

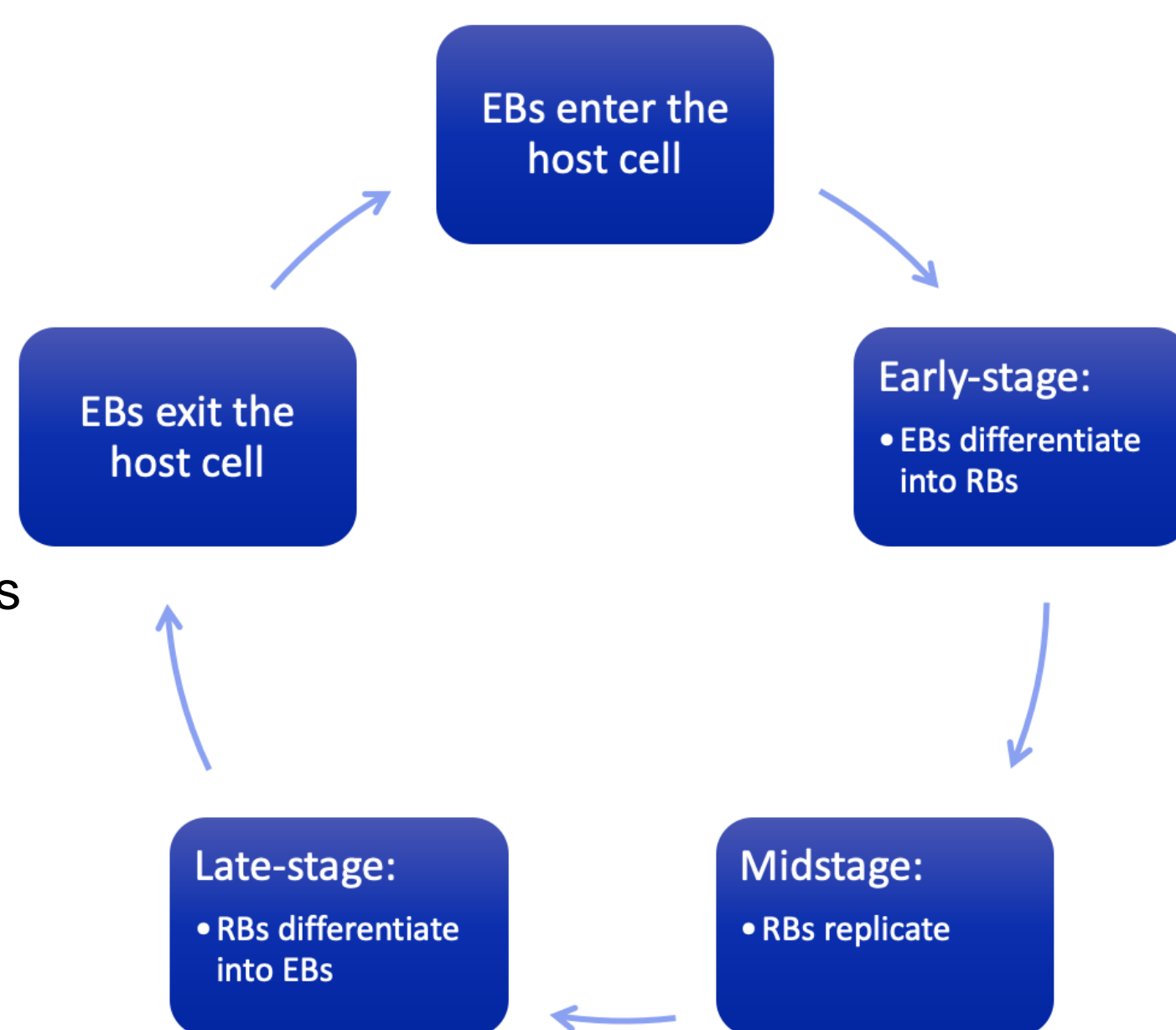
# Investigating the Role of *LtuA* during the Developmental Cycle of *Chlamydia trachomatis*

Amanda Waelde<sup>1</sup>, Kathryn Weidner<sup>2</sup>, Dr. Li Shen<sup>3</sup>  
University of Notre Dame<sup>1</sup>, LSUHSC School of Medicine<sup>2</sup>, LSUHSC<sup>3</sup>

## Introduction

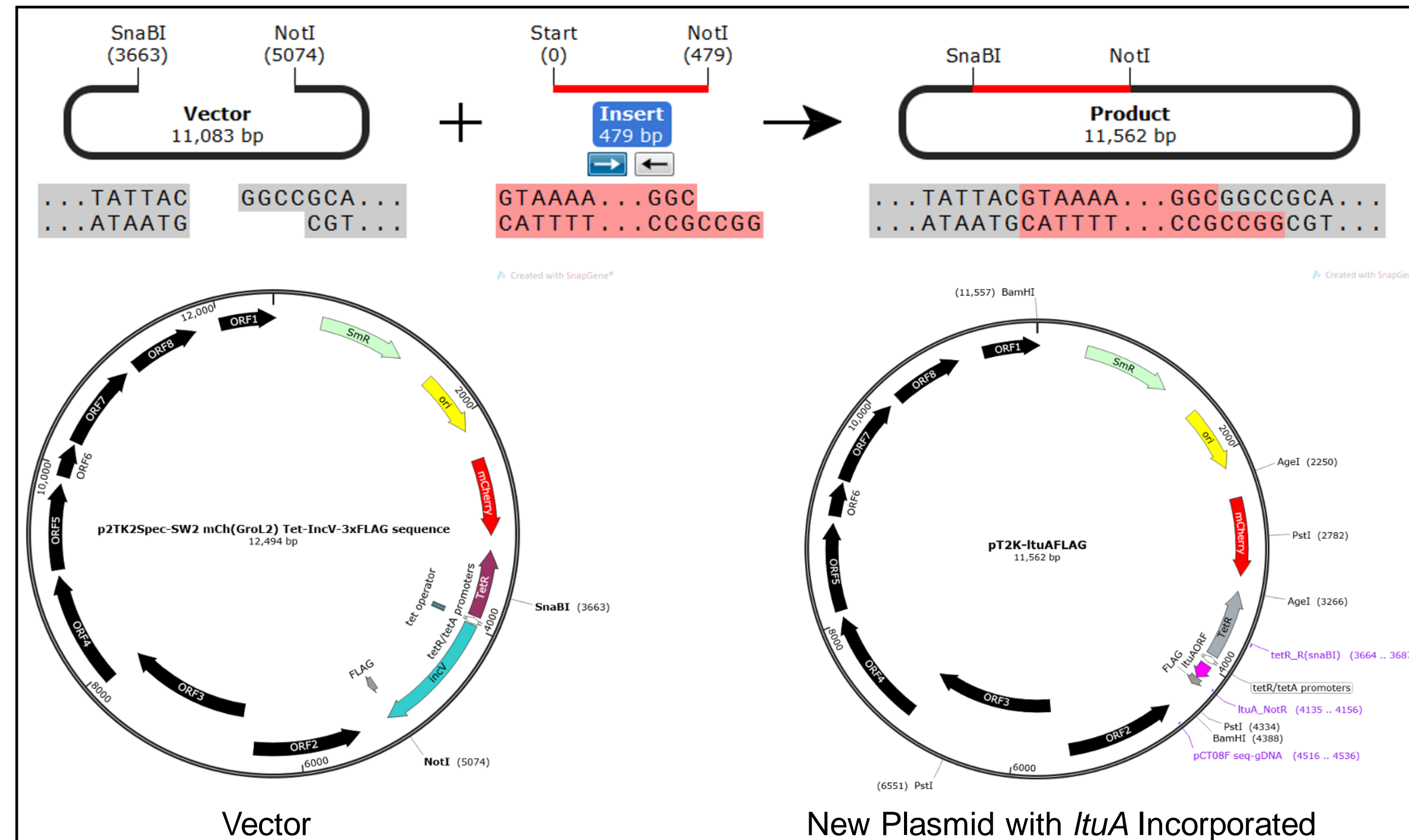
*Chlamydia trachomatis* is a Gram-negative obligate intracellular pathogen. It is the leading infectious cause of blindness and the most common sexually transmitted bacterium. No vaccine currently exists.

During its characteristic developmental cycle, *C. trachomatis* alternates between an infectious, non-replicative form called as an elementary body (EB) and a non-infectious, replicative form called a reticulate body (RB). This development occurs in three stages. EBs transition to RBs in the early stage, RBs multiply in the middle stage, and RBs differentiate into EBs in the late stage. Growth at each stage correlates with a unique pattern of gene expression.



