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SUPPORTING INFECTIOUS DISEASE RESEARCH

Staphylococcus aureus (MRSA), Strain COL Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 21

Catalog No. NR-19517

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

For research use only. Not for use in humans.

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> 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 <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[®] Technology Manual</u> for additional details.

Plate orientation and viability were confirmed for NR-19517.

Material Provided:

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

Packaging/Storage:

NR-19517 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:

<u>Media</u>:

LB broth or agar containing 50 µg/mL kanamycin Incubation: Temperature: 37°C Atmosphere: Aerobic <u>Propagation</u>: 1. Scrape top of frozen well with a pipette tip and streak onto

- Scrape top of frozen well with a pipette tip and streak onto agar plate.
- 2. Incubate the plates at 37°C for 1 day.

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 21, NR-19517."

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 (BMBL)</u>. 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Disclaimers:

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license is required. U.S. Government contractors may need a license before first commercial sale.

References:

 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." <u>J. Bacteriol.</u> 187 (2005): 2426-2438. PubMed: 15774886.

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



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Table 1: Staphylococcus aureus, Strain COL Gateway [®] Clones, Plate 21 (ZSAJU) ¹										
Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage				
4169	A01	1228	SACOL0065	conserved hypothetical protein	YP_184970.1	4.806188925				
4171	A02	1228	SACOL0549	tetrapyrrole methylase family protein	YP_185437.1	5.46009772				
4173	A03	1228	SACOL1066	fmt protein	YP_185930.1	4.475570033				
4176	A04	1228	SACOL1837	S-adenosylmethionine synthetase	YP_186668.1	4.813517915				
4177	A05	1228	SACOL2124	conserved hypothetical protein	YP_186939.1	5.258143322				
4179	A06	1228	SACOL2170	transporter, putative	YP_186982.1	5.460912052				
4182	A07	1228	SACOL2471	transporter, putative	YP_187268.1	4.108306189				
4185	A08	1231	SACOL1900	DNA repair exonuclease family protein	YP_186726.1	5.538586515				
4187	A09	1234	SACOL0392	NADH-dependent flavin oxidoreductase, Oye family	YP_185284.1	5.576985413				
4190	A10	1234	SACOL1861	type I restriction-modification enzyme, S subunit, EcoA family	YP_186689.1	4.691247974				
