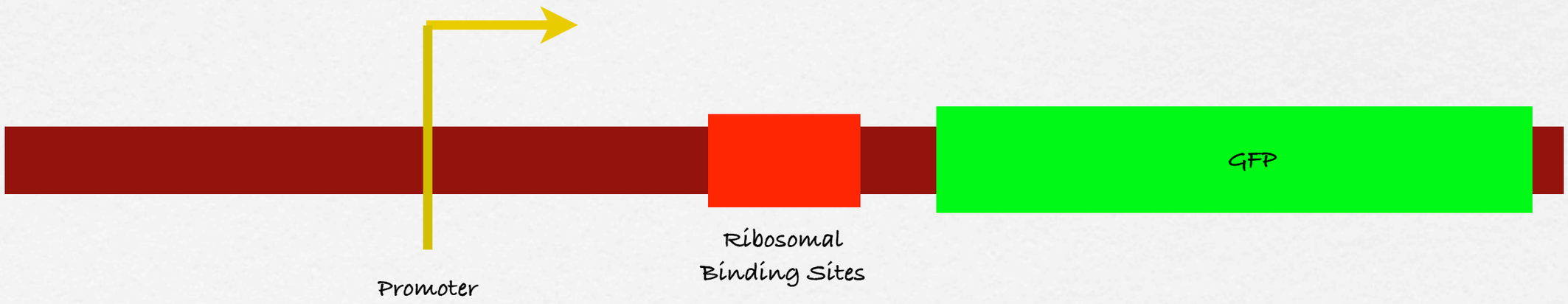
Synthetic Biology for

Our Project and Inverse PCR

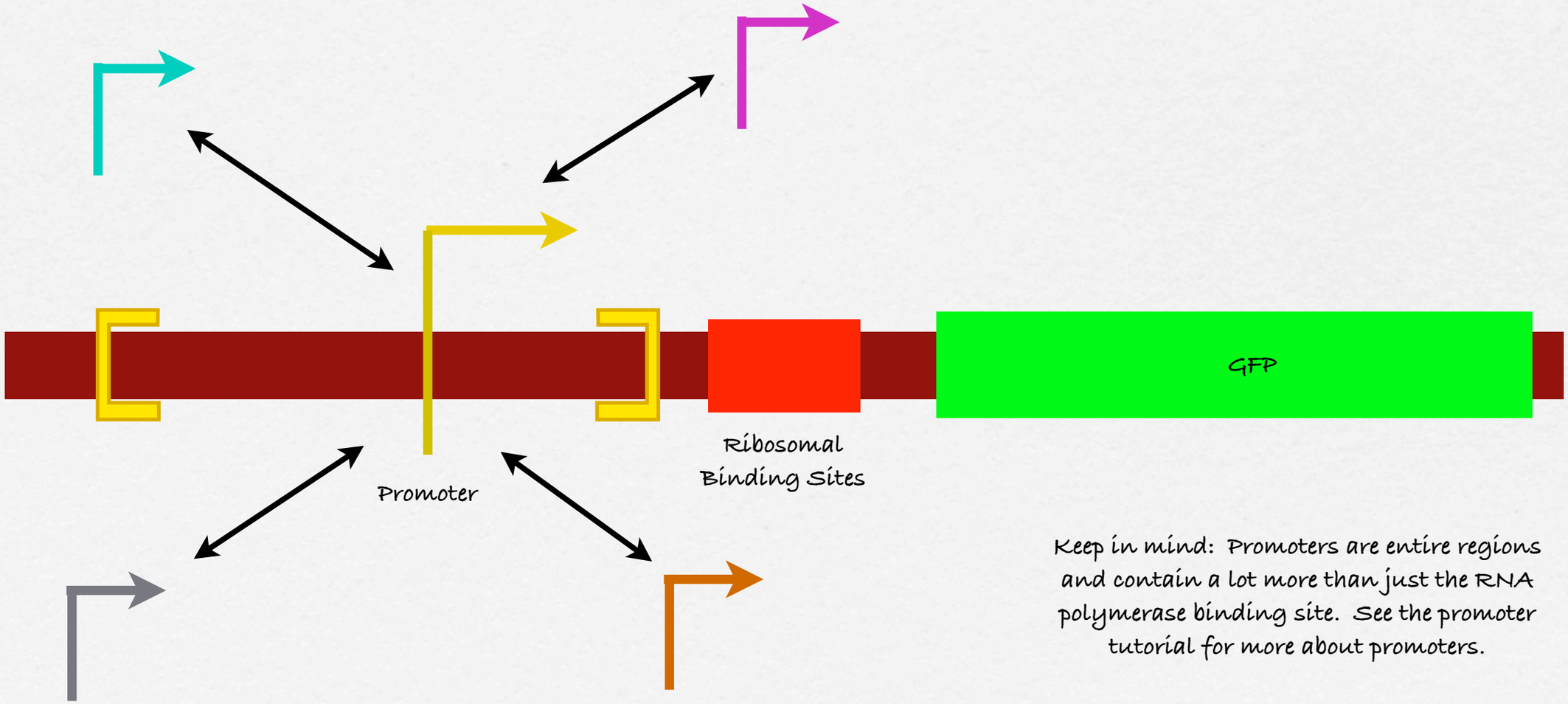
Review: Transcription

- RNA polymerase binds to the promoter region of the DNA
- RNA polymerase reads 3' to 5' (synthesizing 5' to 3' as it goes), making a mRNA transcript of the gene



