# The Role of recA in the Natural Transformation of *Aeromonas salmonicida*

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Biology Research

## Abstract

The genus *Aeromonas* is a gram-negative group of bacteria that are ubiquitous in various fresh water bodies. *Aeromonas* has also been found to be an agent of several gastrointestinal diseases and are often opportunistic pathogens. A characteristic of *Aeromonas* that has recently been discovered is natural transformation, which is the ability to take up environmental DNA and incorporate it into the bacterium's genome. However, natural transformation has only recently been found to occur in the species *Aeromonas salmonicida.* Preliminary studies indicate that the gene *recA*, along with several other genes of interest, might be necessary for natural transformation to occur in this species. The gene *recA* encodes a DNA repair protein that is activated in the presence of DNA fragments and assists in the ligation of the free DNA ends. In order to investigate the role of *recA* in natural transformation of *A. salmonicida*, we will knock out the *recA* gene with a gentamycin-resistance cassette through mutation with a process called splice overlap extension PCR (SOE-PCR). Then, screening of the mutant species will occur to detect a lost ability to transform free DNA, thus indicating *recA*'s role in natural transformation in *Aeromonas salmonicida.* This research is part of a larger investigation of multiple genes that preliminary studies suggest might be involved in natural transformation of this species.

## Introduction

*Aeromonas salmonicida* are gram-negative rods found in aquatic environments and have been found to cause opportunistic infections in the intestines of humans. The genus *Aeromonas* possesses a characteristic known as natural transformation, which is the ability to uptake and incorporate plasmids into the bacterium's genome. Natural transformation, a type of horizontal gene transfer, is achieved when the aeromonad is subjected to environmentally stressful conditions, such as antibiotics, that place selective pressures on the organism. The stress, in turn, induces the aeromonad to competence, or the readiness of a bacterium to transform DNA. Antibiotics have been shown to induce competence in several bacterial species, which raises the issue of spreading antibiotic resistance among both bacteria of the same and different species—an increasing concern among health care professionals and microbiologists. This process is referred to as "natural," since this genus and related genera possess the genes needed for the uptake and incorporation of free DNA into the bacterial genome. The implications of this research will further our understanding of the phenomena of natural transformation and assist in efforts to approach the growing concerns associated with this process. The *recA* gene is a widely distributed gene that is involved in DNA repair frequently associated with UV degradation of DNA. The goal of this research is to establish *RecA*'s involvement in *Aeromonas'* ability to naturally transform environmental DNA.

## Acknowledgements

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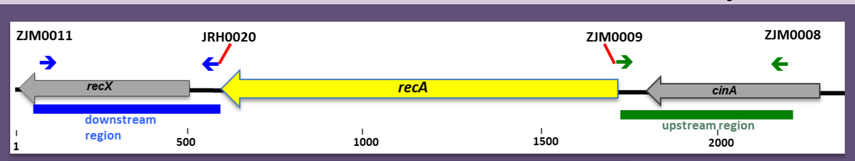


**Figure 1.** Map of primers for amplification of wild-type *A. salmonicida recA* gene as well as the upstream and downstream flanking regions.


**Figure 2.** Map of *A. salmonicida recA* gene replaced with the Gentamycin resistance cassette.


**Figure 3.** Nucleotide alignment of SOE PCR Gentamycin resistance mutant fragment ABC.

## Materials and Methods

A Splice Overlap Extension (SOE) PCR protocol was designed to create a DNA fragment to transform into the wild-type *Aeromonas salmonicida* , which would replace the *recA* gene with a gentamycin resistance cassette (Figure 4). Primers were designed by former Huddleston lab members to amplify 500bp of flanking sequences upstream and downstream of the *recA* gene. ZJM008 and ZJM009 were used to amplify the upstream flanking region "A" and JRH0020 and ZJM0011 were used in amplification of the downstream flanking region "C" (Figure 1). The gentamycin resistance cassette "B" was amplified with primers JRH0010 and JRH0011. After amplification of all 3 regions, SOE PCR was performed to combine products "A" and "B" using primers ZJM0011 and JRH0011 to form a newly constructed product "AB." Then, another SOE PCR reaction was performed to combine products "AB" and "C" using primers ZJM0008 and ZJM0011 to attempt to create the final product "ABC," to be used in the transformation of *A. salmonicida*. Finally, due to an interesting position of the "ABC" product band after gel electrophoresis, the sequence was sent to Yale to be sequenced to determine the integrity of the SOE PCR product before beginning the transformation protocols.

