



Determining the effect of *clpA* in the natural transformation ability of *Aeromonas salmonicida*

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Aeromonas

- Gram negative, rod-shaped Bacteria
- Ubiquitous in aquatic environments
- Opportunistic pathogen
- 5 micrometers long



Gene Transfer

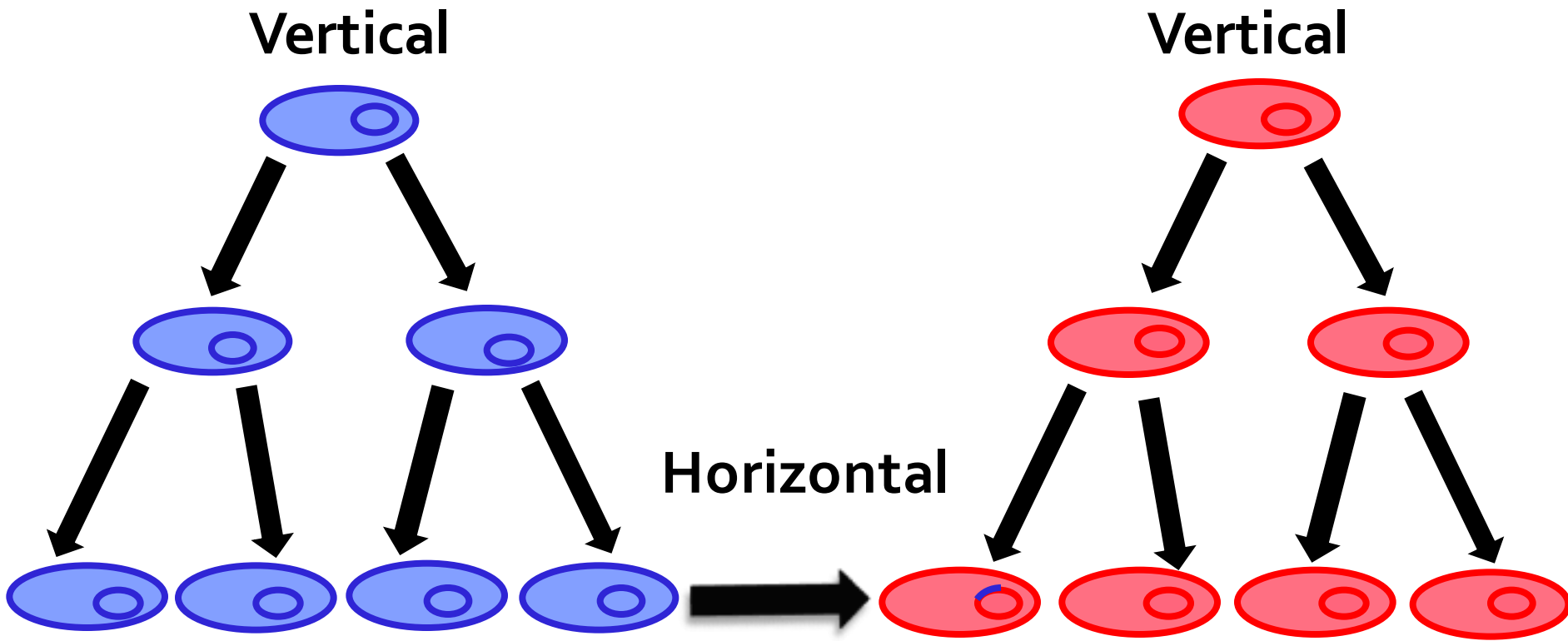


Figure constructed by K. Clemons and J. Huddleston, 2014

Natural Transformation

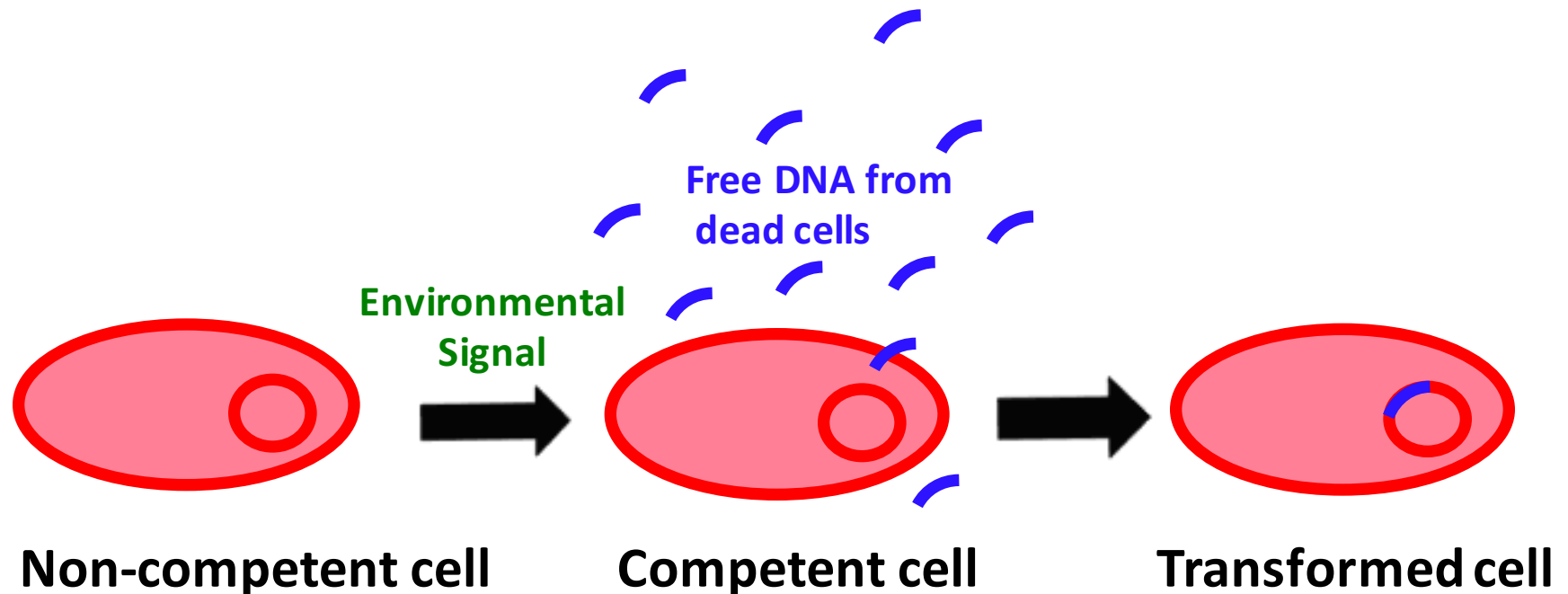
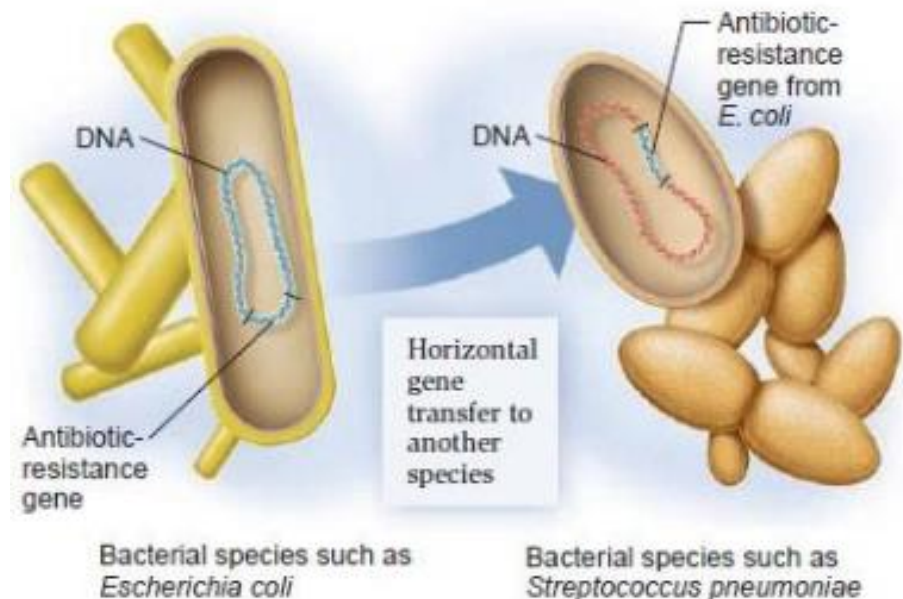