Our Project

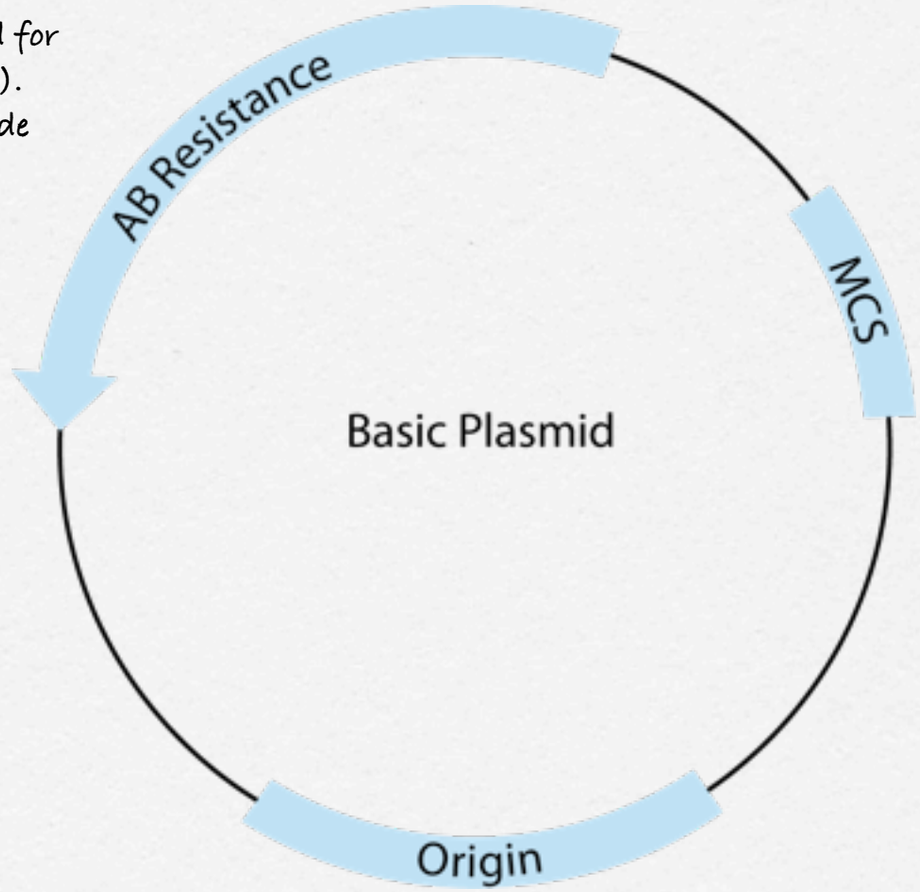
- We want to swap out the promoter for other promoter regions with different activators and repressors



Keep in mind: Promoters are entire regions and contain a lot more than just the RNA polymerase binding site. See the promoter tutorial for more about promoters.

