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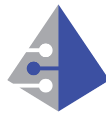

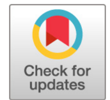

Gateway Entry Vector Library of *Wolbachia pipientis* Candidate Effectors from Strain *w*Mel

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ABSTRACT *Wolbachia pipientis* is an intracellular symbiont that modifies host biology using a type IV secretion system to inject bacterial effectors into the host cytoplasm. We utilized a bioinformatics approach to predict *Wolbachia* effectors and cloned the candidates into an entry vector, which can be utilized for subsequent analyses.

Wolbachia pipientis is the most prevalent infection on Earth and is increasingly promoted for its use in disease vector control (1). Due to both the direct effects that *Wolbachia* may have on the transmission of human pathogens (2) and the myriad effects *Wolbachia* has on insect populations (3), it is important that we identify the mechanisms for symbiosis between *Wolbachia* spp. and their hosts. Although the type IV secretion system has long been hypothesized to be involved in host interaction (4), we conducted the first large-scale screen for effector proteins likely used by *Wolbachia* to manipulate host cell biology (5). Our research generated a set of candidate effectors, publicly available as a resource for further studies. The generation of the plasmid library is described as follows.

Wolbachia open reading frames from the *w*Mel genome were subjected to a BLAST search against the NCBI nr database (accessed April 2012) using TBLASTN v2.2.25+ with default options. In addition, we also performed a search of the Pfam-A database (v26.0) using hmmscan v3.0 with default options (http://hmmer.org), identifying *Wolbachia* proteins with homologies to domains enriched for eukaryote membership. In addition to proteins with eukaryotic homologies, we also included *Wolbachia* proteins specific to the genus. We then culled the proteins that were predicted to be made up of <200 amino acids in order to enrich the data set for true open reading frames.

We targeted the resulting 164 loci from the *w*Mel genome for amplification using modified forward primers to facilitate cloning by means of the Invitrogen Gateway pENTR/D-TOPO system (see reference 5 for more detail). As described in the user manual, blunt-end PCR products were directionally cloned into the pENTR/D-TOPO vector using the TOPO cloning reaction (Fig. 1A) and transformed into Invitrogen One Shot Top10 chemically competent *E. coli* cells using standard protocols. Transformants were plated on selective plates containing LB medium supplemented with kanamycin (LB_{kan}). Colonies were selected and positive transformants were sequence verified to confirm that the protein products were in frame and correctly cloned.

A total of 108 pENTR/D-TOPO clones (in 100 μl of LB_{kan} with 25% glycerol) are included in the plasmid library on two 96-well plates (see Fig. 1B for insert accession numbers and locations on plates). Plates are stored at −80°C.

Data availability. Requests for the resource should be directed to the corresponding author, Irene L. G. Newton (irnewton@indiana.edu).