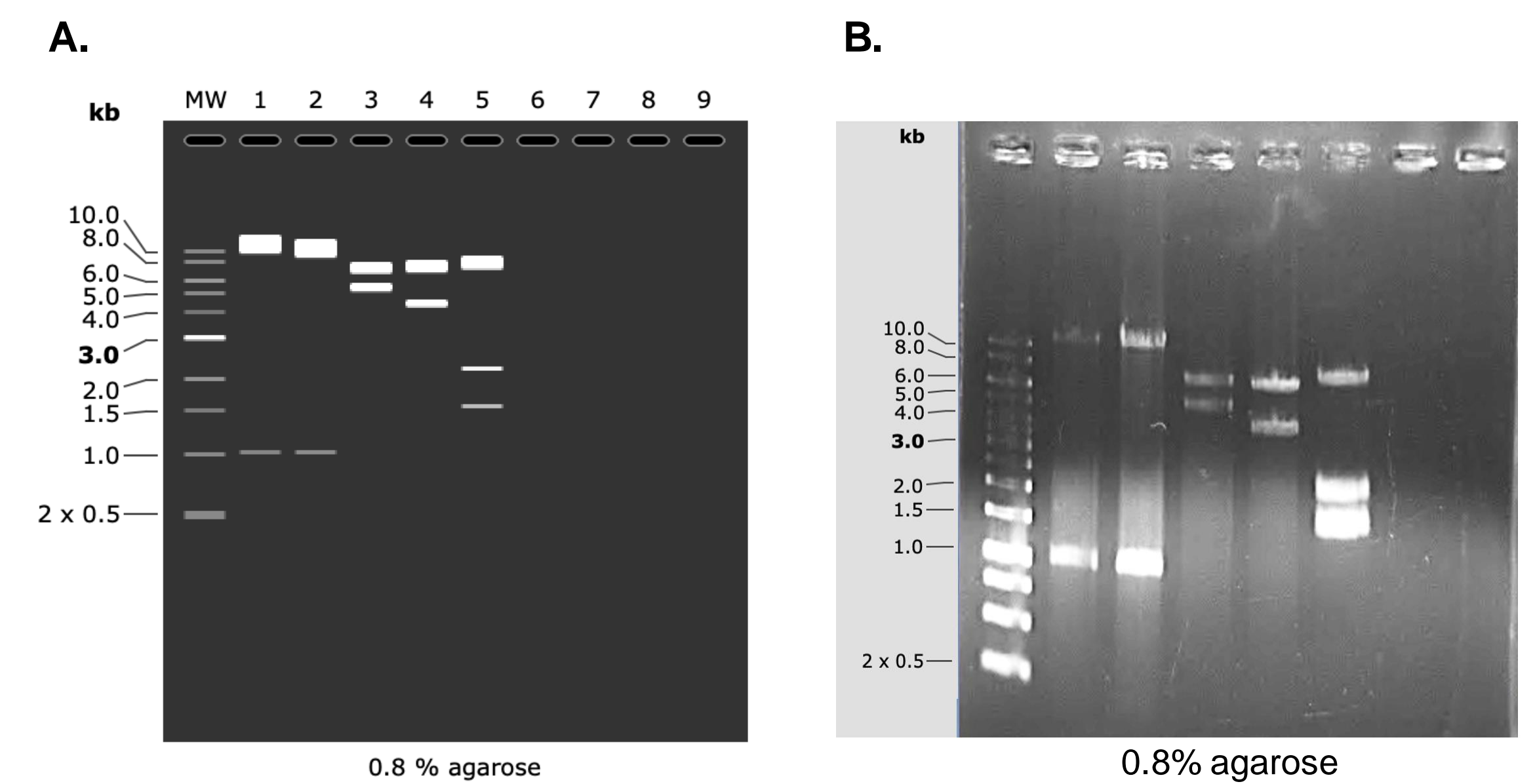
Historically, *C. trachomatis* has been difficult to study due to the lack of genetic tools available to manipulate chlamydial genes, so little is known about how its developmentally regulated gene expression occurs. *LtuA* has been shown to be expressed at the transcriptional level in the late stage, but the function of *LtuA* is unknown. Here, we sought to determine the function of *LtuA* by creating a plasmid containing *LtuA* under the control of a tetracycline-inducible promoter. We hypothesized that we could successfully incorporate *LtuA* into an existing *E. coli-Chlamydia* shuttle plasmid.

## Cloning strategy



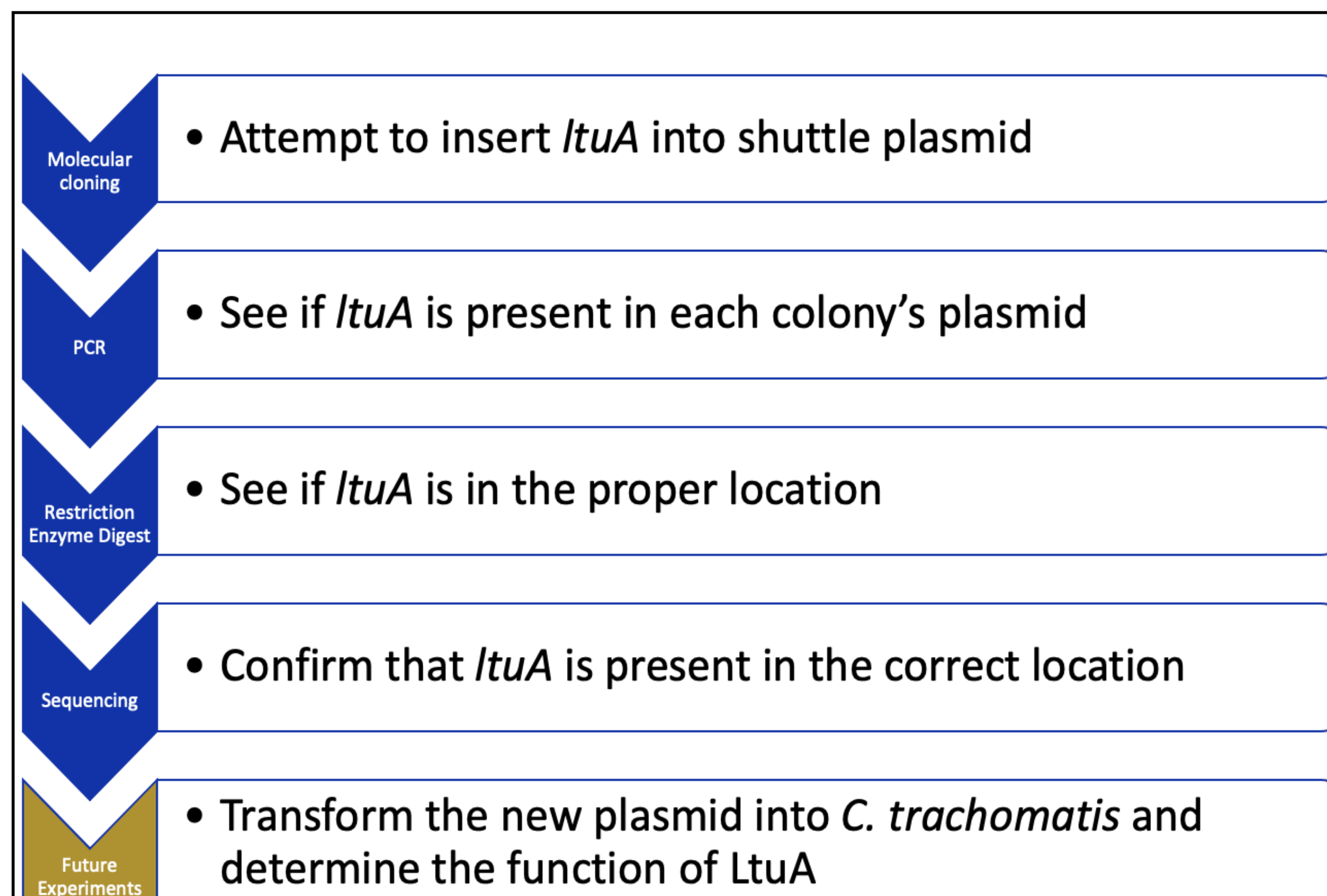
Molecular cloning was used to attempt to incorporate *LtuA* into an existing *E. coli-Chlamydia* shuttle plasmid, p2TK2Spec-SW2 mCh(GroL2) Tet-IncV-3xFLAG, shown at the left. The theoretical resulting plasmid, pT2K-LtuAFLAG, is shown at the right. However, due to the nature of the molecular cloning procedure, it is possible for many other products to form, so testing had to be done to determine which colonies had successfully incorporated *LtuA* into the correct location.

