

Supporting Information

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Table S1. Transfer frequency of the 30 *Erm^R* insertions

Insertion	Coordinates of the <i>Erm^R</i> Insertion on the NEM316 Chromosome (bp)	Transfer Frequency ($\times 10^{-8}$)	Standard Deviation
I-1	144,753	ND*	—
I-2	297,448	0.11	0.10
I-3	332,423	ND	—
I-4	376,913	ND	—
I-5	656,967	0.17	0.09
I-6	658,284	0.69	0.23
I-7	761,268	2.35	0.56
I-8	818,056	1.25	0.16
I-9	821,759	1.03	0.23
I-10	846,720	0.09	0.09
I-11	967,298	0.06	0.06
I-12	986,348	0.87	0.11
I-13	1,064,640	1.71	0.18
I-14	1,073,073	0.06	0.04
I-15	1,080,795	0.12	0.08
I-16	1,124,410	0.06	0.01
I-17	1,159,993	0.05	0.03
I-18	1,213,207	2.33	0.25
I-19	1,272,413	1.28	0.31
I-20	1,304,385	0.06	0.04
I-21	1,355,312	ND	—
I-22	1,458,647	ND	—
I-23	1,527,989	0.03	0.03
I-24	1,619,215	0.04	0.03
I-25	1,665,129	ND	—
I-26	1,784,976	ND	—
I-27	1,836,262	ND	—
I-28	1,928,182	0.01	0.02
I-29	1,942,706	ND	—
I-30	2,105,767	ND	—

*No transconjugant detected ($<10^{-11}$)

Table S2. Conserved regions among the eight *S. agalactiae* chromosomes

Reference	Region of Low Polymorphism Shared by the Indicated Genome
2603 V/R	<p>18RS21 Region 1: 1348.5–1065.5 Region 2: 1232.5–1241 CJB111 Region 1: 44–173.5 Region 2: 180–433 Region 3: 480–494.5 Region 4: 499.5–674.5 Region 5: 717–1029 Region 6: 1232.5–1241 Region 7: 1348.5–1369 Region 8: 1608.5–1688 A909 Region 1: 154.5–173.5 Region 2: 180–411 Region 3: 476–674.5 Region 4: 698.5–1019 Region 5: 1348.5–1369 Region 6: 1494–1728.5 H36B Region 1: 154.5–173.5 Region 2: 180–411 Region 3: 459–674.5 Region 4: 698.5–1019 Region 5: 1348.5–1369 Region 6: 1422.5–1435 Region 7: 1483–1487.5 Region 8: 1640–1728.5 NEM316 Region 1: 1798.5–16.5 Region 2: 44–53 Region 3: 297–328.5 Region 4: 336.5–411 Region 5: 512–674.5 Region 6: 717–795 Region 7: 987.5–1065.5 Region 8: 1228–1241 Region 9: 1348.5–1369 Region 10: 1422–1495 Region 11: 1507.5–1522 Region 12: 1541–1572.5 Region 13: 1678–1688 515 Region 1: 1798.5–44 Region 2: 512–529.5 Region 3: 534.5–547.5 Region 5: 599.5–674.5 Region 6: 987.5–1052.5 Region 7: 1288–1241 Region 8: 1348.5–1384.5 Region 9: 1422–1495 Region 10: 1507.5–1522 Region 11: 1547–1572.5 Region 12: 1678.5–1688 COH1 Region 1: 433–438.5 Region 2: 445–455.5 Region 3: 599.5–700.5 Region 4: 947–1019 Region 5: 1232.5–1335 Region 6: 1348.5–1369 Region 7: 1424–1448</p>

