

1 Supplementary Materials

- 2 **1. Supplementary Methods.** This file contains additional information about materials
3 and methods used in this study.
- 4 **2. Supplementary Results.** This file contains additional analyses and results relevant to
5 this study. Part A: Description of the pSK1-like plasmid variants; Part B: Investigation
6 of the association between plasmid evolution and biocide tolerance.
- 7 **3. Supplementary Dataset.** This file summarises the relevant isolates demographics and
8 WGS information including sequence data accession numbers for all isolates included
9 in this study.
- 10 **4. Supplementary Tables.** This file contains additional tables which provide information
11 about the distribution of gentamicin and chlorhexidine susceptibility in the ST239
12 MRSA population.
- 13 **5. Supplementary Figure S1. pSK1 plasmid gene presence and synteny.** (A)
14 Maximum likelihood phylogenetic tree inferred from 3,883 core genome SNPs
15 illustrates the population structure of ST239 *S. aureus* in Australia. Tips are coloured
16 based on location (refer to key). Branches with < 70% bootstrap support are coloured
17 red. (B) Coloured blocks represent the identification of a pSK1 gene orthologue, using
18 a 95% amino acid identity threshold (excluding insertion sequences). Box length is
19 reflective of gene length and ordered based on pSK1 (Figure S2A). Boxes are linked if
20 orthologs were found to be syntenic. Coloured boxes reflect the six identified patterns.
- 21 **6. Supplementary Figure S2. Structure of pSK1, pTW20_1 and pBPH2003 plasmids.**
22 Plasmid genes have been coloured based on defined plasmid regions. Insertion
23 sequences (IS) are coloured grey (IS256) and black (IS257), with target site
24 duplications (TSD) illustrated: arrows indicate upstream/downstream sequences,
25 orientation, and are coloured to represent unique sequences (refer to key). (A) pSK1;
26 sequence and annotations are that previously published, accession NC_014369 (1). (B)
27 pTW20_1; sequence and annotations are that previously published, accession
28 NC_017352 (2). (C) pBPH2003; a pTW20_1-like plasmid representative of that
29 harboured in isolates from the Asian-Australian ST239 clade.
- 30 **7. Supplementary Figure S3. Modelling temporal-association in phenotypic**
31 **susceptibility data.** Graphs depict linear models developed to explore the potential
32 association between gentamicin MIC and chlorhexidine MIC and MBC with the year
33 in which isolates were recovered. The dotted line indicates the smoothed mean MIC
34 and the bold line indicates the fitted linear model. Four populations were tested (from
35 top to bottom): (i) All ST239 MRSA (n = 211), (ii) the Asian-Australian clade (n = 88),
36 (iii) the Australian clade (n = 123), and (iv) the pSK1-like plasmid harbouring
37 population (n = 91). Note: reference JKD6008 (Australian clade) was not tested.
- 38 **8. Supplementary Figure S4. Exploration of gene presence/absence signatures**
39 **associated with phenotypic chlorhexidine tolerance.** The graphs illustrate the
40 findings from two Discriminant Analysis of Principle Components (DAPC) models,
41 used to investigate genetic signatures in the ST239 MRSA population associated with
42 chlorhexidine MIC. The first model (top panel) examined all accessory gene
43 orthologues (clustered at 95% amino acid homology), and in the second model (bottom

44 panel) gene orthologues associated with either pSK1 or pBPH2003 (representative of
45 the pTW20_1-like plasmid recovered from the Asian-Australian clade) were excluded.
46 The prior hypothesised sub-divisions represent the phenotypic CHX MIC values for the
47 population, as indicated in the key. (A) Scatter plots illustrate the clustering of isolates
48 across the two most discriminant functions. The density plots illustrate the same data
49 in a one-dimensional format for the first (B) and second (C) most discriminant
50 functions. (D) Loading plots illustrate the contributions of all gene orthologues to the
51 DAPC models, with the most highly contributing variables (> 0.01) coloured based on
52 whether they represent a plasmid-associated (green) or chromosomal gene orthologue
53 (blue). (E) Membership graphs illustrate the membership profiles for all isolates,
54 aligned to a maximum likelihood phylogenetic tree for the ST239 population. The
55 probability index indicates the likelihood of assigning an isolate back to one of the
56 hypothesised sub-divisions.

57 **9. Supplementary Figure S5. Exploration of mutations associated with phenotypic**
58 **chlorhexidine tolerance.** (A) Manhattan plot illustrates the results of three Genome
59 Wide Association Studies (GWAS) conducted to identify core genome SNPs associated
60 with CHX MIC, tested at three thresholds: $\text{MIC} \geq 3 \text{ mg/L}$, $\geq 4 \text{ mg/L}$ or $= 6 \text{ mg/L}$. Red
61 line indicates the threshold for significance (with Bonferroni correction). (B) Maximum
62 likelihood phylogenetic tree is that from Figure S1, and adjacent are the phenotypic
63 CHX MIC and MBC values (coloured yellow to red with increasing value). (C) The
64 presence (blue) or absence (white) of the 50 most significantly associated mutations
65 detected at each phenotypic threshold are illustrated (refer to key).

66

67 **Supplementary Methods**

68 **Bacterial Isolates.** This study utilised a collection of 212 Australian ST239 *S. aureus*, selected
69 from available collections for temporal and geographic diversity. Isolates were recovered
70 between 1980 and 2012 from the Australian states of Victoria (n = 123), New South Wales (n
71 = 62), Queensland (n = 22), South Australia (n = 2), and Western Australia (n = 1). Two
72 isolates, including reference *S. aureus* JKD6008, were recovered in New Zealand (3). Bacterial
73 isolates and corresponding whole genome sequence (WGS) data were sourced from:

- 74 • The complete genome of JKD6008 was sourced from (4).
- 75 • 73 isolates were sourced from (5). Six isolates from the original study were excluded
76 because they represented secondary samples from patients already represented in the
77 collection. These isolates have only been included in the generation of the global
78 phylogenetic model (described below).
- 79 • 104 isolates were sourced from the collection of the Australian and New Zealand
80 Cooperative on Outcome in Staphylococcal Sepsis (ANZCOSS) study (6). All ST239
81 from unique patients selected for the ANZCOSS sub-study were included (7).
- 82 • 16 isolates were sourced from the collection of the Vancomycin Efficacy in
83 Staphylococcal Sepsis in Australasia (VANESSA) study (8). All ST239 isolates from
84 unique patients were included.
- 85 • 18 isolates were sourced from the historic collections (1989-1994) housed at the
86 Microbiological Diagnostic Unit Public Health Laboratory, Melbourne, Australia. All
87 ST239 isolates from unique patients in which a viable bacterial isolate could be
88 recovered were included.

89 To generate a global phylogenetic model for the ST239 lineage the Australian ST239 collection
90 was supplemented with the publicly available WGS data for 319 globally diverse ST239
91 isolates, selected to maximise temporal, geographic and genetic diversity of the collection. The
92 WGS data for these isolates was sourced from:

- 93 • The complete genomes of eight ST239 isolates were sourced from (2, 9-15).
- 94 • 174 isolates were sourced from the collections of (16-22). All ST239 isolates that could
95 be accessed from these studies were included.
- 96 • 78 isolates were sourced from the collections of (23, 24). A limited number of isolates
97 were included from both studies, selected to maximise the temporal and geographic
98 diversity of these collections
- 99 • 29 isolates were sourced from the 2015 Australian Staphylococcal Sepsis Outcome
100 Program (ASSOP). Sequence data for all ST239 isolates was kindly provided by the
101 Australian Group on Antimicrobial Resistance (<http://www.agargroup.org/>).
- 102 • A further 24 isolates were sourced from historic collections (1989 – 1994) housed at
103 the Microbiological Diagnostic Unit Public Health Laboratory, Melbourne, Australia.

104 **Whole Genome Sequencing & Sequence Data.** The WGS data for 42 isolates is novel to this
105 study (Supplementary Dataset). Genomic DNA was extracted with the JANUS Automated

106 Workstation, using the Chemagic DNA/RNA kit (Perkin Elmer). Unique dual index libraries
107 were prepared using the Nextera XT DNA preparation kit (Illumina). Libraries were sequenced
108 on a NextSeq platform (Illumina) with 2 x 150 bp chemistry. Long read sequencing was
109 conducted on seven isolates (BPH2003 [pTW20_1-like], BPH2019 [SV3], BPH2056 [SV1],
110 BPH2070 [SV2], BPH2869 [SV6], BPH3244 [SV4], and JKD6009 [SV5]). Genomic DNA
111 was extracted using the DNeasy Blood & Tissue extraction kit (Qiagen) and sequencing was
112 conducted on the Pacific Biosciences RS-II platform with P6-C4 chemistry. WGS data
113 accession numbers can be found in the Supplementary Dataset.

114 **Genome Assembly.** Long-read sequence data was *de novo* assembled in the SMRT Analysis
115 System v2.3.0.140936 (Pacific Biosciences), using the protocol previously described (25).
116 Briefly, raw sequence data was first assembled using the HGAP v3 protocol, with a minimum
117 seed read length of 10,000 bp, genome size of 3 Mb, target coverage of 10 and an overlapper
118 error rate of 0.04. Contigs underwent a second round of error correction using Quiver v1, then
119 were circularised with the overlap removed and orientated to *dnaA* or *repA* in Geneious v8.1.5
120 (Biomatters). Long reads were aligned to the assembly using BridgeMapper v1 and manually
121 checked for assembly errors. The consensus sequence from this alignment was further error
122 corrected with high quality short-read Illumina data using Snippy v3.2
123 (<https://github.com/tseemann/snippy/>), performed in an iterative process until no variants were
124 detected. The final sequences were annotated with Prokka v1.12. Structural comparison of the
125 complete plasmid sequences were conducted using the Artemis Comparison Tool (26), filtering
126 out hits < 100 bp in length.

127 Short read sequence data was *de novo* assembled using SPAdes v3.11.0 (27), excluding the
128 pre-assembly error correction (“--only-assembler”), including post-assembly error correction
129 (“--careful”) and a minimum coverage threshold of five (“--cov-cutoff”) to remove potential
130 contaminants. Assemblies were annotated using Prokka v1.12 (28) and contigs < 200 bp in
131 length were excluded. Contig coverage information was extracted from the assembly data, with
132 the ratio of coverage between chromosome and plasmid associated contigs used to approximate
133 plasmid and gene copy number.

134 **Orthologue Clustering & Gene Synteny.** The presence/absence of plasmid gene orthologues
135 was used to screen short-read assemblies for the presence of a pSK1-like plasmids. The
136 pangenome pipeline Roary (29) was used to perform the ortholog clustering. The protein
137 coding sequences (CDS) identified in the annotated complete genomes and short-read *de novo*
138 assemblies were clustered into orthologous groups at different thresholds of amino acid
139 homology (75%, 80%, 85%, 90%, and 95%), without splitting paralogs (option “-s”). No
140 significant variation was observed in the clustering across the different homology thresholds
141 (data not shown), subsequently all analyses published here use a 95% amino acid homology
142 threshold. Genes were deemed to be syntenic if the CDS were identified as adjacent on a single
143 contig.