## Restriction Enzyme Digests

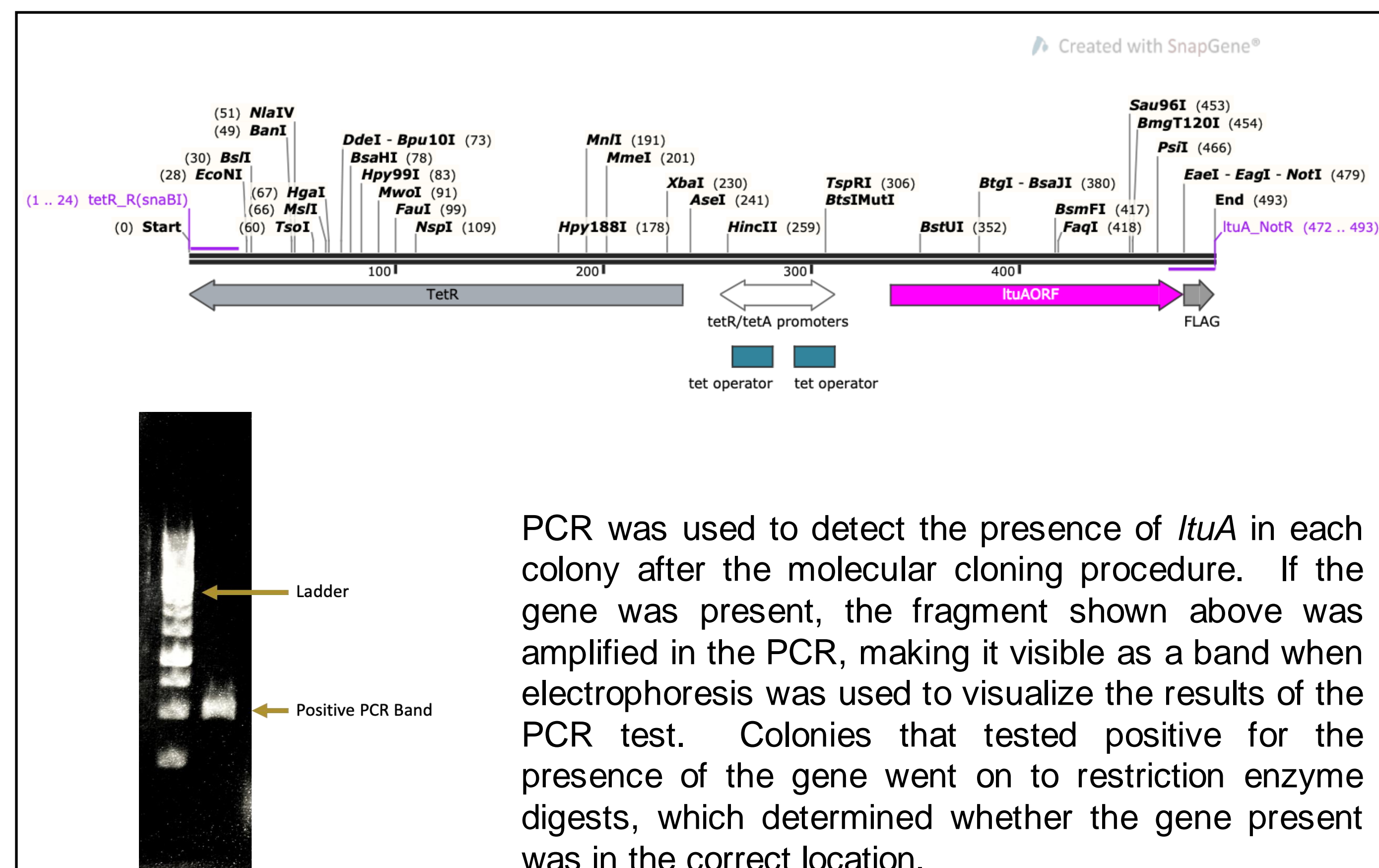


Plasmid DNA was isolated from an overnight culture of each colony that tested positive through PCR. The DNA and a control of the original shuttle plasmid were then cut with restriction enzymes, and the results were visualized through agarose gel electrophoresis. From left to right, the lanes in each image contain a 1 kb DNA ladder, original shuttle plasmid with *AgeI*, new plasmid containing *LtuA* with *AgeI*, original shuttle plasmid with *BamHI*, new plasmid containing *LtuA* with *BamHI*, and new plasmid containing *LtuA* with *PstI*. Part A shows SnapGene's predicted results of the digest if the DNA sequence of the new plasmid from the colony is correct and *LtuA* is present in the correct location. Part B shows the results of the actual gel, matching the predicted results and confirming that *LtuA* was present in the proper location in the plasmid from the colony being tested.

## Methodology



## Polymerase chain reaction (PCR)



PCR was used to detect the presence of *LtuA* in each colony after the molecular cloning procedure. If the gene was present, the fragment shown above was amplified in the PCR, making it visible as a band when electrophoresis was used to visualize the results of the PCR test. Colonies that tested positive for the presence of the gene went on to restriction enzyme digests, which determined whether the gene present was in the correct location.

## Conclusions

**Results:**

- We successfully constructed the plasmid carrying *LtuA* in the correct location.

**Future Experiments:**

- This expression vector will be transformed into *C. trachomatis* cells using our established approach.
- We will assess whether the conditional expression of *LtuA* can be achieved by adding tetracycline at the optimal concentration in the transformed bacteria.
- We will test whether *LtuA* expression may affect the bacterial growth phenotype. Through analysis of growth patterns and the temporary gene expression profile of *C. trachomatis*, we expect to determine the function of *LtuA*.

## References

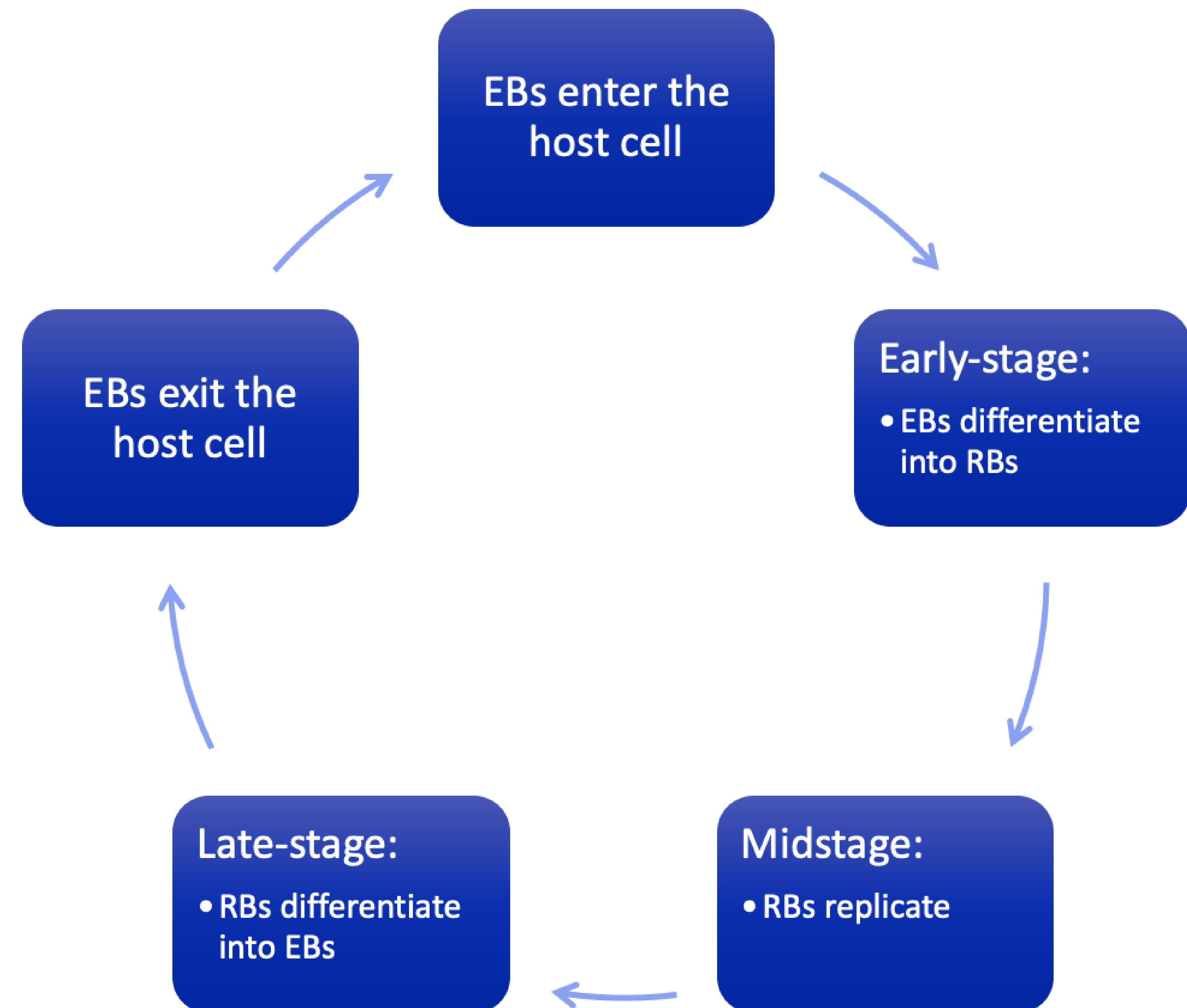
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# Introduction

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Historically, *C. trachomatis* has been difficult to study due to the lack of genetic tools available to manipulate chlamydial genes, so little is known about how its developmentally regulated gene expression occurs. *ItuA* has been shown to be expressed at the transcriptional level in the late stage, but the function of *LtuA* is unknown. Here, we sought to determine the function of *ItuA* by creating a plasmid containing *ItuA* under the control of a tetracycline-inducible promoter. We hypothesized that we could successfully incorporate *ItuA* into an existing *E. coli-Chlamydia* shuttle plasmid.

