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SUPPORTING INFECTIOUS DISEASE RESEARCH

# *Staphylococcus aureus* (MRSA), Strain COL Gateway<sup>®</sup> Clone Set, Recombinant in *Escherichia coli*, Plate 8

# Catalog No. NR-19504

This reagent is the tangible property of the U.S. Government.

# 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 cannot confirm or validate any clone not identified on the plate information table.

The methicillin-resistant *Staphylococcus aureus* (*S. aureus*), strain COL Gateway<sup>®</sup> 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>pDONR<sup>TM</sup>221</u> (<u>Invitrogen<sup>TM</sup></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<sup>®</sup> Clones can be obtained from <u>Invitrogen™</u>. 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™ <u>Gateway<sup>®</sup> Technology Manual</u> for additional details.

# Material Provided:

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

## Packaging/Storage:

NR-19504 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<sup>®</sup> Clone Set, Recombinant in *Escherichia coli*, Plate 8, NR-19504."

#### **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:**

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#### **References:**

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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.

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# Table 1: Staphylococcus aureus, Strain COL Gateway® Clones, Plate 8 (ZSAJH)

Clone	Well	ORF	Locus ID	Description (Gene name)	Accession	Average Depth
	Position	Length			Number	of Coverage
1489	A01	487	SACOL0059	conserved hypothetical protein	YP_184965.1	3.13963039
1491	A02	487	SACOL0631	conserved hypothetical protein	YP_185516.1	2
1493	A03	487	SACOL0755	conserved hypothetical protein	YP_185634.1	3.1724846
1495	A04	487	SACOL1565	arginine repressor	YP_186406.1	2.585215606
1497	A05	487	SACOL1688	conserved hypothetical protein TIGR00256	YP_186527.1	3.162217659
1499	A06	487	SACOL2283	urease accessory protein UreE	YP_187090.1	3.170431212
1501	A07	487	SACOL2391	conserved hypothetical protein	YP_187194.1	2.564681725
1504	A08	490	SACOL0376	prophage L54a, major tail protein, putative	YP_185268.1	2
1506	A09	490	SACOL1921	bacterioferritin comigratory protein	YP_186746.1	2
1507	A10	490	SACOL2052	peptidase, SprT family	YP_186868.1	3.165306122
1512	A11	490	SACOL2361	hypothetical protein	YP_187166.1	3.112244898
1513	A12	490	SACOL2467	lipoprotein, putative	YP_187265.1	3.187755102
1516	B01	490	SACOL2650	transcriptional regulator, putative	YP_187438.1	3.13877551
1517	B02	493	SACOL0273	hypothetical protein	YP_185168.1	3.131845842
1519	B03	493	SACOL0741	conserved hypothetical protein	YP_185620.1	2.559837728
1521	B04	493	SACOL0843	conserved hypothetical protein	YP_185717.1	2
1524	B05	493	SACOL0889	pathogenicity island protein	YP 185760.1	2.563894523
1525	B06	493	SACOL1698	ACT domain protein	YP 186537.1	3.172413793
1527	B07	493	SACOL2661	conserved hypothetical protein	YP 187449.1	3.164300203
1529	B08	496	SACOL0071	conserved hypothetical protein	YP 184976.1	3.1875
1531	B09	496	SACOL0567	transcriptional regulator CtsR	YP 185455.1	2.574596774
1533	B10	496	SACOL1126	conserved hypothetical protein	YP 185990.1	3.16733871
1535	B11	499	SACOL0847	SsrA-binding protein	YP 185721.1	3.180360721
1537	B12	499	SACOL0917	NifU domain protein	YP 185788.1	3.154308617
4500	004	400	04.001.4570	acetyl-CoA carboxylase, biotin carboxyl carrier		0.400000044
1539	C01	499	SACOL1572	protein	YP_186413.1	3.160320641
1541	C02	499	SACOL1768	GAF domain protein	YP 186602.1	2.160320641
1544	C03	499	SACOL1817	6,7-dimethyl-8-ribityllumazine synthase	YP 186649.1	2.561122244
1546	C04	499	SACOL1904	transcriptional regulator, putative	YP 186729.1	3.138276553
4540	005	100		ribosomal-protein-alanine acetyltransferase,		0.000400004
1549	C05	499	SACOL2039	putative	YP_186856.1	3.090180361
1551	C06	499	SACOL2524	transcriptional regulator, MarR family	YP 187318.1	2.561122244
1554	C07	499	SACOL2717	hypothetical protein	YP 187503.1	3.160320641
1555	C08	502	SACOL0229	PTS system, sugar-specific IIA component	YP 185125.1	2.139442231
1557	C09	502	SACOL1284	conserved hypothetical protein	YP 186141.1	3.19123506
1560	C10	502	SACOL1380	conserved hypothetical protein	YP 186233.1	2.577689243
1561	C11	502	SACOL1386	conserved hypothetical protein TIGR00051	YP 186239.1	2.577689243
1563	C12	502	SACOL1627	conserved hypothetical protein TIGR00043	YP 186467.1	2.932270916
1565	D01	502	SACOL2402	conserved hypothetical protein	YP 187205.1	3.157370518
1569	D03	505	SACOL0592	ribosomal protein S7	YP 185478.1	3.138613861
1571	D04	505	SACOL0859	hypothetical protein	YP 185732.1	1.16039604
1573	D05	505	SACOL1064	conserved hypothetical protein	YP 185928.1	2.089108911
1577	D07	505	SACOL1827	RNA polymerase sigma-70 family protein	YP 186659.1	2.17029703
1581	D08	505	SACOL2126	autoinducer-2 production protein LuxS	YP 186941.1	2.411881188
4		=		cvtidine/deoxycvtidylate deaminase family		0.000000
1583	D09	507	SACOL0605	protein, authentic frameshift	N/A	2.585798817