144 **Phylogenetic Analysis.** The Illumina short-read sequence data for the 211 Australian ST239
145 *S. aureus* were mapped to the complete genome of reference JKD6008 (CP002120, (4)), using
146 Snippy v3.2 (<https://github.com/tseemann/snippy/>). An alignment of core genome single

147 nucleotide polymorphisms (SNP) was generated using a minimum coverage of 10 reads, and
148 base-call stringency of 90%. SNPs were deemed core if a base was called for every isolate in
149 the alignment at that site. This alignment was used as the input to generate a maximum
150 likelihood phylogenetic tree with IQ-TREE v1.6.1 (30), modelled with a General Time
151 Reversible (GTR) nucleotide substitution model, empirical base frequencies, a correction for
152 the absence of invariant sites, use of a discrete Gamma model with four rate categories (model
153 option “GTR+F+I+G4”), and 1,000 ultrafast bootstrap replicates (option “-bb 1000”).
154 Two additional mapped alignments were generated: (i) to represent the global ST239 collection
155 (n = 531, mapped to JKD6008), and (ii) to represent the Australian ST239 clade (n = 124,
156 mapped to JKD6008) using the process described above. Recombination was assessed using
157 ClonalFrameML v.1.11 (31). However, as very few recombinant events were detected (data
158 not shown) masking these sites prior to temporal phylogenetic analysis was deemed
159 unnecessary. The package BEAST v2.4.7 (32) was used to construct the time-aware phylogenetic
160 models. From the whole genome alignments, the core genome SNPs were extracted, grouped
161 into a single partition and modelled with the following parameters. The year of isolation was
162 provided as a prior. The GTR model of nucleotide substitution was selected, with the
163 substitution rate estimated using four gamma categories. A relaxed lognormal clock model and
164 a coalescent constant population model were selected (based on previous ST239 studies (5,
165 33)). Invariant sites were included as a constant pattern, weighted based on the nucleotide
166 proportions of reference JKD6008. For the Australian clade, chlorhexidine MIC data was
167 modelled as a discrete trait. Five 50 million step iterations were run and examined for
168 convergence in Tracer v1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>). The burn-in was adjusted
169 for each run to a point at which a stable likelihood was achieved. All runs converged, and none
170 were discarded, based on the comparisons of Bayes Factors, calculated from the harmonic
171 means of the run likelihoods, and the attainment of effective sample sizes (> 200) in all
172 measurements. Runs were joined and re-sampled at a frequency that would achieve ~10,000
173 trees using LogCombiner. Maximum clade credibility (MCC) trees with median node heights
174 were generated in TreeAnnotator.

175 **Genome Wide Association Study (GWAS).** Allelic association was performed using the
176 GWAS tool Plink (34). Core genome SNPs (identified as described above) were used as the
177 input. Three phenotypic divisions were tested, with the tolerant populations defined as a MIC
178 ≥ 3 , ≥ 4 or 6 mg/L. A Bonferroni correction was used to account for multiple hypothesis testing,
179 the significance threshold determined as 0.05/number of SNPs tested.

180 **Discriminant Analysis of Principal Components (DAPC).** DAPC was implemented in R
181 v3.4.2 (<http://www.R-project.org/>) using the package adegenet (35). Presence or absence of the
182 CDS ortholog groups (identified as described above) were used as the input for the DAPC
183 models, filtering out core ortholog groups (present in ≥ 210 isolates) and singletons groups
184 (present in ≤ 3 isolates). Two models were generated: (i) all CDS ortholog groups that met the
185 input criteria, and (ii) the same data set excluding all plasmid-associated CDS. In both models,
186 isolates were assigned to prior populations based on chlorhexidine MIC values (1, 1.5, 2, 3, 4,
187 and 6 mg/L). DAPC models were assessed individually to determine the optimum number of

188 principle components to be retained, based on the a-score and cross validation functions in
189 adegenet.

190 **Antimicrobial Susceptibility Testing.** Phenotypic susceptibility testing for gentamicin and
191 trimethoprim was performed using E-tests (bioMérieux), following the manufacturer's
192 recommendations. *S. aureus* isolate ATCC29213 was used as a control. All results were read
193 in triplicate and interpreted using CLSI guidelines (M100S, 26th Ed).

194 Phenotypic susceptibility testing to chlorhexidine (CHX) was performed by broth
195 microdilution, as per the CLSI protocol for antimicrobial susceptibility testing (M07A10E,
196 January 2015). Chlorhexidine digluconate was sourced from Sigma-Aldrich (#C9394) and
197 diluted in sterile H₂O to 128 mg/L. In microtiter trays (Costar, #3799), serial dilutions of 100µl
198 of CHX in cation adjusted Muller Hinton (CaMH) broth (Oxoid) were generated to a final
199 concentration range of 1.0 - 32 mg/L, tested in 1.5x increments. A second concentration range
200 of 0.25 - 8 mg/L was also used to test isolates found to have MIC values < 2 mg/L. Bacterial
201 isolates, from a fresh overnight plate culture, were resuspended in saline and adjusted to a
202 starting concentration equivalent to a 0.5 McF solution, then diluted 1:100 in CaMH broth. In
203 a 1:1 ratio, 100µl of the bacterial suspension was added to each well, and 200µl to an empty
204 growth control well. Microtiter plates were incubated for 24 hours in a static condition at 37°C.
205 The MIC was interpreted as the well of lowest concentration with no visible growth.

206 To assess viability post CHX exposure (approximate MBC determination), after the MIC was
207 read 10µl from each well in the microtiter plate was sub-cultured to a CaMH agar plate (Oxoid).
208 No CHX inhibitors were added. Plates were incubated for 24 hours at 37°C. The MBC was
209 interpreted as the lowest concentration in which no growth was observed, growth being defined
210 as ≥ 2 colonies. All isolates were tested in biological triplicate. A fourth BMD was performed
211 if the first three replicates were found to have a range in excess of 2 wells in the MIC, or 4
212 wells in the MBC. The median values were used for statistical analysis. Controls were tested
213 regularly and a minimum of twice for every batch performed from a fresh dilution of CHX.
214 Controls included *S. aureus* isolate ATCC29213, JKD6008 and culture-negative wells.

215 All statistical analyses were performed in R v3.4.2 (<http://www.R-project.org/>). Prior to
216 significance testing all MIC and MBC values were transformed to a log₂ scale. Comparison of
217 MIC or MBC values between two population were conducted using a Welsh two sample t-test.
218 Simple linear models were generated to assess temporal trends, comparing year of isolation
219 against log transformed MIC and MBC data using the function "lm". Significance was
220 determined as a p value ≤ 0.05 .

221 **Supplementary Results**

222 **Part A: Description of the pSK1-like plasmid variants**

223 A description of the pSK1-like plasmid variants, as identified through long-read sequencing,
224 has been described in the main text and Figure 2. Provided here is further detail about these
225 plasmid structures and how the insertion sequence (IS) features, specifically the non-coding
226 regions (NCRs), the terminal inverted repeats (TIRs), the target site duplications (TSD), and
227 their location relative to plasmid and chromosomal genes were used to identify which pSK1-
228 like SV was likely carried by each ST239 isolate.

229 **SV1: Absence of Tn4001.** In pSK1, Tn4001 is flanked by inverted copies of IS256 with perfect
230 8 bp TSD (TAAGTAAA). In SV1 only a single copy of this TSD sequence was identified in
231 isolation from an IS256 NCR at the expected plasmid location for Tn4001 (Figure 2A). Tn4001
232 is highly mobile and has been found at multiple chromosomal and plasmid locations (36, 37).
233 In BPH2056 (SV1 representative genome), a chromosomal copy of Tn4001 was identified,
234 flanked by perfect 8 bp TSD (CAAAATCA), located downstream of a hypothetical protein in
235 close proximity to the DNA-directed DNA polymerase III alpha subunit (*dnaE*) gene. Using
236 this variation in TSD patterns flanking Tn4001, it was identified that of the ten short-read
237 assemblies which demonstrated an orthologue pattern consistent with SV1 (Figure S1), five
238 likely carried a plasmid consistent with pSK1 (with Tn4001 located in the plasmid), and the
239 other five likely carried a plasmid consistent with SV1, with a chromosomal copy of Tn4001
240 identified in four isolates.

241 **SV2: IS256-Mediated Chromosomal Integration & Loss of 5,175 bp.** Chromosomal
242 integration of SV2 was mediated by IS256, with two inverted copies flanking the 24,299 bp
243 region, termed IS256-R and IS256-2. A third copy (IS256-L) is present as part of Tn4001
244 (Figure 2B). Additionally, SV2 has a 5,175 bp deletion with loss of six genes when compared
245 to pSK1 (Figure 2B). The upstream TSD for IS256-2 and IS256-R (external to SV2) were
246 identical and represented a novel sequence (GAGTTGAC). A second novel TSD was identified
247 downstream of IS256-2 (internal to SV2, ATGATTTC), with a single copy of the same
248 sequence identified in pSK1 (not adjacent to an IS256 NCR) at the boundary of the region
249 absent in SV2 (Figure 2B). This TSD pattern was identified in all seven short-read assemblies
250 which demonstrated an orthologue pattern consistent with SV2, suggesting that they all carried
251 this integrated plasmid variant.

252 **SV3: IS257-Mediated Deletion of Tn4003 & IS256-Mediated Inversion.** In pSK1, Tn4003 is
253 flanked by direct copies of IS257 with perfect 8 bp TSD (TTTTATAA). In SV3, only a single
254 copy of IS257 was identified, flanked by the same TSD (Figure 2A). Additionally, SV3 also
255 had a 3,488 bp inversion compared to pSK1, flanked by IS256-R and IS256-3 (Figure 2A). The
256 upstream TSD for IS256-R (internal to the inversion) and the downstream TSD for IS256-3
257 (external to the inversion) represented a novel sequence (TTTGAGTT), the former was
258 identified in the reverse complement. A single copy of the same sequence (TTTGAGTT) was
259 identified at the boundary for the inverted segment in pSK1 (Figure 2A, indicated by the dark
260 red circle). The upstream TSD for IS256-3 (internal to the inversion, TTTACTTA) was the

261 same sequence as the upstream TSD for IS256-R in pSK1 (TAAGTAAA), but in the reverse
262 complement (Figure 2A). Utilising the variation in TSD pattern, including the change in
263 sequence orientation and adjacent plasmids genes, it was identified that only seven of the
264 eleven short-read assemblies which demonstrated an orthologue pattern consistent with SV3
265 were likely to carry this plasmid variant.

266 **SV4: IS256-Mediated Chromosomal Integration & Loss of 3,488 bp.** Chromosomal
267 integration of SV4 was mediated by IS256, with two inverted copies flanking the 22,047 bp
268 region. A 3,488 bp region, specifically the region inverted in SV3 was absent (Figure 2B). All
269 internal TSDs were found to correspond with those identified in SV3. The TSD upstream of
270 IS256-R and IS256-3 (external to SV4) were novel (CTTTGTAT) (Figure 2B). This TSD
271 pattern was identified in all four short-read assemblies which demonstrated an orthologue
272 pattern consistent with SV4.