# Methodology

Molecular  
cloning

- Attempt to insert *ltuA* into shuttle plasmid

PCR

- See if *ltuA* is present in each colony's plasmid

Restriction  
Enzyme Digest

- See if *ltuA* is in the proper location

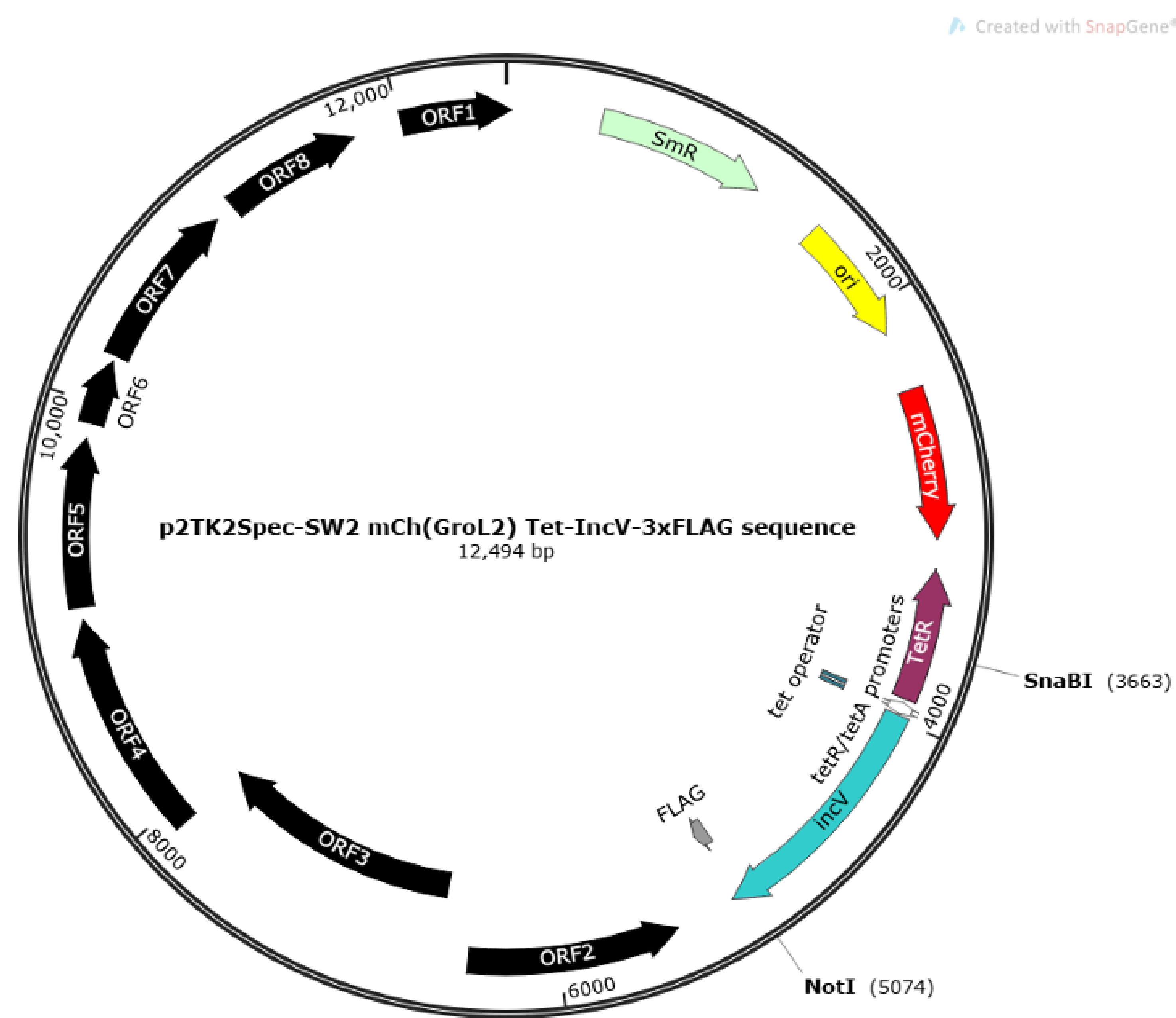
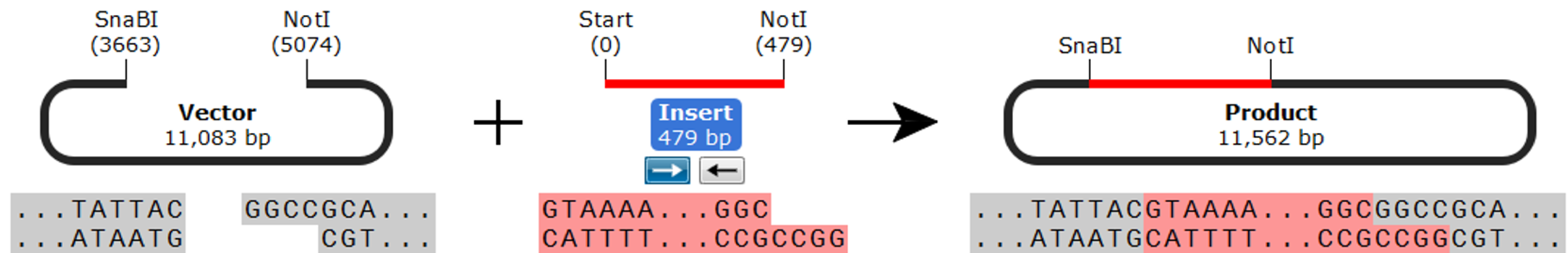
Sequencing

