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SUPPORTING INFECTIOUS DISEASE RESEARCH

Staphylococcus aureus (MRSA), Strain COL Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 22

Catalog No. NR-19518

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For research use only. Not for human use.

Contributor:

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

Manufacturer:

BEI Resources

Product Description:

Production in the 96-well format has increased risk of crosscontamination 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 does not confirm or validate individual mutants provided by the contributor.

The methicillin-resistant *Staphylococcus aureus* (*S. aureus*), strain COL Gateway[®] clone set consists of 25 plates which contain 2343 sequence validated clones from *S. aureus* strain COL cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each open reading frame was constructed in vector <u>pDONRTM221</u> (<u>InvitrogenTM</u>) with a native 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 InvitrogenTM. 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 <u>Gateway[®] Technology Manual</u> 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.

Packaging/Storage:

NR-19518 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.
- 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: *Staphylococcus aureus* (MRSA), Strain COL Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 22, NR-19518."

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

Disclaimers:

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

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References:

1. Gill, S. R., et al. "Insights on Evolution of Virulence and Resistance from the Complete Genome Analysis of an

Early Methicillin-Resistant *Staphylococcus aureus* Strain and a Biofilm-Producing Methicillin-Resistant *Staphylococcus epidermidis* Strain." J. Bacteriol. 187 (2005): 2426-2438. PubMed: 15774886.

ATCC[®] is a trademark of the American Type Culture Collection.



Table 1: Staphylococcus aureus, Strain COL Gateway® Clones, Plate 22 (ZSAJV)

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
4381	A01	1309	SACOL1560	2-oxoisovalerate dehydrogenase, E2 component, dihydrolipoamide acetyltransferase	YP_186401.1	6.28342246
4383	A02	1309	SACOL1682	ATPase, AAA family	YP 186522.1	6.332314744
4385	A03	1309	SACOL1777	serine protease HtrA, putative	YP 186611.1	6.30328495
4387	A04	1312	SACOL2373	conserved hypothetical protein	YP 187177.1	5.598323171
4390	A05	1312	SACOL2382	proton/sodium-glutamate symport protein	YP_187186.1	6.298780488
4391	A06	1312	SACOL2521	transporter, putative	YP 187315.1	5.292682927
4393	A07	1312	SACOL2560	hydroxymethylglutaryl-CoA reductase, degradative	YP_187352.1	5.352896341
4395	A08	1315	SACOL1362	homoserine dehydrogenase	YP 186215.1	6.40608365
4398	A09	1318	SACOL0018	adenylosuccinate synthetase	YP 184929.1	6.3323217
4400	A10	1321	SACOL0009	seryl-tRNA synthetase	YP_184920.1	6.333838002
4401	A11	1321	SACOL1281	membrane-associated zinc metalloprotease, putative	YP_186138.1	5.955336866
4404	A12	1321	SACOL1298	peptidase, M16 family	YP 186155.1	6.305071915
4409	B01	1324	SACOL1922	glutamate-1-semialdehyde-2,1-aminomutase	YP_186747.1	6.019637462
4412	B02	1327	SACOL1104	pyruvate dehydrogenase complex E2 component, dihydrolipoamide acetyltransferase	YP_185968.1	6.010550113
4415	B03	1327	SACOL1699	GTP-binding protein, GTP1/OBG family	YP_186538.1	6.441597589
4421	B04	1327	SACOL2219	preprotein translocase, SecY subunit	YP_187029.1	6.171816127
4423	B05	1330	SACOL0026	conserved hypothetical protein	YP_184937.1	6.515789474
4425	B06	1330	SACOL0553	tRNA(IIe)-lysidine synthetase	YP 185441.1	6.521804511
4429	B07	1330	SACOL0699	penicillin-binding protein 4	YP_185581.1	6.407518797
4431	B08	1330	SACOL1088	conserved hypothetical protein	YP 185952.1	6.166165414
4433	B09	1330	SACOL1969	adenylosuccinate lyase	YP_186793.1	6.354135338
4435	B10	1333	SACOL1504	3-phosphoshikimate 1-carboxyvinyltransferase	YP 186348.1	6.475618905
4439	B11	1336	SACOL1410	femA protein	YP 186262.1	6.504491018
4441	B12	1336	SACOL1722	trigger factor	YP 186560.1	6.221556886
4444	C01	1336	SACOL2128	pyrimidine-nucleoside phosphorylase	YP 186943.1	6.062125749
4445	C02	1339	SACOL0842	enolase	YP 185716.1	6.575056012
4447	C03	1342	SACOL0185	peptide ABC transporter, permease protein	YP 185084.1	6.55290611
4450	C04	1342	SACOL0302	branched-chain amino acid transport system II carrier protein	YP_185195.1	6.347242921
4454	C05	1342	SACOL1211	uracil permease	YP_186074.1	6.342026826
4456	C06	1342	SACOL1229	sun protein	YP_186092.1	6.307004471
4457	C07	1342	SACOL1268	Gid protein	YP_186126.1	6.533532042
4459	C08	1342	SACOL2011	sodium transport family protein	YP_186831.1	6.275707899
4463	C09	1345	SACOL2418	IgG-binding protein SBI	YP 187220.1	6.409665428
4466	C10	1348	SACOL0316	conserved hypothetical protein	YP_185208.1	6.325667656
4467	C11	1348	SACOL1790	UDP-N-acetylmuramatealanine ligase	YP 186623.1	5.699554896
4470	C12	1348	SACOL1951	Mur ligase family protein	YP_186776.1	6.28041543
4471	D01	1348	SACOL2319	Na+/H+ antiporter family protein	YP_187126.1	6.442136499
4473	D02	1351	SACOL0946	Na+/H+ antiporter family protein	YP_185815.1	5.948186529

