Rickettsia prowazekii, Strain Madrid E, Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 8

Catalog No. NR-19456

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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)*, strain Madrid E, Gateway® clone set consists of approximately 750 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-19456.

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-19456 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,* Strain Madrid E, Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 8, NR-19456."

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.

Disclaimers:

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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*, Strain Madrid E, Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 8 (ZRPAH)[1]

Clone	Well Position	Locus ID	Description	ORF Length	Accession Number	Average Depth of Coverage
47162	A01	RP527	hypothetical protein RP527	1354	NP_220900.1	1.384786
47131	A02	RP034	hypothetical protein RP034	1357	NP_220428.1	2.470155
47046	A03	RP068	hypothetical protein RP068	1357	NP_220462.1	1.355195
46946	A04	RP228	tail-specific protease precursor (CTP)	1360	NP_220614.1	4.323529
46906	A05	RP313	proline/BETAIN transporter (proP2)	1363	NP_220696.1	5.250183
47246	A06	RP302	translocation protein TolB	1366	NP_220686.1	3.818448
47183	A07	RP759	tRNA modification GTPase TrmE	1372	NP_221111.1	3.115889
46914	A08	RP808	(dimethylallyl)adenosine tRNA methylthiotransferase	1372	NP_221158.1	1.341837
47090	A09	RP842	trigger factor	1372	NP_221190.1	4.251458
47206	A10	RP753	aspartate kinase	1375	NP_221105.1	4.487273
47002	A11	RP325	glutamyl-tRNA synthetase	1378	NP_220708.1	1.365022
47118	A12	RP546	DNA repair protein RadA	1381	NP_220919.1	1.353367
47251	B01	RP173	signal recognition particle protein	1384	NP_220563.1	2.45448
46879	B02	RP596	UDP-N-acetylmuramoylalanyl-D-glutamyl- 2,6-diaminopimelate--D-alanyl-D-alanyl ligase (murF)	1384	NP_220964.1	2.519509
46978	B03	RP237	response regulator PleD	1387	NP_220622.1	2.471521
47226	B04	RP475	muropeptide permease AmpG	1393	NP_220854.1	1.309404
47098	B05	RP666	cell division protein FtsZ	1393	NP_221028.1	4.003589
47010	B06	RP216	cytochrome D ubiquinol oxidase subunit I	1396	NP_220602.1	2.363181
46999	B07	RP675	exodeoxyribonuclease VII large subunit	1396	NP_221036.1	1.742837
47102	B08	RP448	hypothetical protein RP448	1399	NP_220829.1	2.414582
46885	B09	RP526	transcription termination factor Rho	1408	NP_220899.1	4.365057
46990	B10	RP407	hypothetical protein RP407	1411	NP_220788.1	4.231751
47062	B11	RP805	dihydrolipoamide dehydrogenase	1414	NP_221155.1	1.350071
46955	B12	RP089	3-deoxy-D-manno-octulosonic-acid transferase	1420	NP_220482.1	1.326761
46887	C01	RP571	sodium/pantothenate symporter (panF)	1420	NP_220943.1	4.417606
46911	C02	RP665	fumarate hydratase	1420	NP_221027.1	4.302817
47167	C03	RP074	NAD(P) transhydrogenase subunit BETA (pntB)	1432	NP_220468.1	4.291201
47055	C04	RP294	guanosine pentaphosphate phosphohydrolase (gppA)	1447	NP_220678.1	1.299931
47198	C05	RP623	glutamyl-tRNA synthetase	1447	NP_220990.1	3.866621
46983	C06	RP597	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase	1477	NP_220965.1	1.299255