Figure constructed by K. Clemons and J. Huddleston, 2014

Antibiotic Resistance

- Bacterial exposure to antibiotics (Huddleston, 2014)
- Resistant bacteria survive
- *Aeromonas* causes infections in the human stomach and intestines

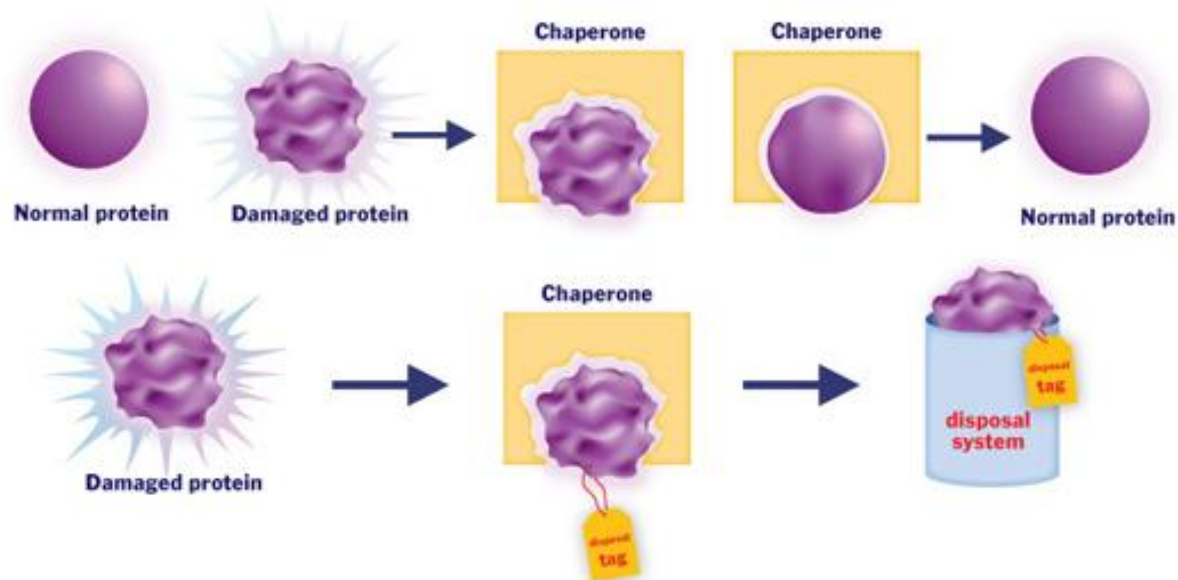


Why study *clpA*?

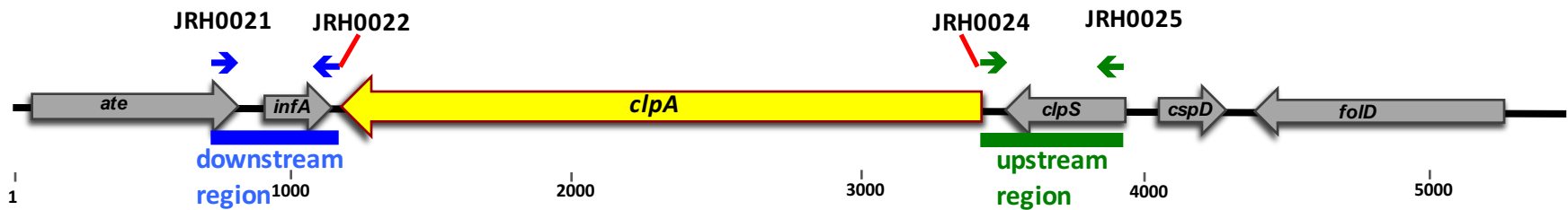
- Random mutagenesis of *Aeromonas*
- Deficient Natural Transformation ability
 - Genes: *ptsI*, *tapY1*, *recBC*, and *clpA/clpS* (Huddleston, 2008)