4191	A11	1234	SACOL1964	Staphylococcus aureus sex pheromone	YP_186788.1	5.534846029				
4194	A12	1237	SACOL0107	pyridoxal-dependent decarboxylase	YP_185011.1	5.452708165				
4195	B01	1237	SACOL0491	cobalamin synthesis protein, putative	YP_185381.1	5.554567502				
4198	B02	1237	SACOL1497	polyA polymerase	YP_186341.1	5.444624091				
4199	B03	1237	SACOL1760	acetate kinase	YP_186594.1	5.035569927				
4201	B04	1237	SACOL2075	cell division protein, FtsW/RodA/SpoVE family	YP_186891.1	5.446240905				
4204	B05	1237	SACOL2155	IS1181, transposase, authentic point mutation	N/A	5.423605497				
4211	B08	1240	SACOL0964	argininosuccinate synthase	YP 185833.1	5.488709677				
4213	B09	1240	SACOL1223	phosphopantothenoylcysteine decarboxylase/phosphopantothenatecysteine ligase	YP_186086.1	5.511290323				
4215	B10	1240	SACOL1428	aspartokinase, alpha and beta subunits	YP_186280.1	5.467741935				
4217	B11	1243	SACOL0944	NADH dehydrogenase, putative	YP_185813.1	5.2614642				
4221	B12	1243	SACOL2340	sodium:glutamate symporter	YP_187146.1	5.420756235				
4223	C01	1243	SACOL2350	tcaB protein	YP_187156.1	4.66773934				
4226	C02	1246	SACOL0213	acyl-CoA dehydrogenase family protein	YP_185112.1	5.376404494				
4229	C03	1246	SACOL1051	isochorismate synthase family protein	YP_185916.1	5.504815409				
4231	C04	1246	SACOL2257	drug transporter, putative	YP_187064.1	5.128410915				
4233	C05	1246	SACOL2437	bicyclomycin resistance protein	YP_187238.1	5.46388443				
4236	C06	1246	SACOL2675	SecY/Sec61-alpha family protein	YP_187463.1	5.453451043				
4238	C07	1249	SACOL0566	nucleoside permease NupC	YP_185454.1	5.444355484				
4239	C08	1249	SACOL0936	DItB protein	YP_185806.1	5.254603683				
4241	C09	1249	SACOL1408	tryptophan synthase, beta subunit	YP_186260.1	5.470776621				
4243	C10	1249	SACOL2477	conserved hypothetical protein	YP_187274.1	5.496397118				
4245	C11	1252	SACOL1065	transcriptional regulator, putative	YP_185929.1	5.39057508				
4247	C12	1252	SACOL1941	YihY family protein	YP_186766.1	5.424920128				
4250	D01	1255	SACOL0070	permease, putative	YP_184975.1	4.956175299				
4251	D02	1255	SACOL0310	nucleoside permease NupC, putative	YP_185202.1	4.706772908				
4253	D03	1255	SACOL0733	sugar efflux transporter, putative	YP_185612.1	5.485258964				
4255	D04	1255	SACOL0885	pathogenicity island protein, integrase	YP_185756.1	5.50438247				
4257	D05	1255	SACOL1658	hypothetical protein	YP_186498.1	4.827091633				
4259	D06	1258	SACOL1764	thiamine biosynthesis protein Thil	YP_186598.1	5.461844197				
4262	D07	1258	SACOL1892	membrane protein, putative	YP_186718.1	5.348171701				
4266	D08	1261	SACOL0806	peptidase T	YP_185680.1	4.749405234				
4267	D09	1261	SACOL1122	cell division protein,FtsW/RodA/SpoVE family	YP_185986.1	5.440126883				