273 **SV5': IS257-Mediated Chromosomal Integration & Loss of 404 bp, SV5: IS257-Mediated**
274 **Inversion & Disruption of Replication Machinery & SV6: IS256-Mediated Deletion of 5,105**
275 **bp.** A hypothesised structure has been developed for SV5', which represents an intermediate
276 structure in the development of SV5 and SV6 (Figure 2). Chromosomal integration in SV5'
277 would likely be mediated by IS257, inserting 9,233 bp upstream of a disrupted copy of the
278 β -haemolysin gene. For compatibility with the recovered SV5 structure, this region would be
279 flanked by IS257-R1 and IS257-R2 from pSK1, located in the same orientation, with absence
280 of the intervening 404 bp region (Figure 2C). All TSD that are internal to SV5' would be
281 unaltered from the sequences identified in pSK1. The TSD downstream of IS257-R2 and
282 IS257-R1 (external to SV5') would represent a novel perfect 8 bp TSD (GTTTTTAC).

283 In SV5, the intermediate SV5' has undergone a 42,660 bp inversion (Figure 2C). This would
284 likely be the result of intramolecular replicative transposition in the inverse orientation (38),
285 and result in the creation of a novel pair of 8 bp TSD (TTCTTATC), located adjacent (in
286 opposite orientations) to both copies of IS257-R2*, one external to the inverted region
287 (adjacent to the unaffected region of ϕ Sa3) and the other one internal (adjacent to the inverted
288 region of ϕ Sa3) (Figure 2C). In addition to the inversion, a 45 bp deletion in *repA* was detected
289 in SV5, causing fragmentation of the gene annotation.

290 In a separate event, the intermediated SV5' structure has undergone an IS256-mediated 5,105
291 bp deletion event, resulting in the 22,520 bp structure SV6 (Figure 2B). All TSD are identical
292 to those presented in the hypothesised SV5' except for one; the upstream TSD for IS256-R
293 (AAATTGTG) is novel. A single copy of this TSD was identified in pSK1, not adjacent to an
294 IS256 NCR at the boundary for the deletion (Figure 2A).

295 Of the remaining 63 short-read assemblies, four had an orthologue pattern consistent with SV3,
296 21 with SV5, and 38 with SV6. Using the variation in TSD patterns described above, it was
297 identified that 24 isolates likely harboured an integrated plasmid consistent with SV5', three
298 with the inverted SV5, and 36 with the shortened SV6. All 63 isolates formed a single
299 monophyletic clade from the expected ancestral node in which SV5' is predicted to have
300 emerged (Figure 3). Further, these classifications were completely congruent with the

301 phylogenetic tree, consistent with the proposed development of these plasmid variants (Figure
302 3). All isolates harbouring an SV5'-like or SV5-like plasmid variant appeared to have disrupted
303 plasmid replication machinery, the large majority demonstrating a fragmented *repA* gene
304 annotation (Figure S1). Two isolates had more extensive deletions, with loss of multiple genes
305 similar to SV6 (Figure 3 & Figure S1). These two isolates appear to represent two further
306 independent IS256-mediated deletion events that have occurred following chromosomal
307 integration. Similarly, four isolates appeared to have undergone an IS257-mediated deletion of
308 Tn4003, with their location in the phylogeny suggestive of two independent events (Figure 3
309 & Figure S1).

310 **Part B: Investigation of the association between plasmid evolution and biocide tolerance**

311 Provided here are the additional investigations undertaken to explore the potential association
312 between the emergence of the pSK1-like plasmid SVs and the development of chlorhexidine
313 tolerance in the ST239 population.

314 **Mutations in *qacAR*.** A comparison of all *qacAR* sequences (n = 89 pSK1-like, n = 67
315 pTW20_1-like) to reference JKD6008 (Australian clade) identified six SNPs resulting in
316 missense mutations: five in *qacA* and one in *qacR*. The most common mutation resulted in a
317 QacA T₃₅₈I amino acid (aa) change. This SNP was identified in all 67 *qacA*-containing Asian-
318 Australian clade isolates and none of the *qacA*-containing Australian clade isolates. As this
319 mutation was invariably associated with one plasmid type, irrespective of MIC, it represented
320 the distinct evolutionary histories of the *qacA* genes in these different plasmid populations and
321 was unlikely to be contributing to phenotypic variation. The only other two commonly
322 observed mutations were identified in the Australian clade and resulted in a QacA H₁₆₄Q or a
323 QacA V₄₀₈I aa change. These mutations were phylogenetically correlated, QacA H₁₆₄Q was
324 identified in 14/18 isolates that harboured SV2, SV3 or SV4, and QacA V₄₀₈I was identified in
325 8/13 isolates that harboured SV3 or SV4. The presence of these mutations did not correlate
326 with CHX phenotype, suggesting that these SNPs were most likely markers of clonal evolution
327 and not significant contributors to the larger shift in CHX tolerance.

328 **Plasmid and *qacA* copy number.** Intermediate sized staphylococcal plasmids like pSK1 are
329 generally considered to be low copy number plasmids (1). Multiple copies of *qacA*, resulting
330 from increased plasmid or gene copy number could potential be contributing to the phenotypic
331 variation observed between the Australian and Asian-Australian clades. To investigate this,
332 *qacA* gene copy number was estimated by comparing the mapped sequence read depth of
333 contigs representing the plasmid regions (as defined in Figure S2) to those representing the
334 chromosome. The ratio of mapped-reads in the plasmid contigs representing region 4
335 (containing *qacAR*) and region 1 (plasmid backbone) were 0.89 (\pm 0.18) in the pSK1-like
336 population and 1.12 (\pm 0.41) in the pBPH2003-like (pTW20_1-like) population. This suggested
337 that each plasmid copy contained only a single copy of *qacA*. The ratio of mapped-reads in
338 these same contigs compared to contigs representing the chromosome (specifically those
339 containing *dnaA*) were 0.68 (\pm 0.28) and 0.77 (\pm 0.24) in the pSK1-like population, and 2.81
340 (\pm 1.32) and 2.69 (\pm 1.33) in the pBPH2003-like population for region 4 and region 1

341 respectively. These ratios suggested that the pSK1-like plasmids were typically single copy in
342 the Australian clade isolates, consistent with most isolates harbouring a chromosomally
343 integrated variant. No significant differences were observed between isolates that harboured
344 an extra-chromosomal or integrated pSK1-like variant. Conversely, the Asian-Australian
345 isolates carried one to four copies of a pBPH2003-like plasmid. As this clade has a lower
346 average MIC to chlorhexidine (Table 2), plasmid copy number did not appear to be contributing
347 to the variation observed in phenotypic CHX susceptibility.

348 ***Discriminant Analysis of Principle Components.*** DAPC is a multivariate technique that can
349 be used to reconstruct population subdivisions hypothesised to be present using genomic data;
350 it finds the smallest number of principle components to build discriminant functions that
351 minimise within group variation and maximise between group variation (39). This approach
352 was utilised to identify potential associations between phenotypic CHX MIC and the presence
353 and/or absence of specific genes. In this analysis the population subdivisions hypothesised to
354 be present in the dataset were the CHX MIC values (1, 1.5, 2, 3, 4, and 6 mg/L). Two models
355 were generated: (i) one dataset representing all accessory genes identified in the ST239
356 collection (n = 212), and (ii) the same dataset with the pSK1 and pBPH2003 (pTW20_1-like)
357 genes excluded.

358 In the first model, broad clusters were able to be resolved that reflected the low (1, 1.5, and 2
359 mg/L), medium (3 mg/L) and high (4 and 6 mg/L) MIC values (Figure S4A, B, C). The top
360 contributing variables in this separation were *qacAR* and the co-located genes in plasmid region
361 4 in the first discriminant function, and the genes specific to pBPH2003 in the second
362 discriminant function (Figure S4D). This finding is consistent with the increase in MIC that
363 was associated with plasmid acquisition and the distinct CHX susceptibility profiles of the
364 pSK1-like and pBPH2003-like populations (Table 2). Furthermore, as all but one of the *qacA*-
365 negative isolates belonged to the Australian clade, the second discriminant function was also
366 reflective of the phylogenetic division in the ST239 population (Figure S4E).

367 In the second model the MIC-reflective clusters were less defined (Figure S4A, B, C). There
368 was some separation based on chromosome-associated gene orthologues, none of which
369 appeared to be associated with efflux systems based on gene annotations (not shown). The
370 membership profiles of the isolates in this model (a measure of assignment probability to the
371 prior hypothesised subdivisions) appeared to be strongly correlated with the phylogeny (Figure
372 S4E), which suggested that this DAPC model was detecting the underlying phylogenetic
373 divisions within the population, which correlated with both CHX MIC and emergence of the
374 pSK1-like plasmid variants.

375 ***Genome Wide Association Studies.*** A GWAS analysis was utilised to explore whether
376 mutation in the core genome were associated with CHX MIC. This dataset was tested at three
377 different phenotypic divisions, with the tolerant population defined as a MIC ≥ 3 , ≥ 4 or 6
378 mg/L. Numerous mutations were identified as significantly associated with MIC after
379 correcting for multiple hypothesis testing (Figure S5A). Examination of the 50 most significant
380 SNPs for each phenotypic division found all to be strongly correlated with the phylogenetic
381 model for the ST239 population (Figure S5B). GWAS approaches are highly susceptible to

382 phylogenetically correlated phenotypes and even if a phylogenetic penalty was applied, it may
383 not be possible to detect a SNP in this dataset that is responsible for increased CHX MICs using
384 this approach.

385 The efflux system encoded by *norA* can actively export CHX and mutations in this gene, and
386 a homologue *norB*, have been associated with enhanced CHX tolerance (40-42). Mutations
387 were detected in both *norA* and *norB* in this dataset, however none were significantly associated
388 with CHX MIC in any phenotypic division tested.

389 **Supplementary Tables**390 **Table S1. Investigation of Gentamicin Resistance in Australian ST239 MRSA**

Isolate Populations (n)	Median (mg/L)		Isolate Populations (n)	Median (mg/L)		P value
		MIC			MIC	
All ST239 (211)						
Australian Clade (123)	10.30	vs	Asian-Australian Clade (88)	179.80		< 0.0001
No AME (91)	0.38	vs	<i>aac(6')-aph(2'')</i> (181)	58.20		< 0.0001
		vs	<i>aadD</i> (73)	15.20		< 0.0001
		vs	<i>aph(3')-III</i> (95)	190.50		< 0.0001
Australian Clade (123)						
No AME (19)	0.39	vs	<i>aac(6')-aph(2'')</i> (99)	21.90		< 0.0001
		vs	<i>aadD</i> (73)	15.20		< 0.0001
		vs	<i>aph(3')-III</i> (8)	164.90		< 0.0001
Asian-Australian Clade (88)						
No AME (1) ^a	0.38	vs	<i>aac(6')-aph(2'')</i> (82)	189.70		-
		vs	<i>aph(3')-III</i> (87)	193.00		-

391 Abbreviations: AME, aminoglycoside modifying enzyme; MIC, minimum inhibitory concentration.

392 ^a Statistical analysis was not performed as only a single isolate was identified harbouring no AME genes.

