

Supplemental Table S1. Primers Used to Confirm NTML Strains.

Primer	Description	Gene
OL338	ATG GGA TCC GTT TCT GCT ATG TAC AAC	Forward <i>brnQ3</i>
OL339	ATG GAA TTC CCT AGG AAA GCG AAG CCA TTC	Reverse <i>brnQ3</i>
OL1573	TAG CGC TGA CGA ATG AAA CTT	Forward <i>sbcC</i>
OL1574	CAT CGC GTT TGC TTT ATT CAT C	Reverse <i>sbcC</i>
OL1633	GAA GGT CAA GCT GTT GAG	Forward <i>msa</i>
OL1634	AAG CAT CAT ATA CAT ACC ACA CT	Reverse <i>msa</i>
OL1635	CCG AAG TGC GAT GTT CAC TT	Forward <i>msrA</i>
OL1636	TCC AAG TGC TAA GAG GTA TAC AG	Reverse <i>msrA</i>
OL1652	GAA GTT GTG CAA GTG ATA CAT G	Forward <i>sacol1602</i>
OL1653	CGA CCG CTC TGT GCG ACT TGC TG	Reverse <i>sacol1602</i>
OL1654	GAG ACG GGA GAT TTC TAC GAG CC	Forward <i>glpF</i>
OL1655	GAG CTT GTT GTT CCT TGG TC	Reverse <i>glpF</i>
OL1656	CAA CAA GAT AAG GAG TGA AC	Forward <i>ebh</i>
OL1657	GTG ATG TCA ATC GGT GCT CCT C	Reverse <i>ebh</i>
OL2074	CAA GGT GCA TGG TTA TAT GTC	Forward <i>sucB</i>
OL2075	CAG CAG TAA GAT ATT TCG ACT AGG	Reverse <i>sucB</i>
OL2089	GAA CCA GGG TGA TGC ATA TCG	Forward <i>ctpA</i>
OL2090	GGA GGC GTG TCA TTA AC	Reverse <i>ctpA</i>
OL2469	GTC GGT GAA GTA CTT GGA ACT GG	Forward <i>hexA</i>
OL2470	CTG TAG CGC CTG CAT CTA TAG C	Reverse <i>hexA</i>
OL2471	GGT GTA TCA CCA GCA AAT GCT CC	Forward <i>tenA</i>
OL2472	GAT CAG TAC CTG CAA TGG TTA ATG C	Reverse <i>tenA</i>
OL2473	CTG TTT ACG CAG CAT ATA CCG CAC	Forward <i>femB</i>
OL2474	GCC TAT AGT ATC AAT AAC TAT AAG CC	Reverse <i>femB</i>
OL2475	GTC AAC TAT CCT AAG CGG AG	Forward <i>pbp2</i>
OL2476	CTG AGG ATT TTA TGT TGA GTG G	Reverse <i>pbp2</i>
OL2477	CAT TGC GGG TCA AGA TGT ATC AG	Forward <i>argJ</i>
OL2478	GAA CGA TAA TGG GGT AGA TGT TG	Reverse <i>argJ</i>
OL2479	GTA CAC ATT AGC TGT GAC TAA TG	Forward <i>sucA</i>
OL2480	GGA ACT TTA ACC TCT GGC ATG AC	Reverse <i>sucA</i>
OL2551	GTC AAG GCG TAG ATA TCC TAT GTA AGG	Forward <i>nupC</i>
OL2552	CAG ACA TAT TGT GCA TGT ATA TCA CC	Reverse <i>nupC</i>
OL2553	TGG TGG TGG GTT TAT TGG	Forward <i>sacol0682</i>
OL2554	CGT GCT AAT GCT AGA TGT	Reverse <i>sacol0682</i>
OL2555	AGA TAA AGA TGG GGA GGA	Forward <i>sacol0684</i>
OL2556	TAC ACT TAA CAC ACC CAC	Reverse <i>sacol0684</i>
OL2557	GGT TGG AAA GGT TGG GTT	Forward <i>sacol0940</i>
OL2558	GTA GTT GGT GAT TGT GCT G	Reverse <i>sacol0940</i>
OL2559	CCT CAT CTA AAC CTA TCG	Forward <i>sacol1349</i>
OL2560	TCC CCT CTT TTC TCT ATT TC	Reverse <i>sacol1349</i>
OL2561	TTT GCC CGT TTC TCT ACT	Forward <i>rpmG</i>
OL2562	CTT CGT AAT CAC CTT TTG CT	Reverse <i>rpmG</i>
OL2563	CGG TGT TGA AGA TAT TGG T	Forward <i>sacol1392</i>
OL2564	GCC ATT CCC TCT TTT TTG T	Reverse <i>sacol1392</i>
OL2565	AGA AGA TGG TAA GAA GGG	Forward <i>licT</i>
OL2566	AAG AGA CGA TGA TAG GTG	Reverse <i>licT</i>
OL2567	GTG AGA ATG ATG GCT TTG	Forward <i>sacol1412</i>
OL2568	GGA TTA GAT GAA GAG GAA GA	Reverse <i>sacol1412</i>
OL2569	ACC CGG CAC AAA TTA CAA	Forward <i>opp2F</i>
OL2570	AAC GGG GTG AAT ATG ATG A	Reverse <i>opp2F</i>
OL2571	CAA CCG GAG AGC AAC AAA	Forward <i>asd</i>
OL2572	GCC AAC ACC CTC AAA TAA A	Reverse <i>asd</i>
OL2573	AAG GCA TCA CAT ATC CAG AA	Forward <i>alr2</i>
OL2574	TAC CAA AGC CAC CAC CAA	Reverse <i>alr2</i>
OL2575	CAA CTC TAA TAG CAA CAC CA	Forward <i>sacol1445</i>
OL2576	CAC ACT CAA TAG CAA CCA	Reverse <i>sacol1445</i>
OL2577	CCG CCA ACT AAT CCA GAA	Forward <i>sacol1447</i>
OL2578	CAC AAA CAA CAC AAC AAG CA	Reverse <i>sacol1447</i>
OL2579	ATA GAA CAT CCC CCC CCA	Forward <i>sacol1452</i>
OL2580	GAC CAA GAC CAA GCA GAA	Reverse <i>sacol1452</i>
OL2581	TTG TTG TTT CGC TTG TGG T	Forward <i>ald</i>
OL2582	GAT GGA TAT GCG GGT GTT	Reverse <i>ald</i>
OL2583	CAC ACC TTC TTC TTA CA C	Forward <i>sacol1479</i>
OL2584	CCA AAT CCT ACT ACA GCA	Reverse <i>sacol1479</i>

