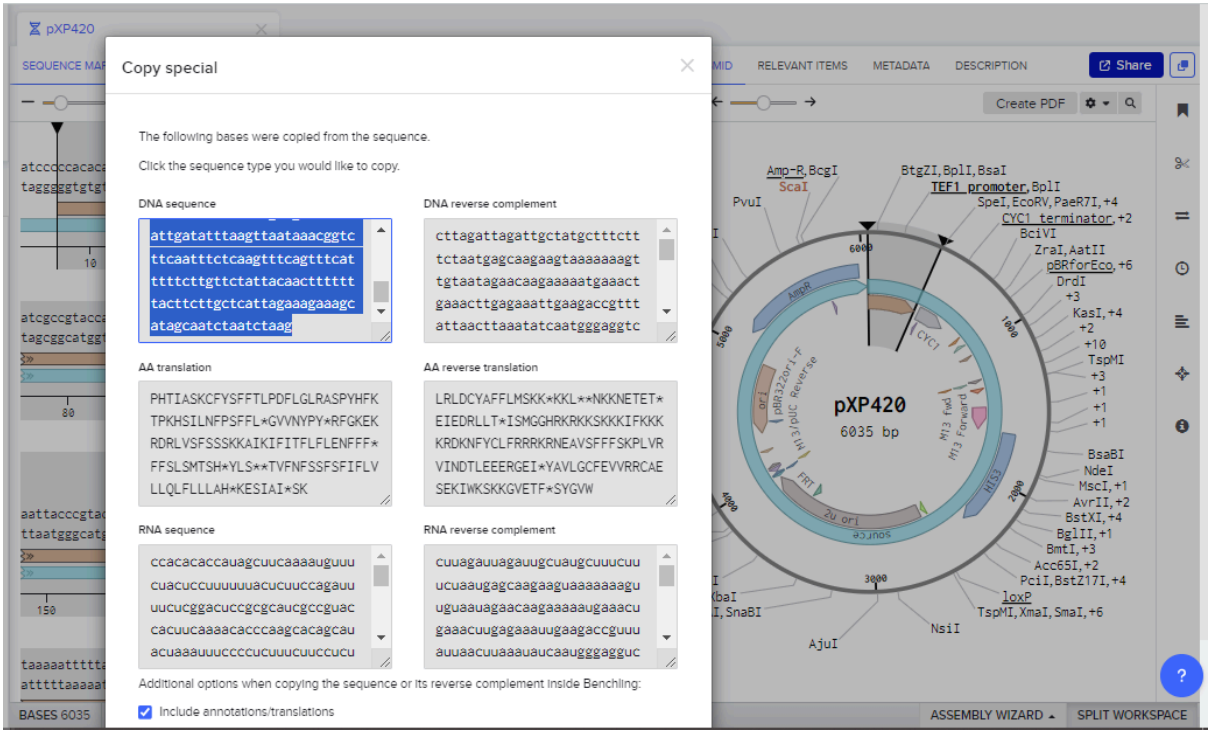
Then, I had to find a restriction enzyme. Which are specific locations along a DNA sequence that serve as recognition and cutting sites for restriction enzymes. When these enzymes are applied, they act like molecular scissors. They open up the DNA, and the cut plasmid, which is then ready to accept and incorporate new DNA sequences. It's preferable to select a solo restriction site to be more specific and prevent cuts at multiple locations that might disrupt the process. That's why the

chosen restriction site was **ScaI**, and also because it has a blunt end, meaning that one doesn't have to worry less about matching sticky ends, as we can insert our sequence directly.