393

394

395 **Table S2. Gentamicin Resistance and Chlorhexidine Tolerance Profiles**

SK1 Plasmids (n)	Gentamicin MIC	Median (mg/L)	
		Chlorhexidine MIC	Chlorhexidine MBC
Structural Variants			
No SK1 Plasmid (32)	1.80	1.80	4.60
SV1 (5)	142.10*	2.80	7.30
pSK1 (5)	9.60	2.40	6.20
SV2 (7)	9.20	3.30	7.30
SV3 (7)	15.90	4.40	9.70
SV4 (4)	15.30	4.90	10.80
SV5' (3)	15.10	5.20	10.50
SV5 (24)	16.00	4.00	8.90
SV6 (36)	21.50	5.00	8.80
Structural Changes Grouped			
Extra-chromosomal (17) ^a	26.10	3.20	7.80
Integrated + <i>repA</i> deletion (25) ^b	19.10	3.70	8.50
Integrated + MG deletion (49) ^c	18.60	4.70	8.60

396 Abbreviations: MIC, minimum inhibitory concentration; MG, multi-gene; SV, pSK1-like structural variant.

397 ^a Includes all isolates classified as harbouring SV1, prototypical SK1 and SV3.398 ^b Includes all isolates classified as SV5' and SV5 with a fragmented pSK1 *repA* sequence identified.399 ^c Includes all isolates classified as SV2, SV4, SV5', SV5 and SV6 with a multi-gene deletion in pSK1 region 1.400 * All five isolates representing SV1 carry *aph(3')-III*.

401

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536

Supplementary Dataset

	ID	Year	Country	State (AUS)	Data Type	Sequence Platform	Sample/Genome Accession	Run Accession	Sequence Reference
1	BPH0468	2005	Australia	QLD	WGS Reads	Illumina MiSeq	SAMEA3212938	ERR732815	Baines. 2015. mBio. PMID: 25736880
2	BPH0475	2003	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212931	ERR732816	Baines. 2015. mBio. PMID: 25736880
3	BPH0488	2003	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212956	ERR732817	Baines. 2015. mBio. PMID: 25736880
4	BPH0491	2005	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212955	ERR732818	Baines. 2015. mBio. PMID: 25736880
5	BPH0494	2005	Australia	QLD	WGS Reads	Illumina MiSeq	SAMEA3212940	ERR732819	Baines. 2015. mBio. PMID: 25736880
6	BPH0496	2004	Australia	QLD	WGS Reads	Illumina MiSeq	SAMEA3212928	ERR732820	Baines. 2015. mBio. PMID: 25736880
7	BPH0499	2005	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212937	ERR732821	Baines. 2015. mBio. PMID: 25736880
8	BPH0502	2003	Australia	WA	WGS Reads	Illumina MiSeq	SAMEA3212919	ERR732822	Baines. 2015. mBio. PMID: 25736880
9	BPH0503	2003	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212967	ERR732823	Baines. 2015. mBio. PMID: 25736880
10	BPH0508	2006	Australia	QLD	WGS Reads	Illumina MiSeq	SAMEA3212893	ERR732824	Baines. 2015. mBio. PMID: 25736880
11	BPH0509	2005	Australia	QLD	WGS Reads	Illumina MiSeq	SAMEA3212911	ERR732825	Baines. 2015. mBio. PMID: 25736880
12	BPH0510	2005	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212957	ERR732826	Baines. 2015. mBio. PMID: 25736880
13	BPH2002	2002	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212904	ERR732827	Baines. 2015. mBio. PMID: 25736880
14	BPH2003	2001	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212913	ERR732828	Baines. 2015. mBio. PMID: 25736880
-	pBPH2003 (BPH2003)	-	-	-	Complete Genome	Pacific Biosciences RS-II	GCA_900607265	-	This Study
-	pBPH2003 (BPH2003)	-	-	-	WGS Long Reads	Pacific Biosciences RS-II	SAMEA5047398	ERR2858549	This Study
15	BPH2004	2001	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212949	ERR732829	Baines. 2015. mBio. PMID: 25736880
16	BPH2005	1999	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212961	ERR732830	Baines. 2015. mBio. PMID: 25736880
17	BPH2006	2001	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212925	ERR732831	Baines. 2015. mBio. PMID: 25736880
18	BPH2007	2001	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212952	ERR732832	Baines. 2015. mBio. PMID: 25736880
19	BPH2008	2000	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212918	ERR732833	Baines. 2015. mBio. PMID: 25736880
20	BPH2009	2000	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212948	ERR732834	Baines. 2015. mBio. PMID: 25736880
21	BPH2010	2001	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212899	ERR732835	Baines. 2015. mBio. PMID: 25736880
22	BPH2011	2001	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212962	ERR732836	Baines. 2015. mBio. PMID: 25736880
23	BPH2012	1999	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212958	ERR732837	Baines. 2015. mBio. PMID: 25736880
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25	BPH2014	1999	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212969	ERR732839	Baines. 2015. mBio. PMID: 25736880
26	BPH2015	2004	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212954	ERR732840	Baines. 2015. mBio. PMID: 25736880
27	BPH2016	2005	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212908	ERR732841	Baines. 2015. mBio. PMID: 25736880
28	BPH2017	2005	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212936	ERR732842	Baines. 2015. mBio. PMID: 25736880
29	BPH2018	2004	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212942	ERR732843	Baines. 2015. mBio. PMID: 25736880
30	BPH2019	2005	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212951	ERR732844	Baines. 2015. mBio. PMID: 25736880
-	SV3 (BPH2019)	-	-	-	Complete Genome	Pacific Biosciences RS-II	GCA_900607255	-	This Study
-	SV3 (BPH2019)	-	-	-	WGS Long Reads	Pacific Biosciences RS-II	SAMEA5047399	ERR2858550	This Study
31	BPH2021	2004	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212903	ERR732845	Baines. 2015. mBio. PMID: 25736880
32	BPH2022	2005	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212945	ERR732846	Baines. 2015. mBio. PMID: 25736880
33	BPH2023	2006	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212915	ERR732847	Baines. 2015. mBio. PMID: 25736880
34	BPH2024	2007	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212944	ERR732848	Baines. 2015. mBio. PMID: 25736880
35	BPH2029	2009	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212905	ERR732852	Baines. 2015. mBio. PMID: 25736880
36	BPH2031	2008	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212965	ERR732853	Baines. 2015. mBio. PMID: 25736880
37	BPH2032	1998	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212926	ERR732854	Baines. 2015. mBio. PMID: 25736880
38	BPH2034	2008	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212917	ERR732856	Baines. 2015. mBio. PMID: 25736880
39	BPH2054	1980	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212883	ERR732860	Baines. 2015. mBio. PMID: 25736880
40	BPH2055	1980	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212885	ERR732861	Baines. 2015. mBio. PMID: 25736880
41	BPH2056	1981	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212902	ERR732862	Baines. 2015. mBio. PMID: 25736880
-	SV1 (BPH2056)	-	-	-	Complete Genome	Pacific Biosciences RS-II	GCA_900607305	-	This Study
-	SV1 (BPH2056)	-	-	-	WGS Long Reads	Pacific Biosciences RS-II	SAMEA5047400	ERR2858551	This Study
42	BPH2057	1981	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212889	ERR732863	Baines. 2015. mBio. PMID: 25736880

43	BPH2059	1981	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212897	ERR732864	Baines. 2015. mBio. PMID: 25736880
44	BPH2068	2009	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212906	ERR732865	Baines. 2015. mBio. PMID: 25736880
45	BPH2070	1998	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212890	ERR732866	Baines. 2015. mBio. PMID: 25736880
-	SV2 (BPH2070)	-	-	-	Complete Genome	Pacific Biosciences RS-II	GCA_900607275	-	This Study
-	SV2 (BPH2070)	-	-	-	WGS Long Reads	Pacific Biosciences RS-II	SAMEA5047401	ERR2858552	This Study
46	BPH2071	1998	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212888	ERR732867	Baines. 2015. mBio. PMID: 25736880
47	BPH2072	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212947	ERR732868	Baines. 2015. mBio. PMID: 25736880
48	BPH2073	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212927	ERR732869	Baines. 2015. mBio. PMID: 25736880
49	BPH2075	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212896	ERR732870	Baines. 2015. mBio. PMID: 25736880
50	BPH2077	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212932	ERR732871	Baines. 2015. mBio. PMID: 25736880
51	BPH2078	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212950	ERR732872	Baines. 2015. mBio. PMID: 25736880
52	BPH2079	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212922	ERR732873	Baines. 2015. mBio. PMID: 25736880
53	BPH2080	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212929	ERR732874	Baines. 2015. mBio. PMID: 25736880
54	BPH2081	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212941	ERR732875	Baines. 2015. mBio. PMID: 25736880
55	BPH2082	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212920	ERR732876	Baines. 2015. mBio. PMID: 25736880
56	BPH2083	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212886	ERR732877	Baines. 2015. mBio. PMID: 25736880
57	BPH2084	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212892	ERR732878	Baines. 2015. mBio. PMID: 25736880
58	BPH2085	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212907	ERR732879	Baines. 2015. mBio. PMID: 25736880
59	BPH2086	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212900	ERR732880	Baines. 2015. mBio. PMID: 25736880
60	BPH2088	1982	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA3212968	ERR732881	Baines. 2015. mBio. PMID: 25736880
61	BPH2090	2012	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212943	ERR732882	Baines. 2015. mBio. PMID: 25736880
62	BPH2094	1998	Australia	NSW	WGS Reads	Ion Torrent PGM	SAMEA3212946	ERR732883	Baines. 2015. mBio. PMID: 25736880
63	BPH2095	2003	Australia	NSW	WGS Reads	Ion Torrent PGM	SAMEA3212887	ERR732884	Baines. 2015. mBio. PMID: 25736880
64	BPH2096	2005	Australia	NSW	WGS Reads	Ion Torrent PGM	SAMEA3212923	ERR732885	Baines. 2015. mBio. PMID: 25736880
65	BPH2097	2007	Australia	NSW	WGS Reads	Ion Torrent PGM	SAMEA3212939	ERR732886	Baines. 2015. mBio. PMID: 25736880
66	BPH2098	2007	Australia	NSW	WGS Reads	Ion Torrent PGM	SAMEA3212884	ERR732887	Baines. 2015. mBio. PMID: 25736880
67	BPH2099	2007	Australia	NSW	WGS Reads	Ion Torrent PGM	SAMEA3212933	ERR732888	Baines. 2015. mBio. PMID: 25736880
68	JKD6000	2002	Australia	VIC	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212909	ERR732889	Baines. 2015. mBio. PMID: 25736880
69	JKD6004	2002	Australia	QLD	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212901	ERR732891	Baines. 2015. mBio. PMID: 25736880
70	JKD6008	2006	New Zealand	-	Complete Genome	Roache 454 GS20, SOLiD	CP002120	-	Howden. 2010. JBact. PMID:20802046
71	JKD6009	2003	New Zealand	-	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212894	ERR732894	Baines. 2015. mBio. PMID: 25736880
-	SV5 (JKD6009)	-	-	-	Complete Genome	Pacific Biosciences RS-II	GCA_900607245	-	This Study
-	SV5 (JKD6009)	-	-	-	WGS Long Reads	Pacific Biosciences RS-II	SAMEA5047402	ERR2858553	This Study
72	JKD6021	2001	Australia	VIC	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212953	ERR732895	Baines. 2015. mBio. PMID: 25736880
73	JKD6052	2004	Australia	QLD	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212916	ERR732899	Baines. 2015. mBio. PMID: 25736880
74	JKD6121	2005	Australia	VIC	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212963	ERR732900	Baines. 2015. mBio. PMID: 25736880
75	BPH2065	1981	Australia	SA	WGS Reads	Illumina NextSeq 500	SAMEA5042664	ERR2855733	This Study
76	BPH2066	1981	Australia	SA	WGS Reads	Illumina NextSeq 500	SAMEA5042665	ERR2855734	This Study
77	BPH2104	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042666	ERR2855735	This Study
78	BPH2106	1992	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042667	ERR2855736	This Study
79	BPH2107	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042669	ERR2855737	This Study
80	BPH2154	1992	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042676	ERR2855744	This Study
81	BPH2161	1992	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042678	ERR2855746	This Study
82	BPH2163	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042679	ERR2855747	This Study
83	BPH2165	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042680	ERR2855748	This Study
84	BPH2203	1992	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042687	ERR2855755	This Study
85	BPH2205	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042689	ERR2855757	This Study
86	BPH2226	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042695	ERR2855763	This Study
87	BPH2228	1990	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA5042696	ERR2855764	This Study
88	BPH2233	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042699	ERR2855767	This Study
89	BPH2234	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042700	ERR2855768	This Study