OL2585	ATC CCA ACA TAC AGC TAC A	Forward <i>sacI1480</i>
OL2586	AAC GAA CCA AAG GGG AAA	Reverse <i>sacI1480</i>
OL2587	CCA CCA GAA ACG CCC ATA	Forward <i>pepT2</i>
OL2588	TGC CAA CAA CAC ACA AAG	Reverse <i>pepT2</i>
OL2589	AAC CGC CAC CAG AAA TAA	Forward <i>sacI1556</i>
OL2590	TGC CAA CAA CAC ACA AAG	Reverse <i>sacI1556</i>
OL2591	CCG CAA AAT CCC CAT CAA	Forward <i>sacI1620</i>
OL2592	CCA TAA CTC ACC CCA ACC	Reverse <i>sacI1620</i>
OL2593	ACC CCT CCT AAA AAC CAA	Forward <i>ribD</i>
OL2594	CAA ATC CAA TGC CTC CAA	Reverse <i>ribD</i>
OL2735	GAA AAA TAG CTA AGG GGA GT	Forward <i>sacI0722</i>
OL2736	TGC TTT GTC TTG GTC ATT C	Reverse <i>sacI0722</i>
OL2737	ACC TCA TCC GCC AAT TAA A	Forward <i>isdB</i>
OL2738	CAT CGT CAC ACT CAT AAC T	Reverse <i>isdB</i>
OL2739	CAA CGG AGG GGT AAA TCA	Forward <i>pepA3</i>
OL2740	AAG ACA CCA CAT AAC GCA	Reverse <i>pepA3</i>
OL2741	CTA ATA GGT GAG GCG CTT	Forward <i>trpF</i>
OL2742	GTC TGC TTT TGC CTC TTT	Reverse <i>trpF</i>
OL2743	CAA TAG CAG TCA GTT TCA AG	Forward <i>pepF2</i>
OL2744	CAG CAA GAC ATT TAG AAC G	Reverse <i>pepF2</i>
OL2745	CAT AGA TGG TGT TGA AGA AG	Forward <i>pstC</i>
OL2746	GGG AGT AAT AGA AAG AGG T	Reverse <i>pstC</i>
OL2747	AAC CTC TTT CTA TTA CTC CC	Forward <i>pstS</i>
OL2748	CCC AGC CAT TTT TGT ATT C	Reverse <i>pstS</i>
OL2749	CCT GCG CCA AAA AAC ATA	Forward <i>norD</i>
OL2750	GGA AGA TGA ACG TGA ACA A	Reverse <i>norD</i>
OL2751	CCA TCT CTA TTT GCC TCC	Forward <i>sacI1476</i>
OL2752	AAG GGT GTG GTA GGT TAA A	Reverse <i>sacI1476</i>
OL2753	AAA CAT AGC TCA TCA CCC	Forward <i>sacI1531</i>
OL2754	CAA TCT GCC TCT CTA ATA C	Reverse <i>sacI1531</i>
OL2755	GGC CCC GGA CTA TAA TTG A	Forward <i>sacI1947</i>
OL2756	GGC CCC AAC AAA GAG AAA	Reverse <i>sacI1947</i>
OL2757	CCG AGA ATC TTT AAC ACC A	Forward <i>ilvB</i>
OL2758	ACA AGG TCG TAG CAT TCA	Reverse <i>ilvB</i>
OL2759	ATT GAT GTT CGT CCG GTT	Forward <i>hmrA</i>
OL2760	GAG TGT AGG GTA AAA GGG AG	Reverse <i>hmrA</i>
OL2761	GGG GAA ATA TGT AAA CGA AC	Forward <i>sacI2143</i>
OL2762	GGC CAA AGT ATT CAA CAA G	Reverse <i>sacI2143</i>
OL2763	AAG ACC GTG ATG AGA AGA	Forward <i>rocF</i>
OL2764	ACA TAG AGC AAA GGG GGA	Reverse <i>rocF</i>