Review: Plasmid Composition

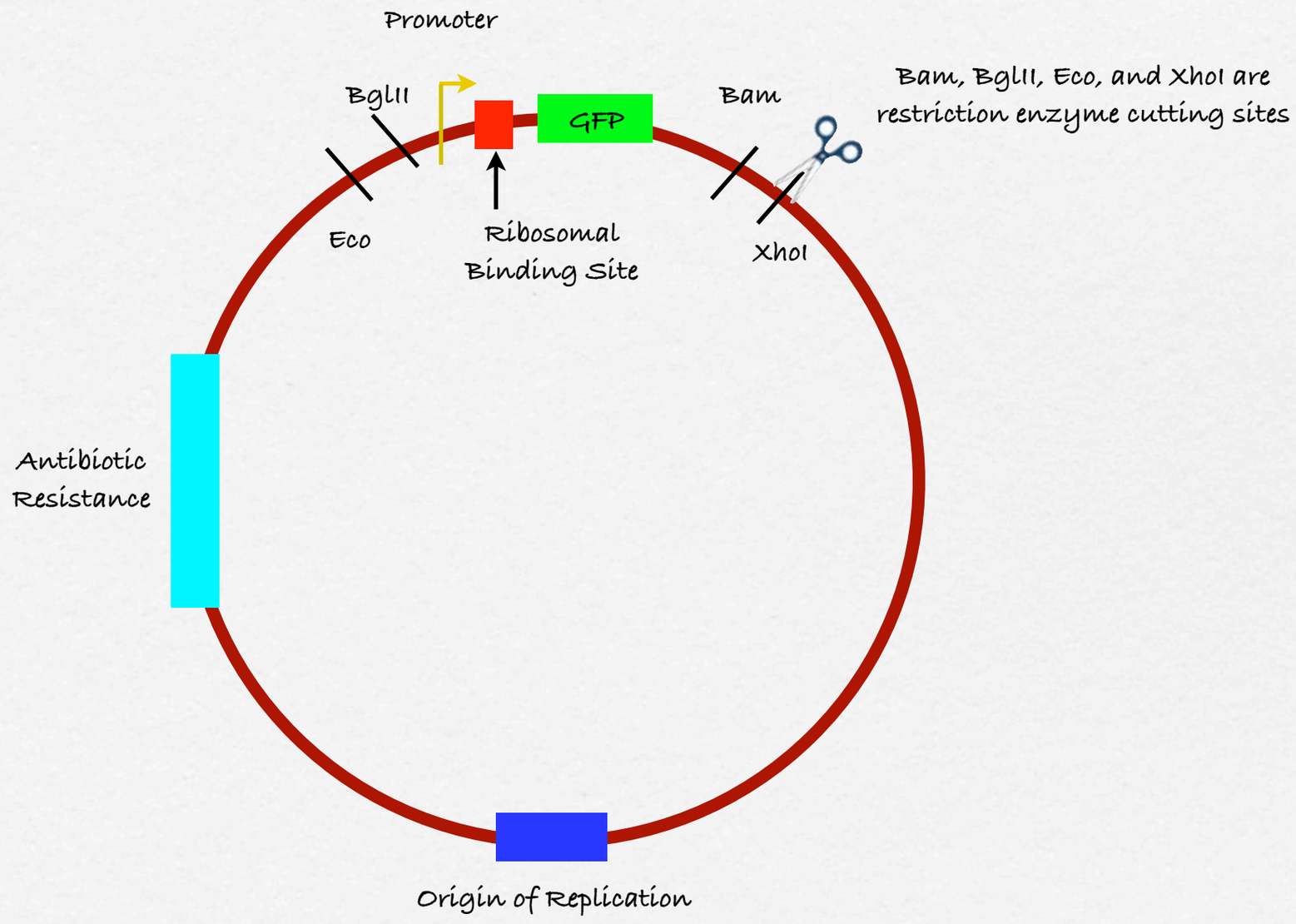
Antibiotic Resistance. Used for selection. (See later slides). Example antibiotics include ampicillin (Amp) and kanamycin (kan)



Multiple Cloning Site. This area contains sites for restriction enzymes to cut and paste in different DNA.

