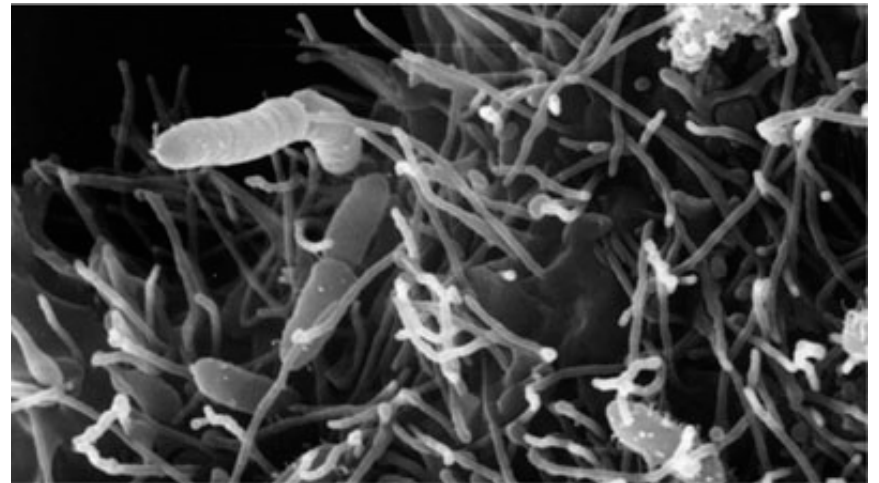


Determining Genes Necessary for Natural Transformation in *Aeromonas salmonicida*

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Aeromonas Genus

- Gram-negative bacteria
- Rod-shaped
- Ubiquitous
- Aquatic habitats
- Facultative anaerobic
- Ferments glucose



Huddleston, J. R. (2008). *Natural transformation in Aeromonas species*. Texas Tech University. Lubbock, TX

Kenyon, C. (2010, July 20). *Aeromonas hydrophilia* adhering to human epithelial cells [Web Photo]. Retrieved from <http://biowiki.kenyon.edu/index.php/Aeromonas>

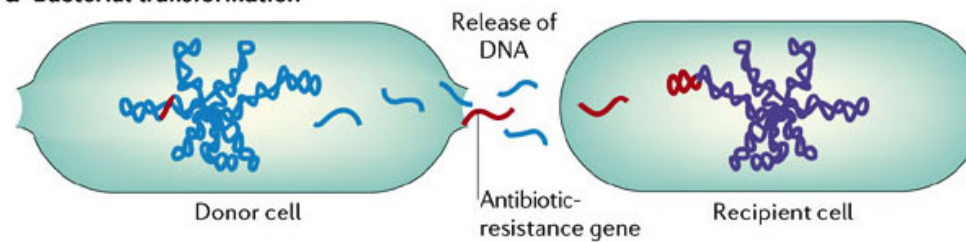
Causative Agent of Many Diseases



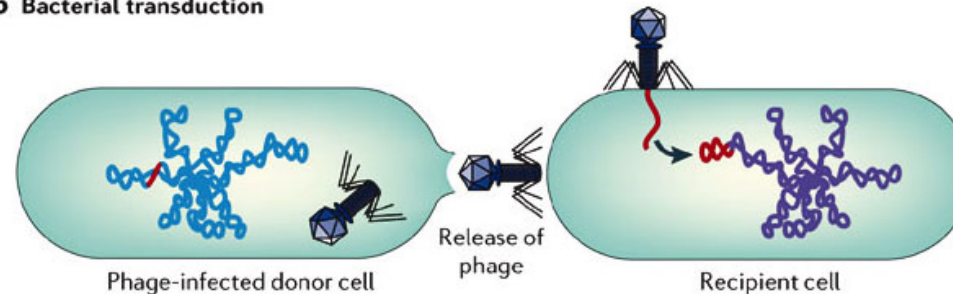
- Infected wounds and ingestion of contaminated water
- Treatment for infections is difficult due to increased antibiotic resistance
- Antibiotic resistance genes pass between individual cells

