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Importancia: Artículo de mayor citación dentro de mis publicaciones. Este artículo fue uno de los primeros esfuerzos de identificar un Orfeoma completo de una bacteria E. coli, permitiendo a otros investigadores continuar con la determinación de genes relevantes en la infección o virulencia.					

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The *Escherichia coli* K-12 ORFeome: a resource for comparative molecular microbiology

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Abstract

Background: Systems biology and functional genomics require genome-wide datasets and resources. Complete sets of cloned open reading frames (ORFs) have been made for about a dozen bacterial species and allow researchers to express and study complete proteomes in a high-throughput fashion.

Results: We have constructed an open reading frame (ORFeome) collection of 3974 or 94% of the known *Escherichia coli* K-12 ORFs in Gateway® entry vector pENTR/Zeo. The collection has been used for protein expression and protein interaction studies. For example, we have compared interactions among YgjD, YjeE and YeaZ proteins in *E. coli*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*. We also compare this ORFeome with other Gateway-compatible bacterial ORFeomes and show its utility for comparative functional genomics.

Conclusions: The *E. coli* ORFeome provides a useful resource for functional genomics and other areas of protein research in a highly flexible format. Our comparison with other ORFeomes makes comparative analyses straighforward and facilitates direct comparisons of many proteins across many genomes.

Background

High-throughput DNA sequencing has increased the number of genome sequences to over 1,000 bacterial species from which we can infer their proteomes and often major parts of their metabolism and regulatory pathways. A systems level understanding of cells, however, will require the functional characterization of these proteins and how they work together. In recent years, a growing number of efforts have used high throughput assays to catalog gene expression, protein interactions, localization and metabolic activities. For many of these studies, the first step is to identify and then clone all the open reading frames (the "ORFeome") encoded by the genome of the organism [1].

Here we describe the construction of a comprehensive *Escherichia coli* ORF collection in a Gateway® [2] entry vector. The library represents 3974 ORFs or 94% of all protein-coding genes. The Gateway® system facilitates the transfer of ORFs into a large range of expression vectors that are suitable for downstream studies. Here we demonstrate the utility of the *E. coli* ORFeome by comparing it to 12 other available microbial ORFeomes and by testing a set of protein-protein interactions among 5 species.

The complete genome sequence of *Escherichia coli* K-12 encodes 4333 protein-coding ORFs [3] (http://cmr.jcvi.org/). Kitagawa et al. previously cloned all the *E. coli* ORFs (the "ASKA library") into an expression vector creating N-terminal 6xHis and C-terminal GFP fusions [4]. However, the ASKA library cannot be used to flexibly transfer ORFs into other expression vectors [5,6]. Libraries of all open reading frames cloned into highly flexible vectors will be needed to take full advantage of the information found in any genome sequence. We transferred the ASKA library [4] into an Gateway® entry vector (pENTR/Zeo) by *Sfi*I restriction enzyme cloning (Figure 1). About 250 *E. coli* clones which were not present in the ASKA library or which were not successfully cloned from the ASKA library into the Gateway® entry

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