Staphylococcus aureus (MRSA), Strain COL Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 24

Catalog No. NR-19520

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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 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 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 pDONR™221 (Invitrogen™) 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 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.

Material Provided:

Every 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-19520 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: *Staphylococcus aureus* (MRSA), Strain COL Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 24, NR-19520."

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. 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.

Support Provided by NIAID

Table 1: *Staphylococcus aureus*, Strain COL Gateway® Clones, Plate 24 (ZSAJX)[1]

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
4899	A01	1612	SACOL2469	glutamate synthase-related protein	YP_187266.1	6.254962779
4901	A02	1618	SACOL0381	conserved hypothetical protein	YP_185273.1	6.640296663
4903	A03	1618	SACOL2063	conserved hypothetical protein	YP_186879.1	6.265142151
4905	A04	1627	SACOL0093	L-lactate permease	YP_184998.1	6.592501537
4912	A05	1633	SACOL2363	L-lactate permease	YP_187168.1	6.545621555
4913	A06	1633	SACOL2476	peptide ABC transporter, peptide-binding protein	YP_187273.1	6.447642376
4915	A07	1633	SACOL2624	acetyl-CoA synthetase, putative	YP_187413.1	5.47458665
4917	A08	1636	SACOL1427	ABC transporter, ATP-binding protein	YP_186279.1	6.375305623
4919	A09	1639	SACOL1773	D-3-phosphoglycerate dehydrogenase	YP_186607.1	5.804148871
4928	A10	1645	SACOL2119	CTP synthase	YP_186934.1	6.193920973
4930	A11	1648	SACOL1190	conserved hypothetical protein	YP_186052.1	6.542475728
4931	A12	1651	SACOL0051	conserved hypothetical protein	YP_184961.1	6.305269534
4933	B01	1651	SACOL2016	chaperonin, 60 kDa	YP_186835.1	5.996365839
4937	B02	1659	SACOL0041	cassette chromosome recombinase B, authentic frameshift	N/A	6.341772152
4939	B03	1659	SACOL2172	IS1272-related, transposase, degenerate	N/A	6.167570826
4941	B04	1666	SACOL0744	ABC transporter, ATP-binding protein, MsbA family	YP_185623.1	6.157863145
4943	B05	1672	SACOL0598	L-ribulokinase, putative	YP_185484.1	6.134569378
4945	B06	1672	SACOL2458	amino acid permease	YP_187256.1	6.234449761
4947	B07	1675	SACOL0173	indole-3-pyruvate decarboxylase	YP_185072.1	5.708656716
4951	B08	1681	SACOL1240	DAK2 domain protein	YP_186100.1	5.887566924
4954	B09	1681	SACOL1384	osmoprotectant transporter, BCCT family	YP_186237.1	6.40928019
4958	B10	1686	SACOL1744	IS1272-related, transposase, degenerate	N/A	6.225385528
4961	B11	1696	SACOL0088	Na/Pi cotransporter family protein	YP_184993.1	6.188679245
4963	B12	1696	SACOL0663	arginyl-tRNA synthetase	YP_185547.1	6.150353774
4965	C01	1696	SACOL1804	polysaccharide biosynthesis protein	YP_186637.1	6.044221698
4967	C02	1696	SACOL2324	urocanate hydratase	YP_187131.1	5.565448113
4969	C03	1699	SACOL1739	sensory box histidine kinase PhoR	YP_186576.1	6.145968217
4971	C04	1699	SACOL2199	acetolactate synthase, catabolic	YP_187010.1	6.092407298
4973	C05	1708	SACOL0745	ABC transporter, ATP-binding protein, MsbA family	YP_185624.1	6.120608899
4977	C06	1708	SACOL1321	aerobic glycerol-3-phosphate dehydrogenase	YP_186176.1	6.201405152
4979	C07	1708	SACOL2364	conserved hypothetical protein	YP_187169.1	5.662763466
4982	C08	1711	SACOL2068	K+-transporting ATPase, A subunit	YP_186884.1	6.397428404
4986	C09	1717	SACOL2612	hydrolase, CocE/NonD family	YP_187401.1	6.38905067
4987	C10	1723	SACOL0242	teichoic acid biosynthesis protein, putative	YP_185138.1	6.074869414
4989	C11	1723	SACOL2042	dihydroxy-acid dehydratase	YP_186859.1	6.145095763
4992	C12	1726	SACOL0367	prophage L54a, terminase, large subunit, putative	YP_185259.1	6.275782155
4993	D01	1729	SACOL0238	teichoic acid biosynthesis protein, putative	YP_185134.1	6.152111047
4995	D02	1729	SACOL1767	conserved hypothetical protein	YP_186601.1	6.067090804
4997	D03	1732	SACOL0520	DNA polymerase III, gamma and tau subunits	YP_185408.1	6.113163972
5000	D04	1732	SACOL1098	metallo-beta-lactamase family protein	YP_185962.1	6.252309469

