

Repetitive Elements and Genomic Rearrangements

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Introduction

This project looks at the activity, effects, and possible genetic manipulations of the insertion sequences commonly found in chromosomal DNA, particularly that of *Escherichia coli*.

Insertion sequences are small portions of the genetic code that are prone to duplications and inversions. In short, insertion sequences are transposable elements. They are frequently seen repeated multiple times throughout one genome.

The insertion sequence IS150 of the DH10B line of *E. coli* is of particular interest to the current study. There are three discrete IS150 locations on DH10B. While other insertion sequences such as IS1A and IS2 are seen with greater frequency, they are not always 100% conserved and leave more room for error.

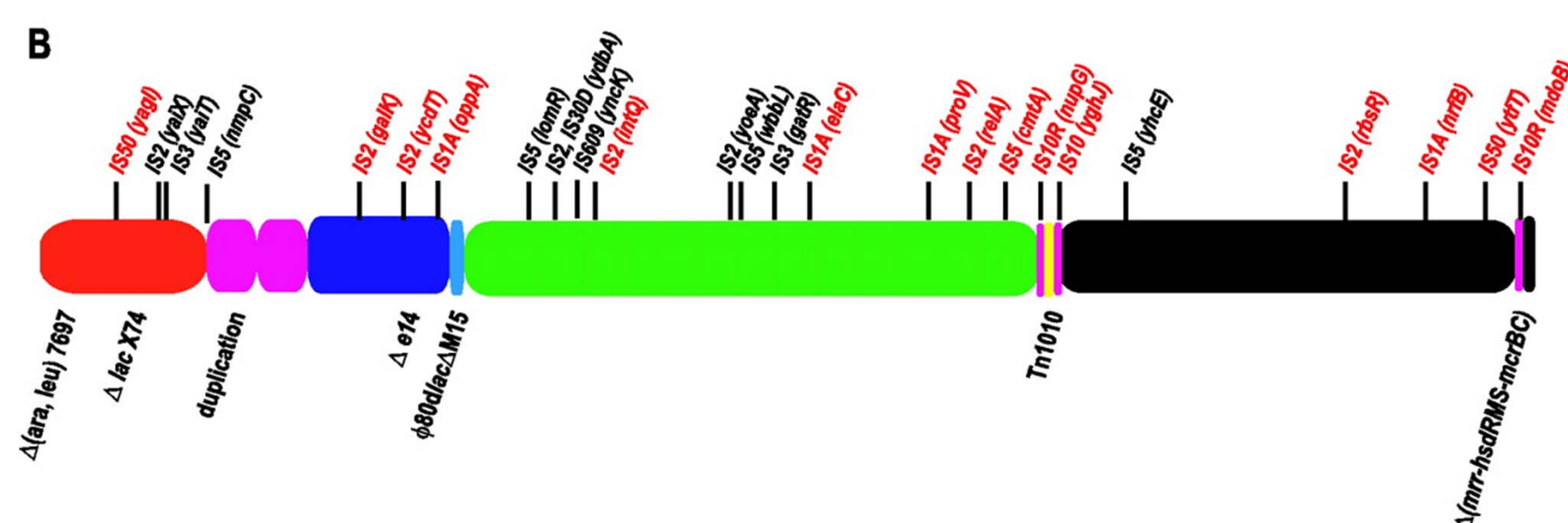


Figure 1. A map of the Insertion sequences present in the DH10B genome. In the current study, IS150 is of greatest interest.
Image Credit: Durfee et al., 2017

Methods

A variety of techniques are used to study, characterize, and modify the *Escherichia coli* MC1061 and DH10B genomes.

The polymerase chain reaction, commonly known as PCR, is the most powerful and widely used amplification technique used in modern laboratories. Primers were developed to select for certain sequences which were amplified exponentially in the laboratory.

Genetic products can be analyzed using gel electrophoresis, which uses electric current to run DNA fragments through agarose gel. This sorts the fragments by size and allows further analysis to occur.

References

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3. Posfai, G., Kolisnichenko, V., Berezcki, Z. & Blattner, F. Markerless gene replacement in Escherichia coli stimulated by a double-strand break in the chromosome. *Nucleic Acids Research* 27, 4409-4415 (1999).