90	BPH2235	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042701	ERR2855769	This Study
91	BPH2236	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042702	ERR2855770	This Study
92	BPH2238	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042703	ERR2855771	This Study
93	BPH2710	2011	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317322	ERR2137022	Publication in process
94	BPH2718	2011	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317330	ERR2137030	Publication in process
95	BPH2723	2011	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317335	ERR2137035	Publication in process
96	BPH2726	2011	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317338	ERR2137038	Publication in process
97	BPH2747	2011	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317359	ERR2137059	Publication in process
98	BPH2779	2011	Australia	VIC	WGS Reads	Illumina HiSeq	SAMEA104317390	ERR2137090	Publication in process
99	BPH2780	2011	Australia	VIC	WGS Reads	Illumina HiSeq	SAMEA104317391	ERR2137091	Publication in process
100	BPH2781	2011	Australia	VIC	WGS Reads	Illumina HiSeq	SAMEA104317392	ERR2137092	Publication in process
101	BPH2787	2011	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA104317398	ERR2137098	Publication in process
102	BPH2798	2011	Australia	VIC	WGS Reads	Illumina HiSeq	SAMEA104317409	ERR2137109	Publication in process
103	BPH2824	2011	Australia	VIC	WGS Reads	Illumina HiSeq	SAMEA104317431	ERR2137131	Publication in process
104	BPH2827	2011	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA104317434	ERR2137134	Publication in process
105	BPH2869	2012	Australia	NSW	WGS Reads	Illumina MiSeq	SAMEA104317472	ERR2137172	Publication in process
-	SV6 (BPH2869)	-	-	-	Complete Genome	Pacific Biosciences RS-II	GCA_900607295	-	This Study
-	SV6 (BPH2869)	-	-	-	WGS Long Reads	Pacific Biosciences RS-II	SAMEA104317472	ERR2858554	This Study
106	BPH2896	2012	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317497	ERR2137197	Publication in process
107	BPH2946	2012	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317543	ERR2137243	Publication in process
108	BPH2947	2012	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA104317544	ERR2137244	Publication in process
109	BPH3202	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317594	ERR2137294	Publication in process
110	BPH3208	2007	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317600	ERR2137300	Publication in process
111	BPH3220	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317612	ERR2137312	Publication in process
112	BPH3234	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317626	ERR2137326	Publication in process
113	BPH3244	2007	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317636	ERR2137336	Publication in process
-	SV4 (BPH3244)	-	-	-	Complete Genome	Pacific Biosciences RS-II	GCA_900607285	-	This Study
-	SV4 (BPH3244)	-	-	-	WGS Long Reads	Pacific Biosciences RS-II	SAMEA104317636	ERR2858555	This Study
114	BPH3250	2007	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317642	ERR2137342	Publication in process
115	BPH3254	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317646	ERR2137346	Publication in process
116	BPH3260	2007	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317652	ERR2137352	Publication in process
117	BPH3262	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317654	ERR2137354	Publication in process
118	BPH3266	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317658	ERR2137358	Publication in process
119	BPH3272	2007	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317664	ERR2137364	Publication in process
120	BPH3276	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317668	ERR2137368	Publication in process
121	BPH3284	2008	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317676	ERR2137376	Publication in process
122	BPH3306	2007	Australia	QLD	WGS Reads	Illumina NextSeq 500	SAMEA104317698	ERR2137398	Publication in process
123	BPH3324	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317716	ERR2137416	Publication in process
124	BPH3328	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317720	ERR2137420	Publication in process
125	BPH3332	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317724	ERR2137424	Publication in process
126	BPH3344	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317736	ERR2137436	Publication in process
127	BPH3346	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317738	ERR2137438	Publication in process
128	BPH3348	2007	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317740	ERR2137440	Publication in process
129	BPH3356	2007	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317748	ERR2137448	Publication in process
130	BPH3360	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317752	ERR2137452	Publication in process
131	BPH3362	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317754	ERR2137454	Publication in process
132	BPH3366	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317758	ERR2137458	Publication in process
133	BPH3368	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317760	ERR2137460	Publication in process
134	BPH3370	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317762	ERR2137462	Publication in process
135	BPH3372	2008	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317764	ERR2137464	Publication in process
136	BPH3376	2007	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA104317768	ERR2137468	Publication in process

188	BPH3662	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325216	ERR2145175	Publication in process
189	BPH3664	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325218	ERR2145177	Publication in process
190	BPH3666	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325220	ERR2145179	Publication in process
191	BPH3668	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325222	ERR2145181	Publication in process
192	BPH3670	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325224	ERR2145183	Publication in process
193	BPH3672	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325226	ERR2145185	Publication in process
194	BPH3674	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325228	ERR2145187	Publication in process
195	BPH3676	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325230	ERR2145189	Publication in process
196	BPH3678	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325232	ERR2145191	Publication in process
197	BPH3680	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325234	ERR2145193	Publication in process
198	BPH3682	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325236	ERR2145195	Publication in process
199	BPH3684	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325238	ERR2145197	Publication in process
200	BPH3686	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325240	ERR2145199	Publication in process
201	BPH3688	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325242	ERR2145201	Publication in process
202	BPH3692	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325246	ERR2145205	Publication in process
203	BPH3694	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325248	ERR2145207	Publication in process
204	BPH3696	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325250	ERR2145209	Publication in process
205	BPH3698	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325252	ERR2145211	Publication in process
206	BPH3700	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325254	ERR2145213	Publication in process
207	BPH3702	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325256	ERR2145215	Publication in process
208	BPH3704	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325258	ERR2145217	Publication in process
209	BPH3706	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325260	ERR2145219	Publication in process
210	BPH3708	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325262	ERR2145221	Publication in process
211	BPH3710	2008	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325264	ERR2145223	Publication in process
212	BPH3712	2007	Australia	NSW	WGS Reads	Illumina NextSeq 500	SAMEA104325266	ERR2145225	Publication in process

Additional WGS Data

213	JKD6001	2003	Australia	VIC	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212898	ERR732890	Baines. 2015. mBio. PMID: 25736880
214	JKD6005	2002	Australia	QLD	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212895	ERR732892	Baines. 2015. mBio. PMID: 25736880
215	JKD6007	2002	Australia	QLD	WGS Reads	Illumina MiSeq	SAMEA3212912	ERR732893	Baines. 2015. mBio. PMID: 25736880
216	JKD6022	2001	Australia	VIC	WGS Reads	Illumina MiSeq	SAMEA3212914	ERR732896	Baines. 2015. mBio. PMID: 25736880
217	JKD6023	2001	Australia	VIC	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212964	ERR732897	Baines. 2015. mBio. PMID: 25736880
218	JKD6051	2003	Australia	QLD	WGS Reads	Illumina Genome Analyzer Iix	SAMEA3212960	ERR732898	Baines. 2015. mBio. PMID: 25736880
219	BPH2108	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042670	ERR2855738	This Study
220	BPH2109	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042671	ERR2855739	This Study
221	BPH2113	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042672	ERR2855740	This Study
222	BPH2119	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042673	ERR2855741	This Study
223	BPH2120	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042674	ERR2855742	This Study
224	BPH2139	1992	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042675	ERR2855743	This Study
225	BPH2157	1992	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042677	ERR2855745	This Study
226	BPH2167	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042681	ERR2855749	This Study
227	BPH2168	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042682	ERR2855750	This Study
228	BPH2196	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042683	ERR2855751	This Study
229	BPH2198	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042684	ERR2855752	This Study
230	BPH2199	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042685	ERR2855753	This Study
231	BPH2202	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042686	ERR2855754	This Study
232	BPH2204	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042688	ERR2855756	This Study
233	BPH2207	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042690	ERR2855758	This Study
234	BPH2210	1990	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042691	ERR2855759	This Study
235	BPH2213	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042692	ERR2855760	This Study
236	BPH2219	1991	Australia	TAS	WGS Reads	Illumina NextSeq 500	SAMEA5042693	ERR2855761	This Study
237	BPH2222	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042694	ERR2855762	This Study

