

Figure S1

Reverting the non-synonymous SNP present within the M3 *nra* allele results in a small, but reproducible, increase in pilus expression. Western blot analysis. The parental M3 isolate (MGAS10870) and derivatives M3Δnra, M3ΔmsmR, M3nra^{FIXED}, and M3nra^{FIXED}ΔmsmR were evaluated for pilus expression. Cell wall proteins were isolated from exponential phase cultures and assayed for reactivity to an anti-pilus (T3) antibody. Data is representative of three separate experiments.

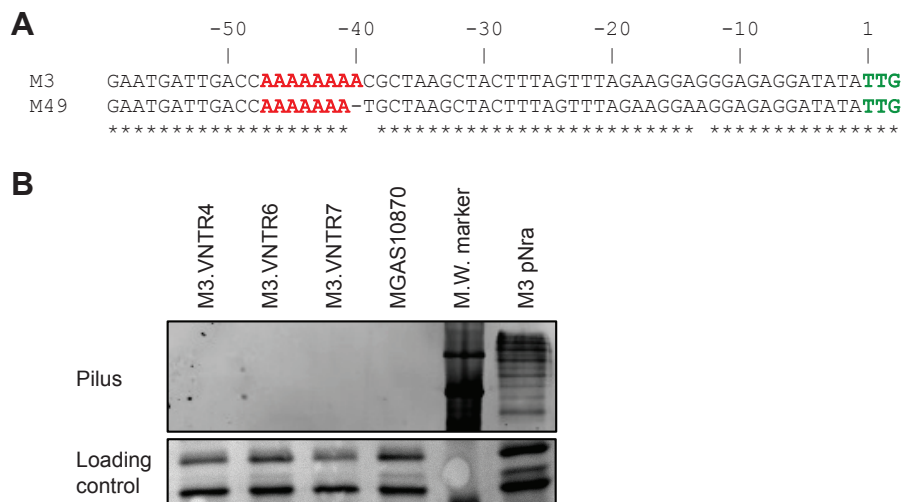


Figure S2

A VNTR located within the *nra.cpa* intergenic region does not influence M3 GAS pilus expression.

(A) Schematic of the polynucleotide VNTR (red nucleotides) upstream of *cpa* in M3 and M49 GAS. The *cpa* translational start codon (TTG) is shown in green. (B) Western blot analysis of pilus expression in parental M3 isolate MGAS10870 and altered (8A to 7A) VNTR mutant derivatives (note that M3.VNTR4, M3.VNTR6, and M3.VNTR7 are independently created derivatives).

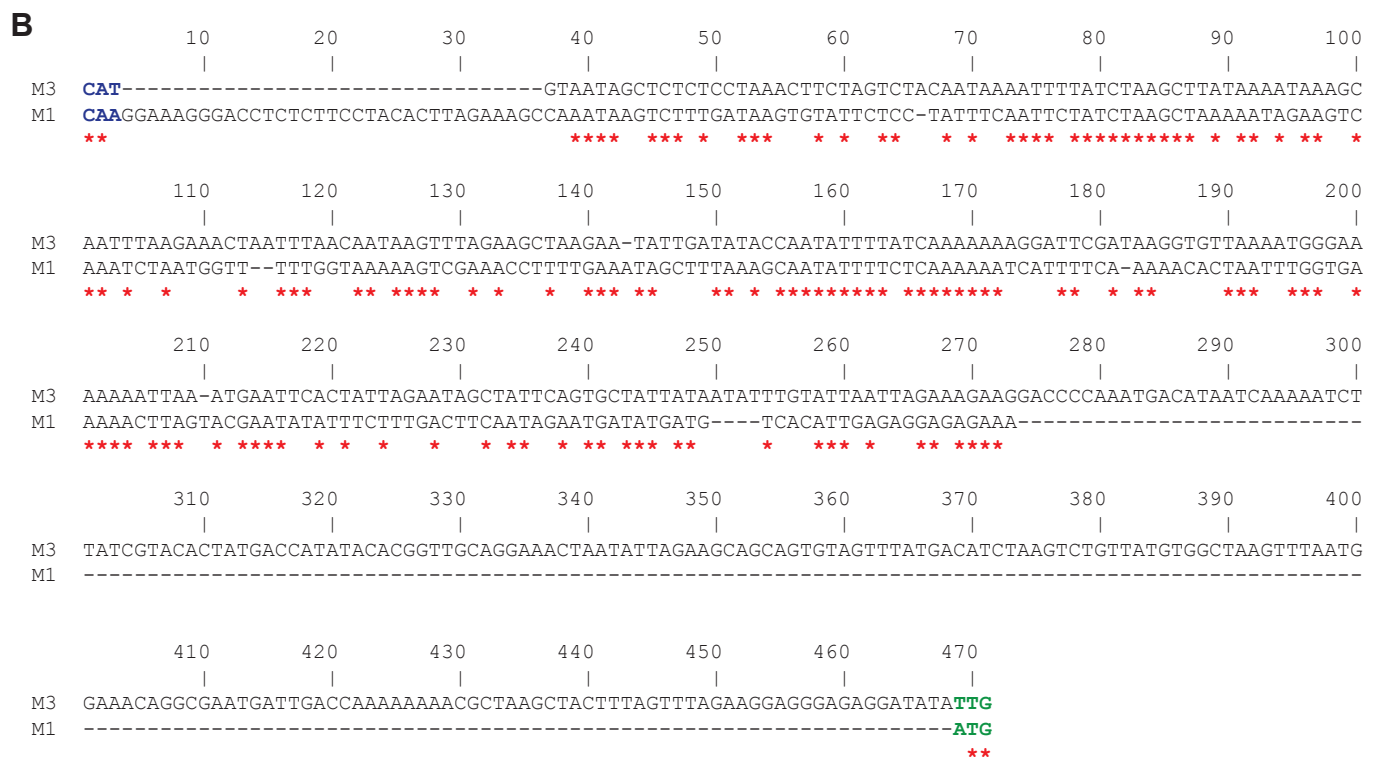
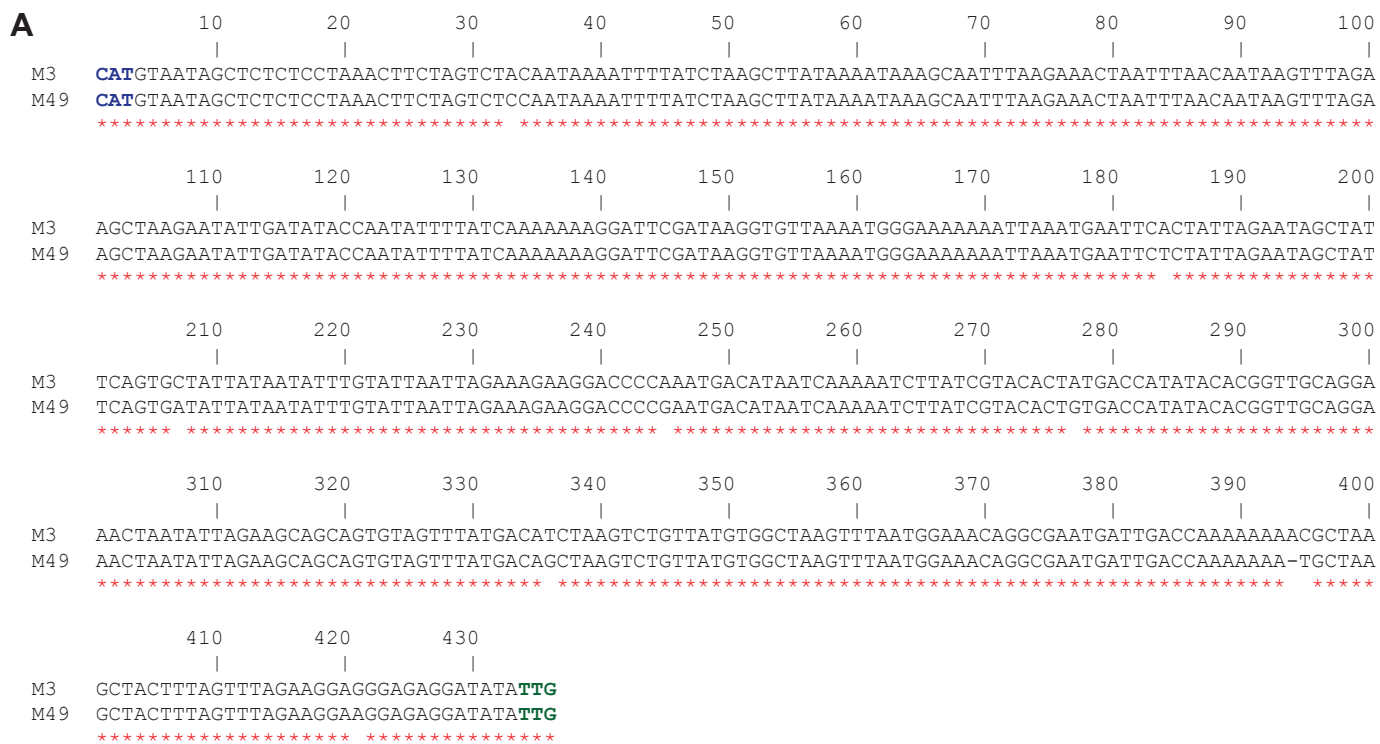


