Polymorphic Locus SerMT1 for Sequence-based Typing of *Serratia marcescens*

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ABSTRACT

Introduction: Outbreaks of pathogenic agents within healthcare facilities may have sporadic or nosocomial sources. Effective intervention requires distinguishing between these sources, which in turn requires epidemiological investigation in conjunction with typing of pathogen isolates. For the latter, established methods range from ribotyping and pulse-field gel electrophoresis to multilocus sequence typing and whole genome sequencing. Due to technical complexities and costs associated with these methods, however, typing is rarely pursued. Polymorphic locus sequence typing (PLST) addresses these issue by employing conventional PCR and Sanger sequencing; the key to PLST is its focus on a tandem repeat-containing locus exhibiting maximal variation due to combinations of single nucleotide polymorphisms and insertions/deletions. Recent studies described identification of *Candida glabrata* PLST locus CgMT-C and its use in uncovering nosocomial outbreaks of this opportunistic yeast (Katiyar & Edlind, 2021). The bacterium *Serratia marcescens* has been implicated in numerous outbreaks, particularly among neonates and the immunocompromised. (Johnson & Quach, 2017; Diekema et al., 2019; Johnson et al., 2020). Relatedly, *Serratia* species have the ability to colonize the hospital environment (e.g., sinks), although few studies have definitively established an outbreak-environment link.

Methods: To extend PLST to *S. marcescens*, tandem repeats were bioinformatically identified in the genome sequence of a representative strain and used with flanking sequences as queries in BLASTN searches of the GenBank nr/nt *Serratia* genome database including 97 *S. marcescens*, 15 *S. plymuthica*, 8 *S. liquefaciens*, and 18 additional *Serratia spp.* strains. One locus designated SerMT1 was present in all strains and exhibited high variability associated with 6-19 copies of a consensus PVVEPE-encoding repeat within signal recognition particle receptor gene *ftsY*. Primers corresponding to conserved flanking sequences were designed and used to amplify and sequence SerMT1 from colony lysates (Tris-EDTA suspensions, 100ºC, 10 min) of *S. marcescens* strains 0262/ATCC43862 and 0354/ATCC8100 and *S. liquifaciens* strain 0838/ATCC 27592 (Microbiologics, St. Clound, MN).

Results: SerMT1 was downloaded from all 138 strains, aligned using Clustal-Omega, and phylogenetically analyzed using DNA parsimony (dnapars in Phylip package). This analysis resolved 112 total alleles and yielded an impressively high Simpson's Diversity Index of 0.998. Six clusters of 2 to 4 *S. marcescens* strains shared SerMT1 sequence; for 4 clusters, epidemiologic relatedness was supported by their GenBank annotations (e.g., UMH-10/UMH-11 isolated the same month and WVU001/WVU003 isolated 2 days apart at their respective facilities). In the laboratory, the locus was readily amplified and sequenced from crude lysates of 3 *Serratia spp.* strains; bioinformatic analysis confirmed their expected identity or relatedness to GenBank strains. In agreement with WGS-SNP data for isolates from a Miami, FL outbreak (Jimenez et al., 2020), SerMT1 identified 3 distinct clusters, but with enhanced resolution of cluster 1.

Conclusion: SerMT1 PLST provides an affordable, user-friendly new tool for epidemiologic investigation of *S. marcescens* outbreaks.

RESULTS

Fig. 1. Standard methods for typing *S. marcescens* are technically complex, time consuming, and costly