238	BPH2230	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042697	ERR2855765	This Study
239	BPH2232	1991	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042698	ERR2855766	This Study
240	BPH2240	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042704	ERR2855772	This Study
241	BPH2242	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042705	ERR2855773	This Study
242	BPH2243	1989	Australia	VIC	WGS Reads	Illumina NextSeq 500	SAMEA5042706	ERR2855774	This Study
243	S20209-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
244	S20305-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
245	S20603-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
246	S20607-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
247	S20608-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
248	S20610-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
249	S20613-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
250	S20617-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
251	S20622-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
252	S20623-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
253	S20629-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
254	S20826-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
255	S23616-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
256	S23619-2015	2015	Australia	NSW	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
257	S31902-2015	2015	Australia	VIC	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
258	S31904-2015	2015	Australia	VIC	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
259	S31913-2015	2015	Australia	VIC	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
260	S31918-2015	2015	Australia	VIC	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
261	S32313-2015	2015	Australia	VIC	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
262	S41210-2015	2015	Australia	QLD	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
263	S51001-2015	2015	Australia	NT	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
264	S51003-2015	2015	Australia	NT	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
265	S51006-2015	2015	Australia	NT	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
266	S51007-2015	2015	Australia	NT	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
267	S51008-2015	2015	Australia	NT	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
268	S51014-2015	2015	Australia	NT	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
269	S51024-2015	2015	Australia	NT	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
270	S51408-2015	2015	Australia	SA	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
271	S51504-2015	2015	Australia	SA	WGS Reads	Illumina MiSeq	-	-	agargroup.org.au/agar-surveys
272	TW20	2003	United Kingdom	-	Complete Genomes	-	FN433596, FN433597, FN433598	-	Holden. 2010. JBact. PMID:19948800
273	Z172	2010	Taiwan	-	Complete Genomes	Illumina/Solexa + PacBio	CP006838, CP006839, CP006840	-	Chen. 2013. GenomeA. PMID:PMC3853063
274	T0131	2006	China	-	Complete Genomes	Roche 454 / Solexa	CP002643	-	Li. 2011. JBact. PMID:21551295
275	BMB9393	1993	Brazil	-	Complete Genome	454 GS FLX Titanium	CP005288, CP005289	-	Costa. 2013. GenomeA. PMID:23929475
276	XN108	-	China	-	Complete Genomes	Ion Torrent PGM	CP007447	-	Zhang. 2014. GenomeA. PMID:25059856
277	GV69	1996	Brazil	-	Complete Genomes	454 GS FLX Titanium	CP009681; SAMN03144721	SRR2601051	Botelho. 2016. StandGenSci. PMID:27152133
278	HCI1335	2001	Brazil	-	Complete Genomes	454 GS FLX Titanium	CP012012	-	Botelho. 2016. GenBioEvol. PMID:27635055
279	M92	-	Canada	-	Complete Genomes	PacBio RS-II	CP015447	-	McClure. 2017. GenomeA. PMID:28596402
280	2A8	2001	Czech Republic	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029549	ERR064912	Castillo-Ramirez. 2012. GB. PMID:23270620
281	3HK	2000	Czech Republic	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029566	ERR064907	Castillo-Ramirez. 2012. GB. PMID:23270620
282	AGT1	1997	Argentina	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029527	ERR064926	Castillo-Ramirez. 2012. GB. PMID:23270620
283	AGT9	1997	Argentina	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029509	ERR064923	Castillo-Ramirez. 2012. GB. PMID:23270620
284	AGT67	1997	Argentina	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029555	ERR064924	Castillo-Ramirez. 2012. GB. PMID:23270620
285	AGT120	1998	Argentina	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029554	ERR064919	Castillo-Ramirez. 2012. GB. PMID:23270620
286	ANS46	1982	Australia	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029537	ERR064898	Castillo-Ramirez. 2012. GB. PMID:23270620
287	BK2421	1996	United States	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029510	ERR064899	Castillo-Ramirez. 2012. GB. PMID:23270620
288	BRA2	1997	Brazil	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029514	ERR064914	Castillo-Ramirez. 2012. GB. PMID:23270620

289	BRA36	1997	Brazil	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029559	ERR064917	Castillo-Ramirez. 2012. GB. PMID:23270620
290	BZ48	1997	Brazil	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029508	ERR064918	Castillo-Ramirez. 2012. GB. PMID:23270620
291	CHI59	1998	China	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029524	ERR064937	Castillo-Ramirez. 2012. GB. PMID:23270620
292	CHI61	1998	China	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029538	ERR064938	Castillo-Ramirez. 2012. GB. PMID:23270620
293	CHL1	1997	Chile	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029526	ERR064915	Castillo-Ramirez. 2012. GB. PMID:23270620
294	CHL151	1998	Chile	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029539	ERR064916	Castillo-Ramirez. 2012. GB. PMID:23270620
295	D71	1996	Germany	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862562	ERR024980	Castillo-Ramirez. 2012. GB. PMID:23270620
296	D90	1996	Germany	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862585	ERR025801	Castillo-Ramirez. 2012. GB. PMID:23270620
297	DEN907	2001	Denmark	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029548	ERR064948	Castillo-Ramirez. 2012. GB. PMID:23270620
298	DEU2	2009	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862577	ERR026652	Castillo-Ramirez. 2012. GB. PMID:23270620
299	DEU3	2009	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862574	ERR026653	Castillo-Ramirez. 2012. GB. PMID:23270620
300	DEU5	2009	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862580	ERR026655	Castillo-Ramirez. 2012. GB. PMID:23270620
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305	DEU12	2009	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862553	ERR026651	Castillo-Ramirez. 2012. GB. PMID:23270620
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307	DEU15	2008	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862558	ERR026665	Castillo-Ramirez. 2012. GB. PMID:23270620
308	DEU16	2008	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862557	ERR026666	Castillo-Ramirez. 2012. GB. PMID:23270620
309	DEU17	2008	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862559	ERR026667	Castillo-Ramirez. 2012. GB. PMID:23270620
310	DEU19	2008	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862561	ERR026668	Castillo-Ramirez. 2012. GB. PMID:23270620
311	DEU20	2008	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862560	ERR026669	Castillo-Ramirez. 2012. GB. PMID:23270620
312	DEU23	2008	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862541	ERR026662	Castillo-Ramirez. 2012. GB. PMID:23270620
313	DEU25	2008	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862542	ERR026663	Castillo-Ramirez. 2012. GB. PMID:23270620
314	DEU27	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862536	ERR026677	Castillo-Ramirez. 2012. GB. PMID:23270620
315	DEU28	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862537	ERR026678	Castillo-Ramirez. 2012. GB. PMID:23270620
316	DEU29	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862538	ERR026679	Castillo-Ramirez. 2012. GB. PMID:23270620
317	DEU30	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862531	ERR026680	Castillo-Ramirez. 2012. GB. PMID:23270620
318	DEU35	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862662	ERR026683	Castillo-Ramirez. 2012. GB. PMID:23270620
319	DEU36	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862661	ERR026684	Castillo-Ramirez. 2012. GB. PMID:23270620
320	DEU37	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862660	ERR026674	Castillo-Ramirez. 2012. GB. PMID:23270620
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329	DEU50	2006	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862566	ERR026687	Castillo-Ramirez. 2012. GB. PMID:23270620
330	ES26	1996	Spain	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862530	ERR024981	Castillo-Ramirez. 2012. GB. PMID:23270620
331	FFP103	1990	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029533	ERR064932	Castillo-Ramirez. 2012. GB. PMID:23270620
332	GRE4	1998	Greece	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029513	ERR064904	Castillo-Ramirez. 2012. GB. PMID:23270620
333	GRE18	1998	Greece	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029546	ERR064902	Castillo-Ramirez. 2012. GB. PMID:23270620
334	GRE108	1998	Greece	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029521	ERR064936	Castillo-Ramirez. 2012. GB. PMID:23270620
335	GRE317	1999	Greece	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029564	ERR064903	Castillo-Ramirez. 2012. GB. PMID:23270620
336	H202	2006	Thailand	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862584	ERR025802	Castillo-Ramirez. 2012. GB. PMID:23270620
337	H211	2006	Denmark	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862528	ERR024972	Castillo-Ramirez. 2012. GB. PMID:23270620
338	H216	2006	Denmark	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862587	ERR025803	Castillo-Ramirez. 2012. GB. PMID:23270620
339	H24	2005	Egypt	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862527	ERR024973	Castillo-Ramirez. 2012. GB. PMID:23270620