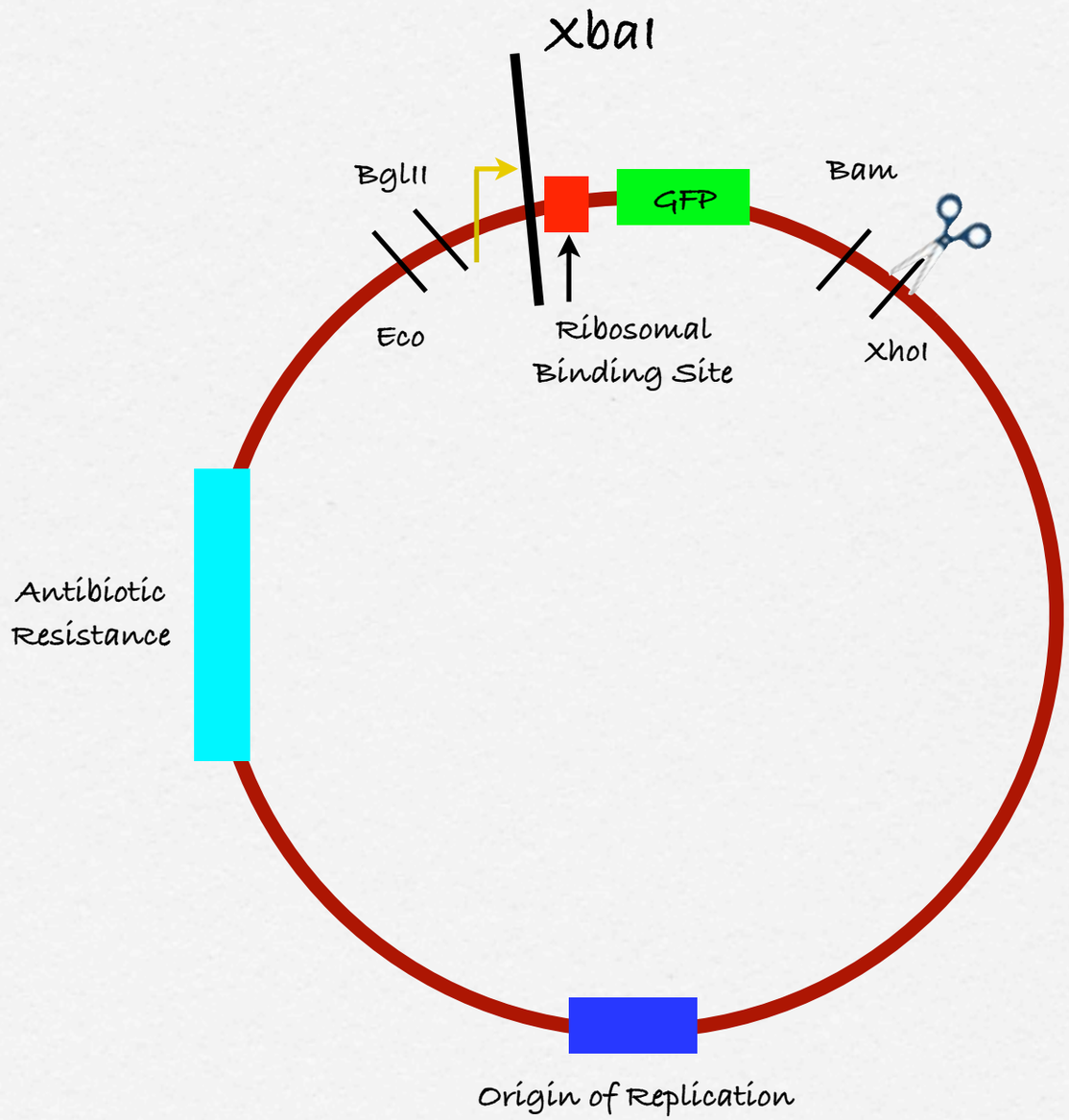
Origin of Replication. This is important so that when bacteria replicate, the plasmid is also replicated.

IGEM Plasmid



Bam, BglII, Eco, and XhoI are restriction enzyme cutting sites

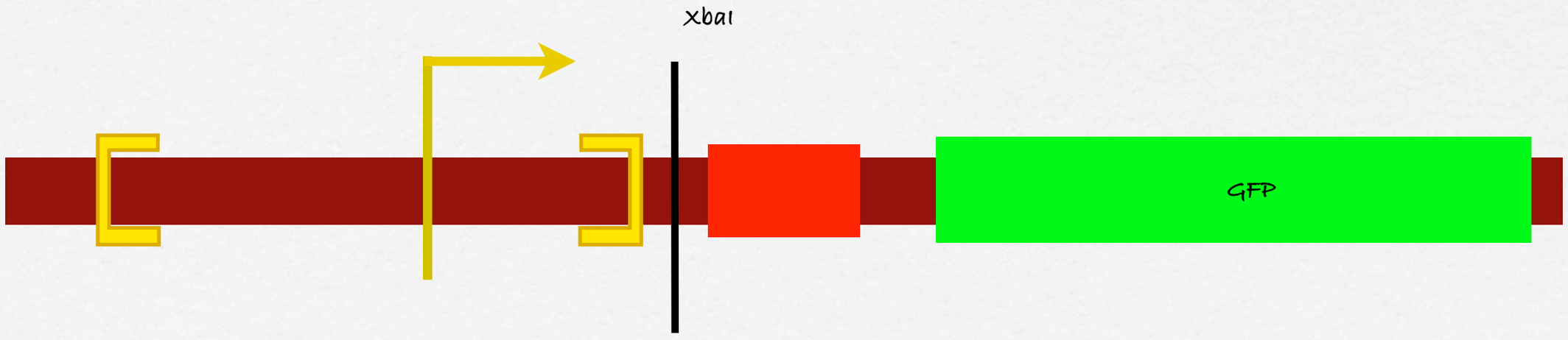
Our Plasmid



We want to insert a new restriction enzyme site, Xba1, between the promoter and the ribosomal binding site