Supplemental Table S2. Proteins identified as binding to the *sigS* promoter region in a biotin pull down assay.

Accession #	Gene	Function
SACOL0009	<i>serS</i>	Seryl-tRNA synthetase
SACOL0435	<i>ychF</i>	GTP-binding protein
SACOL0476	<i>hsdM</i>	Type I restriction-modification system M subunit
SACOL0477		Putative restriction/modification system specificity protein
SACOL0562	<i>lysS</i>	Lysyl-tRNA synthetase
SACOL0570	<i>clpC</i>	ATP-dependent Clp Protease ATP-binding subunit
SACOL0588	<i>rpoB</i>	DNA-directed RNA polymerase subunit beta
SACOL0589	<i>rpoC</i>	DNA-directed RNA polymerase subunit beta'
SACOL0793		Ribonucleoside-diphosphate reductase, beta subunit
SACOL1215	<i>carB</i>	Carbamoyl-phosphate synthase large chain
SACOL1267	<i>topA</i>	DNA topoisomerase 1
SACOL1271	<i>hslU</i>	ATP-dependent protease ATPase subunit
SACOL1390	<i>parC</i>	DNA topoisomerase IV, subunit A
SACOL1494	<i>asnS</i>	Asparaginyl-tRNA synthetase
SACOL1614	<i>nfo</i>	Probable endonuclease 4
SACOL1681	<i>cymR</i>	Rrf2 family protein
SACOL1729	<i>thrS</i>	Threonyl-tRNA synthetase
SACOL1737	<i>polA</i>	DNA polymerase I
SACOL1759		Putative universal stress protein
SACOL1790	<i>murC</i>	UDP-N-acetylmuramate-L-alanine ligase
SACOL2213	<i>rpoA</i>	DNA-directed RNA polymerase subunit alpha

Supplemental Table S3. Tn551 Insertions Resulting in Increased Expression of *sigS*.