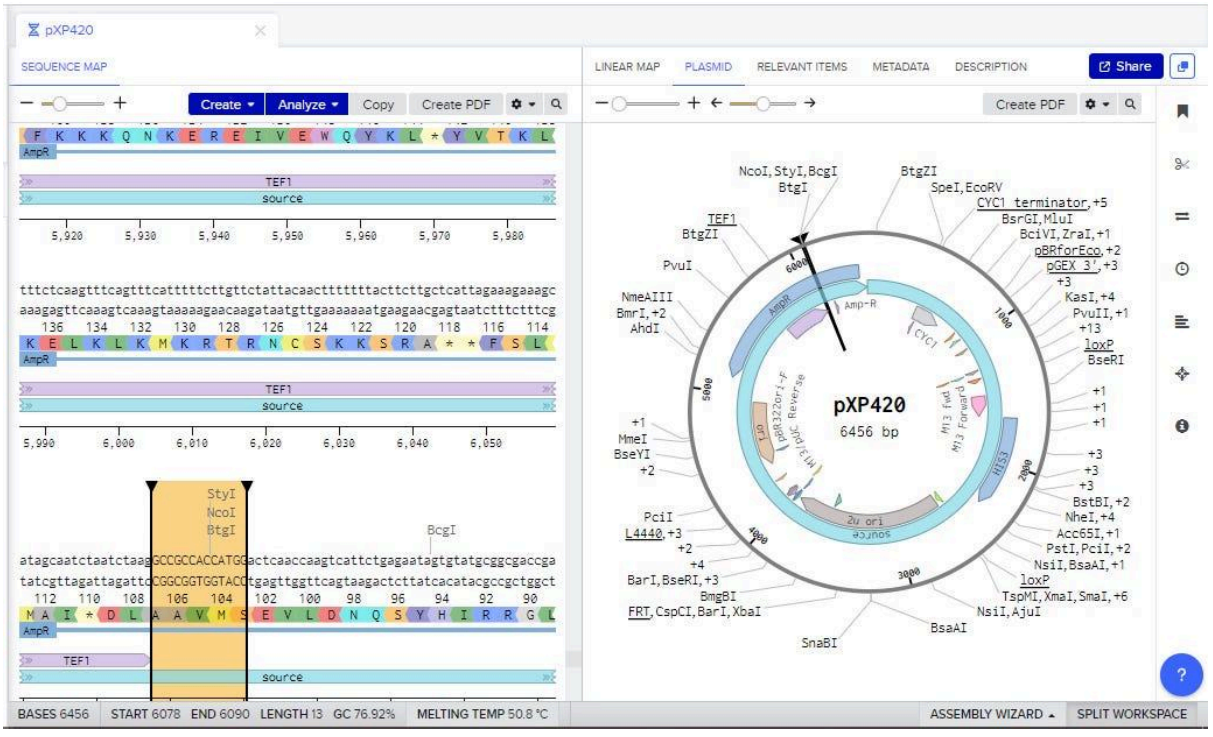


Step 3

Next, we have to replicate the DNA sequence of the **TEF1 promoter**, known for its specificity to eukaryotic organisms, especially yeast cells. This promoter governs the initiation and regulation of the transcription process for a specific target gene, as it acts as a transcription elongation factor. Additionally, the replication of this promoter is necessary as GFP, our target gene, is considered an mRNA, and what the TEF1 promoter does is provide the necessary signals for the proper initiation of transcription by ensuring that the genetic information in our target gene is accurately transcribed into GFP mRNA, allowing for the subsequent synthesis of the Green Fluorescent Protein.



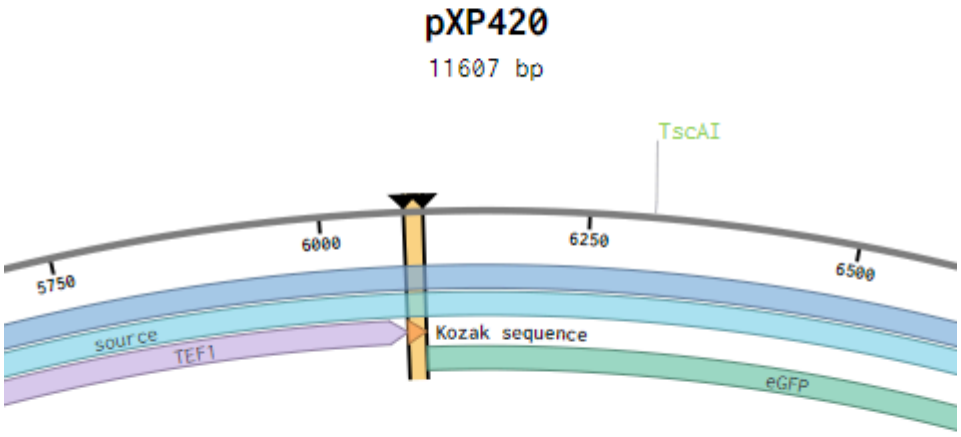
Then, this sequence was inserted precisely where our chosen restriction site is located. Consequently, the ScaI sequence is overwritten, essentially replaced by the TEF1 promoter sequence. Once we do this, we make an annotation and make the other promoter not visible.