- Confirm that *ltuA* is present in the correct location

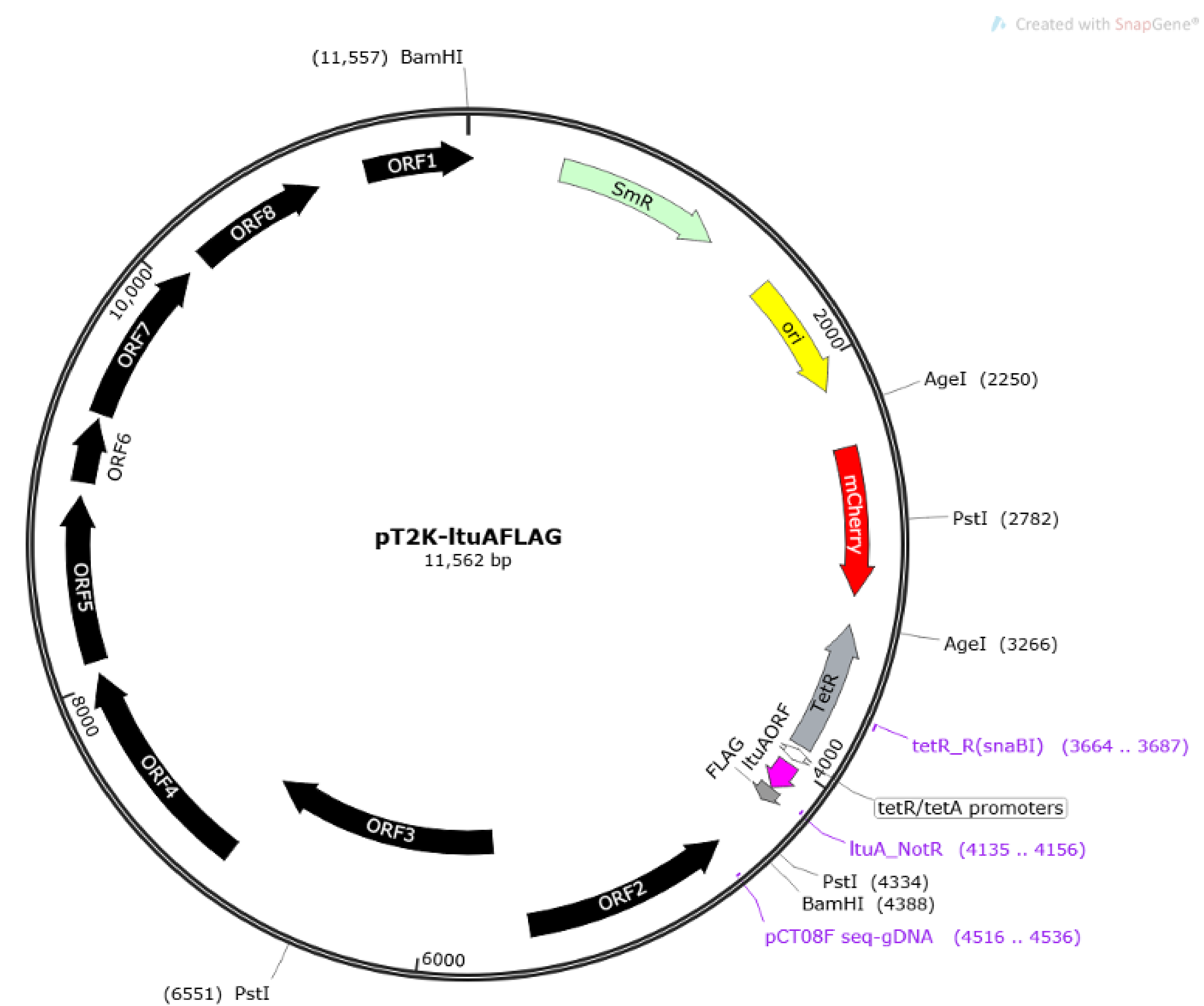
Future  
Experiments

- Transform the new plasmid into *C. trachomatis* and determine the function of LtuA

# Cloning



Vector



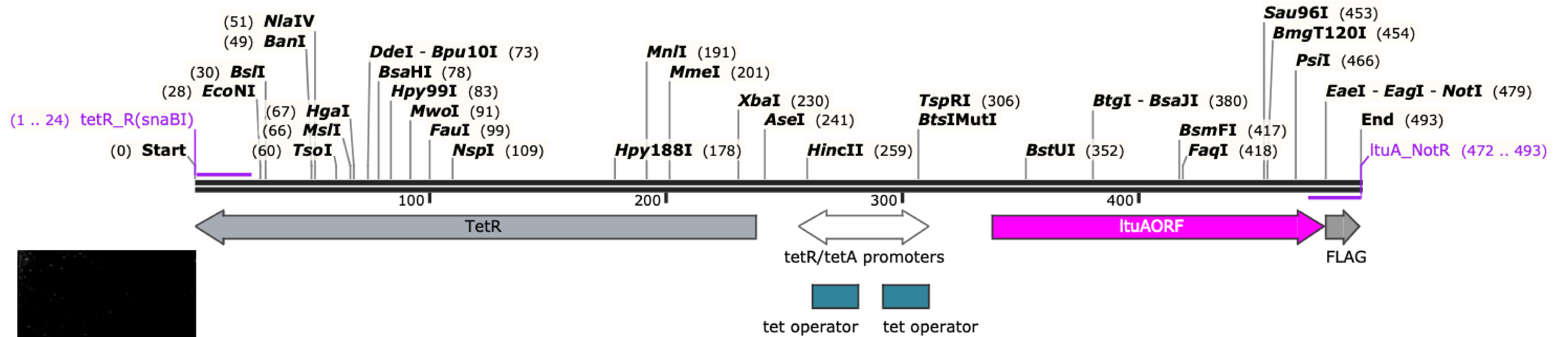
New Plasmid with *ItuA* Incorporated

Molecular cloning was used to attempt to incorporate *ItuA* into an existing *E. coli-Chlamydia* shuttle plasmid, p2TK2Spec-SW2 mCh(GroL2) Tet-IncV-3xFLAG, shown at the left. The theoretical resulting plasmid, pT2K-ItuAFLAG, is shown at the right. However, due to the nature of the molecular cloning procedure, it is possible for many other products to form, so testing had to be done to determine which colonies had successfully incorporated *ItuA* into the correct location.