Figure S3
Comparison of the *nra-cpa* intergenic region in M3 GAS with the equivalent regions in M1 and M49 GAS.
 Conserved nucleotides are highlighted by red asterisks. The *nra/rofA* start codons are shown in blue, while the *cpa* start codon is shown in green. (A) Comparison between M3 and M49 GAS. (B) Comparison between M3 and M1 GAS.

Gene name in M1	Gene name in M3	Function of encoded protein	Fold change in transcript level for MGAS10870 (M3) relative to MGAS2221 (M1)
<i>M5005_Spy_0144</i>	<i>SpyM3_0132</i>	hypothetical protein	637.00
<i>hasB</i>	<i>hasB</i>	UDP-glucose 6-dehydrogenase	230.00
<i>hasA</i>	<i>hasA</i>	hyaluronan synthase	188.33
<i>hasC</i>	<i>hasC</i>	UDP-glucose pyrophosphorylase	137.00
<i>M5005_Spy_0143</i>	<i>SpyM3_0131</i>	hypothetical protein	106.00
<i>mac</i>	<i>SpyM3_0583</i>	hypothetical protein	89.60
<i>scpC</i>	<i>scpC</i>	cell envelope proteinase	66.40
<i>M5005_Spy_0352</i>	<i>SpyM3_0305</i>	hypothetical protein	48.33
<i>M5005_Spy_0354</i>	<i>SpyM3_0307</i>	hypothetical protein	20.86
<i>spn</i>	<i>spn</i>	NAD glycohydrolase	15.17
<i>slo</i>	<i>slo</i>	streptolysin O	14.17
<i>M5005_Spy_0353</i>	<i>SpyM3_0306</i>	hypothetical protein	14.13
<i>ifs</i>	<i>ifs</i>	hypothetical protein	13.84
<i>sse</i>	<i>sse</i>	esterase	13.50
<i>spyA</i>	<i>SpyM3_0304</i>	hypothetical protein	12.08
<i>M5005_Spy_1417</i>	<i>SpyM3_1209</i>	hypothetical protein	11.00
<i>M5005_Spy_1731</i>	<i>SpyM3_1739</i>	hypothetical protein	11.00
<i>M5005_Spy_0115</i>	<i>SpyM3_0105</i>	hypothetical protein	9.00
<i>M5005_Spy_1820</i>	<i>SpyM3_1821</i>	hypothetical protein	8.88
<i>M5005_Spy_1431</i>	<i>SpyM3_1426</i>	hypothetical protein	8.50
<i>M5005_Spy_1274</i>	<i>SpyM3_1195</i>	hypothetical protein	8.40
<i>M5005_Spy_1784</i>	<i>SpyM3_1786</i>	PTS system transporter protein II	8.20
<i>M5005_Spy_1787</i>	<i>SpyM3_1789</i>	hypothetical protein	8.00
<i>arcA</i>	<i>arcA</i>	arginine deiminase	7.75
<i>M5005_Spy_1821</i>	<i>SpyM3_1822</i>	hypothetical protein	7.60
<i>cadC</i>	<i>cadX</i>	cadmium efflux system accessory protein	7.57
<i>arcB</i>	<i>arcB</i>	ornithine carbamoyltransferase	7.50
<i>M5005_Spy_1418</i>	<i>SpyM3_1210</i>	hypothetical protein	7.50
<i>dexS</i>	<i>dexS</i>	dextran glucosidase	7.50
<i>M5005_Spy_1425</i>	<i>SpyM3_1420</i>	hypothetical protein	7.33
<i>M5005_Spy_0777</i>	<i>sclB</i>	hypothetical protein	7.23
<i>cadD</i>	<i>cadD</i>	cadmium resistance protein	7.14
<i>M5005_Spy_1435</i>	<i>SpyM3_1430</i>	hypothetical protein	7.13
<i>M5005_Spy_1426</i>	<i>SpyM3_1421</i>	tail protein - phage associated	7.00
<i>M5005_Spy_1434</i>	<i>SpyM3_1429</i>	hypothetical protein	7.00
<i>M5005_Spy_1271</i>	<i>SpyM3_1192</i>	hypothetical protein	6.50
<i>M5005_Spy_1272</i>	<i>SpyM3_1193</i>	arginine repressor	6.50
<i>M5005_Spy_1428</i>	<i>SpyM3_1423</i>	hypothetical protein	6.50
<i>M5005_Spy_1819</i>	<i>SpyM3_1820</i>	hypothetical protein	6.08
<i>M5005_Spy_0854</i>	<i>SpyM3_0790</i>	Na ⁺ driven multidrug efflux pump	6.00
<i>M5005_Spy_1049</i>	<i>SpyM3_0976</i>	hypothetical protein	5.83
<i>M5005_Spy_1429</i>	<i>SpyM3_1424</i>	hypothetical protein	5.83
<i>M5005_Spy_1427</i>	<i>SpyM3_1422</i>	hypothetical protein	5.80
<i>M5005_Spy_1430</i>	<i>SpyM3_1425</i>	hypothetical protein	5.75
<i>M5005_Spy_1432</i>	<i>SpyM3_1427</i>	hypothetical protein	5.62
<i>arcC</i>	<i>arcC</i>	carbamate kinase	5.00
<i>ska</i>	<i>ska</i>	streptokinase A precursor	4.63
<i>M5005_Spy_1433</i>	<i>SpyM3_1428</i>	hypothetical protein	4.60
<i>M5005_Spy_1556</i>	<i>SpyM3_1583</i>	hypothetical protein	4.35
<i>M5005_Spy_0652</i>	<i>SpyM3_0570</i>	hypothetical protein	4.31
<i>M5005_Spy_1824</i>	<i>SpyM3_1826</i>	hypothetical protein	4.17
<i>M5005_Spy_0716</i>	<i>SpyM3_0629</i>	hypothetical protein	4.14
<i>M5005_Spy_0717</i>	<i>SpyM3_0630</i>	hypothetical protein	3.91
<i>M5005_Spy_0706</i>	<i>SpyM3_0619</i>	ABC transporter (binding protein)	3.82
<i>scpA</i>	<i>scpA</i>	C5A peptidase precursor	3.82
<i>M5005_Spy_1678</i>	<i>SpyM3_1693</i>	transport accessory protein	3.77
<i>M5005_Spy_0080</i>	<i>SpyM3_0072</i>	histidine triad (HIT) protein	3.76
<i>M5005_Spy_1528</i>	<i>fhuD.2</i>	ferrichrome ABC transporter (ferrichrome-binding protein)	3.75
<i>M5005_Spy_0281</i>	<i>SpyM3_0243</i>	hypothetical protein	3.72
<i>M5005_Spy_1441</i>	<i>SpyM3_1436</i>	terminase small subunit - phage associated	3.67
<i>M5005_Spy_1437</i>	<i>SpyM3_1432</i>	hypothetical protein	3.63