Step 4

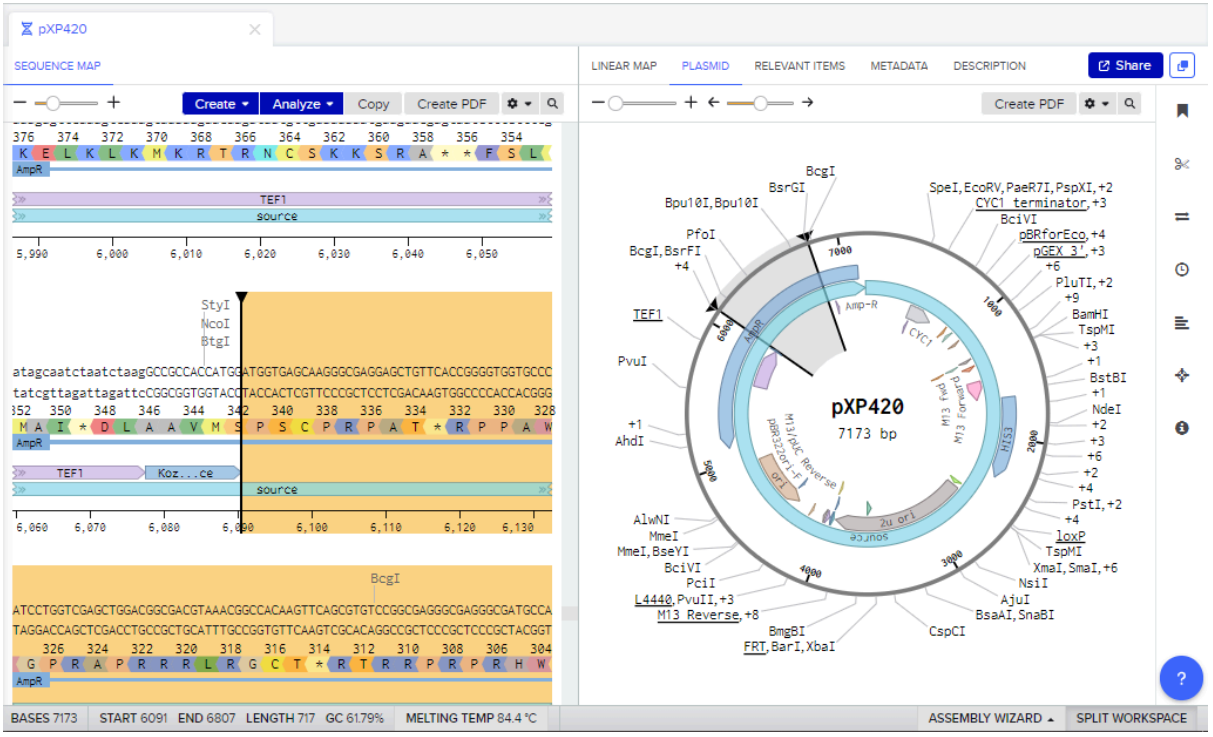
Afterward, we incorporated a **Kozak** sequence right after the TEF1 promoter, to facilitate the translation process, meaning the conversion of DNA into protein, which occurs in the TEF1 promoter. Since GFP is a protein, it's essential to introduce the Kozak sequence to enable the plasmid to identify and process it.

Note: In the video, there was an issue with the Kozak sequence as it included an extra Guanine (GG), which caused a misalignment with the GFP gene. The GFP gene utilized cannot start with guanine, so using the provided Kozak sequence would disrupt the correct reading frame of the gene, leading to potential functional problems in the resulting protein. To avoid this, a different Kozak sequence was utilized: ggatccacgattaaaagaATG. This sequence ensures proper alignment and avoids the issue of starting with a guanine.