Reference	Region of Low Polymorphism Shared by the Indicated Genome
18RS21	<p>Region 8: 1499.5–1679.5 Region 9: 1702.5–1798.5 Region 10: 2100–2114 Region 11: 2119–2128.5</p> <p>CJB111 Region 1: 44–173.5 Region 2: 180–433 Region 3: 480.5–494.5 Region 4: 499.5–674.5 Region 5: 717–1029 Region 6: 1066–1081 Region 7: 1103.5–1124 Region 8: 1232.5–1241 Region 9: 1348.5–1369 Region 10: 1608.5–1688</p> <p>A909 Region 1: 154.5–173.5 Region 2: 180–411 Region 3: 476–674.5 Region 4: 698.5–1019 Region 5: 1124–1132.5 Region 6: 1157–1167.5 Region 7: 1342–1369 Region 8: 1494–1728.5</p> <p>H36B Region 1: 154.5–173.5 Region 2: 180–411 Region 3: 459–674.5 Region 4: 698.5–1019 Region 5: 1103.5–1132.5 Region 6: 1342–1369 Region 7: 1422–1435 Region 8: 1483–1487.5 Region 9: 1640–1728.5</p> <p>NEM316 Region 1: 1798.5–16.5 Region 2: 44–53 Region 3: 297–328.5 Region 4: 336.5–411 Region 5: 512–674.5 Region 6: 717–795 Region 7: 987.5–1081 Region 8: 1103.5–1167.5 Region 9: 1232.5–1241 Region 10: 1348.5–1369 Region 11: 1422–1495 Region 12: 1507.5–1522 Region 13: 1541–1572.5 Region 14: 1678–1688</p> <p>515 Region 1: 1798.5–44 Region 2: 512–529.5 Region 3: 534.5–547.5 Region 4: 599.5–674.5 Region 5: 987.5–1052.5 Region 6: 1069.5–1081 Region 7: 1124–1132.5 Region 8: 1157–1167.5 Region 9: 1232.5–1241 Region 10: 1342–1384.5 Region 11: 1422–1495 Region 12: 1507.5–1522 Region 13: 1547–1572.5</p>

Reference	Region of Low Polymorphism Shared by the Indicated Genome
CJB111	Region 14: 1678–1688 COH1 Region 1: 433–438.5 Region 2: 445–455.5 Region 3: 599.5–700.5 Region 4: 946.5–1019 Region 5: 1103.5–1145.5 Region 6: 1232.5–1369 Region 7: 1424–1448 Region 8: 1499.5–1679.5 Region 9: 1702.5–1798.5 Region 10: 2100–2114 Region 11: 2119–2128.5
	A909 Region 1: 154.5–411 Region 2: 480–494.5 Region 3: 499.5–700.5 Region 4: 717–1019 Region 5: 1319.5–1335 Region 6: 1348.5–1495 Region 7: 1608.5–1688 Region 8: 1728.5–1878.5 Region 9: 2019–2115.5 H36B
	Region 1: 154.5–411 Region 2: 480–494.5 Region 3: 499.5–700.5 Region 4: 717–1019 Region 5: 1103.5–1124 Region 6: 1319.5–1335 Region 7: 1348.5–1369 Region 8: 1487.5–1608.5 Region 9: 1640–1688 Region 10: 1728.5–1878.5 Region 11: 2019–2115.5
	NEM316 Region 1: 44–53 Region 2: 297–328.5 Region 3: 336.5–411 Region 4: 512–795 Region 5: 987.5–1029 Region 6: 1065.5–1124 Region 7: 1232.5–1243.5 Region 8: 1319.5–1369 Region 9: 1678–1688 515
	Region 1: 512–529.5 Region 2: 534.5–547.5 Region 3: 599.5–700.5 Region 4: 987.5–1029 Region 5: 1069.5–1081 Region 6: 1232.5–1243.5 Region 7: 1319.5–1335 Region 8: 1348.5–1369 Region 9: 1541–1547 Region 10: 1678–1688 COH1
	Region 1: 0–9 Region 2: 599.5–674.5 Region 3: 946.5–1019 Region 4: 1103.5–1124 Region 5: 1233–1241 Region 6: 1348.5–1369