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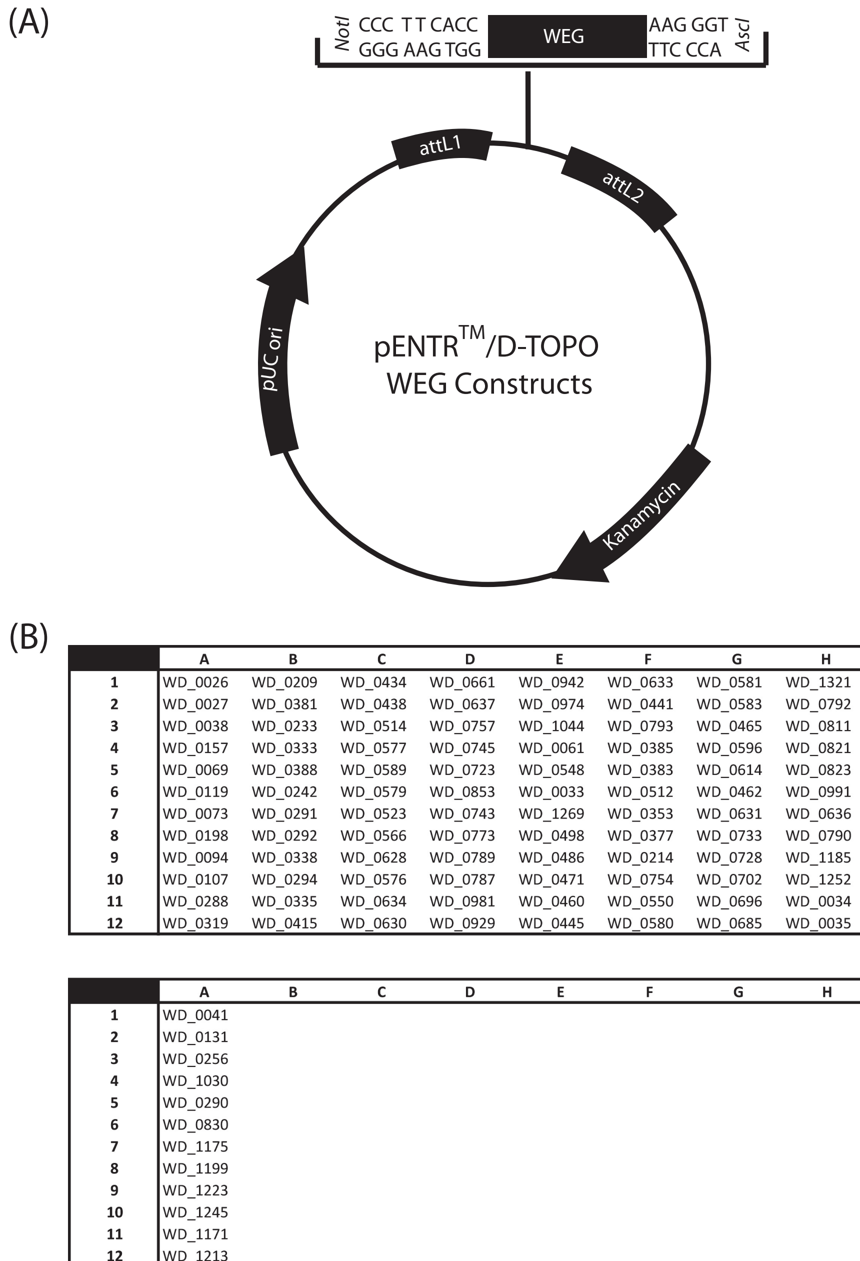

	A	B	C	D	E	F	G	H
1	WD_0026	WD_0209	WD_0434	WD_0661	WD_0942	WD_0633	WD_0581	WD_1321
2	WD_0027	WD_0381	WD_0438	WD_0637	WD_0974	WD_0441	WD_0583	WD_0792
3	WD_0038	WD_0233	WD_0514	WD_0757	WD_1044	WD_0793	WD_0465	WD_0811
4	WD_0157	WD_0333	WD_0577	WD_0745	WD_0061	WD_0385	WD_0596	WD_0821
5	WD_0069	WD_0388	WD_0589	WD_0723	WD_0548	WD_0383	WD_0614	WD_0823
6	WD_0119	WD_0242	WD_0579	WD_0853	WD_0033	WD_0512	WD_0462	WD_0991
7	WD_0073	WD_0291	WD_0523	WD_0743	WD_1269	WD_0353	WD_0631	WD_0636
8	WD_0198	WD_0292	WD_0566	WD_0773	WD_0498	WD_0377	WD_0733	WD_0790
9	WD_0094	WD_0338	WD_0628	WD_0789	WD_0486	WD_0214	WD_0728	WD_1185
10	WD_0107	WD_0294	WD_0576	WD_0787	WD_0471	WD_0754	WD_0702	WD_1252
11	WD_0288	WD_0335	WD_0634	WD_0981	WD_0460	WD_0550	WD_0696	WD_0034
12	WD_0319	WD_0415	WD_0630	WD_0929	WD_0445	WD_0580	WD_0685	WD_0035

	A	B	C	D	E	F	G	H
1	WD_0041							
2	WD_0131							
3	WD_0256							
4	WD_1030							
5	WD_0290							
6	WD_0830							
7	WD_1175							
8	WD_1199							
9	WD_1223							
10	WD_1245							
11	WD_1171							
12	WD_1213							

FIG 1 (A) Plasmid map for pENTRTM/D-TOPO (Invitrogen) constructs containing *Wolbachia* eukaryote-like genes (WEGs) and (B) organization of insert library with *Wolbachia* WEG accession numbers indicated.

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