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Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
4475	D03	1351	SACOL0975	CoA-disulfide reductase	YP 185843.1	6.116950407
4477	D04	1351	SACOL2113	transcription termination factor Rho	YP 186928.1	5.803849001
4479	D05	1354	SACOL0926	5-nucleotidase family protein	YP 185796.1	6.360413589
4481	D06	1354	SACOL1197	cell division protein	YP 186059.1	5.921713442
4487	D07	1354	SACOL2599	conserved domain protein	YP 187390.1	6.062038405
4490	D08	1357	SACOL0640	pyridine nucleotide-disulfide oxidoreductase	YP 185525.1	6.27266028
4493	D09	1360	SACOL0487	hypothetical protein	YP 185377.1	6.495588235
4495	D10	1363	SACOL0046	metallo-beta-lactamase family protein	YP 184956.1	6.305209098
4497	D11	1366	SACOL0966	glucose-6-phosphate isomerase	YP 185835.1	6.038799414
4499	D12	1369	SACOL0021	yycH protein	YP 184932.1	6.451424397
4501	E01	1369	SACOL0064	metallo-beta-lactamase family protein	YP 184969.1	6.355003652
4503	E02	1369	SACOL0275	conserved hypothetical protein	YP 185170.1	4.877282688
4505	E03	1369	SACOL2242	xanthine/uracil permease family protein	YP 187052.1	6.368882396
4507	E04	1372	SACOL0501	sodium-dependent transporter, putative	YP 185389.1	5.397230321
4515	E07	1375	SACOL1329	glutamine synthetase FemC	YP 186184.1	5.509818182
4519	E09	1378	SACOL1443	branched-chain amino acid transport system II carrier protein	YP_186295.1	4.763425254
4530	E10	1381	SACOL1633	conserved hypothetical protein	YP 186473.1	6.086169442
4531	E11	1381	SACOL1719	glutamyl-tRNA reductase	YP 186557.1	6.332367849
4535	F01		SACOL0762	NO CLONE IS NOT AVAILABLE ¹	YP 185639.1	
4537	F02	1384	SACOL1196	UDP-N-acetylmuramoylalanineD-glutamate ligase	YP_186058.1	4.895953757
4539	F03	1387	SACOL0122	tetracycline resistance protein, putative	YP_185026.1	5.434751262
4541	F04	1387	SACOL0543	UDP-N-acetylglucosamine pyrophosphorylase	YP_185431.1	5.044700793
4545	F05	1387	SACOL2459	para-nitrobenzyl esterase	YP_187257.1	5.248017304
4547	F06	1390	SACOL0171	branched-chain amino acid transport system II carrier protein	YP_185070.1	5.948201439
4549	F07	1390	SACOL0405	MATE efflux family protein	YP_185297.1	5.905755396
4552	F08	1390	SACOL1450	sensor histidine kinase ArlS	YP_186302.1	6.2
4553	F09	1390	SACOL1571	acetyl-CoA carboxylase, biotin carboxylase	YP_186412.1	5.103597122
4559	F10	1393	SACOL1030	sodium transport family protein	YP_185895.1	5.601579325
4561	F11	1393	SACOL1578	FtsK/SpoIIIE family protein	YP_186418.1	4.677674085