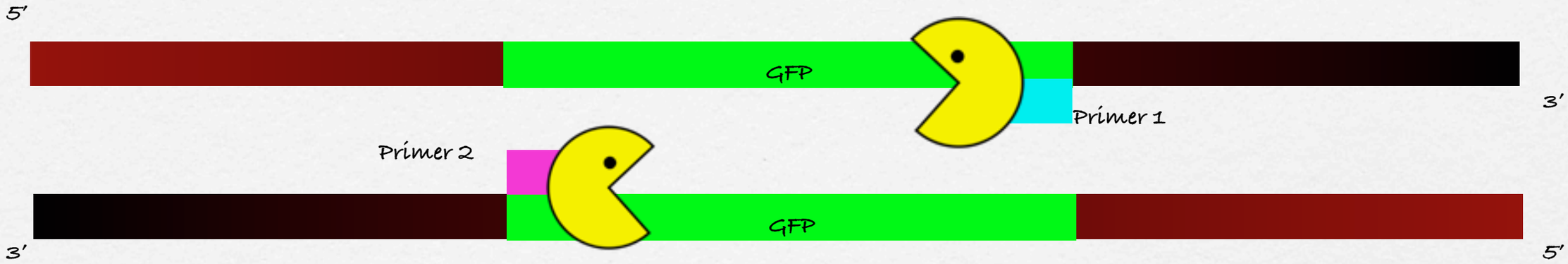
Inserting the XbaI Restriction Site

- We need to insert a restriction enzyme site into the DNA
- Normally to insert something, we use restriction enzymes/ligation to do so, but we don't have a restriction enzyme site for this (that's why we're adding it)
- We will accomplish this using inverse PCR



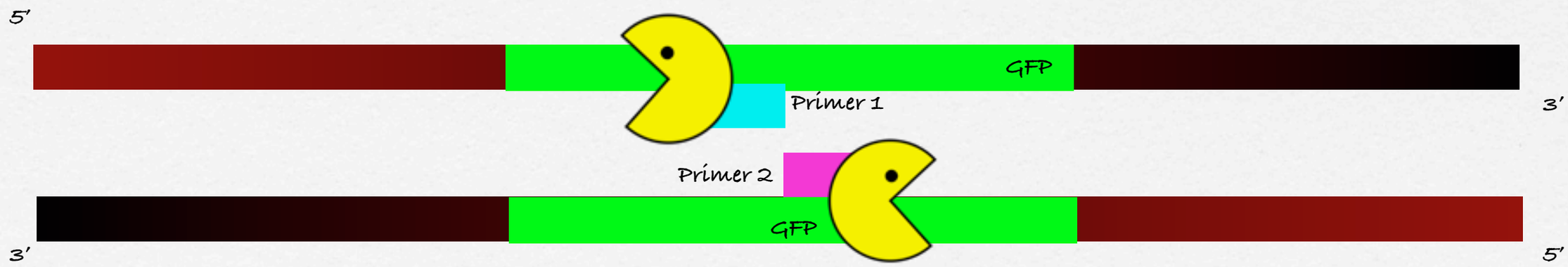
Normal PCR vs. Inverse PCR

Normal PCR



Normal PCR amplifies a target gene. The polymerases act in a criss-cross fashion with both directions going over the target gene.