Reference	Region of Low Polymorphism Shared by the Indicated Genome
A909	<p>Region 7: 1608.5–1679.5</p> <p>H36B Region 1: 1640–459 Region 2: 476–1103.5 Region 3: 1124–1132.5 Region 4: 1184.5–1369 Region 5: 1487.5–1495</p> <p>NEM316 Region 1: 297–328.5 Region 2: 336.5–411 Region 3: 512–700.5 Region 4: 717–795 Region 5: 987.5–1019 Region 6: 1124–1132.5 Region 7: 1157–1167.5 Region 8: 1311.5–1335 Region 9: 1348.5–1369 Region 10: 1507.5–1522 Region 11: 1541–1572.5 Region 12: 1678–1688</p> <p>515 Region 1: 512–529.5 Region 2: 534.5–547.5 Region 3: 599.5–700.5 Region 4: 987.5–1019 Region 5: 1124–1132.5 Region 6: 1157–1167.5 Region 7: 1311.5–1369 Region 8: 1507.5–1522 Region 9: 1547–1572.5 Region 10: 1678–1688</p> <p>COH1 Region 1: 599.5–674.5 Region 2: 946.5–1019 Region 3: 1124–1132.5 Region 4: 1342–1369 Region 5: 1499.5–1679.5 Region 6: 1702.5–1728.5</p>
H36B	<p>NEM316 Region 1: 297–328.5 Region 2: 336.5–411 Region 3: 512–700.5 Region 4: 717–795 Region 5: 987.5–1019 Region 6: 1103.5–1132.5 Region 7: 1311.5–1335 Region 8: 1348.5–1369 Region 9: 1422–1435 Region 10: 1483–1487.5 Region 11: 1678–1688</p> <p>515 Region 1: 512–529.5 Region 2: 534.5–547.5 Region 3: 599.5–700.5 Region 4: 987.5–1019 Region 5: 1124–1132.5 Region 6: 1311.5–1369 Region 7: 1422–1435 Region 8: 1483–1487.5 Region 9: 1541–1547 Region 10: 1678–1688</p> <p>COH1</p>

Reference	Region of Low Polymorphism Shared by the Indicated Genome
NEM316	Region 1: 599.5–674.5 Region 2: 946.5–1019 Region 3: 1103.5–1132.5 Region 4: 1342–1369 Region 5: 1424–1435 Region 6: 1640–1679.5 Region 7: 1702.5–1728.5
515	515 Region 1: 1547–16.5 Region 2: 218.5–228 Region 3: 443–447 Region 4: 470.5–529.5 Region 5: 534.5–547.5 Region 6: 599.5–700.5 Region 7: 795–1052.5 Region 8: 1069.5–1081 Region 9: 1124–1132.5 Region 10: 1157–1167.5 Region 11: 1228–1335 Region 12: 1348.5–1369 Region 13: 1422–1541 COH1 Region 1: 470.5–503 Region 2: 599.5–674.5 Region 3: 987.5–1019 Region 4: 1103.5–1132.5 Region 5: 1232.5–1241 Region 6: 1348.5–1369 Region 7: 1424–1448 Region 8: 1507.5–1522 Region 9: 1541–1572.5 Region 10: 2100–2114 Region 11: 2119–2128.5 COH1 Region 1: 392.5–407.5 Region 2: 470.5–503 Region 3: 599.5–674.5 Region 4: 987.5–1019 Region 5: 1124–1132.5 Region 6: 1232.5–1241 Region 7: 1342–1369 Region 8: 1424–1448 Region 9: 1507.5–1522 Region 10: 1547–1572.5 Region 11: 2100–2114 Region 12: 2119–2128.5

The chromosomal coordinates relative to strain 2603 V/R in kb are indicated. Previously described genomic islands (absent in at least one strain) and ribosomal RNA operons (conserved in all strains) are not taken into account.