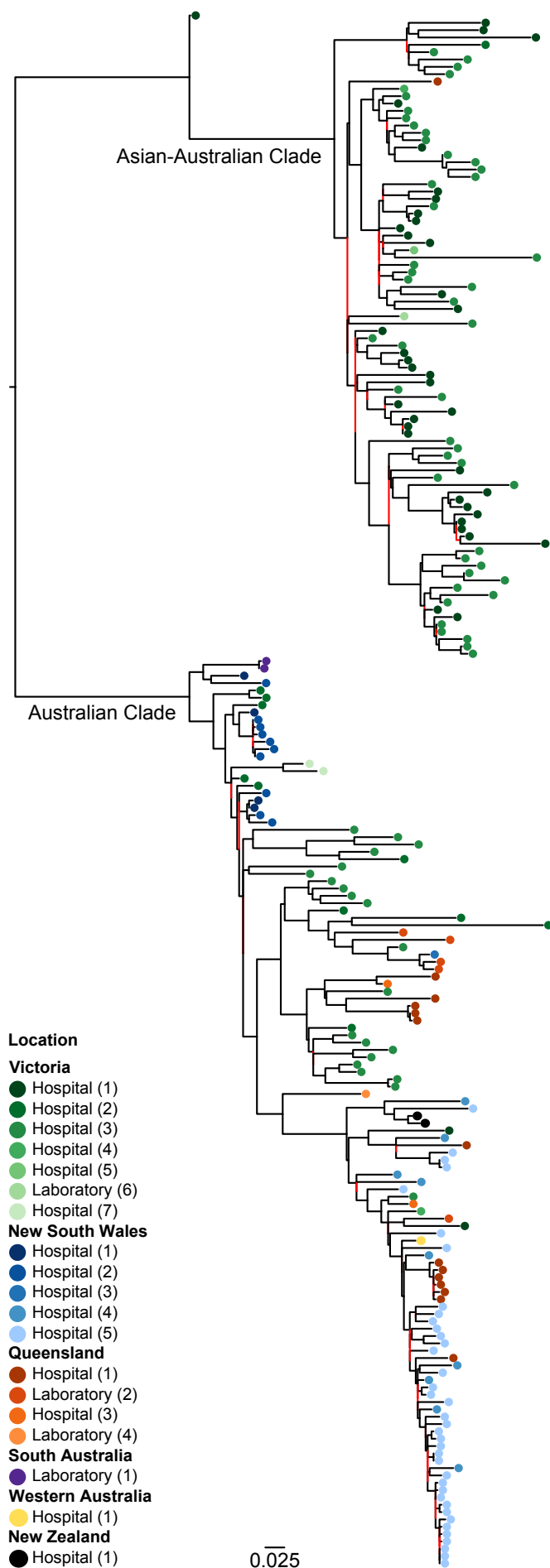
340	H482	2007	Romania	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862595	ERR024983	Castillo-Ramirez. 2012. GB. PMID:23270620
341	HDG2	1992	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029561	ERR064930	Castillo-Ramirez. 2012. GB. PMID:23270620
342	HGSA9	1997	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029534	ERR064921	Castillo-Ramirez. 2012. GB. PMID:23270620
343	HGSA142	2003	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029517	ERR064922	Castillo-Ramirez. 2012. GB. PMID:23270620
344	HSA10	1992	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029520	ERR064931	Castillo-Ramirez. 2012. GB. PMID:23270620
345	HSA11	1992	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029542	ERR064929	Castillo-Ramirez. 2012. GB. PMID:23270620
346	HSJ216	1997	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029550	ERR064920	Castillo-Ramirez. 2012. GB. PMID:23270620
347	HU106	1996	Hungary	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029518	ERR064927	Castillo-Ramirez. 2012. GB. PMID:23270620
348	HU109	1996	Hungary	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029567	ERR064909	Castillo-Ramirez. 2012. GB. PMID:23270620
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350	HU5	2006	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862625	ERR026706	Castillo-Ramirez. 2012. GB. PMID:23270620
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352	HU7	2006	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862623	ERR026708	Castillo-Ramirez. 2012. GB. PMID:23270620
353	HU8	2006	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862622	ERR026709	Castillo-Ramirez. 2012. GB. PMID:23270620
354	HU9	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862627	ERR026710	Castillo-Ramirez. 2012. GB. PMID:23270620
355	HU11	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862634	ERR026701	Castillo-Ramirez. 2012. GB. PMID:23270620
356	HU13	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862636	ERR024668	Castillo-Ramirez. 2012. GB. PMID:23270620
357	HU14	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862637	ERR024672	Castillo-Ramirez. 2012. GB. PMID:23270620
358	HU15	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862630	ERR024673	Castillo-Ramirez. 2012. GB. PMID:23270620
359	HU16	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862631	ERR024674	Castillo-Ramirez. 2012. GB. PMID:23270620
360	HU17	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862632	ERR024675	Castillo-Ramirez. 2012. GB. PMID:23270620
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362	HU23	2009	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862643	ERR024679	Castillo-Ramirez. 2012. GB. PMID:23270620
363	HU25	1993	Brazil	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029569	ERR024670	Castillo-Ramirez. 2012. GB. PMID:23270620
364	HU26	2009	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862644	ERR024671	Castillo-Ramirez. 2012. GB. PMID:23270620
365	HUR18	1997	Hungary	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029551	ERR064910	Castillo-Ramirez. 2012. GB. PMID:23270620
366	HUSA304	1993	Hungary	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029531	ERR064928	Castillo-Ramirez. 2012. GB. PMID:23270620
367	ICP5011	1993	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029545	ERR064933	Castillo-Ramirez. 2012. GB. PMID:23270620
368	ICP5014	1993	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029543	ERR064934	Castillo-Ramirez. 2012. GB. PMID:23270620
369	ICP5062	1993	Portugal	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029562	ERR064935	Castillo-Ramirez. 2012. GB. PMID:23270620
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372	IU4	2006	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862648	ERR024687	Castillo-Ramirez. 2012. GB. PMID:23270620
373	IU6	2006	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862651	ERR024688	Castillo-Ramirez. 2012. GB. PMID:23270620
374	IU7	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862650	ERR024689	Castillo-Ramirez. 2012. GB. PMID:23270620
375	IU9	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862617	ERR024691	Castillo-Ramirez. 2012. GB. PMID:23270620
376	IU10	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862652	ERR024692	Castillo-Ramirez. 2012. GB. PMID:23270620
377	IU11	2007	Turkey	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862653	ERR024682	Castillo-Ramirez. 2012. GB. PMID:23270620
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381	LHH1	1994	United States	-	WGS Reads	Illumina HiSeq 2000	SAMEA1029523	ERR064900	Castillo-Ramirez. 2012. GB. PMID:23270620
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383	LIT76	1996	Lithuania	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862593	ERR025805	Castillo-Ramirez. 2012. GB. PMID:23270620
384	LIT89	1996	Lithuania	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862592	ERR025796	Castillo-Ramirez. 2012. GB. PMID:23270620
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386	M278	2005	Portugal	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862548	ERR024991	Castillo-Ramirez. 2012. GB. PMID:23270620
387	M418	2006	India	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862549	ERR024992	Castillo-Ramirez. 2012. GB. PMID:23270620
388	M592	2006	Syria	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862550	ERR024993	Castillo-Ramirez. 2012. GB. PMID:23270620
389	M705	2007	Thailand	-	WGS Reads	Illumina Genome Analyzer II	SAMEA862613	ERR024958	Castillo-Ramirez. 2012. GB. PMID:23270620
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444	352_05	2005	Singapore	-	WGS Reads	Illumina Genome Analyzer II	SAMEA800280	ERR029467	Hsu. 2015. GenBiol. PMID:25903077
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487	TTSH2_03	2003	Singapore	-	WGS Reads	Illumina Genome Analyzer II	SAMEA800226	ERR030255	Hsu. 2015. GenBiol. PMID:25903077
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506	WAU_31	1997	Singapore	-	WGS Reads	Illumina Genome Analyzer II	SAMEA1021970	ERR053037	Hsu. 2015. GenBiol. PMID:25903077
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509	T9_N3	2008	Thailand	-	WGS Reads	Illumina Genome Analyzer II	SAMEA958822	ERR023888	Tong. GenRes. 2015. PMID:25491771
510	T234_W2	2008	Thailand	-	WGS Reads	Illumina Genome Analyzer II	SAMEA958806	ERR023872	Tong. GenRes. 2015. PMID:25491771
511	T71_N2	2008	Thailand	-	WGS Reads	Illumina Genome Analyzer II	SAMEA958988	ERR033836	Tong. GenRes. 2015. PMID:25491771
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527	BSAC_st697	2003	England	-	WGS Reads	Illumina HiSeq 2000	SAMEA1464583	ERR129279	Reuter. GenRes. 2016. PMID:26672018
528	D8	2007	Gambia	-	WGS Reads	Illumina MiSeq	SAMEA3727072	ERR1213802	Senghore. AppEnvMicro. 2016. PMID:27474712
529	DS_009	2011	Thailand	-	WGS Reads	Illumina HiSeq 2000	SAMEA3448838	ERR1069915	Moradigaravand. mBio. 2017. PMID: 28679748
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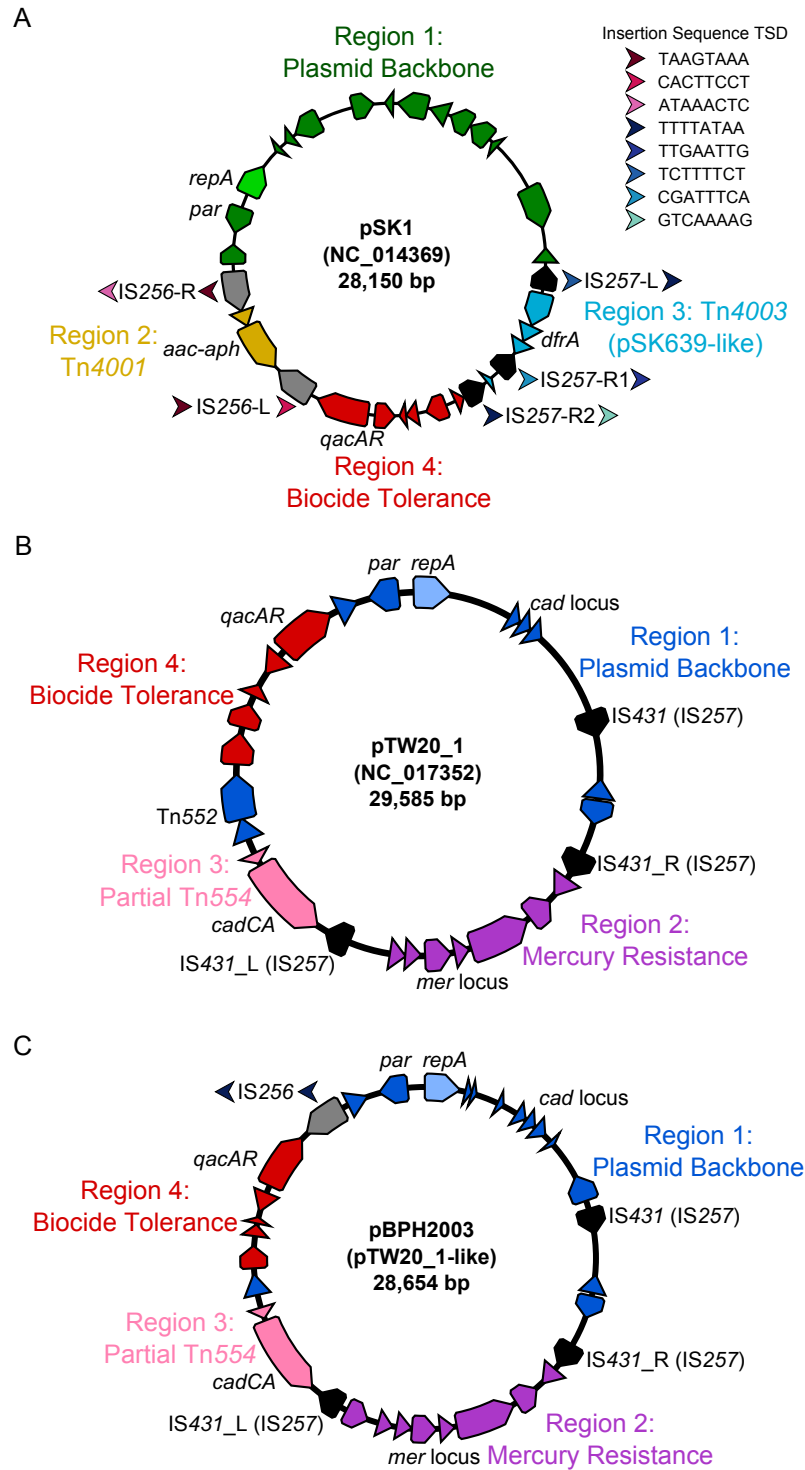
A



B

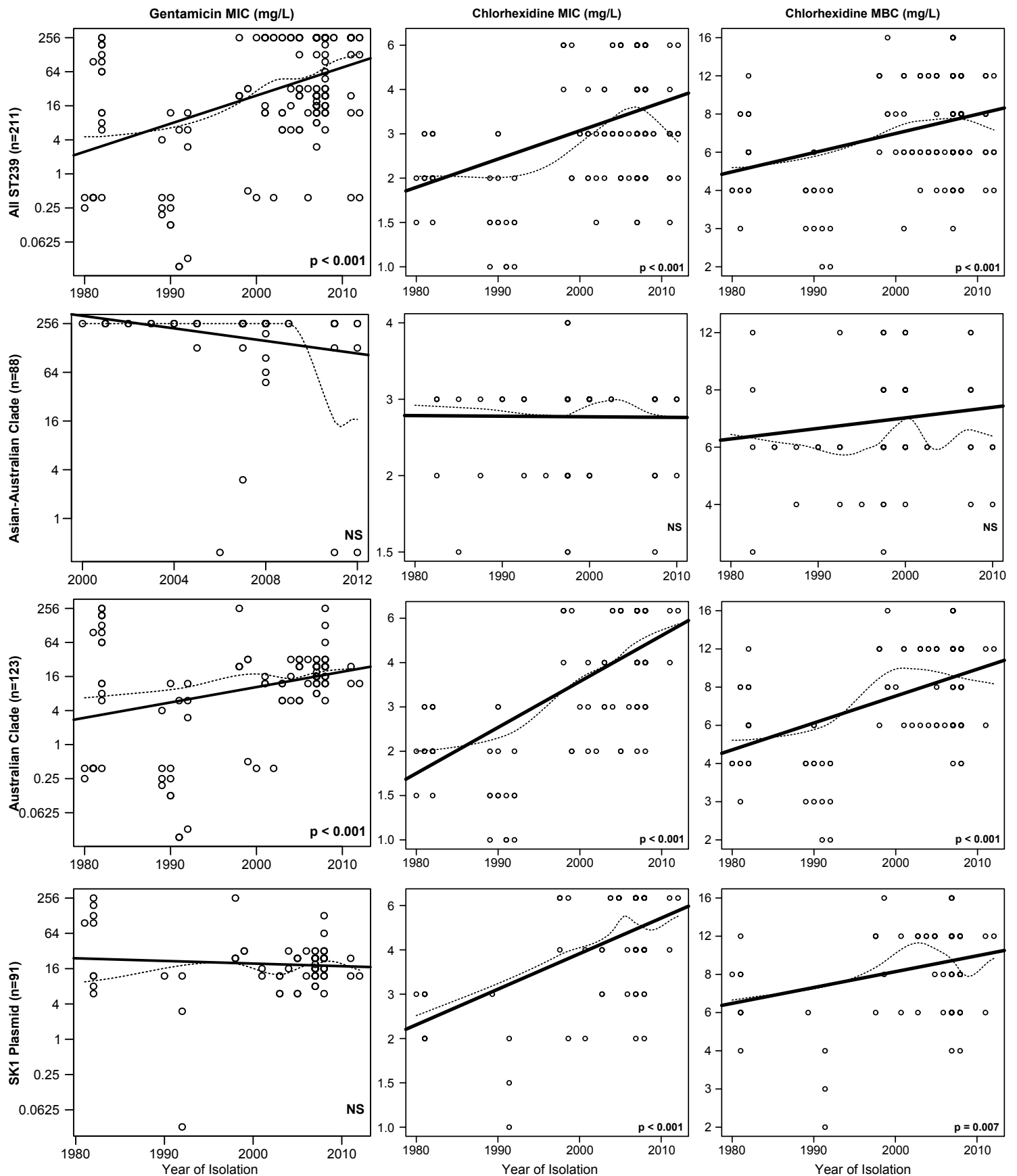


Supplementary Figure S1. pSK1 plasmid gene presence and synteny. (A) Maximum likelihood phylogenetic tree inferred from 3,883 core genome SNPs illustrates the population structure of ST239 *S. aureus* in Australia. Tips are coloured based on location (refer to key). Branches with < 70% bootstrap support are coloured red. (B) Coloured blocks represent the identification of a pSK1 gene orthologue, using a 95% amino acid homology threshold (excluding insertion sequences). Box length is reflective of gene length and ordered based on pSK1 (Figure 2A). Boxes are linked if orthologues were found to be syntenic. Coloured boxes reflect the six identified patterns.