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
5004	D05	1735	SACOL1782	formate--tetrahydrofolate ligase	YP_186615.2	6.306628242
5006	D06	1738	SACOL1282	prolyl-tRNA synthetase	YP_186139.1	6.144418872
5008	D07	1741	SACOL1783	acetyl-CoA synthetase	YP_186616.1	6.269959793
5012	D08	1747	SACOL0416	conserved domain protein	YP_185308.1	6.237550086
5013	D09	1747	SACOL1153	DNA-dependent DNA polymerase family X	YP_186016.1	6.11219233
5015	D10	1747	SACOL2708	ABC transporter, ATP-binding protein	YP_187494.1	5.557527189
5017	D11	1750	SACOL0996	oligopeptide ABC transporter, oligopeptide-binding protein	YP_185864.1	5.978285714
5019	D12	1750	SACOL2282	urease, alpha subunit	YP_187089.1	5.978285714
5021	E01	1753	SACOL1092	phosphoenolpyruvate-protein phosphotransferase	YP_185956.1	6.144894467
5026	E02	1756	SACOL0243	glycosyl transferase, group 2 family protein	YP_185139.1	6.235193622
5027	E03	1762	SACOL0700	ABC transporter, permease/ATP-binding protein	YP_185582.1	6.026106697
5031	E04	1768	SACOL2430	ABC transporter, permease/ATP-binding protein	YP_187232.1	5.793552036
5035	E05	1771	SACOL1924	toxin exporting ABC transporter, permease/ATP-binding protein, putative	YP_186749.1	5.508187465
5037	E06	1774	SACOL2553	pyruvate oxidase	YP_187345.1	5.708568207
5039	E07	1780	SACOL0058	conserved hypothetical protein	YP_184964.1	5.375842697
5041	E08	1786	SACOL1534	sensor histidine kinase SrrB	YP_186376.1	5.150615901
5044	E09	1789	SACOL0102	siderophore biosynthesis protein, lucC family	YP_185007.1	6.735606484
5045	E10	1789	SACOL0245	sensor histidine kinase LytS	YP_185141.1	5.536053661
5047	E11	1792	SACOL1745	pyruvate kinase	YP_186581.1	5.347098214
5049	E12	1792	SACOL2169	aerobactin biosynthesis protein, lucA/lucC family	YP_186981.1	4.618303571
5051	F01	1794	SACOL0774	para-aminobenzoate synthase, component I, putative, authentic frameshift	N/A	5.114827202
5053	F02	1795	SACOL1308	pyruvate ferredoxin oxidoreductase, alpha subunit	YP_186165.1	5.643454039
5055	F03	1798	SACOL0207	conserved domain protein	YP_185106.1	6.426028921
5057	F04	1798	SACOL2431	ABC transporter, permease/ATP-binding protein	YP_187233.1	6.166852058
5059	F05	1801	SACOL1159	succinate dehydrogenase, flavoprotein subunit	YP_186022.1	5.494725153
5061	F06	1801	SACOL1685	aspartyl-tRNA synthetase	YP_186524.1	6.540810661
5064	F07	1804	SACOL0043	conserved hypothetical protein	YP_184953.1	6.588137472
5066	F08	1804	SACOL2043	acetolactate synthase, large subunit, biosynthetic type	YP_186860.1	6.697339246
5067	F09	1810	SACOL0089	antigen, 67 kDa	YP_184994.1	6.648618785
5069	F10	1810	SACOL0187	lipoprotein, putative	YP_185086.1	6.346961326
5071	F11	1813	SACOL0105	siderophore biosynthesis protein, lucC family	YP_185009.1	6.484280199
5074	F12	1816	SACOL0780	ATP-dependent DNA helicase RecQ	YP_185655.1	6.188325991
5075	G01	1816	SACOL1157	excinuclease ABC, C subunit	YP_186020.1	6.46530837
5077	G02	1834	SACOL1619	DNA primase	YP_186459.1	6.428571429
5081	G03	1843	SACOL1005	oligoendopeptidase F	YP_185873.1	6.33478025
5083	G04	1843	SACOL2501	phosphoglucomutase/phosphomannomutase family protein	YP_187296.1	6.542593597
5087	G05	1849	SACOL0978	membrane protein, putative	YP_185846.1	6.1595457
5089	G06	1849	SACOL1419	oligoendopeptidase F, putative	YP_186271.1	5.996755003
5091	G07	1858	SACOL0139	capsular polysaccharide biosynthesis protein Cap5D	YP_185039.1	6.414962325
5095	G08	1861	SACOL0020	sensory box histidine kinase YycG	YP_184931.1	5.834497582
5097	G09	1864	SACOL2443	membrane protein, putative	YP_187244.1	6.292918455
5100	G10	1867	SACOL1637	dnaK protein	YP_186477.1	6.674343867
5107	G11	1882	SACOL1118	GTP-binding protein TypA	YP_185982.1	6.308714134
5109	G12	1882	SACOL1577	conserved hypothetical protein	YP_186417.1	6.341657811
5113	H01	1894	SACOL2666	N-acetylmuramoyl-L-alanine amidase domain protein	YP_187454.1	6.309398099
5115	H02	1909	SACOL2662	transcriptional antiterminator, BglG family	YP_187450.1	5.118910424

Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
5117	H03	1912	SACOL0779	ABC transporter, ATP-binding protein	YP_185654.1	6.185146444
5119	H04	1912	SACOL2737	glucose inhibited division protein A	YP_187523.1	6.290794979
5122	H05	1915	SACOL2639	sulfite reductase (NADPH) flavoprotein alpha-component	YP_187427.1	6.380678851
5124	H06	1915	SACOL2725	ABC transporter, permease protein	YP_187511.1	6.169190601
5127	H07	1924	SACOL0720	ABC transporter, permease protein	YP_185601.1	6.34043659
5129	H08	1927	SACOL2520	membrane protein, putative	YP_187314.1	5.83601453
5133	H09		SACOL0209	No Clone Available	YP_185108.1	
5136	H10	1945	SACOL0383	conserved hypothetical protein	YP_185275.1	6.311568123
5138	H11	1963	SACOL2036	ABC transporter, ATP-binding protein	YP_186853.1	6.133978604
5139	H12	1966	SACOL2347	drug resistance transporter, EmrB/QacA subfamily	YP_187153.1	6.092573754

[1]25 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).

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