Table S3. Bacterial strains and plasmids

Strains and Plasmids	Relevant Genotype and Resistance Profile* or Characteristics
Strains	
NEM316	ST23; III; Isolated from neonate [†]
A909RF	ST7; Ia; Isolated from neonate [‡] , spontaneous mutant for Rif and Fus
A909RF <i>recA</i> ⁻	<i>recA</i> ⁻ ; Tet
BM110	ST17; III; Tet; Isolated from neonate [§]
BM132	ST19; III; Rif, Fus
304-36	ST67; NT ^b ; Bovine mastitis; France
411-07	ST61; NT; Bovine mastitis; France
553-08	ST2; NT; Goat mastitis; France
CCH268	ST10; III; Carriage, adult; France
Dak30	ST26; V; Carriage, adult; Senegal
Ban43	ST328; V; Carriage, adult; Central African Republic
2.22	ST246; Ib; Fish; Israël ^{**} , ^{††}
043-14	ST340; NT; Bovine mastitis; Great Britain
SS1218	ST260; Ib; Bigmouth Buffalo; USA
NEM1575	ST17; III; Neonate; France
Mad74	ST2; Ia; Carriage, adult; Spain
Ban29	ST188; III; Carriage adult; Central African Republic
CCH263	ST23; Ia; Urine, adult; France
CCH350	ST23; NT; Blood, adult; France
Plasmids	
pGhost8	Tet; Thermosensitive derivative of pGK12 ^{‡‡}
pCAM45	Em, Km; ColE1 replicon, thermosensitive derivative of pGK12, Himar1 ^{§§}

*Abbreviations: Em, resistance to erythromycin; Fus, resistance to fusidic acid; Km, resistance to kanamycin; NT, non-typeable; Rif, resistance to rifampicin; Tet, resistance to tetracycline.

[†]Glaser P, *et al.* (2002) Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. *Mol Microbiol.* 45:1499–1513.

[‡]Tettelin H, *et al.* (2005) Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: Implications for the microbial "pan-genome". *Proc Natl Acad Sci U S A* 102:13950–13955.

[§]Stalhammar-Carlemalm M, Stenberg L, Lindahl G. (1993) Protein rib: A novel group B streptococcal cell surface protein that confers protective immunity and is expressed by most strains causing invasive infections. *J Exp Med* 177:1593–1603.

^{||}Horodniceanu T, Bougueleret L, El-Solh N, Bouanchaud DH, Chabbert YA. (1979) Conjugative R plasmids in *Streptococcus agalactiae* (group B). *Plasmid* 2:197–206.

^{||}Brochet M, *et al.* (2006) Genomic diversity and evolution within the species *Streptococcus agalactiae*. *Microbes Infect* 8:1227–1243.

^{**}Eldar A, *et al.* (1997) Restriction fragment length polymorphisms of 16S rDNA and of whole rRNA genes (ribotyping) of *Streptococcus iniae* strains from the United States and Israel. *FEMS Microbiol Lett* 151:155–62.

^{††}Vandamme P, Devriese LA, Pot B, Kersters K, Melin P. (1997) *Streptococcus difficile* is a nonhemolytic group B, type Ib *Streptococcus*. *Int J Syst Bacteriol* 47:81–85.

^{‡‡}Biswas I, Gruss A, Ehrlich SD, Maguin E. (1993) High-efficiency gene inactivation and replacement system for gram-positive bacteria. *J Bacteriol* 175:3628–3635.

^{§§}May JP, Walker CA, Maskell DJ, Slater JD. (2004) Development of an *in vivo* Himar1 transposon mutagenesis system for use in *Streptococcus equi* subsp. *equi*. *FEMS Microbiol Lett* 238:401–409.

