Creation of a Translational Fusion of IncV-β-lactamase to Improve the Study of Chlamydial Type III Secretion *in situ*

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Introduction

Chlamydia trachomatis is an obligate intracellular bacterium that uses a type III secretion system (T3SS), along with multiple others, to release anti-host-effector proteins into infected cells for benefit of this pathogen. However, little is known about how *C. trachomatis* controls its important secretion process, in part, due to historic lack of genetic tool to manipulate gene in *Chlamydia*. Traditionally, the effector proteins need to be overexpressed for assessment using a surrogate T3SS. Recent studies have developed a way to more directly assess secretion in *C. trachomatis* infected cells. One such advancement is the use of the fusion of effector to β-lactamase (Bla) as a reporter system combining with Förster resonance energy transfer (FRET) for assessment of effector secretion *in situ*. Here, we present the creation of a recombinant gene construct that allows for conditionally inducible expression of the effector-*bla* fusion in order to probe the dynamics of the secretion process.

Methods and materials

pIncV was prepared from *E. coli* cells and digested with NotI and SpeI.

***bla* encoding an ampicillin resistant gene was amplified with PCR and digested with NotI and SpeI.**

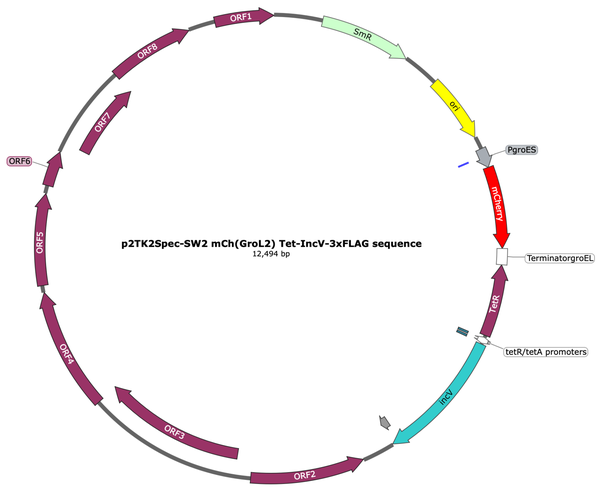
The above DNA fragments were then ligated and used to transform *E.coli* DH5-α cell. Transformants were selected on spectinomycin containing LB agar plate.

Colony PCR was done to amplify the inserted *bla* in of our new plasmid

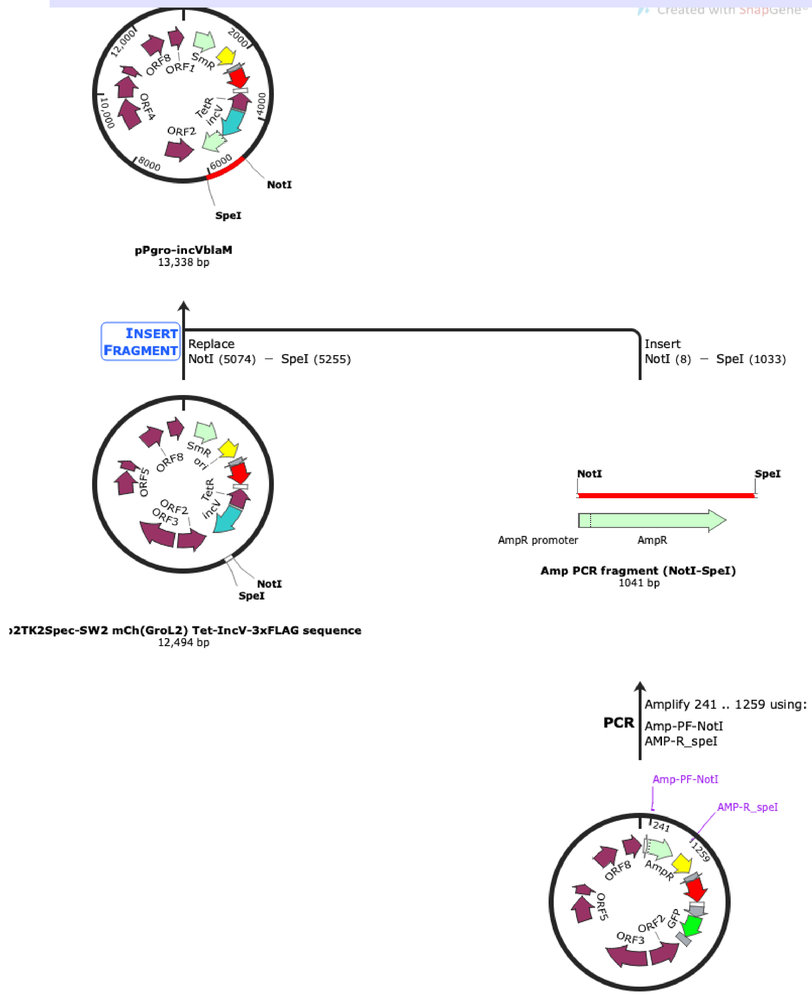
PCR products were examined on 0.8% agarose gel electrophoresis