Horizontal Gene Transfer in Prokaryotes

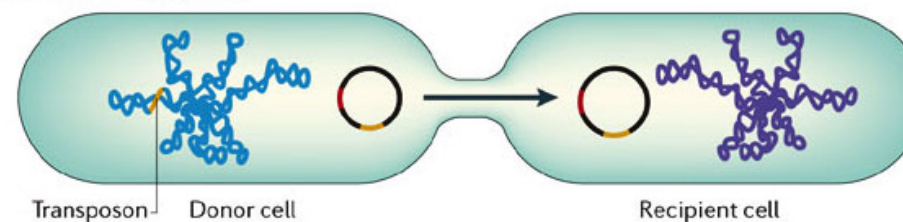
a Bacterial transformation



b Bacterial transduction



c Bacterial conjugation





Natural Transformation

The mechanism:

- DNA release
- DNA dispersal
- DNA persistence
- Recipient competence
- DNA binding, fragmentation, uptake
- DNA expression



Big Picture

The goal of this research is to understand the genetic processes by which *Aeromonas* bacteria transform extracellular DNA.

Process:

1. Gene Acquisition
2. Gene Sequencing
3. Gene Replacement
4. Cell Screening
5. Restoration of Gene Function

Hypothesis

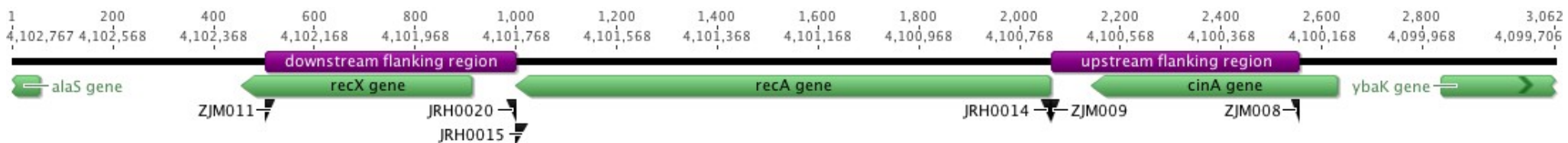
- *clpA/clpS* and *recA* encode proteins that are necessary for natural transformation to occur in *Aeromonas salmonicida*.

Genes of Interest: *clpA/clpS*

- Encode a caseinolytic protease
- Molecular chaperone that degrades damaged proteins during a stress response

Gene of Interest: *recA*

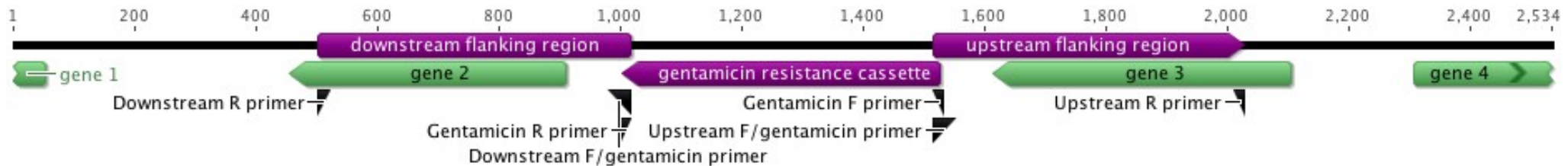
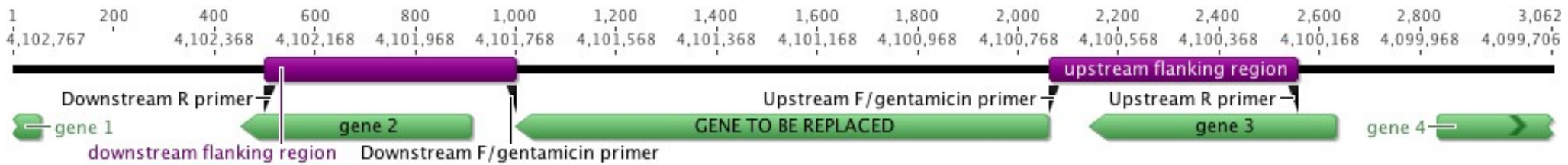
- Encodes a protein that initiates recombination of broken DNA by ligating the ends back together.