<i>spy0843 from SF370</i>	<i>SpyM3_0569</i>	surface antigen	3.62
<i>M5005_Spy_0707</i>	<i>SpyM3_0620</i>	ABC transporter (permease)	3.55
<i>M5005_Spy_0543</i>	<i>adcA</i>	adhesion protein	3.50
<i>M5005_Spy_1279</i>	<i>SpyM3_1200</i>	hypothetical protein	3.48
<i>M5005_Spy_0403</i>	<i>SpyM3_0344</i>	hypothetical protein	3.33
<i>M5005_Spy_1843</i>	<i>SpyM3_1843</i>	hypothetical protein	3.31
<i>mutY</i>	<i>mutY</i>	A/G-specific adenine glycosylase	3.19
<i>M5005_Spy_1525</i>	<i>cycD</i>	ABC transporter ATP-binding protein	3.17
<i>M5005_Spy_1156</i>	<i>SpyM3_1082</i>	hypothetical protein	3.14
<i>M5005_Spy_1822</i>	<i>SpyM3_1823</i>	hypothetical protein	3.00
<i>M5005_Spy_1530</i>	<i>SpyM3_1561</i>	hypothetical protein	3.00
<i>M5005_Spy_1785</i>	<i>SpyM3_1787</i>	transcriptional repressor	3.00
<i>braB</i>	<i>braB</i>	branched-chain amino acid transport protein	3.00
<i>rpmG</i>	<i>rpmG</i>	50S ribosomal protein L33	2.94
<i>scrB</i>	<i>scrB</i>	sucrose-6-phosphate hydrolase	2.86
<i>fhuC</i>	<i>fhuC.2</i>	ferrichrome transport ATP-binding protein	2.75
<i>M5005_Spy_0270</i>	<i>atmA</i>	ABC transporter substrate-binding protein	2.64
<i>M5005_Spy_1313</i>	<i>SpyM3_1293</i>	beta-glucosidase	2.60
<i>M5005_Spy_0647</i>	<i>SpyM3_0566</i>	glycerophosphodiester phosphodiesterase	2.58
<i>sodA</i>	<i>sodM</i>	superoxide dismutase	2.54
<i>M5005_Spy_1454</i>	<i>SpyM3_1446</i>	hypothetical protein	2.50
<i>M5005_Spy_1691</i>	<i>rgfB</i>	RgfB protein	2.50
<i>M5005_Spy_0085</i>	<i>SpyM3_0077</i>	DNA binding protein	2.50
<i>mnmA</i>	<i>mnmA</i>	tRNA-specific 2-thiouridylase MnmA	2.46
<i>pcp</i>	<i>pcp</i>	pyrrolidone-carboxylate peptidase	2.44
<i>M5005_Spy_0646</i>	<i>SpyM3_0565</i>	ABC transporter permease	2.43
<i>M5005_Spy_1391</i>	<i>SpyM3_1478</i>	hypothetical protein	2.41
<i>M5005_Spy_0976</i>	<i>SpyM3_0900</i>	hypothetical protein	2.37
<i>M5005_Spy_1386</i>	<i>SpyM3_1473</i>	hypothetical protein	2.34
<i>M5005_Spy_0402</i>	<i>SpyM3_0343</i>	hypothetical protein	2.33
<i>M5005_Spy_1667</i>	<i>SpyM3_1682</i>	hypothetical protein	2.33
<i>coaA</i>	<i>coaA</i>	pantothenate kinase	2.33
<i>M5005_Spy_0186</i>	<i>SpyM3_0157</i>	regulatory protein RofA related	2.33
<i>folD</i>	<i>folD</i>	bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase/ 5,10-methylene	2.31
<i>M5005_Spy_0338</i>	<i>SpyM3_0295</i>	arsenate reductase	2.29
<i>M5005_Spy_0275</i>	<i>SpyM3_0237</i>	serine/threonine transporter SstT	2.28
<i>M5005_Spy_1558</i>	<i>SpyM3_1585</i>	hypothetical protein	2.25
<i>M5005_Spy_0645</i>	<i>SpyM3_0564</i>	ABC transporter ATP-binding protein	2.22
<i>M5005_Spy_0856</i>	<i>SpyM3_0792</i>	ABC transporter (binding protein)	2.21
<i>M5005_Spy_1453</i>	<i>SpyM3_1445</i>	hypothetical protein	2.20
<i>adk</i>	<i>adk</i>	adenylate kinase	2.19
<i>M5005_Spy_1280</i>	<i>yesM</i>	two-component sensor histidine kinase	2.19
<i>M5005_Spy_0534</i>	<i>SpyM3_0458</i>	acetoin reductase	2.19
<i>M5005_Spy_1515</i>	<i>SpyM3_1547</i>	hypothetical protein	2.18
<i>dinP</i>	<i>dinP</i>	DNA polymerase IV	2.18
<i>M5005_Spy_0500</i>	<i>SpyM3_0425</i>	N-acetylmuramoyl-L-alanine amidase	2.18
<i>pepO</i>	<i>pepO</i>	endopeptidase O	2.17
<i>M5005_Spy_1579</i>	<i>SpyM3_1605</i>	repressor	2.16
<i>M5005_Spy_0312</i>	<i>SpyM3_0271</i>	23S rRNA methyltransferase	2.15
<i>M5005_Spy_1465</i>	<i>SpyM3_1264</i>	hypothetical protein	2.09
<i>aroB</i>	<i>aroB</i>	3-dehydroquinate synthase	2.09
<i>M5005_Spy_0501</i>	<i>SpyM3_0426</i>	hypothetical protein	2.06
<i>M5005_Spy_1235</i>	<i>SpyM3_1158</i>	phosphomannomutase	2.04
<i>M5005_Spy_0849</i>	<i>rluD</i>	ribosomal large subunit pseudouridine synthase	2.03
<i>mtsC</i>	<i>mtsC</i>	integral membrane protein MtsC, ABC transporter (permease protein)	2.02
<i>M5005_Spy_0493</i>	<i>SpyM3_0419</i>	hypothetical protein	2.02
<i>M5005_Spy_1416</i>	<i>SpyM3_1412</i>	cell wall hydrolase, lysin - phage associated	2.00
<i>lacE</i>	<i>lacE</i>	PTS system lactose-specific transporter subunit IIBC	2.00
<i>M5005_Spy_1086</i>	<i>SpyM3_1008</i>	hypothetical protein	2.00
<i>M5005_Spy_1094</i>	<i>SpyM3_1020</i>	hypothetical protein	2.00
<i>gidA</i>	<i>gidA</i>	tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA	2.00
<i>M5005_Spy_1348</i>	<i>SpyM3_1383</i>	permease	-2.00
<i>cfa</i>	<i>cfa</i>	cAMP factor	-2.00
<i>M5005_Spy_0978</i>	<i>SpyM3_0903</i>	Na(+)-linked D-alanine glycine permease	-2.00
<i>M5005_Spy_1347</i>	<i>SpyM3_1382</i>	3-hydroxybutyrate dehydrogenase	-2.00
<i>M5005_Spy_1722</i>	<i>SpyM3_1730</i>	hypothetical protein	-2.00