Accession Number	NE Number ^a	Tn551 Insertion Site	Gene	Hits ^b	Unique ^c
DNA metabolism: DNA replication, recombination and repair					
SACOL0566	NE544	Nucleoside permease	<i>nupC</i>	1	1
SACOL1315	NE974	DNA mismatch repair protein	<i>hexA</i>	1	1
SACOL1382	NE1451	Exonuclease	<i>sbcDC</i>	3	2
SACOL1413	N/A	Hypothetical protein (putative helicase)		1	1
SACOL1479	NE789	Putative, 5'-3' exonuclease		2	1
Regulators					
SACOL0513	NE1883	Transcriptional regulatory protein	<i>glcT</i>	1	1
SACOL1136	N/A	Hypothetical Protein (nucleic acid binding domain)		1	1
SACOL1393	NE1415	Transcriptional antiterminator LicT, putative	<i>licT</i>	3	1
SACOL1451	NE1684	Response regulator	<i>arlR</i>	1	1
SACOL1436	NE9	Modulator of SarA	<i>Msa</i>	1	1
SACOL2086	NE1218	Transcriptional regulator, TenA family	<i>tenA</i>	1	1
SACOL2188	NE436	Lactose phosphotransferase system repressor	<i>lacR</i>	5	1
Toxin production and resistance					
SACOL1441	N/A	Tellurite resistance protein, putative		3	1
Cell envelope associated					
SACOL1411	N/A	Aminoacyltransferase	<i>femB</i>	1	1
SACOL1472	NE1	Cell wall associated fibronectin-binding protein	<i>ebh</i>	6	2
Transporters					
SACOL0682	NE1139	Putative antiporter		1	1
SACOL0684	NE1292	Na ⁺ /H ⁺ antiporter, MnhE component, putative		2	1
SACOL0993	NE1633	Oligopeptide ABC transporter, ATP-binding protein	<i>oppD2</i>	1	1
SACOL1319	NE1580	Glycerol uptake facilitator protein	<i>glpF</i>	5	1
SACOL1392	NE142	Sodium:alanine symporter family protein		3	1
SACOL1414	NE1609	Peptide ABC transporter, ATP-binding protein	<i>opp2F</i>	4	1
SACOL1416	N/A	Peptide ABC-transporter, permease protein, putative		1	1
SAACOL1443	NE44	Branched-chain amino acid transport system II carrier protein	<i>brnQ3</i>	3	1
Amino acid biosynthesis					
SACOL1680	NE595	Glutamate N-acetyltransferase/amino-acid acetyltransferase	<i>argJ</i>	2	1
SACOL1439	NE809	Hypothetical protein (Aspartate aminotransferase superfamily)		1	1
SACOL1429	NE1144	Aspartate-semialdehyde dehydrogenase	<i>asd</i>	1	1
SACOL1434	NE799	Alanine racemase family protein/diaminopimelate decarboxylase	<i>alr2</i>	1	1
SACOL1448	NE1391	Dihydroipoamide succinyltransferase	<i>sucB</i>	1	1
SACOL1449	NE547	2-Oxoglutarate dehydrogenase, E1 component	<i>sucA</i>	3	1
SACOL1478	NE1136	Alanine dehydrogenase	<i>ald</i>	1	1
SACOL2045	NE1177	Ketol-acid reductoisomerase		1	1
Protein synthesis and modification					
SACOL1369	NE1904	50S ribosomal protein l33	<i>rpmG</i>	4	1
SACOL1397	NE1813	Peptide methionine sulfoxide reductase	<i>msrA</i>	4	1
SACOL1455	NE847	Carboxyl-terminal protease	<i>ctpA</i>	1	1
SACOL1480	NE582	Hypothetical protein (ribosome associated GTPase domain)		1	1
Unknown function					
SACOL1452	NE577	PAP2 family protein		1	1
SACOL1602	NE377	Metallo-β-lactamase family protein		1	1
Hypothetical proteins					
SACOL0940	NE1505	Hypothetical protein (No conserved domains)		1	1
SACOL1469	N/A	Hypothetical protein (No conserved domains)		1	1
SACOL1556	NE1346	Hypothetical protein (No conserved domains)		1	1
SACOL2642	N/A	Hypothetical protein (No conserved domains)		1	1
INTERGENIC					
SACOLL1369/ SACOL1370	N/A	50S ribosomal protein L33/30S ribosomal protein S14	<i>rpmG/</i>	1	1
SACOL1396/ SACOL1397	N/A	<i>fmtC</i> protein/peptide methionine sulfoxide reductase	<i>fmtC/ msrA</i>	1	1
SACOL1412/ SACOL1413	N/A	Hydrolase-related protein/hypothetical protein (putative helicase)		2	1
SACOL1442/ SACOL1443	N/A	IS1272-related, transposase, degenerate/Branched-chain amino acid transport system II carrier protein	<i>/brnQ3</i>	2	1
SACOL1445/	N/A	CbbQ/NirQ/NorQ/GpvN family protein/ Conserved		1	1

SACOL1446		hypothetical protein			
SACOL1447/ SACOL1448	N/A	Conserved hypothetical protein/dihydroipoamide acyltransferase		1	1
SACOL1449/ SACOL1450	N/A	2-oxoglutarate dehydrogenase, E1 component/Sensor histidine kinase	<i>sucA/ arlS</i>	2	1
SACOL1451/ SACOL1452	N/A	DNA-binding response regulator ArlR/PAP2 family protein	<i>arlR/</i>	1	1
SACOL1555/ SACOL1556	N/A	Peptidase, M20/M25/M40 family/hypothetical protein	<i>pepT2/</i>	1	1
SACOL1620/ SACOL1621	N/A	Conserved hypothetical protein/CBS domain protein		1	1
SACOL1820/ SACOL1821	N/A	Riboflavin biosynthesis protein RibD/Conserved hypothetical protein	<i>ribD/</i>	1	1
SACOL1893/ SACOL1894	N/A	ABC transporter, ATP-binding protein/HIT family protein		1	1

^aNE numbers are the identification number in the Nebraska Transposon Mutant Library.