Acknowledgements

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MC1061 and DH10B Cell Lines

MC1061 is a strain of *Escherichia coli* that has been modified in the laboratory (Kolisnychenko et al., 2002). It is used by scientists in the laboratory because it has the ability to take in very large plasmids, boasts a high efficiency in transformation reactions, and lacks of common defensive mechanisms (Durfee et al., 2017). The DH10B sub-strain was developed from the MC1061 cell line using a series of recombinations in the lab. The DH10B line is notable for its ability to maintain large plasmids and its efficiency when used in genetic engineering techniques.

DH10B is also known for the prevalence of insertion sequences which cause the strain to have a mutation rate more than 13 times higher than comparable wildtype stains.

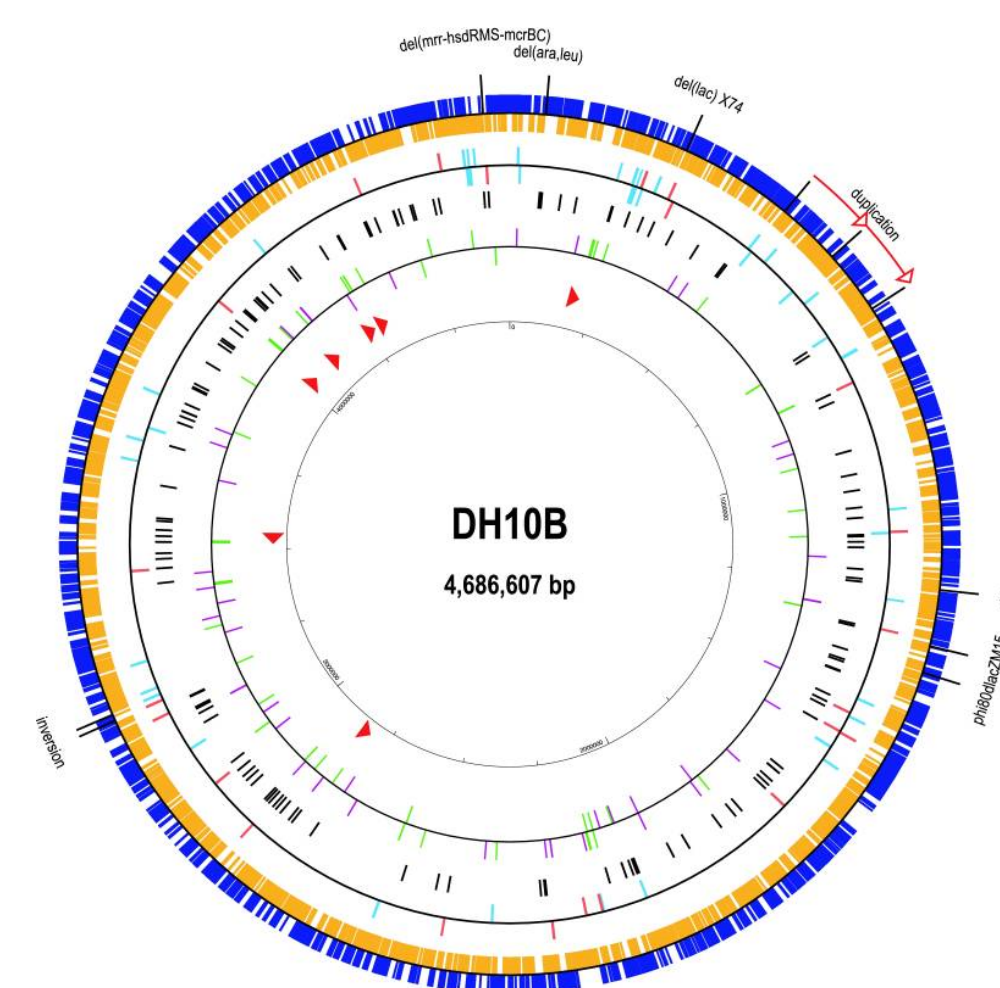


Image Credit: Durfee et al., 2017

Possibilities

Using genetic modification techniques, it is possible to further engineer the DH10B sub-strain and make it more stable while retaining the beneficial properties that have made it ideal for lab work.

Currently, the methods used to obtain these results require repetitive, stepwise use of existing protocols. Many of these techniques make use of plasmids (Posfai et al., 1999). Eventually a protocol can be developed to remove multiple insertion sequences for the *Escherichia coli* genome simultaneously by targeting multiple related loci.

Continued genetic modifications of *E. coli* strains can be designed such that the strain is more stable, better able to survive in varying environments, and otherwise easier for scientists to work with.