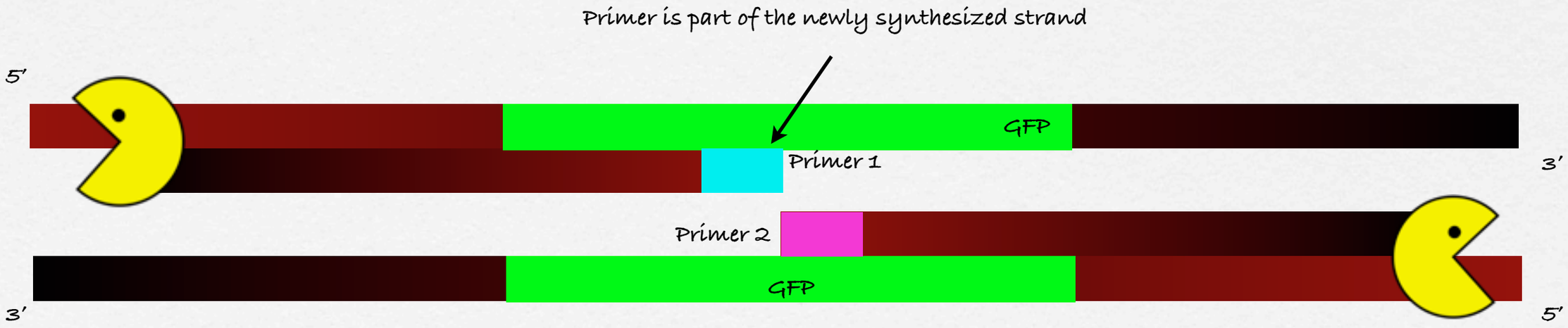
Inverse PCR



Inverse PCR amplifies outward. Since the plasmid is circular, inverse PCR will amplify the entire plasmid.

Adding the XbaI Site

- In PCR, the primers are part of the newly synthesized strands
- We can therefore design our primers to add in the XbaI site



Our Primers

- Our Primers will include 3 distinct regions: spacer, XbaI site, and genome matching site
- Keep in mind: there will need to be two primers (forward and reverse) and they will have the same architecture, but flipped

Forward Primer

Spacer (6 bp) | XbaI site (6 bp) | Genome matching site (~20 bp)