^bHits refer to the total number of Tn551 insertion sites identified for a particular gene.

^cThe unique number of insertions sites refers to those insertions that are a result of distinctive insertion events.

N/A means there was no NTML strain for this gene

Supplemental Table S4. Tn551 Insertions Resulting in Decreased Expression of *sigS*.

Accession Number	NE Number ^a	Tn551 Insertion Site	Gene	Hits ^b	Unique ^c
Regulators					
SACOL1393	NE1415	Transcriptional antiterminator, <i>licT</i> putative	<i>licT</i>	3	1
SACOL1398	N/A	Transcriptional regulator, putative		1	1
Cell Envelope Associated					
SACOL1138	NE1102	LPXTG cell wall surface anchor protein	<i>isdB</i>	1	1
SACOL1531	NE707	Lipoprotein, putative		1	1
Transporters					
SACOL0722	NE187	Phosphate transporter family protein		1	1
SACOL1424	NE1459	Phosphate ABC transporter, phosphate binding protein	<i>pstS</i>	2	1
SACOL1443	NE44	Branched chain amino acid transport system II carrier protein	<i>brnQ3</i>	7	1
SACOL1476	NE211	Amino acid permease		1	1
Amino Acid Biosynthesis					
SACOL1407	NE1215	5'Phosphoribosyl anthranilate isomerase	<i>trpF</i>	1	1
SACOL2043	NE1166	Acetolactate synthase, large subunit	<i>ilvB</i>	1	1
SACOL2154	NE134	Arginase	<i>rocF</i>	1	1
Protein Synthesis and Modification					
SACOL1402	NE1306	Glutamyl aminopeptidase, putative	<i>pepA3</i>	1	1
SACOL1419	NE195	Oligoendopeptidase F, putative	<i>pepF2</i>	1	1
SACOL2125	NE600	Peptidase, M20/M25/M40 family protein	<i>hmrA</i>	1	1
Unknown Function					
SACOL1412	NE1611	Hydrolase-related protein		2	1
SACOL1452	NE577	PAP2 family protein		1	1
Hypothetical Proteins					
SACOL1344	N/A	Hypothetical protein		3	2
SACOL1418	N/A	Conserved hypothetical protein		1	1
SACOL1444	NE143	Conserved hypothetical protein		3	2
SACOL1947	NE1706	Conserved hypothetical protein		3	1
SACOL2143	NE1104	Conserved hypothetical protein		2	1
INTERGENIC					
SACOL0610/ SACOL0611	N/A	SdrE protein/glycosyl transferase, group 1 family protein	<i>sdrE/</i>	1	1
SACOL1348/ SACOL1349	N/A	Conserved hypothetical protein/conserved hypothetical protein		1	1
SACOL1369/ SACOL1370	N/A	Ribosomal protein L33/ribosomal protein S14	<i>rpmG1/</i> <i>rpsN1</i>	1	1
SACOL1398/ SACOL1399	N/A	Transcriptional regulator, putative/ 4-oxalocrotonate tautomerase	<i>/dmpl</i>	1	1
SACOL1407/ SACOL1408	N/A	5'Phosphoribosyl anthranilate isomerase/tryptophan synthase, beta subunit	<i>trpF/tr</i> <i>pB</i>	1	1
SACOL1411/ SACOL1412		FemB protein/hydrolase related protein	<i>femB/</i>	1	1
SACOL1441/ SACOL1442	N/A	Tellurite resistance protein, putative/ IS1272-related transposase		1	1
SACOL1443/ SACOL1444	N/A	Branched-chain amino acid transport system II protein/Conserved hypothetical protein	<i>brnQ3/</i>	1	1
SACOL1447/ SACOL1448	N/A	Conserved hypothetical protein/2-oxoglutarate dehydrogenase, E2 component	<i>/sucB</i>	1	1
SACOL1468/ SACOL1469	N/A	Membrane protein, putative/hypothetical protein		1	1
SACOL1711/ SACOL1712	N/A	DNA 3-methyladenine glycosylase /arbB protein putative		1	1
SACOL1913/ SACOL1914	N/A	RNA methyltransferase/Iron-sulfur cluster, binding protein		1	1

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^cThe unique number of insertions sites refers to those insertions that are a result of distinctive insertion events.

N/A means there was no NTML strain for this gene