Step 5

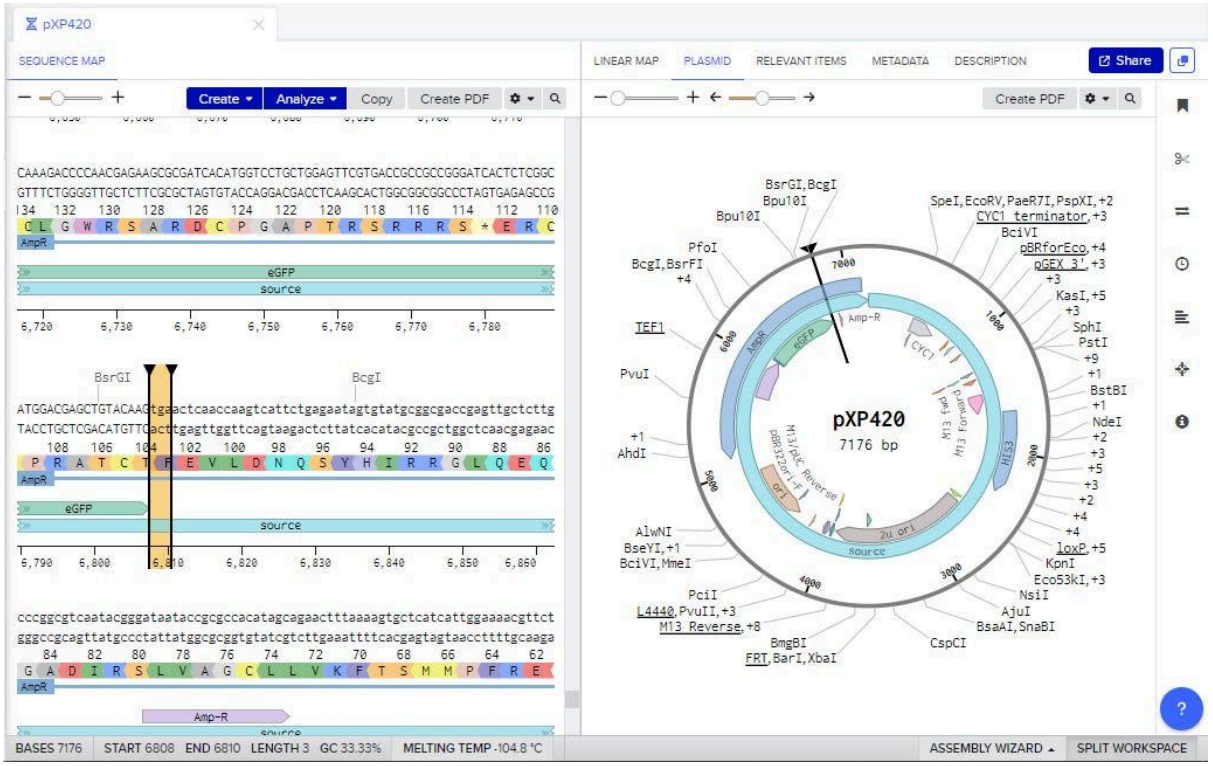
Afterward, we add the GFP to our plasmid by copying the gene's sequence and inserting it at the end of our Kozak sequence, and then we make an annotation.



Step 6

To prevent endless translation, we use the **stop codon TGA**, and we write it after our GFP sequence.

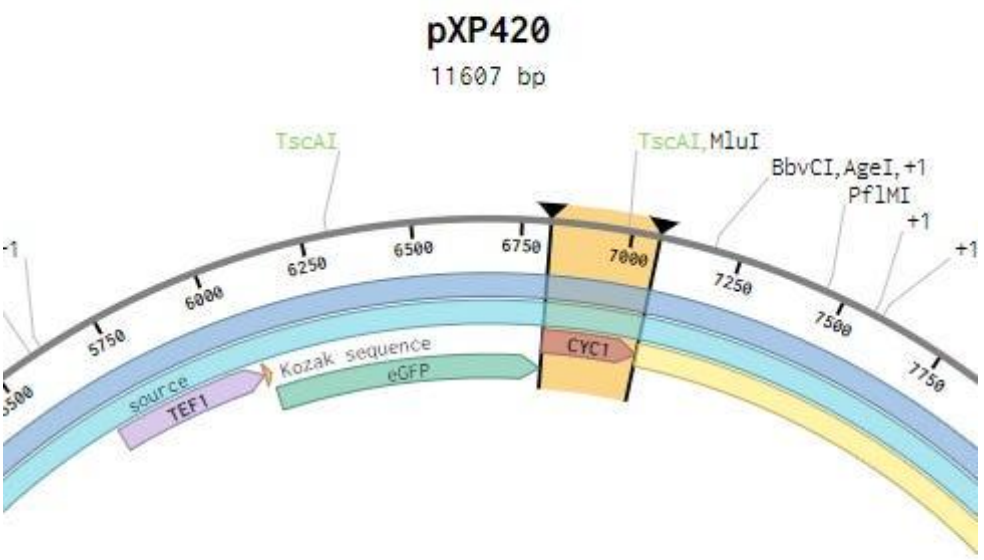
A stop codon is a set of three nucleotides in DNA or messenger RNA (mRNA) that signals the cell to halt protein synthesis.



Step 7

After this, we copy the **CYC1 terminator** sequence, which you can find in the plasmid, and paste it after the stop codon. Subsequently, we make an annotation and also turn off the previous CYC1 terminator not visible to avoid any confusion.

What the CYC1 terminator does is mark where the transcription process should end.