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# **Product Information Sheet for NR-19504**

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Clone	Well Position	ORF Length	Locus ID	Description (Gene name)	Accession Number	Average Depth of Coverage
1586	D10	508	SACOL0092	conserved hypothetical protein	YP 184997.1	3.153543307
1587	D11	508	SACOL0728	conserved hypothetical protein	YP 185609.1	1.580708661
1589	D12	508	SACOL0767	conserved hypothetical protein	YP 185644.1	3.161417323
1593	E02	508	SACOL1705	hypothetical protein	YP 186544.1	2.137795276
1596	E03	508	SACOL2158	conserved hypothetical protein	YP 186970.1	2.582677165
1597	E04	508	SACOL2300	conserved hypothetical protein	YP 187107.1	3.023622047
1599	E05	508	SACOL2438	endopeptidase, putative	YP 187239.1	2.356299213
1601	E06	509	SACOL2015	integrase/recombinase-related protein, authentic	N/A	2.561886051
1603	E07	511	SACOL0560	hydroxymethyldihydropteridine pyrophosphokinase	YP_185448.1	2.483365949
1605	E08	511	SACOL0657	acetyltransferase, GNAT family, authentic point mutation	N/A	2.575342466
1607	E09	511	SACOL1325	glutathione peroxidase	YP 186180.1	3.170254403
1609	E10	511	SACOL1665	transcription elongation factor GreA	YP 186505.1	2.753424658
1611	E11	511	SACOL2683	peptide methionine sulfoxide reductase, putative	YP 187470.1	3.154598826
1613	E12	514	SACOL0025	conserved hypothetical protein	YP 184936.1	3.085603113
1615	F01	514	SACOL0951	Na+/H+ antiporter. MnhE component	YP 185820.1	1.968871595
1618	F02	514	SACOL1461	dihydrofolate reductase	YP 186310.1	2
1619	F03	514	SACOI 1841	nucleoside triphosphatase YtkD	YP 186672.1	2,426070039
1621	F04	514	SACOI 2055	anti-sigma B factor	YP 186871.1	2,453307393
1623	F05	514	SACOI 2064	membrane protein, putative	YP 186880.1	3.147859922
1625	F06	517	SACOL 0077	hypothetical protein	YP 184982 1	2 181818182
1628	F07	517	SACOL 0684	Na+/H+ antiporter MnhF component putative	YP 185566 1	2 574468085
1629	F08	517	SACOL0756	ebsC protein	YP 185635 1	3 145067698
1633	F09	517	SACOL 1120	conserved hypothetical protein	YP 185984 1	2 566731141
1635	F10	517	SACOL1134	lipopolysaccharide core biosynthesis protein	YP 185998 1	2 145067698
1637	F11	517	SACOL 1845		YP 186676 1	3 114119923
1642	F12	520	SACOL 0510	acetyltransferase GNAT family	YP 185398 1	3 015384615
1643	G01	520			YP 185701 1	3 173076923
1040	001	020	0/1002002/	molybdonterin-guanine dinucleotide biosynthesis	11_100701.1	0.170070020
1647	G02	520	SACOL2265	protein B	YP_187072.1	3.167307692
1649	G03	520	SACOL2567	hypothetical protein	YP_187359.1	2.823076923
1651	G04	523	SACOL0297	conserved hypothetical protein	YP_185191.1	2.571701721
1654	G05	523	SACOL0734	conserved hypothetical protein	YP_185613.1	2
1655	G06	523	SACOL1227	polypeptide deformylase	YP_186090.1	3.143403442
1657	G07	523	SACOL2519	conserved hypothetical protein	YP_187313.1	3.135755258
1659	G08	523	SACOL2580	hypothetical protein	YP_187372.1	2.585086042
1662	G09	523	SACOL2583	acetyltransferase, GNAT family	YP_187375.1	2
1663	G10	526	SACOL0282	conserved hypothetical protein TIGR01741	YP_185177.1	2.171102662
1666	G11	526	SACOL0289	conserved hypothetical protein	YP_185183.1	2.570342205
1669	G12	526	SACOL1314	conserved hypothetical protein	YP_186170.1	3.153992395
1673	H01	526	SACOL2343	conserved hypothetical protein	YP_187149.1	2.58365019
1675	H02	526	SACOL2541	acetyltransferase, GNAT family	YP_187333.1	3.13878327
1677	H03	529	SACOL0166	conserved hypothetical protein	YP_185065.1	2.514177694
1680	H04	529	SACOL0807	conserved hypothetical protein	YP_185681.1	3.153119093
1682	H05	529	SACOL0871	acetyltransferase, putative	YP_185743.1	-
1683	H06	529	SACOL1762	thiol peroxidase, putative	YP_186596.1	3.15879017
1685	H07	529	SACOL2062	conserved hypothetical protein	YP_186878.1	2.826086957
1687	H08	529	SACOL2267	molybdenum cofactor biosynthesis protein C	YP_187074.1	1.56710775
1690	H09	530	SACOL0288	hypothetical protein, authentic frameshift	N/A	3.164150943
1691	H10	532	SACOL0172	isochorismatase	YP_185071.1	3.148496241
1693	H11	532	SACOL0710	conserved hypothetical protein	YP_185592.1	2.34962406
1695	H12	532	SACOL1048	acetyltransferase, GNAT family	YP_185913.1	3.152255639

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