

## **Supplementary Information Guide**

### **Supplementary Figures.**

Figure S1. Creation of mutants.

Figure S2. Pathway distribution of essential and non-essential genes in *S. sanguinis*.

### **Supplementary Tables.**

Table S1. Genes in *S. sanguinis* and primers used.

Table S2. Microarray analysis of gene expression in *S. sanguinis*.

Table S3. Summary of all mutants in *S. sanguinis*.

Table S4. Functional summary of essential genes in *S. sanguinis*.

Table S5. Growth comparison of mutants in CDM and BHI media.

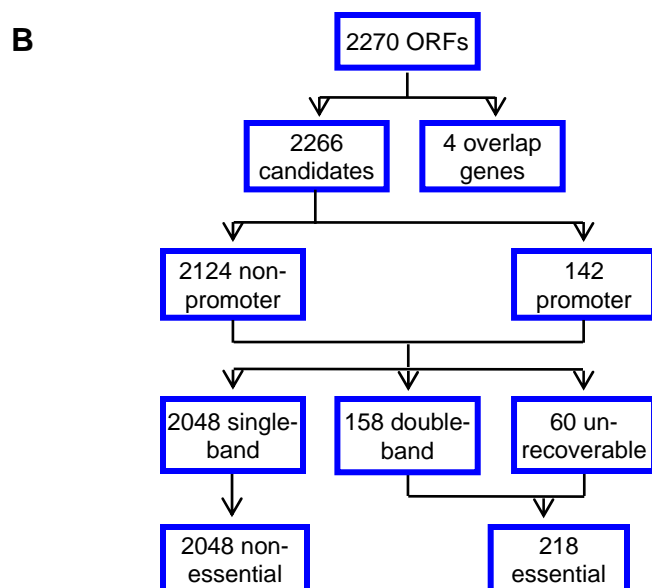
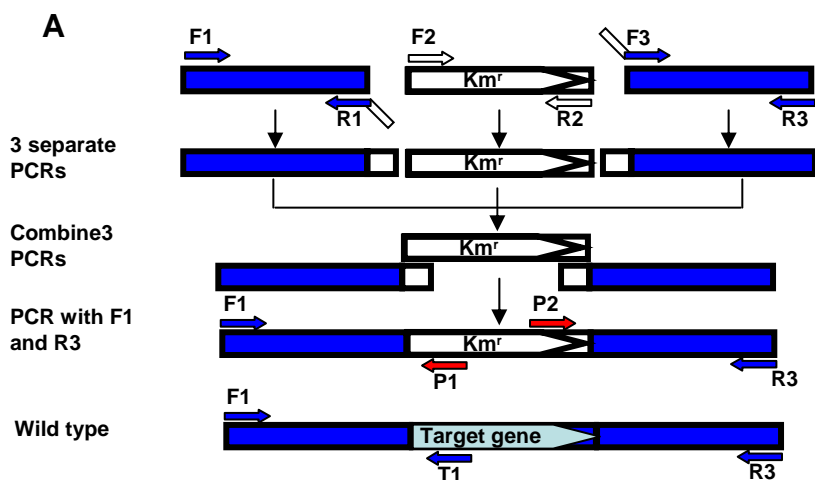
Table S6. Gene comparisons among streptococci.