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Clone	Well Position	Locus ID	Description	ORF Length	Accession Number	Average Depth of Coverage
47034	C07	RP151	aspartyl/glutamyl-tRNA amidotransferase subunit B	1486	NP_220542.1	1.251009
47114	C08	RP265	isocitrate dehydrogenase	1486	NP_220650.1	2.2786
46977	C09	RP291	VIRB10 protein (virB10)	1486	NP_220675.1	4.333782
47038	C10	RP283	putative monovalent cation/H+ antiporter subunit D	1495	NP_220667.1	2.274247
47070	C11	RP542	replicative DNA helicase	1495	NP_220915.1	3.854181
46938	C12	RP793	NADH dehydrogenase subunit M	1510	NP_221143.1	1.240397
47110	D01	RP821	hypothetical protein RP821	1513	NP_221170.1	4.171183
47087	D02	RP152	aspartyl/glutamyl-tRNA amidotransferase subunit A	1516	NP_220543.1	1.205145
46970	D03	RP602	patatin B1 precursor (pat1)	1519	NP_220970.1	2.229098
47222	D04	RP247	UDP-N-acetylmuramate--L-alanine ligase	1522	NP_220632.1	1.13929
47023	D05	RP366	apolipoprotein N-acyltransferase	1525	NP_220749.1	3.877377
46930	D06	RP229	histidine kinase sensor protein (barA)	1528	NP_220615.1	3.295812
47230	D07	RP053	ADP,ATP carrier protein	1531	NP_220447.1	3.856303
46934	D08	RP142	leucyl aminopeptidase	1537	NP_220533.1	1.207547
47210	D09	RP739	ADP,ATP carrier protein	1537	NP_221091.1	1.216005
47239	D10	RP477	ADP,ATP carrier protein	1540	NP_220856.1	1.185714
46994	D11	RP590	virulence factor MVIN (mviN)	1558	NP_220959.1	1.19448
47083	D12	RP509	phosphomannomutase (exoC)	1561	NP_220885.1	1.718129
46927	E01	RP683	methionyl-tRNA synthetase	1561	NP_221044.1	2.15631
47122	E02	RP382	hypothetical protein RP382	1567	NP_220763.1	1.150606
46943	E03	RP314	alkaline protease secretion protein AprE	1570	NP_220697.1	1.592214
47078	E04	RP500	ADP,ATP carrier protein	1573	NP_220876.1	4.902098
47126	E05	RP803	F0F1 ATP synthase subunit alpha	1573	NP_221153.1	5.331214
47094	E06	RP282	putative monovalent cation/H+ antiporter subunit D	1576	NP_220666.1	3.857234
47150	E07	RP619	propionyl-COA carboxylase BETA chain precursor (pccB)	1579	NP_220986.1	4.937302
47059	E08	RP476	lipopolysaccharide 1,2-glucosyltransferase (rfaJ)	1588	NP_220855.1	2.670655
47191	E09	RP586	preprotein translocase subunit SecD	1591	NP_220955.1	1.686989
46924	E10	RP847	bifunctional N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase/tRNA (m7G46) methyltransferase	1591	NP_221195.1	4.423633
47220	E11	RP157	multidrug resistance protein B (emrB)	1594	NP_220548.1	5.033877
47234	E12	RP329	hypothetical protein RP329	1597	NP_220712.1	4.99186
46950	F01	RP855	hypothetical protein RP855	1597	NP_221203.1	4.336255
46966	F02	RP371	lysyl-tRNA synthetase	1621	NP_220754.1	3.951265
46874	F03	RP405	cytochrome c oxidase polypeptide I	1639	NP_220786.1	4.253813
46902	F04	RP865	DNA polymerase III subunits gamma and tau	1660	NP_221213.1	4.195783
47106	F05	RP214	ABC transporter ATP-binding protein	1681	NP_220600.1	3.992861
47066	F06	RP789	hypothetical protein RP789	1696	NP_221139.1	3.585495
47242	F07	RP182	DNA repair protein RECN (recN)	1699	NP_220571.1	4.691583
46960	F08	RP478	hypothetical protein RP478	1699	NP_220857.1	4.187758
46918	F09	RP048	putative inner membrane protein translocase component YidC	1717	NP_220442.1	3.931275
47186	F10	RP441	hypothetical protein RP441	1717	NP_220822.1	4.848573
46894	F11	RP567	penicillin binding protein (pbpA2)	1720	NP_220939.1	3.586047

Clone	Well Position	Locus ID	Description	ORF Length	Accession Number	Average Depth of Coverage
46898	F12	RP612	hypothetical protein RP612	1741	NP_220980.1	4.497415
47138	G01	RP810	glutathione-regulated potassium-efflux system protein KEFB (kefB)	1762	NP_221160.1	5.150312
47255	G02	RP065	arginyl-tRNA synthetase	1765	NP_220459.1	1.012465
47020	G03	RP315	alkaline protease secretion ATP-binding protein AprD	1786	NP_220698.1	4.568309
47214	G04	RP378	CTP synthetase	1795	NP_220761.1	4.358774
47485	G05	RP528	single-stranded-DNA-specific exonuclease RECJ (recJ)	1795	NP_220901.1	4.759331
47466	G06	RP563	hypothetical protein RP563	1801	NP_220935.1	1.024431
47414	G07	RP565	penicillin-binding protein (pbpA1)	1819	NP_220937.1	3.71138
47307	G08	RP128	succinate dehydrogenase flavoprotein subunit	1825	NP_220520.1	4.424658
47262	G09	RP145	aspartyl-tRNA synthetase	1852	NP_220536.1	4.043197
47387	G10	RP513	ribonucleotide-diphosphate reductase subunit alpha	1858	NP_220889.1	4.370829
47299	G11	RP205	multidrug resistance transporter ATM1	1864	NP_220593.1	4.348712
47458	G12	RP859	DNA primase	1885	NP_221207.1	3.341645
47362	H01	RP017	hypothetical protein RP017	1918	NP_220412.1	3.567779
47268	H02	RP185	molecular chaperone DnaK	1918	NP_220574.1	4.205422
47294	H03	RP858	RNA polymerase sigma factor RpoD	1942	NP_221206.1	4.378991
47343	H04	RP043	cell division protein FTSH (ftsH)	1948	NP_220437.1	4.300308
47258	H05	RP540	primosome assembly protein PriA	1981	NP_220913.1	4.199394
47350	H06	RP849	glycyl-tRNA synthetase subunit beta	1981	CAA15273.1	3.53054
47411	H07	RP400	putative soluble lytic murein transglycosylase precursor (slt)	1990	NP_220781.1	4.224121
47279	H08	RP792	NADH dehydrogenase subunit L	1996	NP_221142.1	4.242986
47402	H09	RP691	hypothetical protein RP691	2005	NP_221052.1	4.05187
47499	H10	RP203	excinuclease ABC subunit B	2023	NP_220591.1	4.054375
47290	H11	RP618	propionyl-COA carboxylase alpha chain precursor (pccA)	2032	NP_220985.1	4.068898
47338	H12	RP105	hypothetical protein RP105	2053	NP_220497.1	4.953726

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

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