4564	F12	1393	SACOL2073	UDP-N-acetylmuramoyl-tripeptideD-alanyl-D- alanine ligase	YP_186889.1	6.137114142
4565	G01	1393	SACOL2427	adenosylmethionine8-amino-7-oxononanoate aminotransferase	YP_187229.1	6.262024408
4567	G02	1393	SACOL2514	gluconate transporter, permease protein	YP_187308.1	5.155061019
4569	G03	1393	SACOL2669	conserved hypothetical protein	YP_187457.1	5.065326633
4571	G04	1395	SACOL2205	conserved hypothetical protein, degenerate	N/A	5.665232975
4575	G05	1396	SACOL1094	cytochrome d ubiquinol oxidase, subunit l	YP_185958.1	5.406876791
4577	G06	1396	SACOL1661	acetyl-CoA carboxylase, biotin carboxylase, putative	YP_186501.1	5.783667622
4581	G07	1396	SACOL1957	RNA methyltransferase, TrmA family	YP_186782.1	6.196991404
4583	G08	1399	SACOL0572	DNA repair protein RadA	YP_185458.1	5.165832738
4585	G09	1402	SACOL1253	signal recognition particle protein	YP_186112.1	4.94935806
4587	G10	1405	SACOL2048	3-isopropylmalate dehydratase, large subunit	YP_186865.1	5.592882562
4591	G11	1405	SACOL2636	citrate transporter, permease protein	YP_187424.1	6.250533808
4593	G12	1408	SACOL0751	deoxyribodipyrimidine photolyase, putative	YP_185630.1	6.228693182
4595	H01	1408	SACOL1874	epidermin leader peptide processing serine protease EpiP	YP_186702.1	6.318892045
4597	H02	1408	SACOL2133	conserved hypothetical protein	YP_186948.1	5.573863636
4600	H03	1408	SACOL2359	sensor histidine kinase	YP_187164.1	5.524147727
4601	H04	1411	SACOL0261	drug transporter, putative	YP_185156.1	6.228915663
4603	H05	1411	SACOL0621	substrateCoA ligase, putative	YP_185506.1	5.549255847

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Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
4606	H06	1414	SACOL0200	phosphoglycerate transporter family protein	YP_185099.1	5.89533239
4607	H07	1414	SACOL0963	argininosuccinate lyase	YP_185832.1	5.445544554
4615	H08	1414	SACOL2738	tRNA modification GTPase TrmE	YP_187524.1	6.084158416
4621	H09	1417	SACOL1360	aspartate kinase	YP_186213.1	4.87791108
4623	H10	1417	SACOL2352	tcaA protein	YP_187158.1	6.141848977
4625	H11	1420	SACOL1013	magnesium transporter	YP_185881.1	6.102816901
4627	H12	1420	SACOL1908	fumarate hydratase, class II	YP_186733.1	6.068309859

¹25 clones in the *Staphylococcus aureus* (MRSA), Strain COL Gateway[®] Clone Set (Plates 1-25), Recombinant in *Escherichia coli*, have been physically removed from the clone set due to international distribution limitations set by U.S. Department of Commerce restrictions (Commerce Control List).