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SUPPORTING INFECTIOUS DISEASE RESEARCH

Yersinia pestis, Strain KIM, Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 9

Catalog No. NR-19605

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Contributor:

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

Manufacturer:

BEI Resources

Product Description:

The Yersinia pestis (Y. pestis), strain KIM, Gateway[®] clone set consists of 43 plates (plate 2 of this clone set has been discontinued) which contain more than 3600 sequence validated clones from Y. pestis, strain KIM cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each open reading frame was constructed in vector <u>pDONRTM221</u> (InvitrogenTM) with an ATG start codon and a TAG 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 <u>Invitrogen</u>TM. Recombination was facilitated through an *att*B substrate (*att*B-PCR product or a linearized *att*B expression clone) with an *att*P substrate (pDONRTM221) 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 InvitrogenTM Gateway[®] Technology Manual for additional details.

Material Provided:

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

<u>Note:</u> 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 cannot confirm or validate any clone not identified on the plate information table.

Packaging/Storage:

NR-19605 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 18 to 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Yersinia pestis*, Strain KIM, Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 9, NR-19605."

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. <u>Biosafety in</u> <u>Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see <u>www.cdc.gov/biosafety/publications/bmbl5/index.htm</u>.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

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References:

- Deng, W., et al. "Genome Sequence of Yersinia pestis KIM." <u>J. Bacteriol.</u> 184 (2002): 4601-4611. PubMed: 12142430.
- Lindler, L. E., et al. "Complete DNA Sequence and Detailed Analysis of the Yersinia pestis KIM5 Plasmid Encoding Murine Toxin and Capsular Antigen." <u>Infect.</u> <u>Immun.</u> 66 (1998): 5731-5742. PubMed: 9826348.

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Clone	Well Position	Locus ID ¹	Description (Gene name)	ORF Length	Accession Number	Average Depth of Coverage
36700	A01	NTL02YP3054	hypothetical protein	393	AAM86650.1	3.967667436
36724	A02	NTL02YP3072	hypothetical protein	399	AAM86668.1	3
36756	A03	NTL02YP3061	colanic acid synthesis regulator	408	AAM86657.1	3.995535714
36761	A04	NTL02YP0185	hypothetical phage protein	411	AAM83781.1	3.458980044
38402	A05	NTL02YP3073	acetyl CoA carboxylase, carboxytransferase component, alpha subunit	960	AAM86669.1	5.051
38439	A06	NTL02YP1961	putative muconate cycloisomerase I	975	AAM85557.1	3.916256158
38605	A08	NTL02YP3058	ATP-binding component of a transporter	1032	AAM86654.1	3.572761194
38629	A09	NTL02YP0200	regulator of penicillin binding protein 3	1044	AAM83796.1	3.519372694
38650	A10	NTL02YP1974	fermentative D-lactate dehydrogenase, NAD-dependent	1050	AAM85570.1	4.386238532
38688	A11	NTL02YP0869	DNA-dependent ATPase, DNA- and ATP- dependent coprotease	1071	AAM84465.1	5.96669667
38752	A12	NTL02YP3042	hypothetical protein	1095	AAM86638.1	6.449339207
38925	B01	NTL02YP0203	putative reductase	1185	AAM83799.1	3.266938776
36771	B02	NTL02YP1972	hypothetical protein	414	AAM85568.1	4.533039648
36820	B03	NTL02YP0874	hypothetical protein	429	AAM84470.1	2.886993603
36857	B04	NTL02YP3065	hypothetical protein	441	AAM86661.1	3.702702703
36898	B05	NTL02YP1973	heat shock protein	453	AAM85569.1	2.914807302
37020	B06	NTL02YP0197	rod shape-determining protein	489	AAM83793.1	3.988657845
37104	B07	NTL02YP0876	hypothetical protein	516	AAM84472.1	5.588129496
37241	B08	NTL02YP0870	regulator, OraA protein	564	AAM84466.1	6.240066225
38932	B09	NTL02YP3076	tetraacyldisaccharide-1-P sythetase	1185	AAM86672.1	5.342857143
39265	B10	NTL02YP3071	cell cycle protein	1383	AAM86667.1	3.598735067
39348	B11	NTL02YP0191	PmbA/TIdD family protein	1446	AAM83787.1	3.721399731
39358	B12	NTL02YP3052	transcriptional regulator for nitrite reductase (cytochrome c552)	1452	AAM86648.1	3.754021448
39463	C01	NTL02YP1286	putative periplasmic binding transport protein	1554	AAM84882.1	3.618569636
39486	C02	NTL02YP0875	gamma-glutamate-cysteine ligase	1599	AAM84471.1	3.812690665
39539	C03	NTL02YP1958	transcriptional regulator of aroF, aroG, tyrA and	1668	AAM85554.1	3.760538642
37255	C05	NTL02YP3057	putative phosphatase	567	AAM86653.1	5.561779242
37251	C06	NTL02YP0873	putative phosphatase	567	AAM84469.1	6.546952224
37282	C07	NTI 027P1060	thial perovidase	576	AAM85556 1	5 321/28571