Map of cloning vector

E. coli-C. trachomatis shuttle vector p2TK2Spec-SW2 mCh(GroL2) Tet-IncV-3xFLAG was used as the backbone for cloining .

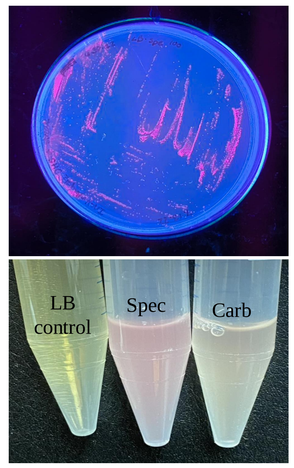


Cloning strategy



Results

Growth of *E. coli* cells carrying pP*gro*-incV*bla*M



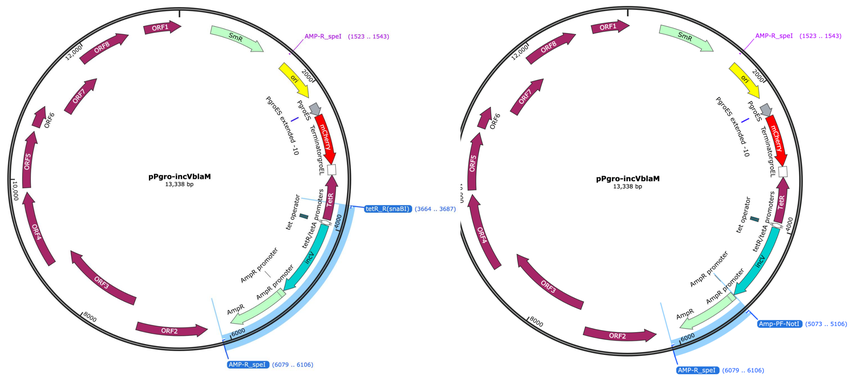
Growth of transformed *E. coli* on Spectinomycin containing plates;

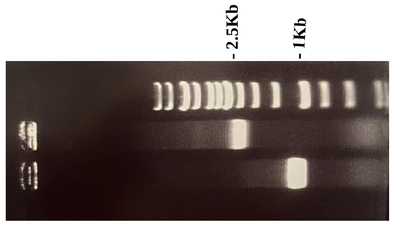
Expression of mCherry (red) was visualized under UV light.

Growth of transformed *E.coli* in Carbenicillin and Spectinomycin containing LB broth;

The growth of *E. coli* in Carbenicillin suggests expression of Ampicillin resistance gene.

PCR was used to examine gene of interest in *E. coli*





-Examining PCR products ; evidence of each fragment in 0.8% agarose gel

Conclusion

The construct pP*gro*-incVblaM was accomplished through cloning and transformation; we can conclude that our recombinant gene construct was successfully transformed because of the phenotypically expressed mCherry gene and Ampicillin resistance gene. In the future, this method will be used to introduce new genes into Chlamydia cells to study and influence its type three secretion system.

References

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