Rickettsia prowazekii Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 9

Catalog No. NR-19457

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Contributor:

Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

Manufacturer:

BEI Resources

Product Description:

Clone plates are replicated using a BioMek® FX robot. Production in the 96-well format has increased risk of cross-contamination between adjacent wells. Individual clones should be purified (e.g. single colony isolation and purification using good microbiological practices) and sequence-verified prior to use. BEI Resources only confirms the clone plate orientation and viability of randomly picked clones. BEI Resources does not confirm or validate individual clone identities provided by the contributor.

The *Rickettsia prowazekii* (*R. prowazekii*) Gateway® clone set consists of approximately 748 sequence validated clones from *R. prowazekii*, strain Madrid E cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each open reading frame was constructed in vector pDONR™221 (Invitrogen™) with an ATG start codon and no stop codon. The sequence was validated by full length sequencing of each clone with greater than 1X coverage and a mutation rate of less than 0.2%. Detailed information about each clone is shown in Table 1.

Information related to the use of Gateway® Clones can be obtained from Invitrogen™. Recombination was facilitated through an *att*B substrate (*att*B-PCR product or a linearized *att*B expression clone) with an *att*P substrate (pDONR™221) to create an *att*L-containing entry clone. The entry clone contains recombinational cloning sites, *att*L1 and *att*L2 to facilitate gene transfer into a destination vector, M13 forward and reverse priming sites for sequencing and a kanamycin resistance gene for selection. Please refer to the Invitrogen™ Gateway® Technology Manual for additional details.

Plate orientation and viability were confirmed for NR-19457.

Material Provided:

Each inoculated well of the 96-well plate contains approximately 60 µL of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

Packaging/Storage:

NR-19457 was packaged aseptically in a 96-well plate. The product is provided frozen and should be stored at -80°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:

Media:
LB broth or agar containing 50 µg/mL kanamycin.
Incubation:
Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 37°C.
Atmosphere: Aerobic
Propagation:

1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plates at 37°C for 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Rickettsia prowazekii* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 9, NR-19457."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Andersson, S. G., et al. "The Genome Sequence of *Rickettsia prowazekii* and the Origin of Mitochondria." Nature 396 (1998): 133-140. PubMed: 9823893.

Support Provided by NIAID

Table 1: *Rickettsia prowazekii* Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 9 (ZRPAI)[1]

Clone	Well Position	Locus ID	Description	ORF Length	Accession Number	Average Depth of Coverage
47519	A01	RP797	NADH dehydrogenase subunit G	2062	NP_221147.1	4.872454
47383	A02	RP256	ribonuclease E (rne)	2086	NP_220641.1	4.674017
47514	A03	RP281	protease II	2089	NP_220665.1	4.154619
47370	A04	RP720	NAD-dependent DNA ligase LigA	2104	NP_221075.1	4.826046
47326	A05	RP006	hypothetical protein RP006	2155	NP_220402.1	2.970302
47282	A06	RP674	hypothetical protein RP674	2170	NP_221035.1	4.869124
47360	A07	RP560	3-hydroxyacyl-CoA dehydrogenase	2197	NP_220931.1	4.133364
47406	A08	RP593	ATP-dependent DNA helicase RECG (recG)	2209	NP_220962.1	4.765505
47492	A09	RP823	cell division protein FTSK homolog (ftsK)	2269	NP_221172.1	4.144116
47333	A10	RP504	polynucleotide phosphorylase/polyadenylase	2272	NP_220880.1	4.62456
47494	A11	RP160	outer membrane protein OMP1 (omp1)	2341	NP_220550.1	4.449381
47420	A12	RP326	DNA topoisomerase I	2365	NP_220709.1	4.314588
47319	B01	RP103	type IV secretion system ATPase VirB4	2452	NP_220495.1	4.316069
47473	B02	RP580	DNA gyrase subunit B	2458	NP_220951.1	3.648495
47435	B03	RP687	valyl-tRNA synthetase	2479	NP_221048.1	4.149657
47513	B04	RP418	phenylalanyl-tRNA synthetase subunit beta	2482	NP_220799.1	3.870266
47447	B05	RP421	leucyl-tRNA synthetase	2521	NP_220802.1	3.992067
47503	B06	RP347	outer membrane assembly protein (asmA)	2542	NP_220730.1	4.708891
47431	B07	RP780	UDP-glucose 6-dehydrogenase (Udg)	2542	NP_221130.1	4.833596
47440	B08	RP776	DNA polymerase I	2638	NP_221127.1	4.589841
47334	B09	RP349	hypothetical protein RP349	2641	NP_220732.1	4.184021
47354	B10	RP799	aconitate hydratase	2671	NP_221149.1	4.107076
47463	B11	RP298	DNA mismatch repair protein MutS	2710	NP_220682.1	4.497048
47399	B12	RP206	DNA gyrase subunit A	2752	NP_220594.1	4.478198
47346	C01	RP465	alkaline phosphatase synthesis sensor protein PhoR	2800	NP_220845.1	4.600357
47311	C02	RP180	2-oxoglutarate dehydrogenase E1 component	2845	NP_220570.1	3.786292
47367	C03	RP106	hypothetical protein RP106	2950	NP_220498.1	4.27322
47390	C04	RP170	acriflavin resistance protein D	3061	NP_220560.1	4.511598
47289	C05	RP598	transcription-repair coupling factor	3397	NP_220966.1	4.288195
47275	C06	RP108	hypothetical protein RP108	3502	NP_220500.1	4.163621
47451	C07	RP785	VIRB4 protein precursor (virB4)	3544	NP_221135.1	4.947799
47474	C08	RP704	cell surface antigen (sca5)	4966	NP_221064.1	3.867902

[1]All information in this table was provided by J. Craig Venter Institute at the time of deposition.

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