Table 1: Yersinia pestis, Strain KIM, Gateway[®] Clone Set, Recombinant in Escherichia coli, Plate 9 (UYPVI)

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Clone	Well Position	Locus ID ¹	Description (Gene name)	ORF Length	Accession Number	Average Depth of Coverage
37289	C08	NTL02YP1288	hypothetical protein	579	AAM84884.1	2.802907916
37395	C12	NTL02YP3066	hypothetical protein	615	AAM86662.1	2.839694656
38116	D01	NTL02YP1928	N-ethylmaleimide reductase	858	AAM85524.1	3.628062361
38126	D02	NTL02YP1937	pyridoxal kinase 2 / pyridoxine kinase	861	AAM85533.1	3.613762486
38162	D03	NTL02YP3018	succinyl-CoA synthetase, alpha subunit	873	AAM86614.1	3.380065717
38192	D04	NTL02YP3028	hypothetical protein	882	AAM86624.1	1.60845987
38188	D05	NTL02YP0845	probable 3-hexulose-6-phosphate isomerase	882	AAM84441.1	4.590021692
38222	D06	NTL02YP1945	homolog of Salmonella peptide transport permease protein	891	AAM85541.1	5.175080559
38293	D07	NTL02YP0855	anaerobic dimethyl sulfoxide reductase subunit C	924	AAM84451.1	1.613070539
38352	D08	NTL02YP0866	putative transcriptional regulator	945	AAM84462.1	3.665989848
38418	D09	NTL02YP1946	peptide ABC transport inner membrane permease protein	966	AAM85542.1	4.588469185
36246	D10	NTL02YP1951	phage shock protein B	228	AAM85547.1	3
36285	D11	NTL02YP1953	phage shock protein	243	AAM85549.1	3
36340	D12	NTL02YP1939	hypothetical protein	264	AAM85535.1	3.740131579
36407	E01	NTL02YP3031	hypothetical protein	285	AAM86627.1	5.270769231
36449	E02	NTL02YP0858	cytochrome b(562)	303	AAM84454.1	3.78425656
36462	E03	NTL02YP0842	hypothetical protein	306	AAM84438.1	2.75433526
38481	E04	NTL02YP3041	hypothetical protein	990	AAM86637.1	4.178640777
38475	E05	NTL02YP0847	putative inner membrane permease protein of ABC transporter	990	AAM84443.1	3.355339806
38493	E06	NTL02YP1944	putative ATP-binding protein of peptide ABC transport system	993	AAM85540.1	3.343659245
38766	E07	NTL02YP0850	putative solute-binding periplasmic protein of ABC transporter	1104	AAM84446.1	3.277972028
38852	E08	NTL02YP0863	sugar (D-ribose?) transport system permease protein	1146	AAM84459.1	4.3153457
38870	E09	NTL02YP1933	hypothetical protein	1155	AAM85529.1	4.09958159
36580	E10	NTL02YP3024	succinate dehydrogenase, hydrophobic subunit	348	AAM86620.1	3.75
36590	E11	NTL02YP3030	small membrane protein A	351	AAM86626.1	4
36737	E12	NTL02YP3013	hypothetical protein	402	AAM86609.1	4
36873	F01	NTL02YP1957	hypothetical protein	447	AAM85553.1	1.310061602
36872	F02	NTL02YP1927	lactoylglutathione lyase	447	AAM85523.1	4.72073922
36948	F03	NTL02YP1932	putative outer membrane protein	468	AAM85528.1	3.214566929
38894	F04	NTL02YP3019	succinyl-CoA synthetase, beta subunit	1167	AAM86615.1	3.186412593
38996	F05	NTL02YP3020	2-oxoglutarate dehydrogenase (dihydrolipoyltranssuccinase E2 component)	1224	AAM86616.1	3.201740506
39292	F07	NTL02YP1954	putative enzyme	1398	AAM85550.1	3.621696801
39320	F08	NTL02YP0865	hypothetical protein	1425	AAM84461.1	3.899658703
37160	F10	NTL02YP3032	hypothetical protein	537	AAM86628.1	3.109185442
37240	F11	NTL02YP0857	cytochrome b(561)	564	AAM84453.1	3.612582781
37333	F12	NTL02YP1940	hypothetical protein	591	AAM85536.1	4.624405705
39394	G01	NTL02YP3037	hypothetical protein	1491	AAM86633.1	3.577400392
39420	G02	NTL02YP0848	putative ATP-binding protein of ABC transport system	1515	AAM84444.1	3.760771704
39430	G03	NTL02YP0862	putative ATP-binding protein of ABC transport system	1524	AAM84458.1	3.598465473