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Clone	Well Position	ORF Length	Locus ID	Description	Accession Number	Average Depth of Coverage
4269	D10	1261	SACOI 2453	amino acid ABC transporter ATP-binding protein	YP 1872521	5 395717684
4271	D11	1264	SACOL 0415	Dvp-tvpe peroxidase family protein	YP 185307 1	5 393196203
4273	D12	1264	SACOL0701	nucleoside permease NupC, putative	YP 185583.1	5.484177215
4275	E01	1264	SACOL1659	conserved hypothetical protein	YP 186499.1	5.416139241
4277	E02	1264	SACOL1749	NADP-dependent malic enzyme, putative	YP 186585.1	5.524525316
4279	E03	1267	SACOL2334	conserved hypothetical protein	YP 187141.1	5,505919495
4281	E04	1270	SACOL2657	arginine deiminase	YP 187445.1	5,446456693
4283	E05	1273	SACOL0116	hypothetical protein	YP 185020.1	5,488609584
4286	E06	1273	SACOL 0368	prophage L 54a portal protein HK97 family	YP 185260 1	5 352710134
4287	E07	1273	SACOI 1326	GTP-binding protein putative	YP 186181 1	5 417910448
4291	E08	1273	SACOL 2105	serine hydroxymethyltransferase	YP 186920 1	5 44854674
4293	E09	1273	SACOL 2323	imidazolonepropionase	YP 187130 1	5 355852317
4295	E00	1273	SACOL 2689	intercellular adhesion protein A	YP 187476 1	5 418695994
4299	F11	1276	SACOL0343	prophage L54a, replicative DNA belicase, putative	YP 185235 1	5 394200627
4301	F12	1276	SACOL 0916	cysteine desulfurase. SufS subfamily	YP 185787 1	5 442789969
1001		1270	SACOLA000			0.112100000
4303	F01	1276	3	plasmid recombination enzyme		5.45846395
4305	F02	1279	SACOL0961	glutamate dehydrogenase, NAD-specific	YP_185830.1	4.826426896
4307	F03	1279	SACOL0988	3-oxoacyl-(acyl-carrier-protein) synthase II	YP_185856.1	4.709147772
4309	F04	1279	SACOL1265	endopeptidase resistance gene	YP_186123.1	5.451915559
4311	F05	1279	SACOL1503	TPR domain protein	YP_186347.1	5.366692729
4313	F06	1279	SACOL1876	epidermin biosynthesis protein EpiC, authentic point mutation	N/A	5.388584832
4318	F07	1285	SACOL0163	drug transporter, putative	YP_185062.1	4.572762646
4319	F08	1285	SACOL1251	cell division protein FtsY, putative	YP_186110.1	5.357198444
4321	F09	1285	SACOL2031	ammonium transporter family protein	YP_186849.1	5.33229572
4323	F10	1285	SACOL2409	fmhA protein	YP 187212.1	5.339299611
4325	F11	1291	SACOL0103	drug transporter, putative	YP_185008.1	6.298993029
4327	F12	1291	SACOL1937	aminopeptidase PepS	YP_186762.1	6.336948102
4329	G01	1291	SACOL2702	histidinol dehydrogenase	YP_187488.1	6.355538342
4331	G02	1294	SACOL0232	PTS system, sorbitol-specific IIC component	YP_185128.1	6.390262751
4333	G03	1294	SACOL1411	femB protein	YP_186263.1	6.35316847
4335	G04	1294	SACOL2019	sdrH protein, putative	YP_186838.1	5.5
4337	G05	1294	SACOL2116	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2	YP_186931.1	6.343122102
4339	G06	1294	SACOL2266	molybdopterin biosynthesis MoeA protein, putative	YP_187073.1	6.230293663
4343	G07	1297	SACOL0150	capsular polysaccharide biosynthesis protein Cap5O	YP_185050.1	6.240555127
4345	G08	1297	SACOL1400	ImpB/MucB/SamB family protein	YP_186252.1	6.33461835
4347	G09	1297	SACOL1686	histidyl-tRNA synthetase	YP_186525.1	6.117193524
4349	G10	1297	SACOL1721	ATP-dependent Clp protease, ATP-binding subunit ClpX	YP_186559.1	6.064764842
4351	G11	1297	SACOL1778	tyrosyl-tRNA synthetase	YP_186612.1	6.32767926
4353	G12	1300	SACOL1297	conserved hypothetical protein	YP_186154.1	5.268461538
4355	H01	1300	SACOL1435	diaminopimelate decarboxylase	YP_186287.1	6.335384615
4357	H02	1300	SACOL2092	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	YP_186907.1	6.28
4360	H03	1300	SACOL2253	femX protein	YP_187061.1	6.311538462
4361	H04	1303	SACOL0194	maltose ABC transporter, permease protein	YP_185093.1	6.304681504
4363	H05	1303	SACOL0459	xanthine permease	YP_185349.1	6.306983883
4365	H06	1303	SACOL1448	2-oxoglutarate dehydrogenase, E2 component, dihydroipoamide succinyltransferase	YP_186300.1	6.34228703
4368	H07	1303	SACOL1667	peptidase, U32 family	YP 186507.1	6.256331543
4369	H08	1303	SACOL1741	isocitrate dehydrogenase, NADP-dependent	YP 186578.1	6.307751343
4372	H09	1303	SACOL1805	conserved hypothetical protein TIGR00275	YP 186638.1	6.228702993
4374	H10	1303	SACOL2050	threonine dehydratase	YP 186867.1	6.253261704
4376	H11	1306	SACOL0193	maltose ABC transporter, maltose-binding protein, putative	 YP_185092.1	5.797090352
4377	H12	1306	SACOL1709	folylpolyglutamate synthase/dihydrofolate synthase	YP 186548.1	6.30704441

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