



The Expanding Molecular Genetics Tool Kit in *Chlamydia*

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ABSTRACT *Chlamydia* has emerged as an important model system for the study of host pathogen interactions, in part due to a resurgence in the development of tools for its molecular genetic manipulation. An additional tool, published by Keb et al. (G. Keb, R. Hayman, and K. A. Fields, *J. Bacteriol.* 200:e00479-18, 2018, <https://doi.org/10.1128/JB.00479-18>), now allows for custom genetic engineering of genomic regions that were traditionally recalcitrant to genetic manipulation, such as genes within operons. This new method will be an essential instrument for the elucidation of *Chlamydia*-host interactions.

KEYWORDS *Chlamydia* genetics, *Chlamydia trachomatis*

For decades, *Chlamydia trachomatis*, the causative agent of ocular and venereal diseases, remained frustratingly intractable to any form of stable genetic manipulation, with only a few reports describing the successful transformation of *Chlamydia* species prior to 2007 (1, 2). However, the observation that *Chlamydia* readily exchanges DNA by lateral gene transfer (LGT) (3, 4) in the setting of coinfections paved the way for experimental system where mutations can be passed from one strain to another and thus enable genotype-phenotype associations (5, 6). Point mutations derived spontaneously or after exposure to mutagens provide hypo- and hypermorphic alleles (5) and temperature-sensitive mutations (7), further expanding the emerging genetic toolkit available to study *C. trachomatis*.

A turning point in *Chlamydia* genetics was the finding by Clarke and colleagues that *C. trachomatis* can be stably transformed with a chimeric plasmid derived from the *Chlamydia* "cryptic" plasmid and a ColE1 plasmid after CaCl₂ treatment and selection with penicillin G (8). The observation that *C. trachomatis* is transformable then led to the use of suicide vectors to deliver other tools for genome engineering, such as group II introns for targeted insertional disruption of *Chlamydia* genes (9, 10). This tool, known as TargeTron, is used broadly in many bacterial species but relies on gene-specific predictive algorithms for target design that vary in efficiency and specificity. Insertional mutagenesis with TargeTron-based vectors invariably will have polar effects if insertions occur at the 3' end of operons, making it more difficult to assign specific function to individual genes within an operon.

Mueller and colleagues (12) developed an alternative strategy for targeted mutagenesis called fluorescence-reported allelic exchange mutagenesis (FRAEM). In this system, open reading frames are replaced by allelic exchange with a selectable marker (12). The FRAEM plasmid vector provides the substrate for allelic exchange in the form of a *gfp-blaM* cassette flanked by 3 kb of DNA homologous sequences upstream and downstream of the chromosomal region to be replaced. The elegance of this approach relies on making the FRAEM plasmid conditionally replicative by placing *pgp6*, which controls plasmid maintenance and inheritance (13, 14), under the control of an anhydrous tetracycline (aTet)-inducible genetic circuit. Allelic exchange occurs as a two-step process, where selection for penicillin resistance selects for transformed *Chlamydia* expressing green fluorescent protein (GFP) and mCherry, which is present in the FRAEM

Accepted manuscript posted online 8 October 2018

Citation Valdivia RH, Bastidas RJ. 2018. The expanding molecular genetics tool kit in *Chlamydia*. *J Bacteriol* 200:e00590-18. <https://doi.org/10.1128/JB.00590-18>.

Editor Thomas J. Silhavy, Princeton University

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For the article discussed, see <https://doi.org/10.1128/JB.00479-18>.

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plasmid backbone. These clones are then screened in the absence of aTet for loss of the mCherry marker and maintenance of GFP, which selects for allelic exchange resulting from double-crossover events. In this issue, Keb and colleagues expand the FRAEM system by incorporating flanking *loxP* sites to the *gfp-blaM* cassette and adding a step where a second suicide plasmid encoding Cre recombinase is introduced by LGT (11). As a result, the floxed *gfp-blaM* cassette is rapidly excised from the chromosome, leaving a small *loxP* scar.

The utility of this new system is manifold. An obvious one is the ability to generate markerless gene deletions, opening the door for generating *Chlamydia* strains with multiple gene deletions. This is especially advantageous for *C. trachomatis* given the small number of selectable markers that are currently available. Markerless strains also have the added benefit of limiting any impact the expression of the selectable marker may have on *Chlamydia* fitness. In addition, the broad application of the Cre-*loxP* system, either through FRAEM or TargeTron, opens the possibility for extensive genome editing in *Chlamydia*, allowing the excision of large regions of chromosomal DNA. Such a system might be useful for characterizing the function of chromosomal regions, such as the highly polymorphic plasticity zones, or the family of expanded homologous polymorphic membrane proteins (Pmp) found among various *Chlamydia* species and their contributions to both *Chlamydia* virulence and tissue tropisms (15, 16). The FRAEM-based Cre-*loxP* system should enable the deletion of *Chlamydia* noncoding RNAs and small RNAs, two gene regulatory elements that have remained largely uncharacterized in *Chlamydia* species. Furthermore, the Cre-*loxP* system can be adapted to generate conditional excision of essential genes by controlling the expression of Cre.

Bacterial genes are often organized in polycistronic operons so as to coregulate the synthesis of proteins that perform related functions. The FRAEM-based Cre-*loxP* now enables individualized disruption of genes within operons without polar effects on the transcription of genes in the 3' direction from the targeted gene. Keb and colleagues demonstrate that such an analysis is feasible in *Chlamydia* spp. by utilizing their FRAEM Cre-*loxP* system to delete the gene encoding the secreted *Chlamydia* effector TmeA. Both *tmeA* (CTL0063) and *tmeB* (CTL0064) are transcribed as an operon (17). Excision of the *tmeA* locus by FRAEM revealed a role for TmeA in promoting *Chlamydia* invasion of cultured cells (18). However, the excision of *tmeA* also impacted *tmeB* transcription (12), confounding the role for TmeA in *Chlamydia* invasion. Using their FRAEM Cre-*loxP* system, Keb and colleagues excised *tmeA* without compromising TmeB production and observed the same phenotypes associated with a previous disruption of *tmeA*.

The application of the FRAEM Cre-*loxP* system will have an immediate impact on the field of *Chlamydia* molecular genetics. Together with new reports describing the feasibility of CRISPR/Cas in *Chlamydia* spp. (19), the current genetic toolkit now enables customized cleavage at essentially any locus in the genome, establishing *C. trachomatis* as a versatile model system for the study of host-pathogen interactions and pathogenesis.

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