A. Essential gene presentations among streptococci.

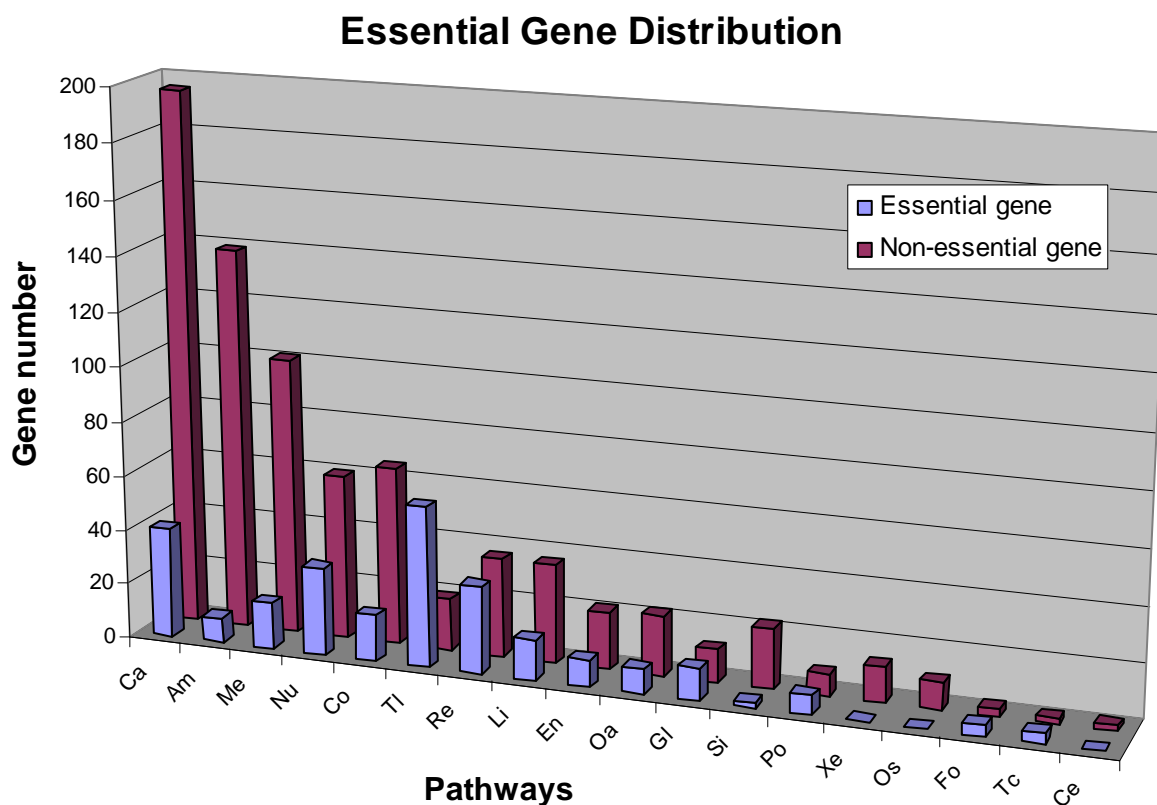
B. Streptococcal gene sequence identities among streptococci

Table S7. Comparison of *S. sanguinis* essential genes with DEG Database.

Table S8. Comparison of *S. sanguinis* essential genes with *S. aureus* and *B. subtilis*.



**Figure S1. Creation of mutants. A. Schematic strategy for replacement mutagenesis by PCR.** PCRs are performed using F1/R1, F2/R2 and F3/R3 primers. The three amplicons are purified and mixed in equal amounts. A final PCR amplicon is obtained using F1/R3. P1 and P2 are sequencing primers. T1 is the internal gene-specific primer. **B. Flowchart of essential and non-essential gene identification.** The numbers of genes at each phase of the study are shown. “Non-promoter” and “promoter” refer to genes mutagenized with the promoterless and promoter-containing constructs, respectively. “Single-band” and “double-band” refer to the number of locus-specific amplicons detected by PCR.



**Figure S2 Pathway distribution of essential and non-essential genes in *S. sanguinis*.**

Genes were classified based on KEGG Pathway Maps. Some genes may have multiple assignments when they involve in different pathways. Ca, Carbohydrate Metabolism; Am, Amino Acid Metabolism; Me, Membrane Transport; Nu, Nucleotide Metabolism; Co, Metabolism of Cofactors and Vitamins; Tl, Translation; Re, Replication and Repair; Li, Lipid Metabolism; En, Energy Metabolism; Oa, Metabolism of Other Amino Acids; Gl, Glycan Biosynthesis and Metabolism; Si, Signal Transduction; Po, Metabolism of Terpenoids and Polyketides; Xe, Xenobiotics Biodegradation and Metabolism; Os, Biosynthesis of Other Secondary Metabolites; Fo, Folding, Sorting and Degradation; Tc, Transcription; Ce, Cell Motility. Some genes involved in multiple pathways were included in each pathway.