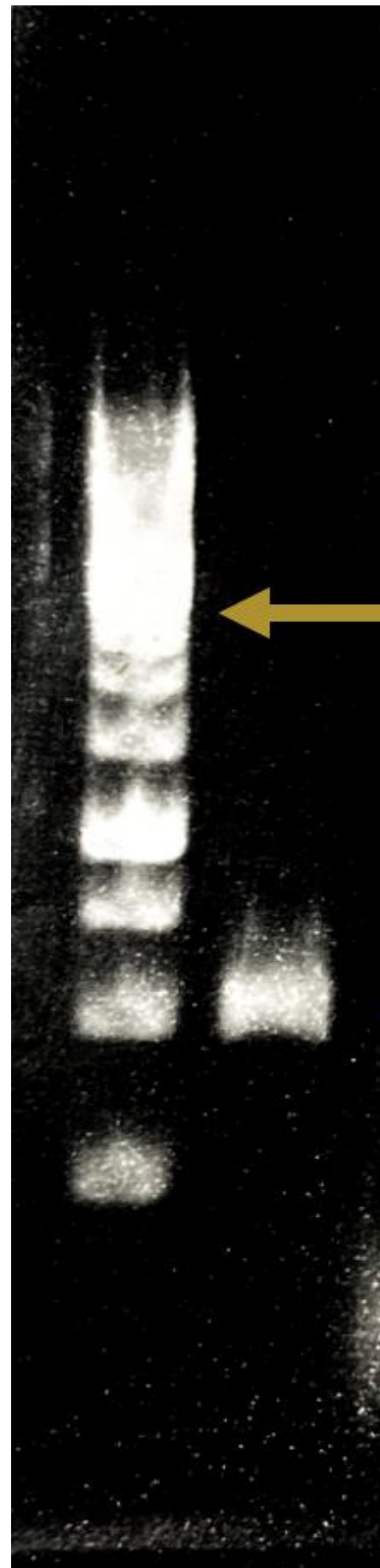


# PCR

Created with SnapGene®



Amplified  
493 bp



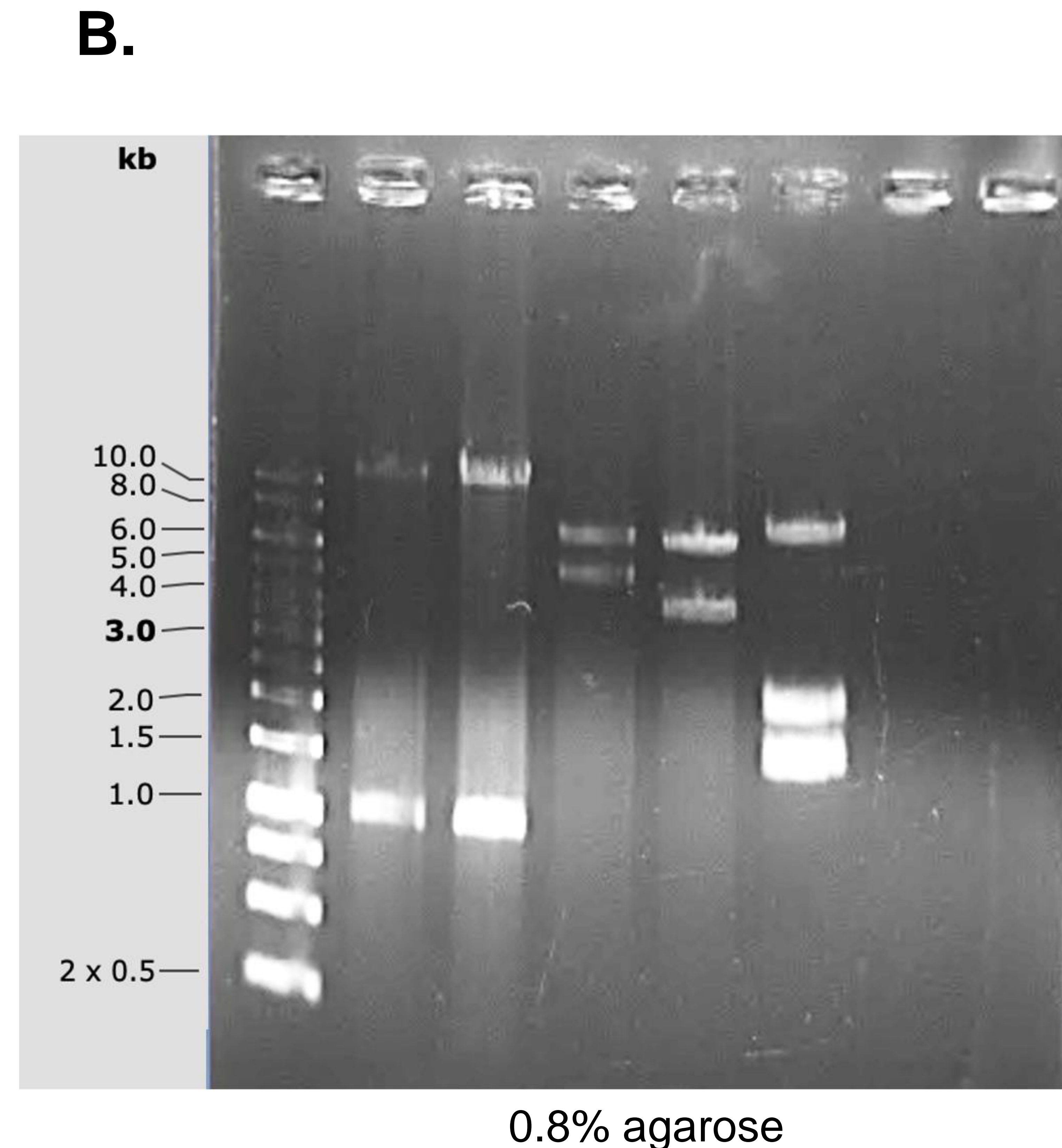
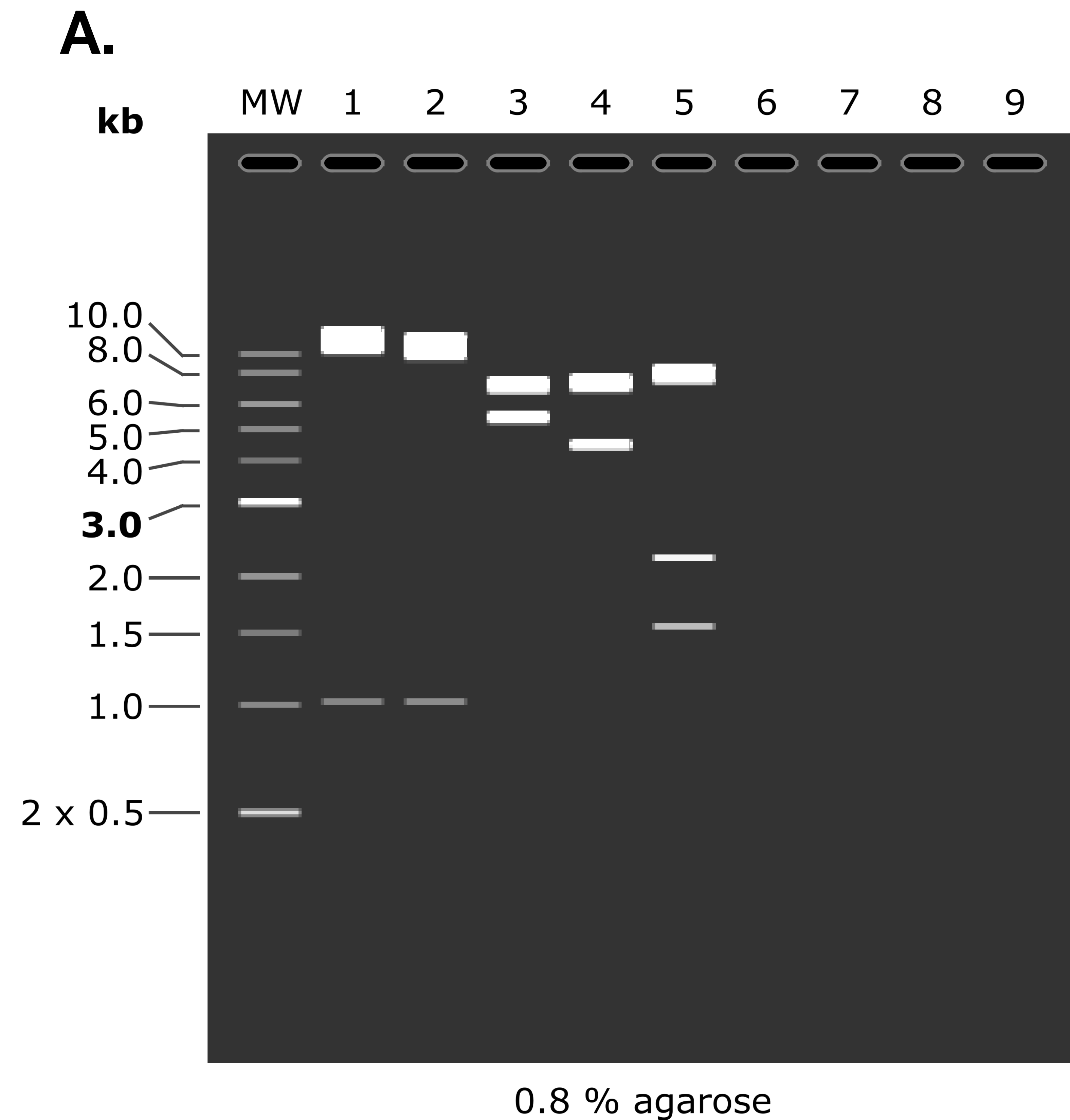
Ladder

Positive PCR Band

PCR was used to detect the presence of *ItuA* in each colony after the molecular cloning procedure. If the gene was present, the fragment shown above was amplified in the PCR, making it visible as a band when electrophoresis was used to visualize the results of the PCR test. Colonies that tested positive for the presence of the gene went on to restriction enzyme digests, which determined whether the gene present was in the correct location.



# Restriction Enzyme Digests



Plasmid DNA was isolated from an overnight culture of each colony that tested positive through PCR. The DNA and a control of the original shuttle plasmid were then cut with restriction enzymes, and the results were visualized through agarose gel electrophoresis. From left to right, the lanes in each image contain a 1 kb DNA ladder, original shuttle plasmid with Agel, new plasmid containing *ItuA* with Agel, original shuttle plasmid with BamHI, new plasmid containing *ItuA* with BamHI, and new plasmid containing *ItuA* with PstI. Part A shows SnapGene's predicted results of the digest if the DNA sequence of the new plasmid from the colony is correct and *ItuA* is present in the correct location. Part B shows the results of the actual gel, matching the predicted results and confirming that *ItuA* was present in the proper location in the plasmid from the colony being tested.