Function of *clpA*

- Molecular chaperone (Hoskins et al., 1998)
- Induced to action by stress



Aeromonas clpA Genome Region

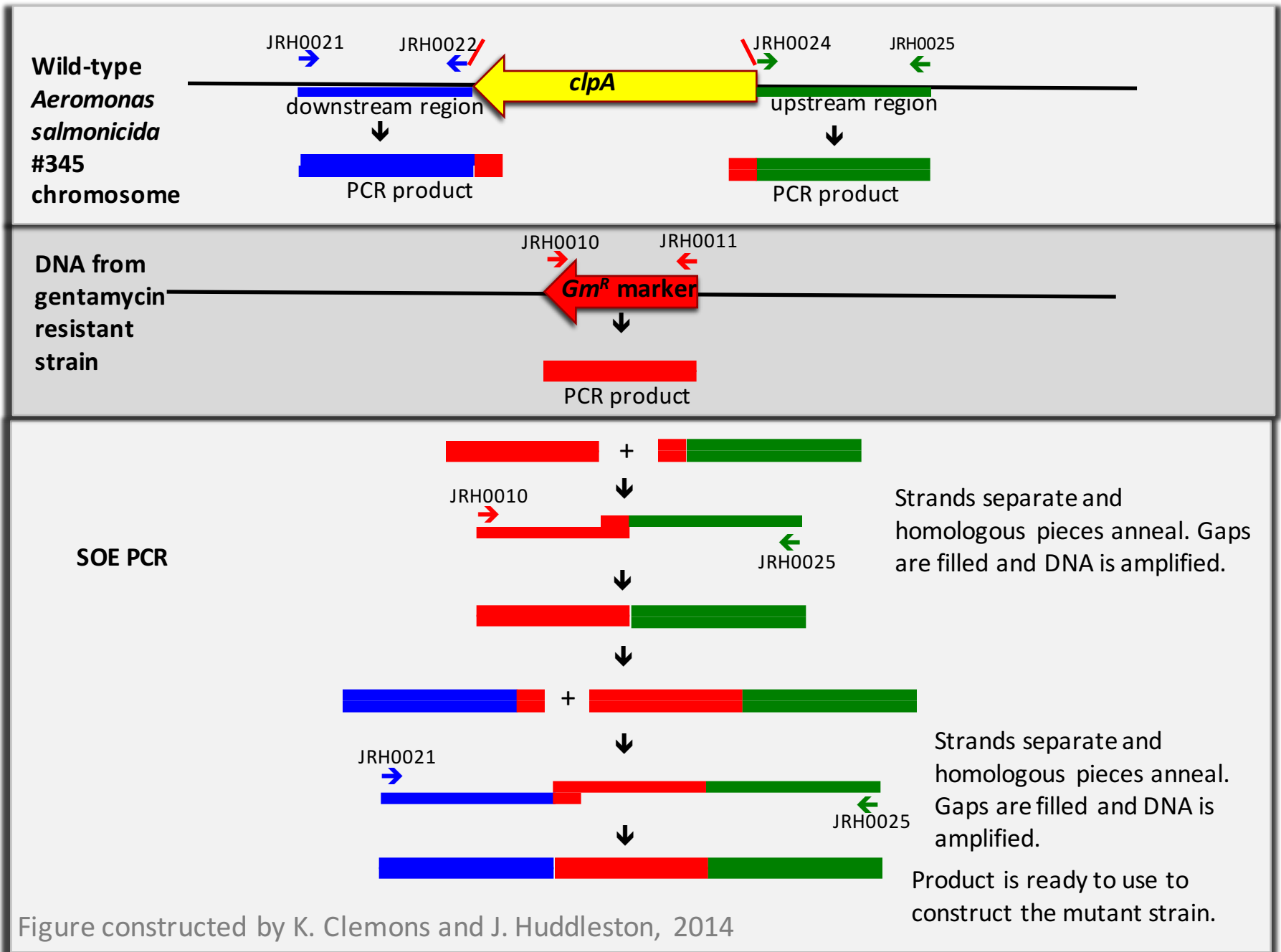


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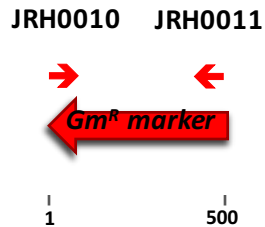
Knock-out Mutants