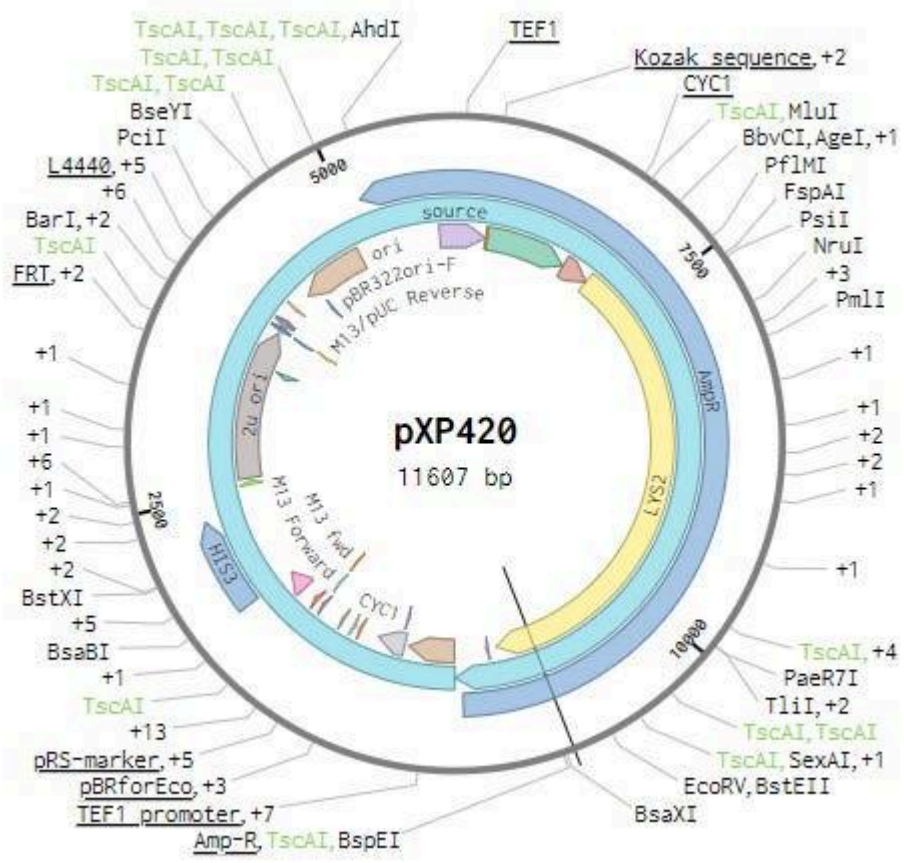
Step 8

Once the previous step is finished, we have to add a selection marker. To determine the appropriate marker, we have to consider the yeast strain we're working with and identify sequences where it might struggle in leucine production. Therefore, we opt for a leucine auxotrophic selection marker, specifically LYS2. This step is essential to ensure the successful development of polymers, such as GFP, in our genetic engineering process. This step is crucial to successfully create the GFP, which are essentially polymers, during our genetic engineering process.

Step 9

Last but not least, we want to have two origin sites, which is where DNA replication begins. Fortunately, the plasmid we used already had two.

After following all these steps, we should end up with this:



Navigating Challenges and Gaining Insights

I ran the simulation multiple times, grappling with unfamiliar terms and steps. This process not only helped me understand gene editing better but also deepened my knowledge of how Benchling operates. I'm thrilled to share the tips and tricks I've gathered along the way.

Challenges: During the simulation, a challenge emerged when I observed that the plasmid used in the tutorial had longer DNA sequences than the one I selected, despite both being called the same and originating from the same source. It took some time to discern that the tutorial creator had copied the TEF1 promoter from a different plasmid and pasted it into the one featured in the video. Once I did this, I considered using the same plasmid, but given that the chosen plasmid in the tutorial lacked one out of two origin sites crucial for DNA replication, I decided to proceed with the pXP420 plasmid.

Insight: This detail wasn't addressed in the videos but rather in the comments. It pointed out a minor error with the Kozak sequence, which I highlighted in the "note" section. What I learned from this is how crucial it is to be careful, attentive,

and patient every time we work with DNA sequences, as a single letter can have a significant impact on the entire process.

Brief Sum Up: gene editing is way cooler than I once imagined. I just made a yeast plasmid to produce GFP, my plasmid it's bright green! And I did this with not much knowledge of the field—simply making a few tweaks and moving some things around. I loved exploring with Benchling and DNA, it's really not that hard to learn new things when you put your mind and effort into it. You won't even notice how much time you spend on it when you enjoy the process. I'll definitely keep working on more simulations like this.

My name is Nayra, I am enthusiastic about science and particularly about gene editing. I believe it can help us solve so many actual environmental issues triggering our planet. So if you have any suggestions, questions or just want to talk, you can email me at: nccnayra@gmail.com, or message me on LinkedIn. Thanks so much for reading!