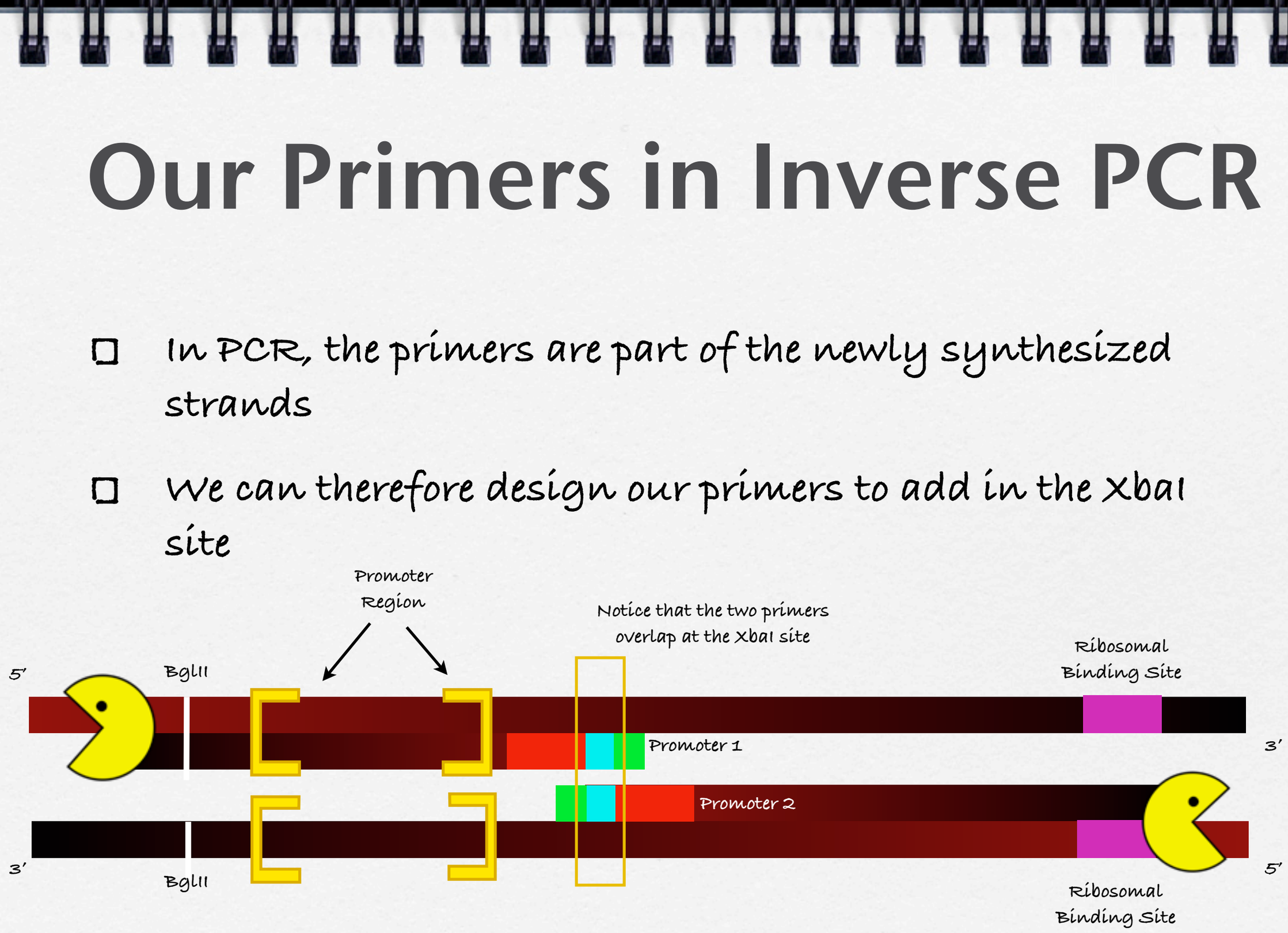
Reverse Primer

The spacer region is for future steps. We need these extra base pairs (bp) for restriction enzymes to attach to.

The genome matching site is what will attach to the DNA during the annealing step of PCR.

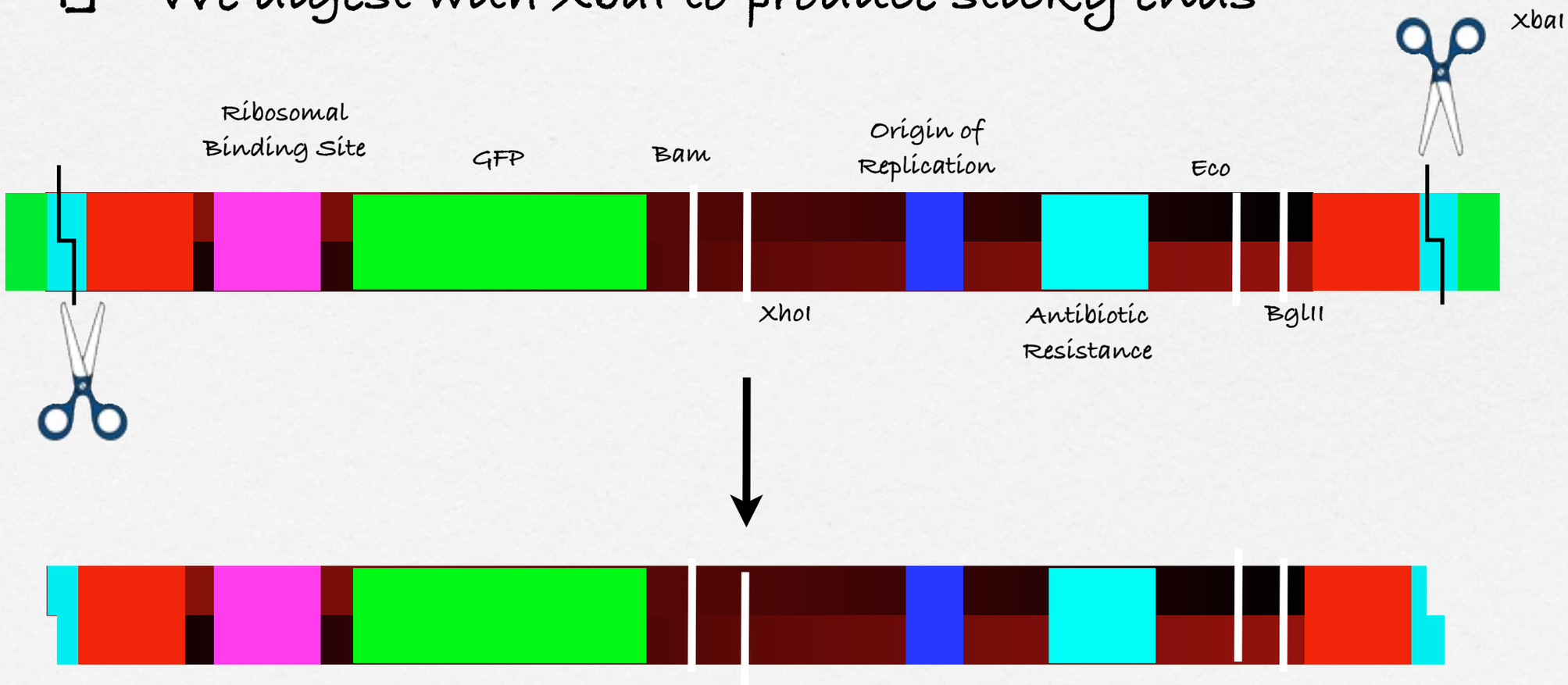
Our Primers in Inverse PCR

- In PCR, the primers are part of the newly synthesized strands
- We can therefore design our primers to add in the XbaI site