Gene Replacement

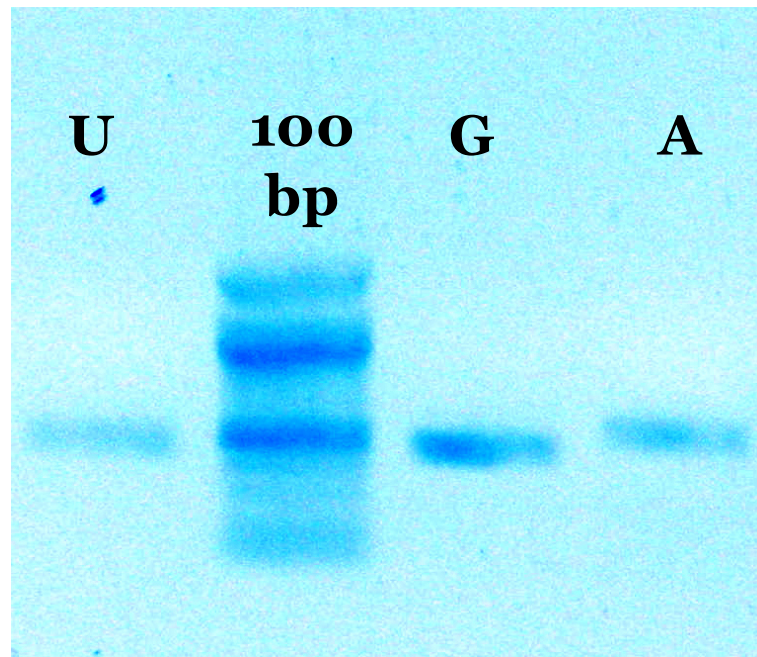
- The genes of interest and a gentamicin resistance cassette, which was used as a marker, were amplified.
- The amplified sequence was removed from the genome of *A. salmonicida* using a technique called splice overlap extension PCR, also known as SOE PCR.

SOE PCR



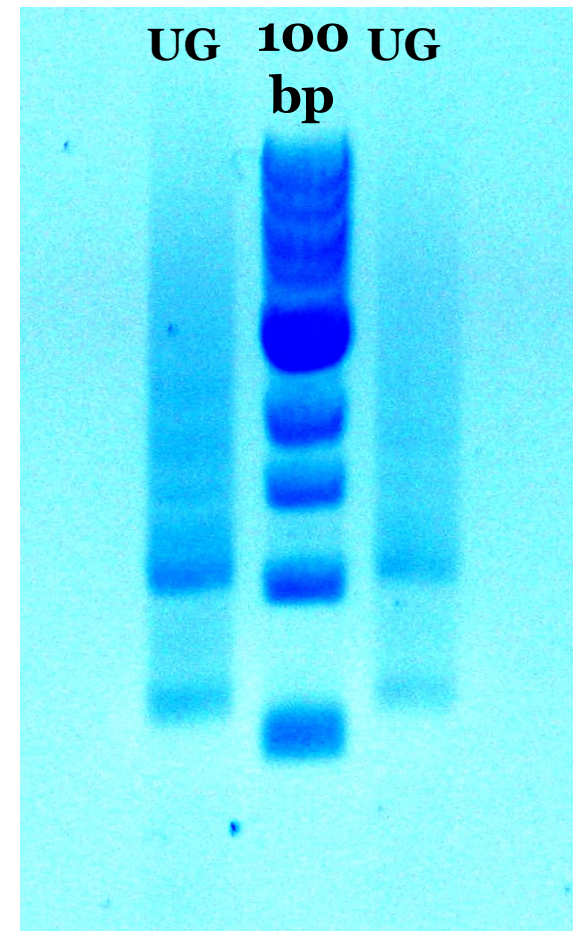
Gel Results

- The upstream and downstream regions of the genes of interest and the gentamicin cassette were successfully amplified individually.