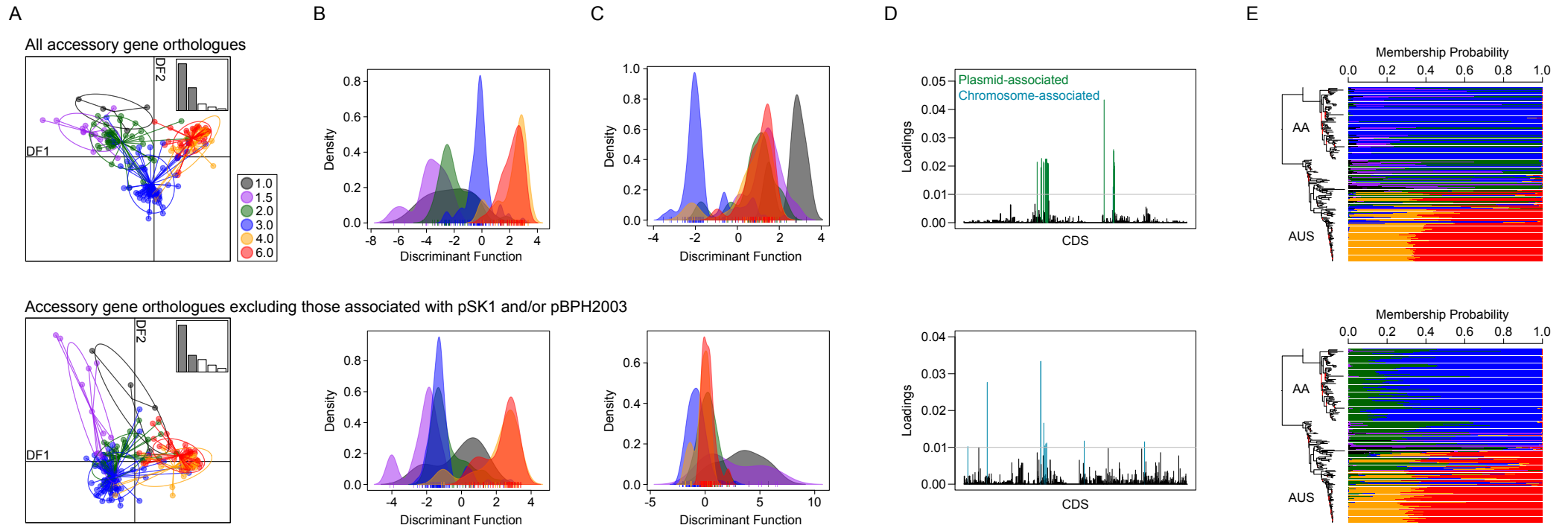
Supplementary Figure S2. Structure of pSK1, pTW20_1 and pBPH2003 plasmids. Plasmid genes have been coloured based on defined plasmid regions. Insertion sequences (IS) are coloured grey (IS256) and black (IS257), with target site duplications (TSD) illustrated: arrows indicate upstream/downstream sequences, orientation, and are coloured to represent unique sequences (refer to key). (A) pSK1; sequence and annotations are that previously published, accession NC_014369. (B) pTW20_1; sequence and annotations are that previously published, accession NC_017352. (C) pBPH2003; a pTW20_1-like plasmid representative of that harboured in isoaltes from the Asian-Australian ST239 clade.

Supplementary Figure 3



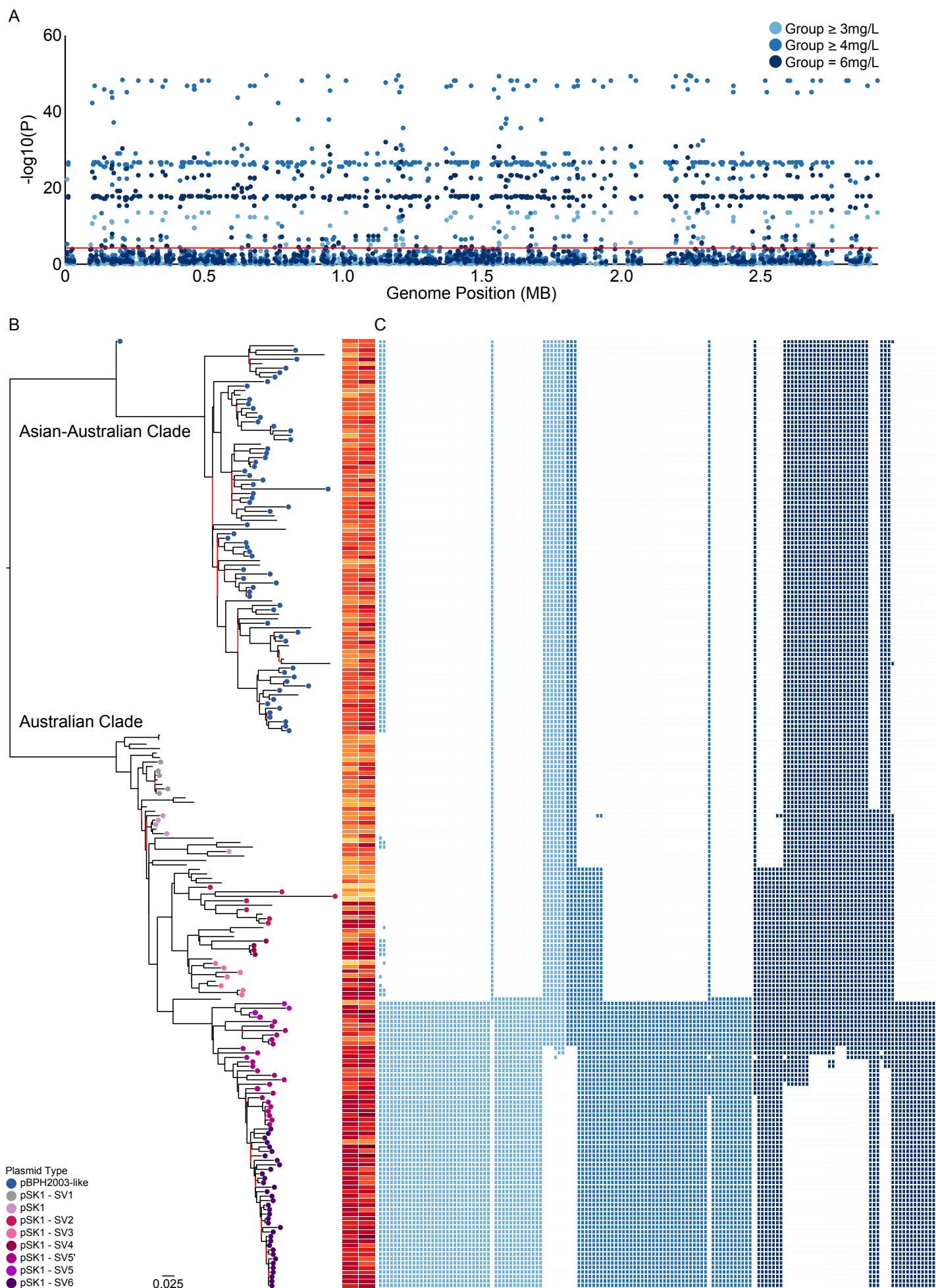
Supplementary Figure S3. Modelling temporal-association in phenotypic susceptibility data. Graphs depict linear models developed to explore the potential association between gentamicin MIC, and chlorhexidine MIC and MBC with the year in which isolates were recovered. The dotted lines indicate the smoothed mean MIC and the bold lines indicate the fitted linear model. Four populations were tested (from top to bottom): (i) All ST239 MRSA (n = 211), (ii) the Asian-Australian clade (n = 88), (iii) the Australian clade (n = 123), and (iv) the pSK1-like plasmid harbouring population (n = 91). Note: reference JKD6008 (Australian clade) was not tested.

Supplementary Figure 4



Supplementary Figure S4. Exploration of gene presence/absence signatures associated with phenotypic chlorhexidine tolerance. The graphs illustrate the findings from two Discriminant Analysis of Principle Components (DAPC) models, used to investigate genetic signatures in the ST239 MRSA population associated with chlorhexidine MIC. The first model (top panel) examined all accessory gene orthologues (clustered at 95% amino acid homology), and in the second model (bottom panel) gene orthologues associated with either pSK1 or pBPH2003 (representative of the pTW20_1-like plasmid recovered from the Asian-Australian clade) were excluded. The prior hypothesised sub-divisions represent the phenotypic CHX MIC values for the population, as indicated in the key. (A) Scatter plots illustrate the clustering of isolates across the two most discriminant functions. The density plots illustrate the same data in a one-dimensional format for the first (B) and second (C) most discriminant functions. (D) Loading plots illustrate the contributions of all gene orthologues to the DAPC models, with the most highly contributing variables (> 0.01) coloured based on whether they represent a plasmid-associated (green) or chromosomal gene orthologues (blue). (E) Membership graphs illustrate the membership profiles for all isolates, aligned to a maximum likelihood phylogenetic tree for the ST239 population. The probability index indicates the likelihood of assigning an isolate back to one of the hypothesised sub-divisions.

Figure S5



Supplementary Figure S5. Exploration of mutations associated with phenotypic chlorhexidine tolerance. (A) Manhattan plot illustrates the results of three Genome Wide Association Studies (GWAS) conducted to identify core genome SNPs associated with CHX MIC, tested at three thresholds: MIC > 3 mg/L, > 4 mg/L or = 6 mg/L. Red line indicates the threshold for significance (with Bonferroni correction). (B) Maximum likelihood phylogenetic tree is that from Figure S1, and adjacent are the phenotypic CHX MIC and MBC values (coloured yellow to red with increasing value). (C) The presence (blue) or absence (white) of the 50 most significantly associated mutations detected at each phenotypic threshold are illustrated.