- Knock-out mutants
- Effects of the knock-out gene
 - Ex. Physical change, disabling a process, decrease in occurrence of a process
- Purpose of one gene in a process

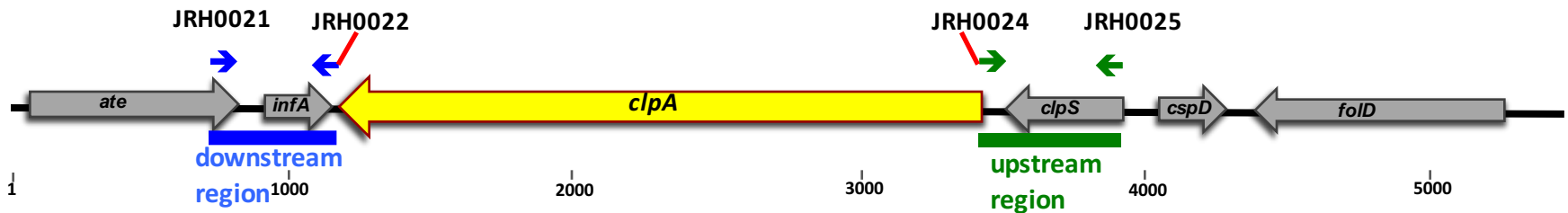
SOE PCR Scheme: Splicing overlap extension polymerase chain reaction



Isolating Segments



Three Segments of Mutant Fragment

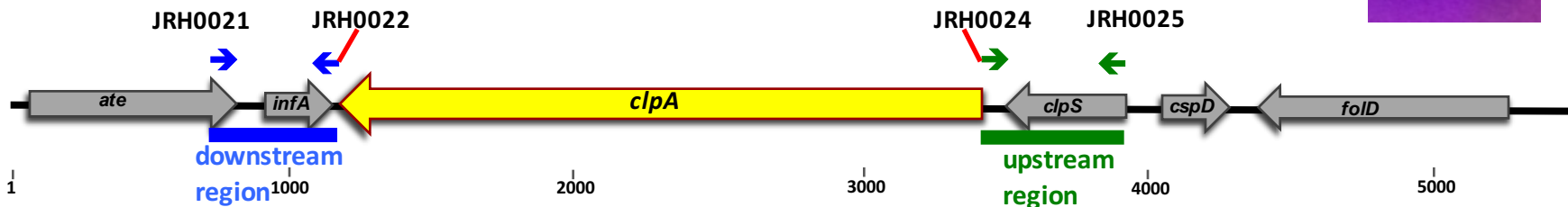
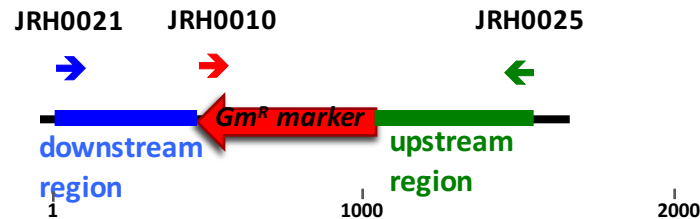
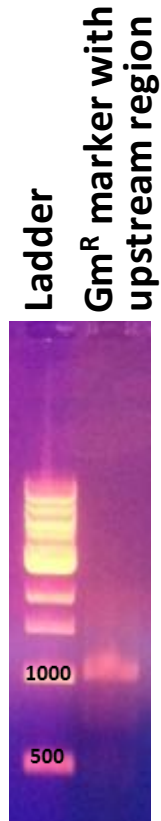