<i>ihk</i>	<i>ihk</i>	histidine kinase	-2.00
<i>dnaK</i>	<i>dnaK</i>	molecular chaperone DnaK	-2.01
<i>pmi</i>	<i>pmi</i>	mannose-6-phosphate isomerase	-2.02
<i>M5005_Spy_0944</i>	<i>SpyM3_0870</i>	16S rRNA m(2)G 1207 methyltransferase	-2.04
<i>M5005_Spy_0836</i>	<i>SpyM3_0773</i>	acid phosphatase/phosphotransferase	-2.05
<i>M5005_Spy_0498</i>	<i>SpyM3_0423</i>	transcriptional regulator	-2.08
<i>M5005_Spy_0676</i>	<i>SpyM3_0589</i>	hypothetical protein	-2.08
<i>M5005_Spy_1790</i>	<i>SpyM3_1792</i>	hypothetical protein	-2.10
<i>tkt</i>	<i>tkt</i>	transketolase	-2.11
<i>M5005_Spy_0197</i>	<i>SpyM3_0164</i>	ABC transporter ATP-binding/membrane spanning protein	-2.11
<i>M5005_Spy_0681</i>	<i>SpyM3_0594</i>	two-component sensory transduction histidine kinase	-2.13
<i>M5005_Spy_1374</i>	<i>SpyM3_1461</i>	hypothetical protein	-2.14
<i>isp</i>	<i>isp</i>	hypothetical protein	-2.16
<i>M5005_Spy_0680</i>	<i>SpyM3_0593</i>	two-component response regulator	-2.16
<i>pfID</i>	<i>pfID</i>	pyruvate formate-lyase	-2.20
<i>mur1.1</i>	<i>mur1.1</i>	peptidoglycan hydrolase	-2.20
<i>M5005_Spy_0852</i>	<i>SpyM3_0788</i>	oxidoreductase, short chain dehydrogenase/reductase	-2.21
<i>mipB</i>	<i>mipB</i>	fructose-6-phosphate aldolase	-2.22
<i>lppC</i>	<i>lppC</i>	acid phosphatase	-2.24
<i>asnA</i>	<i>asnA</i>	asparagine synthetase AsnA	-2.25
<i>sagA</i>	<i>sagA</i>	streptolysin S associated protein	-2.25
<i>nrdD</i>	<i>nrdD</i>	anaerobic ribonucleoside triphosphate reductase	-2.26
<i>uviB</i>	<i>uviB</i>	hypothetical protein	-2.27
<i>M5005_Spy_0549</i>	<i>SpyM3_0472</i>	hypothetical protein	-2.28
<i>M5005_Spy_0457</i>	<i>SpyM3_0394</i>	hypothetical protein	-2.30
<i>M5005_Spy_1306</i>	<i>SpyM3_1287</i>	two-component sensor histidine kinase	-2.33
<i>sdhA</i>	<i>sdhA</i>	L-serine dehydratase alpha subunit	-2.33
<i>M5005_Spy_0571</i>	<i>SpyM3_0489</i>	hypothetical protein	-2.35
<i>nrdG</i>	<i>nrdG</i>	anaerobic ribonucleotide reductase activator	-2.38
<i>M5005_Spy_1726</i>	<i>SpyM3_1734</i>	ABC transporter permease	-2.43
<i>M5005_Spy_1601</i>	<i>SpyM3_1626</i>	hypothetical protein	-2.43
<i>M5005_Spy_0593</i>	<i>SpyM3_0512</i>	hypothetical protein	-2.44
<i>clpL</i>	<i>clpL</i>	ATP-dependent Clp proteinase (ATP-binding subunit)	-2.45
<i>dnaJ</i>	<i>dnaJ</i>	molecular chaperone DnaJ	-2.55
<i>ahpF</i>	<i>ahpF</i>	NADH oxidase/alkyl hydroperoxidase	-2.55
<i>hrcA</i>	<i>hrcA</i>	heat-inducible transcription repressor	-2.58
<i>M5005_Spy_1377</i>	<i>SpyM3_1464</i>	trans-acting positive regulator	-2.67
<i>nrdF</i>	<i>nrdF</i>	ribonucleotide-diphosphate reductase subunit beta	-2.71
<i>purD</i>	<i>purD</i>	phosphoribosylamine--glycine ligase	-2.75
<i>scrA</i>	<i>scrA</i>	PTS system sucrose-specific transporter subunit IIABC	-2.75
<i>sdhB</i>	<i>sdhB</i>	L-serine dehydratase beta subunit	-2.77
<i>M5005_Spy_1343</i>	<i>SpyM3_1377</i>	LysR family transcriptional regulator	-2.80
<i>purF</i>	<i>purF</i>	amidophosphoribosyltransferase	-2.83
<i>M5005_Spy_1144</i>	<i>SpyM3_1070</i>	hypothetical protein	-2.88
<i>M5005_Spy_0202</i>	<i>SpyM3_0169</i>	hypothetical protein	-2.89
<i>M5005_Spy_1373</i>	<i>SpyM3_1460</i>	ABC transporter ATP-binding protein	-2.90
<i>grpE</i>	<i>grpE</i>	heat shock protein GrpE	-2.99
<i>M5005_Spy_0366</i>	<i>SpyM3_0316</i>	hypothetical protein	-3.02
<i>M5005_Spy_0750</i>	<i>SpyM3_0660</i>	ABC transporter ATP-binding protein	-3.04
<i>ropB</i>	<i>ropB</i>	transcription regulator	-3.14
<i>nrdE.1</i>	<i>nrdE.1</i>	ribonucleotide-diphosphate reductase subunit alpha	-3.17
<i>M5005_Spy_1749</i>	<i>SpyM3_1755</i>	pyruvate formate-lyase activating enzyme	-3.17
<i>sloR</i>	<i>sloR</i>	regulatory protein	-3.25
<i>purE</i>	<i>purE</i>	phosphoribosylaminoimidazole carboxylase catalytic subunit	-3.33
<i>M5005_Spy_1786</i>	<i>SpyM3_1788</i>	hypothetical protein	-3.33
<i>nrdI</i>	<i>nrdI</i>	ribonucleotide reductase stimulatory protein	-3.33
<i>M5005_Spy_0572</i>	<i>SpyM3_0490</i>	hypothetical protein	-3.35
<i>int.2</i>	<i>int.2</i>	integrase - phage associated	-3.43
<i>M5005_Spy_1729</i>	<i>SpyM3_1737</i>	hypothetical protein	-3.44
<i>M5005_Spy_1727</i>	<i>SpyM3_1735</i>	ABC transporter ATP-binding protein	-3.60
<i>M5005_Spy_0123</i>	<i>SpyM3_0112</i>	hypothetical protein	-3.63
<i>M5005_Spy_1728</i>	<i>SpyM3_1736</i>	ATP-binding cassette transporter protein	-3.65
<i>metB</i>	<i>metB</i>	cystathionine beta-lyase	-4.00
<i>nagB</i>	<i>nagB</i>	N-acetylglucosamine-6-phosphate isomerase	-4.40
<i>smeZ</i>	<i>smeZ</i>	hypothetical protein	-4.50
<i>secE</i>	<i>secE</i>	preprotein translocase subunit SecE	-4.74