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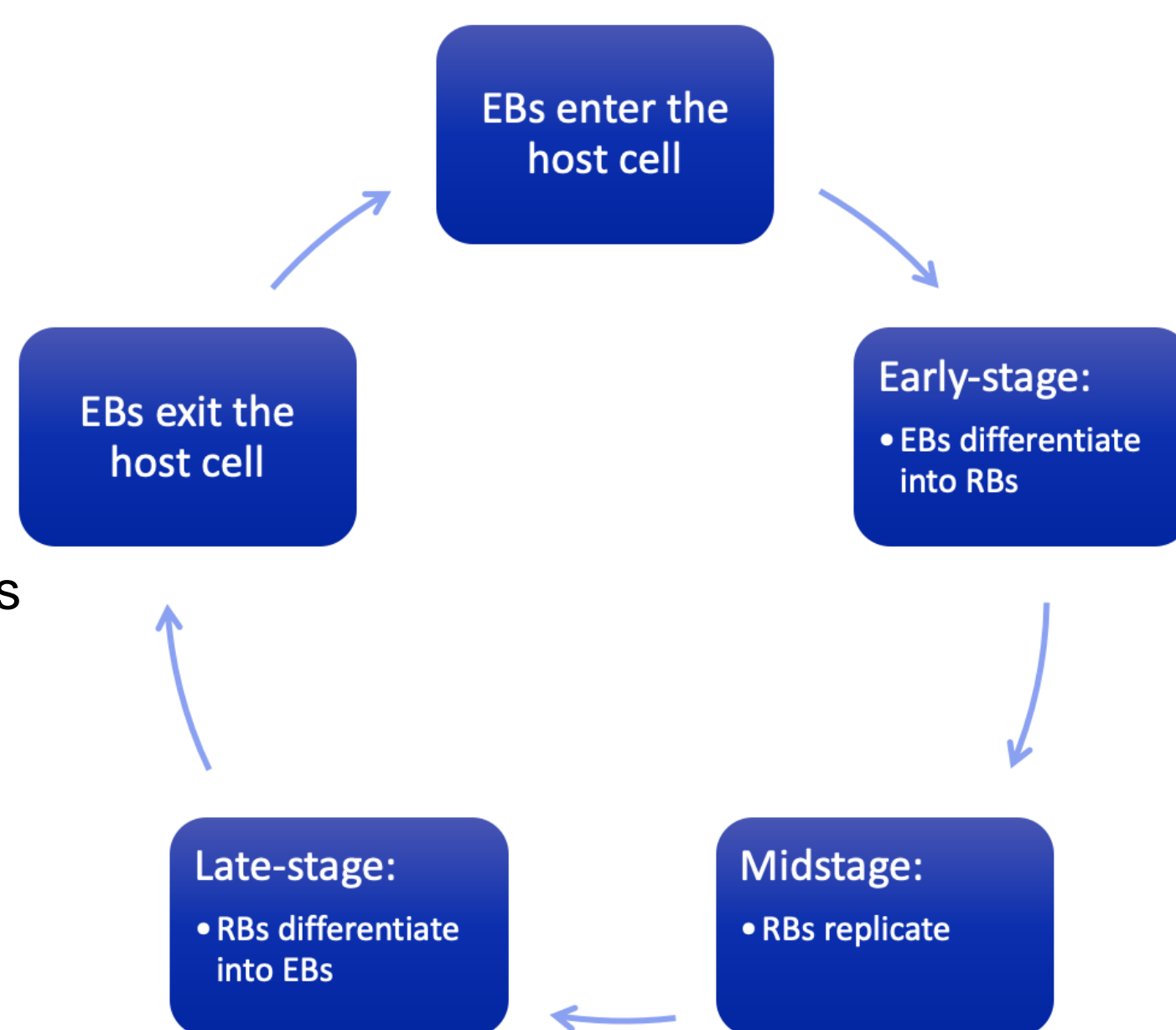
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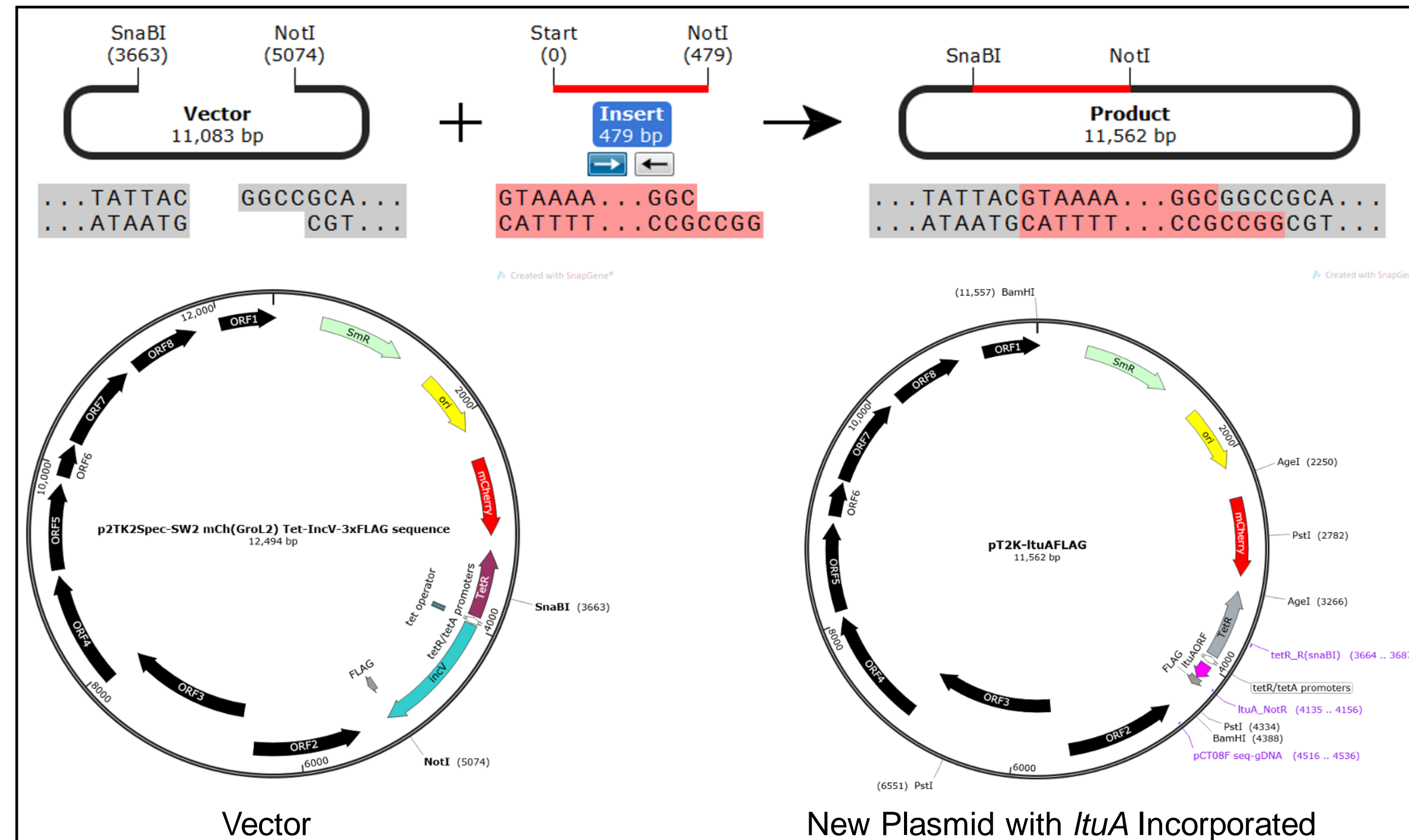
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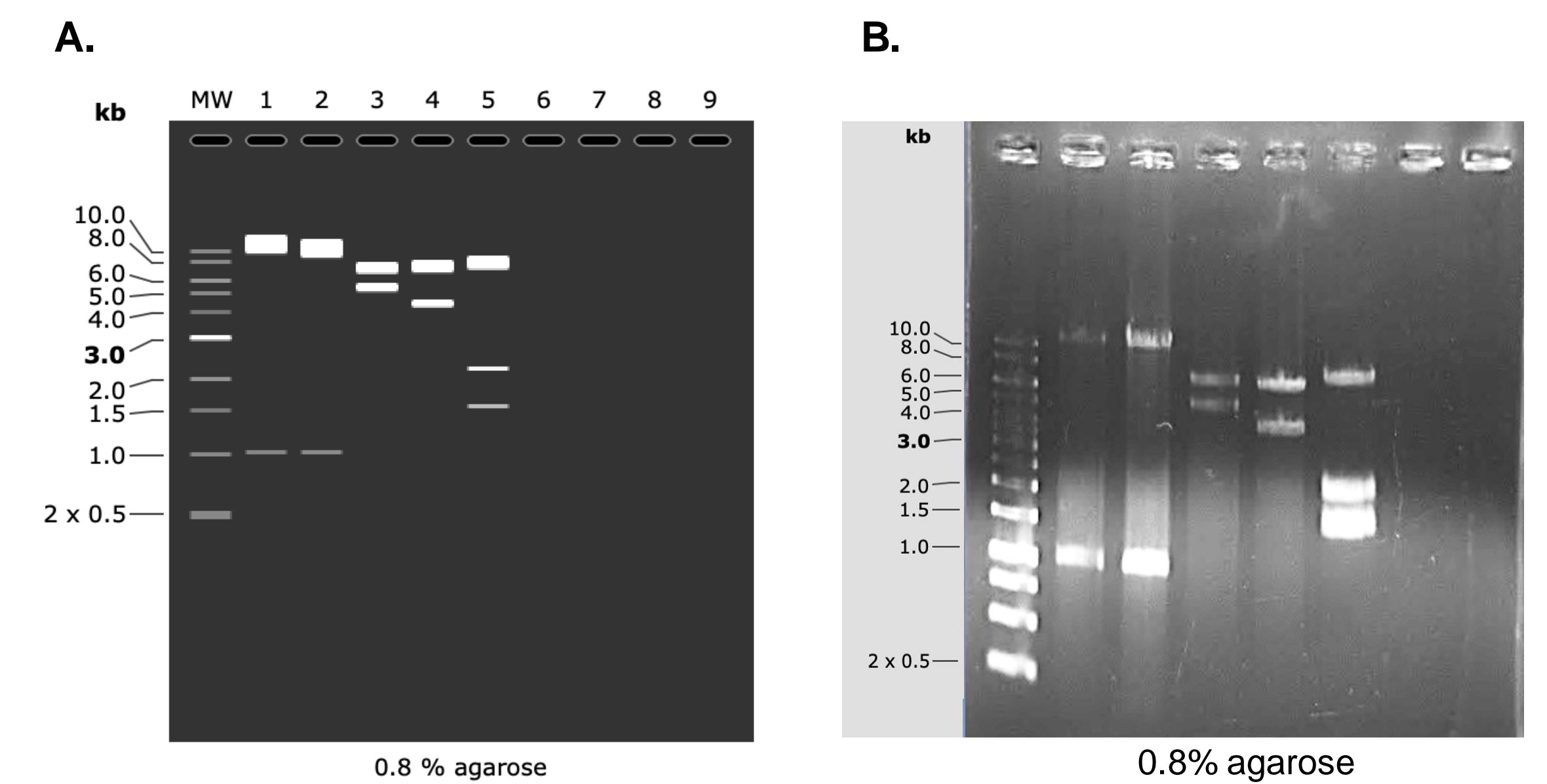