Table S4. Oligonucleotide primers

Name	Forward Probe	Reverse Probe
Determination of the Erm ^R marker insertion		
<i>Himar</i>	CAAACATGAGAGCTTAGTACGTG	AACACACTCTTAAGTTTGCTTC
<i>RecA</i> inactivation		
<i>RecA</i>	GAATTCGGTGATGAACGTCGAA	GGATCCCATACGAGCTTGCAAAC
<i>gbs1121</i> inactivations		
<i>gbs1121</i>	GGATCCATGGCAATCTAGCAGAT	GAATTCCTGCATGCTTGCCGA
Characterization of transconjugants*		
0183	TTGGCTTATGTCAAAGGTT	TATGACCAGAGGTCCCTAGA
0235	CGTACCCTAGTGAGTTGTC	CAGAAACAAGTCGGTCTTTC
0262	ATAAAAGGCGTGCAATTAGA	AACACCAAATTAGCCAAATAA
0286	GATTCAACTTTAGCAGGACG	TTACTCCAAGGGCTATTTCA
0318	CAGAAATGGACAGAACCATT	ACCAAACCACCATAAGAATG
0355	TATTGAAGAAACAAAGCCGT	ATCATAGCGCATACGAGAAT
0358	AATAGCGTTACTGCGGATAC	TTCGTTGATTTACCTTCTAA
0411	GCTCAAGAATGGTTAGATGG	TTTGCTCCAGACTTTTCAT
0423	TTAGGTTTTGTAAACGGCATT	TCATTTCTTACGTGGTCTGA
0442	CCTGGTACTCATGAACGACT	CTTCAATAAACCATCCCCATA
0449	GACATGTGGTGTCCTTGA	AAAGTCTCTATAGCAAACCTCCAT
0502	CTTCTCAAATGAGTCCGTC	GAAACAACCTTCAAACCTGC
0514	TATGGCGTCACAAGACAATA	TGAGTCAGAGACATATTGCG
0535	GGAAAAACAAAAGCAAGTTGA	TCACAACATCCAAAGGAATA
0542	TGGGTTTTGAATTAGAGGAA	TAGAAATAACGGGAAGACGA
0654	TGAACGCATCTGTTCTATTG	TACCAAAGCTGAGCAGTACA
0674	GAAATTACAGGTCCAGCATC	CTAGCCCTACAACCATTTTG
0684	CTTTAGGAGTTCTGCGTCAC	TTTACCTAATTTGCGATGGT
0743	TCTTTAATCGATGCTGGTT	TTGCTTAAGTGTCCAAGGAT
0747	GCACTTGAGTCGAGAACAAT	TTTCTGTTGCGATAGCTTTT
0788	TAAAAGGAGCAAGAGATGGA	ATGCCATCTGAACATCATAAC
0807	TCAAAATGCGGAATTAGACT	CTAAATCACGCGCTAAATCT
0832	AAAGGCACTCAGTCGTAGAG	TATTTATTGACTGGACGCAA
0864	AACCAGGAGTTGATATGTGG	CATAGTTAGCACCATAGGC
0890	GAAAAAGAGCGTGCAGTATT	AGCATAAGCTCCGATGATAA
0900	GGACAAGACTACGAACAAGC	TACCGTATTTGTTACGGAGG
0927	GCAGATAAGGGAGATATCGTT	AAAGAGAGCATCAATCGAAC
0930	GTCTAACTGAGCACGGTTTC	CTCCCATACGAGATGCTAAG
0948	CAAAATGGACTTCGTGCTAT	AGAACGACCAGTTTGAGAAA
0968	TCGGTGATATCATTAAATTCAAA	AGTTGGGCTAACTTTTGATG
1044	ACTGACGCAAATGAATTTTT	AAAGGCCAATTAATGACAAA
1076	ATCGCCCTTGATTTAGTACA	TCTATTCAAAGACCAGGTGA
1082	TGTCAAAATAGGAATGGAGC	TTTCGTTATAGGTCCTCCCA
1091	GAAGCTTTCCGGATTTTTAT	AGCATGAAAGTCAGCATCTT
1105	CAGAATAACCTTCCAAGTGC	CTTGGTAGACATTGTTGGGT
1108	TTAATCAAGCATTACGGACA	ATAAAAGTCACTTCCGCATC
1163	CAAGCTTATTTTCTCATGTTCA	CACCTACTTTTGGCGTATT
1167	TCGAAAAAGCTAGTTCCAAA	TGGAGAAAAACTCAAGATGC
1180	TGACAACCGTTTACTTGAGTT	TAGTTGAGGTGCTATTTTCGAG
1198	GGAAAAACAGCTACAAGTTGG	GCTTCGTAGTCAACATCTGA
1231	GAATCGTTTATCTCCAAGA	CTACCAAAGAAGCCACGATA
1379	TGAAGAATATCAGTTTCCCG	CCATTTGCCAAGGACTAATA
1410	TTTTGGACACCGTACTTTCT	TGTAAACTAGCAGCATCACG
1445	ATGGAGCAAAAGTCCTGTTA	TTTCTAAATGCAAGATGGGT
1494	CACCTTTTAGACGAACGAAC	AACTCAGCCAAAGTACGTGT
1581	TCAGACACAATTTGGTGGTA	TTGTATCAATTTGCACGATGT
1606	GGGTTATCATCACACGATT	GACCTCAAATGGATTAGCTG
1627	GCTATTGGGGTTAGTGACAG	ATCCTTGTGACGATATCCTT
SNP mapping in a collection of 14 additional strains		
0588	CTTCGCTGAATGTTTAGGTT	ATTGCATGTAAATAGCGGTT
0589	TGATGGAAAAGGCTCTCTTA	TTTCACACGTCTATCAACCA
0594	GCTTGCCTTAAACAAATTGA	AATGAAGAGGCCATTACAAC
0595	TTCTTTCCTTACTTGCAGGA	TCTAAAATGATATCCGCAGAA
0596	ACCAAGATGCAACATTTCTT	TTGTTGAATCCTTCAGCTTC
0597	TCAGGCTAAAGATGGAAGG	CATAAGGGATCTCTTCGCTA
0598	CATATTTGGAGACGAGGAAA	TTTGCTGTTTTAGTTGTCCA
0601	GATGTTTCAGCAGGTGTCTT	AAAATTGGCAAGATTATCCA