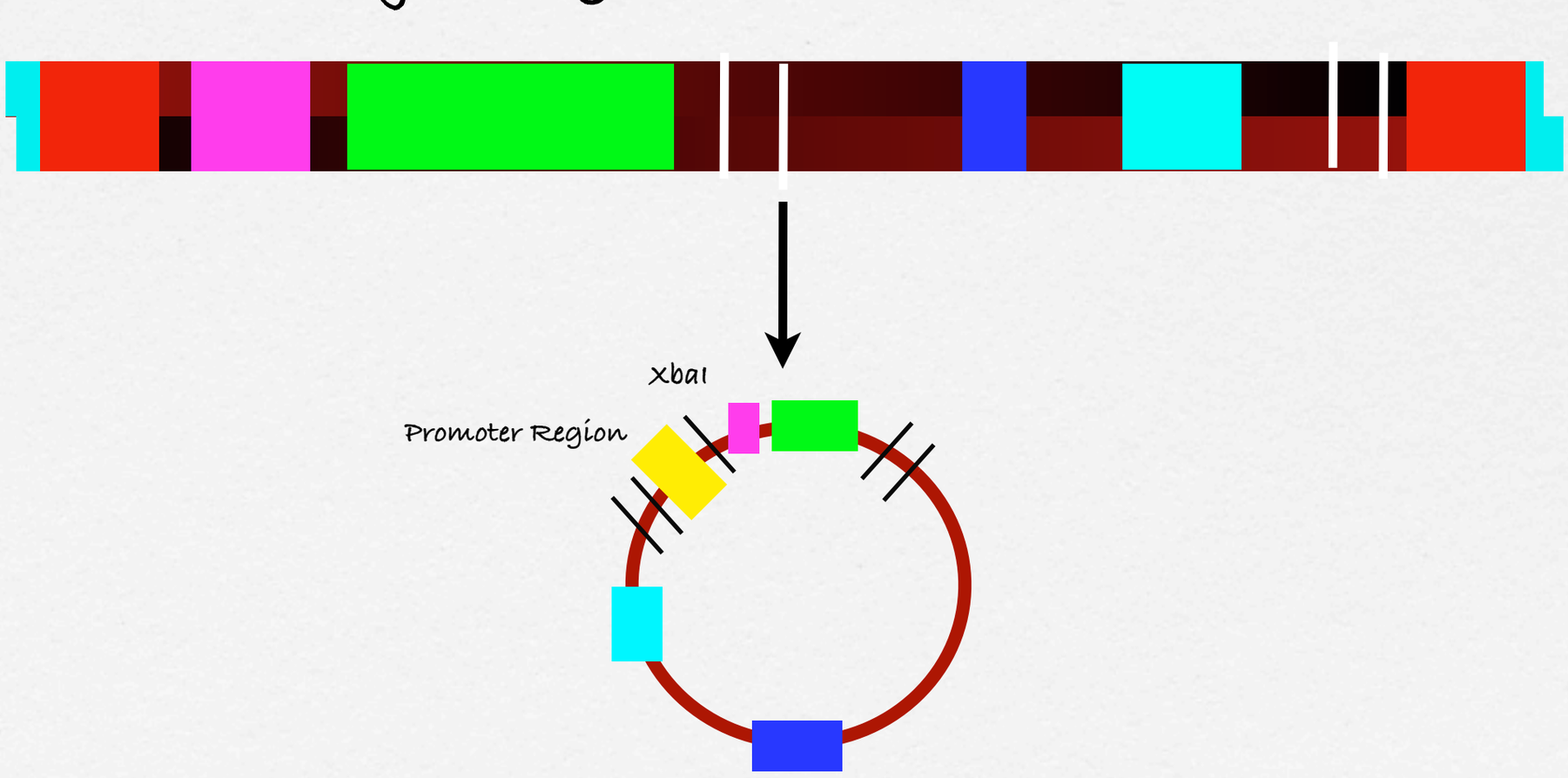
After PCR: Cutting with XbaI

- After we have done PCR, we will have linear strands of DNA with XbaI sites at either end
- We digest with XbaI to produce sticky ends



Ligation and... Plasmid

- Now we add DNA Ligase
- The sticky ends on either side of the plasmid overlap, and will want to join together



Summary

- Our project is on classifying promoter regions
- We want to be able to swap promoter regions, so we are adding a restriction enzyme site to flank only the promoter region; this makes it easier to swap out
- We can insert the restriction enzyme site using Inverse PCR
- Inverse PCR copies the entire plasmid, instead of just the target gene