<i>xpt</i>	<i>xpt</i>	xanthine phosphoribosyltransferase	-4.80
<i>M5005_Spy_0771</i>	<i>SpyM3_0679</i>	hypothetical protein	-5.00
<i>M5005_Spy_1153</i>	<i>SpyM3_1078</i>	cation/potassium uptake protein	-5.50
<i>M5005_Spy_0770</i>	<i>SpyM3_0678</i>	hypothetical protein	-5.67
<i>M5005_Spy_0859</i>	<i>SpyM3_0795</i>	purine (xanthine) permease	-6.65
<i>M5005_Spy_1730</i>	<i>SpyM3_1738</i>	hypothetical protein	-7.03
<i>endoS</i>	<i>endoS</i>	secreted endoglycosidase	-8.25
<i>M5005_Spy_0475</i>	<i>SpyM3_0404</i>	PTS system beta-glucoside-specific transporter subunit IIABC	-9.00
<i>scrK</i>	<i>SpyM3_1567</i>	fructokinase	-10.00
<i>M5005_Spy_0769</i>	<i>SpyM3_0677</i>	hypothetical protein	-11.00
<i>agaS</i>	<i>SpyM3_0468</i>	tagatose-6-phosphate aldose/ketose isomerase	-11.00
<i>M5005_Spy_0544</i>	<i>SpyM3_0467</i>	GntR family transcriptional regulator	-14.00
<i>rofA</i>	<i>nra</i>	negative transcriptional regulator	-29.20
<i>M5005_Spy_0111</i>	<i>SpyM3_0102</i>	hypothetical protein	-230.00
<i>M5005_Spy_0107</i>	<i>SpyM3_0098</i>	collagen binding protein	-324.00
<i>M5005_Spy_0109</i>	<i>SpyM3_0100</i>	hypothetical protein	-417.00
<i>M5005_Spy_0108</i>	<i>SpyM3_0099</i>	hypothetical protein	-463.00

Table S1

Differentially regulated genes between MGAS2221 and MGAS10870, as identified by RNA-Seq.