Name	Forward Probe	Reverse Probe
0602	TTATATTTGGGATTTGGGTG	TGACCCATCGACTAATAAGG
0605	AGGTTTGTAGTCATGGACG	CACAAAAAGCAATTCCTACC
0608	AGCTTCTACTGGTGAACACG	TAGCCCATCTTCAGTACCTT
0610	AGTTACCCGAATTGTTGATG	CCGAATCATGTCTCAGTAT
0612	CCACAGAGAAGGACAGAAAAG	ATGGTATCTGTTCTCGCATC
0615	TTTGGTTTTAGGGATTACGA	TTTTTAGGTTTATCGCCTGT
0628	GCGACTGGAATGAGTATTGT	GTAATTCCAACACAAAACGGT
0630	TCGTTTAATTTTCTTAGCGG	CAATGTAAAAACGGTACCTT
0636	TTCTTATCTTTTAGTGCCG	GTCTGTTTTCTGAATTTTG
0641	GGATTATTGGAGTCGTTTTTG	TCTCCGACTTTCCAATTTAAA
0642	TACCTTTTCATGGAGATTGG	TGGATAGTGATATGCGACTG
0645	GAGGACAGGGTAATGTTTTCAG	AGAAAAGAACGGTTGACTTGA
0646	GCAAGAGCTTTACAAGAGGA	TAAAAAGCTTACGAGCCAAG
0653	AGAGGTTCTCTGAGCAATACA	TGACAAGAACAACAAAACCA
1019	GCGCTATGGTGTTTATCTT	CTATAATCGGTTTTTGCC
1020	CTCCAAGTGTTGAAAGAGC	CTACGCAAAGTTGAAGGTT
1022	AAGCTATCGGACAAATCAAAA	TTTTTGCCATAATCTCCAAT
1024	GAAAATTCAGCTCTCACCAC	GAATTTCTGGTTAACTGCG
1029	GGAAAAGTCTCGATTTGATG	GACTTTTGCCATGCTAATTC
1032	CAAGCTAAAGAAGATTGCGT	CATGTACTACCATGCCACAG
1035	TAATATTAGGGGAGCAAGCA	TCGATAGATAAAGTCCCCCT
1036	CCAACAAGTAACCCAGAGAA	TCTAAAAAGCGGGATACTTG
1038	CCGGTGATTTTATCCATTTA	CAACATCATTTGACGGTGTA
1040	ATGGCAACTACGACAAAAAC	CAACAATGCGATCTAAAAACA
1416	TCTGCATCAGGTGTTGATTA	CATCAAAACGATCCATCTCT
1417	ACCGTATGACTAGCGATGAT	TATCAGGCAGACCTTCTAGC
1418	TTATCACGACTTGAGCAAAA	TCAACCGTTATCTGCCTAAT
1419	TTGATTCAGGACCAGTTCAT	ATGCTTCAGCTTTTATTTGC
1421	CTTCCTATCATGAAGCCAAC	TACCATATTTACGCCGCTCT

*According to NEM316 nomenclature.