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39464	G04	NTL02YP1941	putative transport protein	1554	AAM85537.1	3.669385194
39475	G05	NTL02YP3017	cytochrome d terminal oxidase, polypeptide subunit I	1569	AAM86613.1	3.840894966
39523	G06	NTL02YP1947	peptide-binding periplasmic protein of peptide ABC transport	1647	AAM85543.1	4.643746295
39594	G07	NTL02YP3023	succinate dehydrogenase, flavoprotein subunit	1767	AAM86619.1	3.715550636
37398	G08	NTL02YP0854	anaerobic dimethyl sulfoxide reductase subunit B	618	AAM84450.1	3.914893617
37517	G09	NTL02YP1935	pyridoxinephosphate oxidase	654	AAM85531.1	4.063400576
37562	G10	NTL02YP1950	phage shock protein A	666	AAM85546.1	5.009915014
37698	G11	NTL02YP3022	succinate dehydrogenase, iron sulfur protein	717	AAM86618.1	1.780713342
37853	G12	NTL02YP0852	L-ribulose-5-phosphate 4-epimerase	771	AAM84448.1	6.731196054
37973	H01	NTL02YP0851	transcriptional regulator	810	AAM84447.1	6.616470588
38058	H02	NTL02YP3002	phosphoglyceromutase 1	837	AAM86598.1	5.380843786
38131	H03	NTL02YP2995	repressor of molybdate ABC transporter genes	861	AAM86590.1	5.1809101
38254	H04	NTL02YP1891	hypothetical protein	906	AAM85487.1	5.21141649
38261	H05	NTL02YP0812	ATP-sulfurylase, subunit 2	909	AAM84408.1	5.208640674
38296	H06	NTL02YP2979	putative structural protein	924	AAM86575.1	5.425311203
38321	H07	NTL02YP1919	putative transcriptional regulator LYSR- type	933	AAM85515.1	4.952723535
38456	H08	NTL02YP1889	putative sugar transferase	984	AAM85485.1	4.482421875
38512	H09	NTL02YP0832	putative transcriptional regulator	999	AAM84428.1	4.931665063
38511	H10	NTL02YP0823	RNA polymerase, sigma S (sigma38) factor	999	AAM84419.1	6.111645813
36017	H11	NTL02YP2993	hypothetical protein	153	AAM86589.1	5.683937824
36042	H12	NTL02YP2994	hypothetical protein	159	AAM86591.1	2.869346734

The locus ID numbers given in this table were obtained from JCVI at the time of deposition. Please note that the locus tags have since been updated by JCVI and the corrected locus ID numbers can be obtained from the <u>JCVI-CMR</u> webpage using the locus search option and inputting the above locus IDs.