Strain	Description	Reference
MGAS2221	Clinical M1 GAS isolate recovered in 1988 from Australia	Sumby <i>et al.</i> , 2006. <i>PLoS Pathog.</i> 2(1):e5.
MGAS313	Clinical M1 GAS isolate recovered in the 1980s from Wyoming, USA	Sumby <i>et al.</i> , 2006. <i>PLoS Pathog.</i> 2(1):e5.
MGAS1264	Clinical M1 GAS isolate recovered in 1973 from Denmark	Sumby <i>et al.</i> , 2006. <i>PLoS Pathog.</i> 2(1):e5.
MGAS1508	Clinical M1 GAS isolate recovered in 1985 from the former Czechoslovakia	Sumby <i>et al.</i> , 2005. <i>J Infect Dis</i> 192: 771-82.
MGAS22259	Clinical M1 GAS isolate recovered in 2010 from Alberta, Canada	Cao <i>et al.</i> , 2014. <i>Infect Immun</i> 82:1744-54.
SF370	Clinical M1 GAS isolate recovered in 1985	Ferretti <i>et al.</i> , 2001. <i>Proc Natl Acad Sci USA.</i> 98:4658-63.
MGAS5005	Clinical M1 GAS isolate recovered in 1996 from Ontario, Canada	Sumby <i>et al.</i> , 2005. <i>J Infect Dis</i> 192: 771-82.
MGAS3350	Clinical M1 GAS isolate recovered in 1995 from Minnesota, USA	Cao <i>et al.</i> , 2014. <i>Infect Immun</i> 82:1744-54.
MGAS5456	Clinical M1 GAS isolate recovered in 1994 from Finland	Sumby <i>et al.</i> , 2006. <i>PLoS Pathog.</i> 2(1):e5.
MGAS9127	Clinical M1 GAS isolate recovered in the late 1990's from Alberta, Canada	Sumby <i>et al.</i> , 2005. <i>J Infect Dis</i> 192: 771-82.
MGAS315	Clinical M3 GAS isolate recovered in the 1980s from Texas, USA	Beres <i>et al.</i> , 2002. <i>Proc Natl Acad Sci USA</i> 99:10078-83.
MGAS10870	Clinical M3 GAS isolate recovered in 2002 from Ontario, Canada	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
MGAS491	Clinical M3 GAS isolate recovered pre-1991 from the UK	Cao <i>et al.</i> , 2014. <i>Infect Immun</i> 82:1744-54.
MGAS159	Clinical M3 GAS isolate recovered in the late 1980s from Utah, USA	Cao <i>et al.</i> , 2014. <i>Infect Immun</i> 82:1744-54.
MGAS15049	Clinical M3 GAS isolate recovered in 2006 from Ontario, Canada	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
MGAS182	Clinical M3 GAS isolate recovered in the 1940s from Ottawa, Canada	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
MGAS1254	Clinical M3 GAS isolate recovered in 1937 from New York, USA	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
MGAS1428	Clinical M3 GAS isolate recovered in 1974 from Cottbus, Germany	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
MGAS9056	Clinical M3 GAS isolate recovered in 1998 from Illinois, USA	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
MGAS9507	Clinical M3 GAS isolate recovered in 2001 from Texas, USA	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
MGAS22283	Clinical M3 GAS isolate recovered in 2010 from Alberta, Canada	Beres <i>et al.</i> , 2010. <i>Proc Natl Acad Sci USA.</i> 107:4371-6.
31616/3065	Clinical M3 GAS isolate that originates from the Prague National Reference Centre for Streptococci	-
90-254	Clinical M3 GAS isolate recovered in 1990 from Georgia, USA. Is from a patient with TSL5	-
90-132	Clinical M3 GAS isolate recovered in 1990 from Oregon, USA	-
L-035	Clinical M3 GAS isolate recovered from a case of pharyngitis in 1988 from Ohio, USA	-
PD-824	Clinical M3 GAS isolate recovered from a case of pharyngitis in 1990 from Virginia, USA	-
D944	Clinical M3 GAS isolate from Köln, Germany. Is emm3.92	-
D1216	Clinical M3 GAS isolate from Köln, Germany. Is emm3.23	-
D1253	Clinical M3 GAS isolate from Köln, Germany. Is emm3.92	-
49/123	Clinical M49 GAS isolate from the Czech Republic	Jaffe <i>et al.</i> , 1996. <i>Mol Microbiol.</i> 21:373-84.
591	Clinical M49 GAS isolate recovered from a skin infection in 2004 from Rostock, Germany	Nakata <i>et al.</i> , 2005. <i>Mol Microbiol.</i> 57:786-803.
71-716	Clinical M49 GAS isolate	Podbielski <i>et al.</i> , 1990. <i>Med Microbiol Immunol</i> 179:255-62.
90-053	Clinical M49 GAS isolate recovered in 1990 from Minneapolis, USA	Jaffe <i>et al.</i> , 1996. <i>Mol Microbiol.</i> 21:373-84.
90-397	Clinical M49 GAS isolate recovered in 1990 from Minneapolis, USA	Jaffe <i>et al.</i> , 1996. <i>Mol Microbiol.</i> 21:373-84.
88-299	Clinical M49 GAS isolate recovered in 1988 from Minneapolis, USA	Jaffe <i>et al.</i> , 1996. <i>Mol Microbiol.</i> 21:373-84.
89-288	Clinical M49 GAS isolate recovered in 1989 from Minneapolis, USA	Jaffe <i>et al.</i> , 1996. <i>Mol Microbiol.</i> 21:373-84.
90-306	Clinical M49 GAS isolate recovered in 1990 from Minneapolis, USA	Jaffe <i>et al.</i> , 1996. <i>Mol Microbiol.</i> 21:373-84.
M3 #1 + vector	MGAS22283 containing the pDC123-derived vector pDCBB	This work
M3 #1 + pNra	MGAS22283 containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M3 #2 + vector	MGAS9056 containing the pDC123-derived vector pDCBB	This work
M3 #2 + pNra	MGAS9056 containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M3 #3 + vector	MGAS15049 containing the pDC123-derived vector pDCBB	This work
M3 #3 + pNra	MGAS15049 containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M3 #4 + vector	MGAS10870 containing the pDC123-derived vector pDCBB	This work
M3 #4 + pNra	MGAS10870 containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M3 + vector	MGAS10870 containing the pDC123-derived vector pDCBB	This work
M3 + pNra	MGAS10870 containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M3 + pNra-M3	MGAS10870 containing the pDC123-based plasmid pNra-M3 which harbors the M3 GAS <i>nra</i> allele	This work
M3 + pNra-M18	MGAS10870 containing the pDC123-based plasmid pNra-M18 which harbors the M18 GAS <i>nra</i> allele	This work
M3 + pMsmR	MGAS10870 containing the pDC123-based plasmid pMsmR which expresses a functional MsmR protein from M49 GAS	This work
M3 + pNra ^{FIX}	MGAS10870 containing the pDC123-based plasmid pNra ^{FIX} which expresses a modified M3 GAS Nra protein	This work
M3Δnra	MGAS10870 derivative in which the <i>nra</i> gene was replaced with a spectinomycin resistance cassette	This work
M3Δnra + vector	M3Δnra containing the pDC123-derived vector pDCBB	This work
M3Δnra + pNra	M3Δnra containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M3Δnra + pNra-M3	M3Δnra containing the pDC123-based plasmid pNra-M3 which harbors the M3 GAS <i>nra</i> allele	This work
M3Δnra + pNra-M18	M3Δnra containing the pDC123-based plasmid pNra-M18 which harbors the M18 GAS <i>nra</i> allele	This work
M3Δnra + pMsmR	M3Δnra containing the pDC123-based plasmid pMsmR which expresses a functional MsmR protein from M49 GAS	This work
M3Δnra + pNra ^{FIX}	M3Δnra containing the pDC123-based plasmid pNra ^{FIX} which expresses a modified M3 GAS Nra protein	This work
MGAS8232	Clinical serotype M18 GAS isolate recovered in 1987 from Utah, USA	Smoot <i>et al.</i> , 2002. <i>Proc Natl Acad Sci USA.</i> 99:4668-73.
M18 + vector	MGAS8232 containing the pDC123-derived vector pDCBB	This work
M18 + pNra	MGAS8232 containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M18 + pNra-M3	MGAS8232 containing the pDC123-based plasmid pNra-M3 which harbors the M3 GAS <i>nra</i> allele	This work
M18 + pNra-M18	MGAS8232 containing the pDC123-based plasmid pNra-M18 which harbors the M18 GAS <i>nra</i> allele	This work
M3ΔmsmR	MGAS10870 derivative in which the <i>msmR</i> gene was replaced with a spectinomycin resistance cassette	This work
M3ΔmsmR + vector	M3ΔmsmR containing the pDC123-derived vector pDCBB	This work
M3ΔmsmR + pNra	M3ΔmsmR containing the pDC123-based plasmid pNra which expresses a functional Nra protein from M49 GAS	This work
M3ΔmsmR + pMsmR	M3ΔmsmR containing the pDC123-based plasmid pMsmR which expresses a functional MsmR protein from M49 GAS	This work
M3nra ^{FIXED}	MGAS10870 derivative in which the M3-specific SNP within <i>nra</i> has been altered to that present in other serotypes (C800A)	This work
M3nra ^{FIXED} ΔmsmR	M3nra ^{FIXED} derivative in which the <i>msmR</i> gene has been replaced with a spectinomycin resistance cassette	This work
M3.VNTR4	Derivative of MGAS10870 in which the VNTR within the <i>nra.cpa</i> intergenic regions has 7 A residues not the parental 8	This work
M3.VNTR6	As M3.VNTR4 but independently made	This work
M3.VNTR7	As M3.VNTR4 and M3.VNTR6 but independently made	This work
M3ΔcovR	Derivative of MGAS10870 in which a region of the <i>covR</i> gene has been replaced with a kanamycin resistance cassette	This work
M3ΔcovS	Derivative of MGAS10870 containing a 2 bp deletion within <i>covS</i>	This work
M3rocA ^{FIXED}	Derivative of MGAS10870 in which the mutation within <i>rocA</i> has been fixed (hence strain is <i>rocA+</i>)	Miller <i>et al.</i> , 2015. <i>Mol Microbiol.</i> 98:473-89.
M1ΔcovR	Derivative of MGAS2221 in which a region of the <i>covR</i> gene has been replaced with a kanamycin resistance cassette	Trevino <i>et al.</i> , 2009. <i>Infect Immun</i> 77:3141-9.
M1ΔrocA	Derivative of MGAS2221 in which the <i>rocA</i> gene has been replaced with a spectinomycin resistance cassette	Miller <i>et al.</i> , 2015. <i>Mol Microbiol.</i> 98:473-89.
M1ΔrofA	Derivative of MGAS2221 in which the <i>rofA</i> gene has been replaced with a spectinomycin resistance cassette	Liu <i>et al.</i> , 2012. <i>Mol Microbiol.</i> 86:140-54.