SOE PCR Scheme: Splicing overlap extension polymerase chain reaction

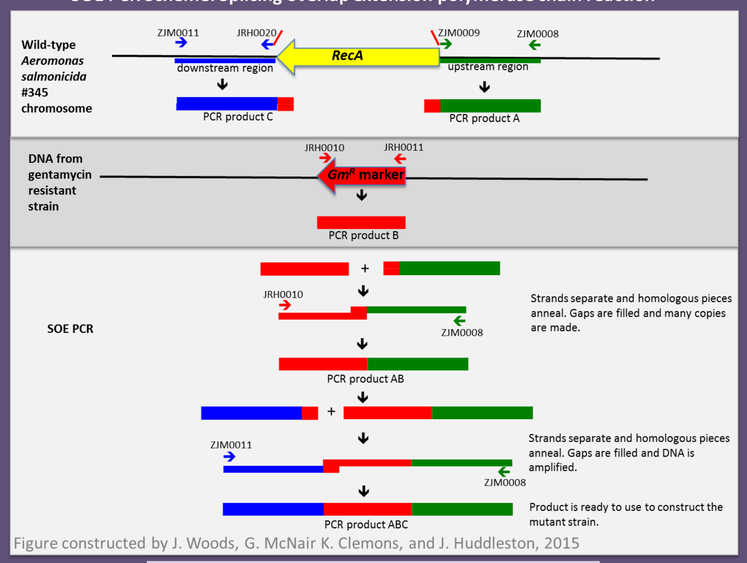


**Figure 4.** SOE PCR scheme for replacing the *recA* gene to create product ABC for the mutant strain.

## Results and Discussion

The production of the SOE PCR mutant fragment for creation of the *recA* mutant is still underway, as we are investigating the reasons the protocol is not working properly. We have consistently created the same incorrect "ABC" fragment as the sequencing alignment shows the upstream genes in the product contain many mismatches. Upon careful analysis, it was found that the mismatched sequence is the same sequence repeated 13 times, and it is the overhang of the primer ZJM0009 that should anneal to the Gentamycin region (Figure 3). It is evident that the downstream region + the Gentamycin resistance cassette is successful; however, the downstream region for unknown reasons under investigation is repeating incorrectly to yield an incorrect mutant fragment. In order to correctly produce our product "ABC," we will reamplify the upstream region with a primer that does not have the Gentamycin overhang. Then, we will reamplify product "A" with the primer ZJM0009 containing the Gentamycin overhang to be used in SOE PCR to create product "AB."

Once this issue is resolved and we can create product "ABC" from SOE PCR, it will be used to transform the wild type *A. salmonicida* into a mutant in which the *recA* gene is replaced by the gentamycin resistance cassette (Figure 2). The mutant strain will then be screened for loss of the ability to perform natural transformation by plating cultures onto plates containing gentamycin and indicated by successful cell growth. Additional confirmation of successful replacement will be demonstrated by the cell's lost ability to grow after being exposed to UV radiation as was previously demonstrated Zack Morgan and Dr. Jennifer Huddleston. After the replacement of *recA* has been confirmed, the culture will be put through a natural transformation assay, which will produce results consistent for an inability to naturally transform. Finally, *recA* will be reintroduced to the cells via a plasmid, and it will be demonstrated through the natural transformation assay that the ability of *Aeromonas salmonicida* to naturally transform DNA has been restored.

## Conclusion

This research project is well under way to show the involvement of *recA* in the natural transformation capabilities of aeromonads. Though still in progress, soon we will begin demonstrating the crucial role of RecA's DNA ligation abilities to naturally transform DNA. For now, the primary goal is to determine and correct the errors which are inhibiting successful production of the mutant "ABC" fragment, which in turn will allow for the final stages of screening for a loss of transformation in *A. salmonicida.* This research, once completed, will contribute to our understanding of the phenomenon of natural transformation, and will thus allow for improved insight into the methods by which bacteria gain resistance to antibiotics.

## Citations

1. J. R. Huddleston. 2008. Natural transformation in Aeromonas species. Texas Tech University:5,75-76.
2. Parker, Jennifer L., Shaw, Jonathan G., 2011. Aeromonas spp. clinical microbiology and disease. Journal of Infect. 62:110-111.