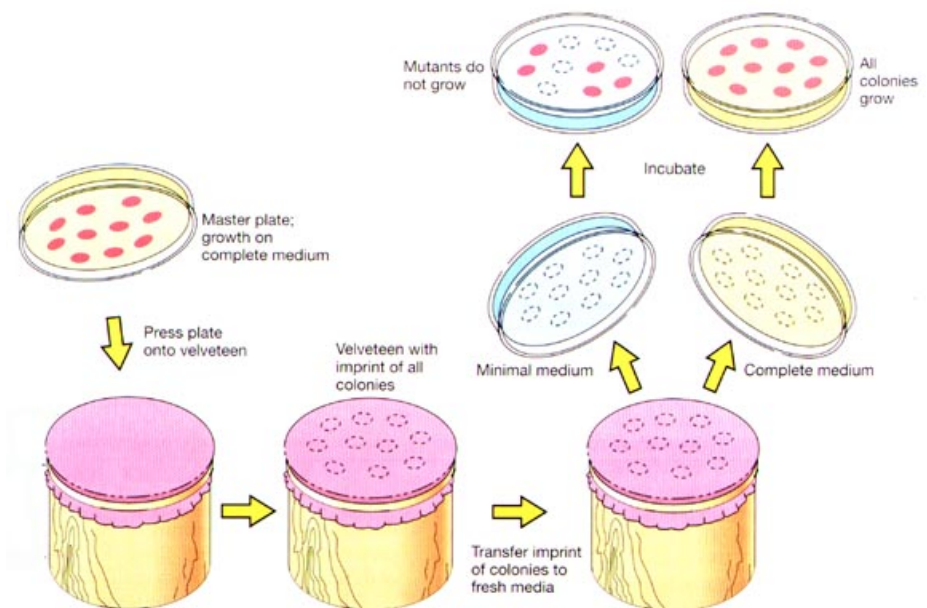
Gel Results

- The upstream region and gentamicin cassette were successfully annealed together. However, not all of the DNA segments annealed properly.



Cell Screening

- The mutated strains of bacteria will then be grown on a gentamicin rich media that will act a screen for strains that did not remove the genes of interest.
- The prototrophs will then be scrutinized to determine the effects of *clpA/clpS* and *recA* on natural transformation.

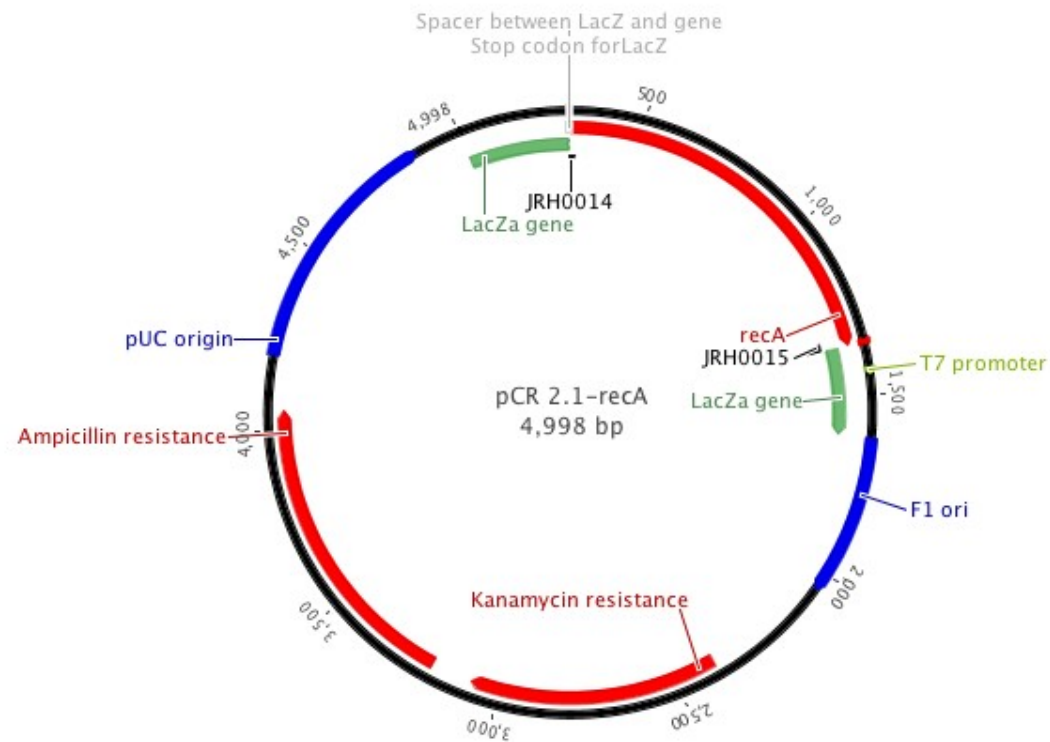




Restoration of Function

- The genes will then be added back into the genome of *Aeromonas* by replacing the gentamicin resistance cassette to confirm the effects of the genes of interest on natural transformation.

Vector 2.1



Conclusion

- Annealing of the upstream-gentamicin cassette complex to the downstream region is still in progress.
- Transformation of the wild-type gene into a vector for amplification is also still in progress.
- The understanding of natural transformation in *A. salmonicida* is crucial in applications such as medicine and microbiology.