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"Creation of a translational fusion of IncV-β-lactamase as a reporter to Improve the Study of Chlamydial type III Secretion System *in situ*"

Abstract

Background: *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 genes 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 fusion of *incV*, an T3SS effector, and *bla* in order to probe the dynamics of the secretion process.

Methods: An *E. coli –C. trachomatis* shuttle plasmid, p2TK2SpecSW2mCh(GroL2)Tet-IncV-3xFLAG, was used as a vector backbone to clone a DNA fragment containing *bla* fused to a *incV* at the 3'. Thus, the incV-bla is under the control of the inducible *tet* promoter. The *bla* gene was amplified by PCR with a specific primer pair. PCR products and the vector plasmid were digested with Not I and Spe I and purified using agarose gel. The NotI-SpeI digested vector and the PCR fragment were ligated. *E. coli* DH5-α cells were transformed with the ligation reaction, followed by selection for spectinomycin resistant colonies. PCR, plasmid restriction enzyme digestion, agarose gel electrophoresis, and DNA sequencing were used to determine the identity of the construct. Bacteria growth in the presence of ampicillin was also examined.

Results: We have successfully constructed the shuttle plasmid containing the *incV-bla* fusion driven by the *tet* system. This expression plasmid will be transformed into *C. trachomatis* cells using our established approach. We will assess whether the conditional expression of *incV-bla* can be achieved by adding inducer in *Chlamydia,* whether IncV secretion can be assessed during infection using FRET, and how secretion of IncV affects the bacterial growth phenotype.

Special Instructions: The abstract is a summary of the project. Do not to exceed one page. Do not change margins, font style or font sizes on this page. **Use this format only- do not modify!!!**