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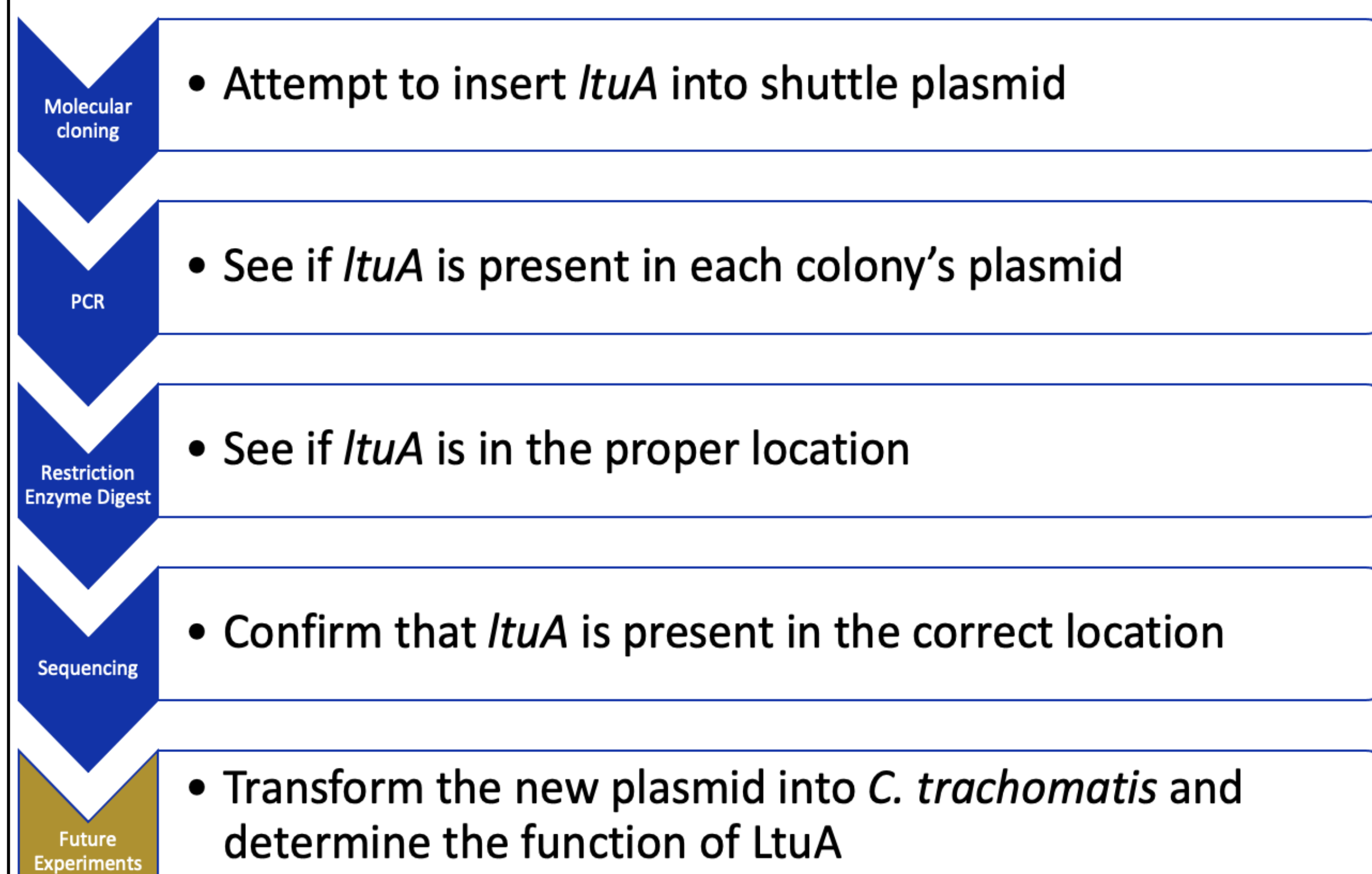
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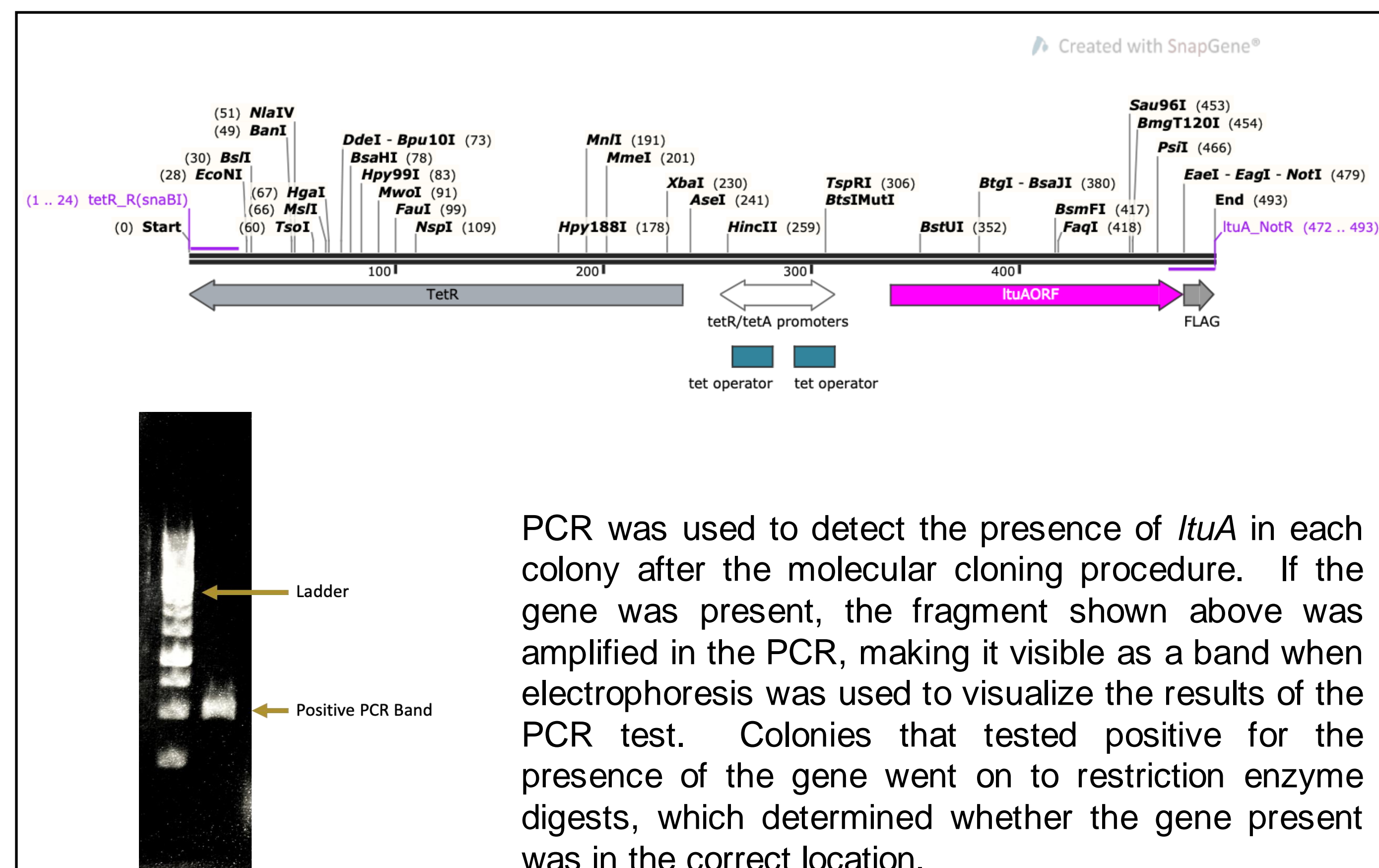


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