Table S2
Overview of the GAS strains used in this study.

Primer/probe name	Sequence	Use
NRATMF	TGTTTAAATGCGGTAGACG	Tagman primer to assay <i>nra</i> mRNA in M3 and M49 GAS
NRATMR	CCACCAATAATTCCTTTGCC	Tagman primer to assay <i>nra</i> mRNA in M3 and M49 GAS
NRATMP	TCCACAGCATATTGAAACTTGCTGCC	Tagman probe to assay <i>nra</i> mRNA in M3 and M49 GAS
PROS.UTMF	TACCACTGGCAATCGTACC	Tagman primer to assay housekeeping gene <i>proS</i> in M1, M3, and M49 GAS
PROS.UTMR	CATTTCAACAGCACCGATCT	Tagman primer to assay housekeeping gene <i>proS</i> in M1, M3, and M49 GAS
PROS.UTMP	CACGCATGATGGTCTGAATTTCTCA	Tagman probe to assay housekeeping gene <i>proS</i> in M1, M3, and M49 GAS
COVRTMF	TGATGAAGCCGTGAGACTAATGT	Tagman primer to assay <i>covR</i> mRNA in M1, M3, and M49 GAS
COVRTMR	CGCACTGTTGGATATAAGATTCTT	Tagman primer to assay <i>covR</i> mRNA in M1, M3, and M49 GAS
COVRTMP	TCTCCGGGCAAAATGACATTCC	Tagman probe to assay <i>covR</i> mRNA in M1, M3, and M49 GAS
ROFATMF	ACGCAGCAAGACAAAAACACTATT	Tagman primer to assay <i>rofA</i> mRNA in M1 GAS
ROFATMR	CCGCTTCCACGATAAAGCTAA	Tagman primer to assay <i>rofA</i> mRNA in M1 GAS
ROFATMP	TCCACCCACCTTAAACCTCTCCTGGTTA	Tagman probe to assay <i>rofA</i> mRNA in M1 GAS
M3.FBABTMF	GCCTGTTATCGAAAGCCTTCCCT	Tagman primer to assay <i>fabB</i> mRNA in M3 GAS
M3.FBABTMR	CCAGGCAGCTGTCTATATAGCTTAAA	Tagman primer to assay <i>fabB</i> mRNA in M3 GAS
M3.FBABTMP	CACTGCAAAAAAATCTGGGTAGATGCACC	Tagman probe to assay <i>fabB</i> mRNA in M3 GAS
M3.CPATMF	TTTAAAGTCTGAGTATGTTCCGGATA	Tagman primer to assay <i>cpa</i> mRNA in M3 GAS
M3.CPATMR	CCTTCCGCATTTCTGATAATGA	Tagman primer to assay <i>cpa</i> mRNA in M3 GAS
M3.CPATMP	TCCCCAAAAACCCAGGAGAAGAGCCTC	Tagman probe to assay <i>cpa</i> mRNA in M3 GAS
M3.FCTATMF	AATAAGCGTGACACTCAAGTTCCA	Tagman primer to assay <i>tee</i> mRNA in M3 and M49 GAS
M3.FCTATMR	AGCCACAATGCTAAGAACTGCAA	Tagman primer to assay <i>tee</i> mRNA in M3 and M49 GAS
M3.FCTATMP	CTGGTGTAGGGACCCCTTGCTCCA	Tagman probe to assay <i>tee</i> mRNA in M3 and M49 GAS
M3.MSMRTMF	AGGCGGTAATGGCTAGATG	Tagman primer to assay <i>msmR</i> mRNA in M3 GAS
M3.MSMRTMR	AGACGACTTGCCAAACAACA	Tagman primer to assay <i>msmR</i> mRNA in M3 GAS
M3.MSMRTMP	TGCTGATCCTGATATCATTATCCGCG	Tagman probe to assay <i>msmR</i> mRNA in M3 GAS
107TMF	AAAAGGGAGTCAGAAAGTAACTGGT	Tagman primer to assay <i>cpa</i> mRNA in M1 GAS
107TMR	CCGGAACCTGTTTGGCATT	Tagman primer to assay <i>cpa</i> mRNA in M1 GAS
107TMP	CGTCAAGCTTTGAGCAACTGATTGATCC	Tagman probe to assay <i>cpa</i> mRNA in M1 GAS
109TMF	ACTACTGTCAACGAGACGGAAATAAG	Tagman primer to assay <i>tee1</i> mRNA in M1 GAS
109TMR	CGTATTGATCCACCTTTATCTGAATT	Tagman primer to assay <i>tee1</i> mRNA in M1 GAS
109TMP	TGACTTTAGTCATCGGTGTGTTCAAAGCTACACC	Tagman probe to assay <i>tee1</i> mRNA in M1 GAS
M28CPATMF	TTGGGACAGAATACCATCCA	Tagman primer to assay <i>cpa</i> mRNA in M49 GAS
M28CPATMR	TCCCAACTCACCAGTACTG	Tagman primer to assay <i>cpa</i> mRNA in M49 GAS
M28CPATMP	TGCATACGAATCAGCTCAACCAATCA	Tagman probe to assay <i>cpa</i> mRNA in M49 GAS
UNR441	AGATCTAAGCAGAACTTCTATACGTTCTGCACTGTCAAAATAGTATTTTACCATTTTCCAG	Used with UNR442 to amplify <i>msmR</i> from M49 GAS
UNR442	GGTTGTACAAATTTATCTGAAACAAAAAGCGCTCTATTTTATAATGAAACTTATTGAC	Used with UNR441 to amplify <i>msmR</i> from M49 GAS