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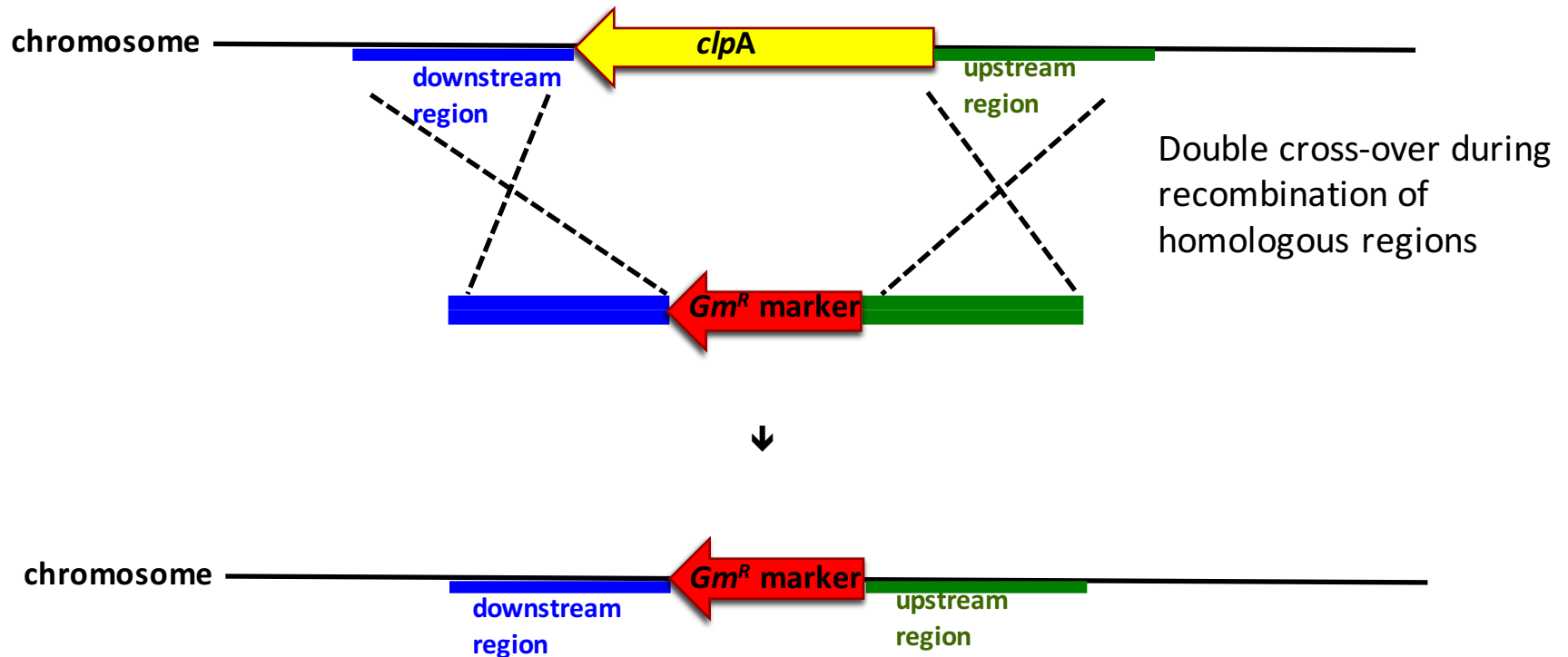
SOE PCR in the Lab

Two Segment Combination

Mutant Fragment



Recombination of SOE PCR Product with the Wild-type Gene Region



- Replacement of *clpA* with the *Gm^R* marker
- The transformation ability of this mutant strain can now be studied and compared to the wild-type strain.

Before and After

Wild-type



Mutant



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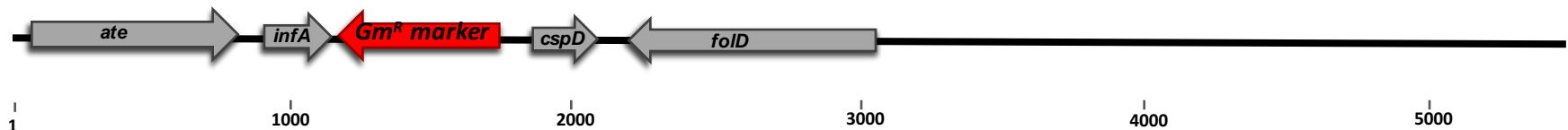
Continuing Research

➤ Transformation of *Aeromonas* with the *clpA* mutant fragment

➤ *Aeromonas clpS* mutant



➤ *Aeromonas clpA/clpS* mutant



Transforming *Aeromonas*

- Understanding function and role of specific genes will enable us to understand natural transformation



References

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