UNR443	AGATCTAAGCAGAACTTCTATACGTTCTGCACTAAACTACACTGCTGCTTCTAATATTAG	Used with UNR444 to clone the <i>nra</i> alleles from M3, M18, and M49 GAS
UNR444	GGTTGTACAAATTTATCTGAAACAAAAAGCGAGAAATGTAGAAGCACATACAACCTGAATC	Used with UNR444 to clone the <i>nra</i> alleles from M3, M18, and M49 GAS
UNR307	GGTACTTGGTACCAAAAATTTG	Used to create GAS strain M3Δnra
UNR308	CTATTTAAATAACAGATTAAAAAATATAATAAACGAGCAATFACAATAAATAACTGACTTTC	Used to create GAS strain M3Δnra
UNR309	GGGATTTCCACATCACCAATC	Used to create GAS strain M3Δnra
UNR310	GTACCAAATAGCATTTTGAATC	Used to create GAS strain M3Δnra
UNR311	GTTATTAGTTATAGTTATTATAACATGTATTCTCTTTTGGACATAAGGCATGTAATAGCTCTC	Used to create GAS strain M3Δnra
UNR312	CAGATCTTAAATAGATTACAG	Used to create GAS strain M3Δnra
UNR313	GAAGTCAGTTATTTATTTGTAATGCTCGTTTATTATAAATTTTTTAACTGTTATTTAAATAG	Used to create GAS strain M3Δnra
UNR314	GAGAGCTATTACATGCCTTATGTCAAAAAAGAGAATACATGTTATAATAACTATAACTAATAAC	Used to create GAS strain M3Δnra
UNR315	CCTAATGCTATTACCAATCAAG	Used to create GAS strain M3ΔmsmR
UNR316	CTATTTAAATAACAGATTAAAAAATATAATAAACACCAGATAAATTTAAGAATTCAAG	Used to create GAS strain M3ΔmsmR
UNR317	GCTATCATTGAAACAAAAGAGAG	Used to create GAS strain M3ΔmsmR
UNR318	GTTATTAGTTATAGTTATTATAACATGTATTCAATCGAATCTAACCATTTGATAGATTGCC	Used to create GAS strain M3ΔmsmR
UNR319	CGTTGCCGATCGTATAAATCTCT	Used to create GAS strain M3ΔmsmR
UNR320	CTTCATATTTATCTATATCTG	Used to create GAS strain M3ΔmsmR
UNR321	CTTGAATCTTAAATATTATCTCGGTTATTATAAATTTTTTAACTGTTATTTAAATAG	Used to create GAS strain M3ΔmsmR
UNR322	GGCAATCTATAAATGGTTAGATTGCAATGTAATAACTATAACTAATAAC	Used to create GAS strain M3ΔmsmR
UNR323	CTTCATATTTATCTATATCTG	Used to create GAS strain M3ΔmsmR
UNR222	GGTAATATAAATTAAGAAAAGATAATCGAGATCTCCTGTGAGAACGTTAACG	Used to create GAS strain M3nra ^{FIXED}
UNR223	CGTTAACGTTCTCACAGGAGATCTCGAATTTCTTTTCTTAATTTATATTACC	Used to create GAS strain M3nra ^{FIXED}
UNR224	TGCCCTATGTCAAAAAGAAAAGGATAGTTTC	Used to create GAS strain M3nra ^{FIXED}
UNR225	GAACATATCCTTTTCTTTTGGACATAAGGCAGAACTAGAGCTTGATGAAAATTTGTTTG	Used to create GAS strain M3nra ^{FIXED}
UNR226	CAAACATTTTCCCTTTATGCTATAATCCGTACGCTAGCAATGTACA	Used to create GAS strain M3nra ^{FIXED}
UNR227	GTAGACGATGACAGCGTGTGTG	Used to create GAS strain M3nra ^{FIXED}
UNR96	GATTATAGCATAAAGGAAAATGTTG	Used to create GAS strain M3nra ^{FIXED}
COVUPUP	GCTGATAAGAAAGAGTTGGTTGAAG	Used with COVR1 to create GAS strain M3ΔcovR
COVR1	TGAGCGCAGGTTTAAATTTAGG	Used with COVUPUP to create GAS strain M3ΔcovR
BBLCS1	CTGCCTTTACACTGATTGCCATAGTTCAAC	Used with BBLCS4 to help create GAS strain M3ΔcovS
BBLCS2	CAAAACAATTTTCTCAAGCTCTAGTTCTAATCTACTTTCAACCTTCATCTGTAATG	Used with BBLCS3 to help create GAS strain M3ΔcovS
BBLCS3	CGAAGAATGTCTGTTATCTGACAATATGTTAGACAAAATG	Used with BBLCS2 to help create GAS strain M3ΔcovS
BBLCS4	CAATTTGTCTAACATATTGTCAGATAACAGACAATTTCTTG	Used with BBLCS1 to help create GAS strain M3ΔcovS
UNR619	GGAAACAGCGAATGATTGACCAAAAAACGCTAAGCTACTTTAGTTAGAAGG	Used with UNR623 to help create GAS strain M3.VNTR
UNR620	CCTTCAAACAAAGTAGCTTAGCGTTTTTGGTCAATCAATCCGCTGTTCC	Used with UNR625 to help create GAS strain M3.VNTR
UNR623	CAAAACAATTTTCTCAAGCTCTAGTTCCCTAAAACGTAAGTCCCATTAGGTAGCTC	Used with UNR619 to help create GAS strain M3.VNTR
UNR625	TGTACAATGCTAGCGTACGATGACAAGGCAAGTAACATATTG	Used with UNR620 to help create GAS strain M3.VNTR

Table S3
Primers and probes used in this study.