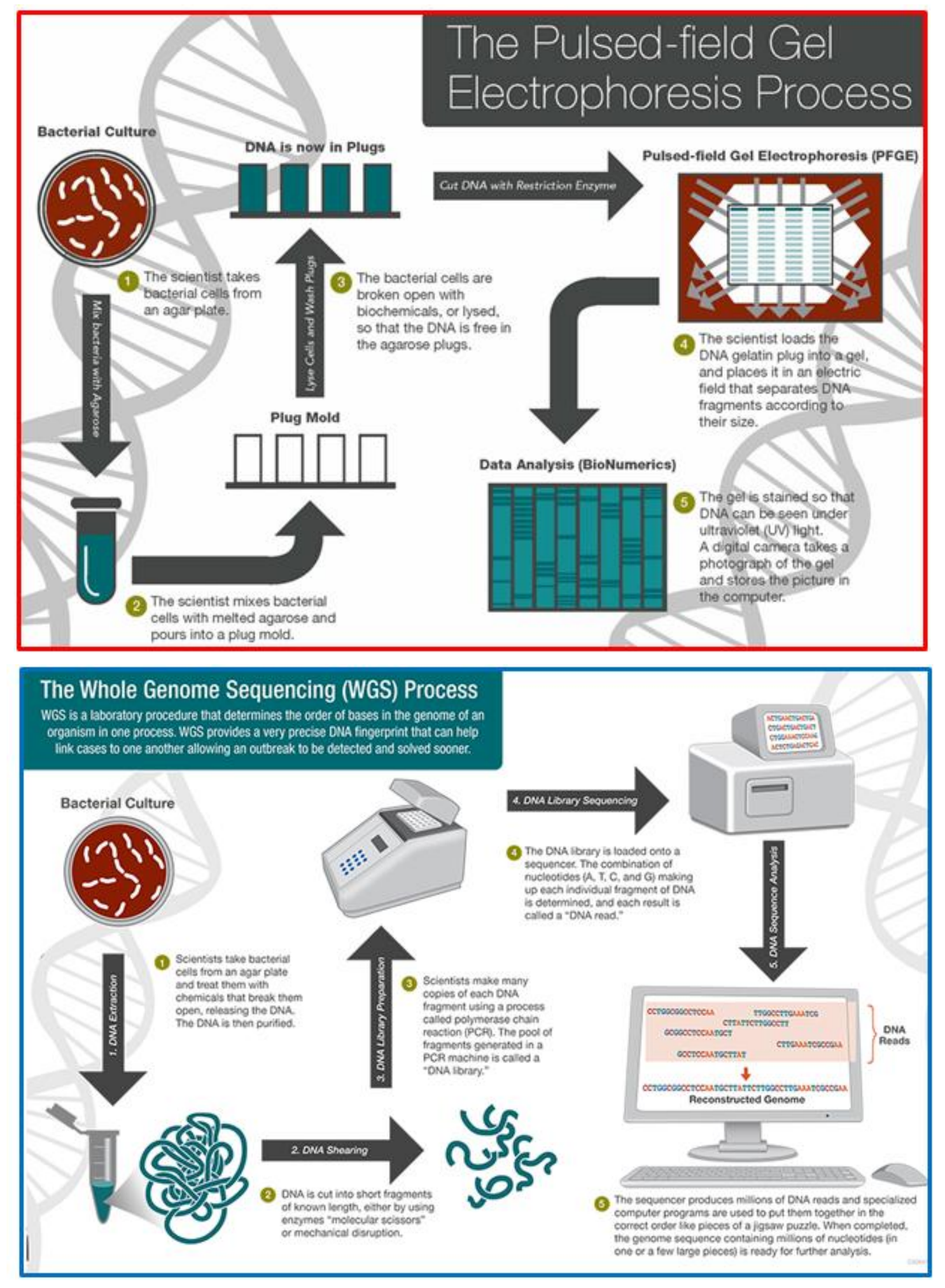

The Pulsed-field Gel Electrophoresis Process
Bacterial Culture
DNA is now in Plugs
Pulsed-field Gel Electrophoresis (PFGE)
Plug Mold
Data Analysis (BioNumerics)
The Whole Genome Sequencing (WGS) Process
Bacterial Culture
DNA Library Preparation
DNA Shearing
Reconstructed Genome

Fig. 2. Identification of PLST locus SerMT1 and application to *S. marcescens* typing: (top) protocols; (bottom left) representative SerMT1 DNA and corresponding *ftsY* protein sequences highlighting the tandem repeats (purple and red/blue) and primer regions (underlined); (bottom right) clustal alignment of SerMT1 repeat regions from representative strains involved in an *S. marcescens* outbreak (see Fig. 6)

- Bioinformatic identification of tandem repeat regions within *S. marcescens* genome sequences
- Evaluation of tandem repeat regions by BLASTN queries of GenBank genome databases
- Selection of SerMT1 locus based on maximal polymorphism, absence of insertion sequences, and inclusion in all genomes
- Design of PCR primers targeting conserved flanking sequences
- Laboratory evaluation: by PCR from colony lysates, gel confirmation, ExoSAP-IT treatment, addition of sequencing primer, and submission for Sanger dideoxynucleotide sequencing
- Sequence analysis: editing as needed based on chromatograms, trimming to selected termini, clustal-omega alignment with representative sequences, dendrogram contruction to identify strain relationships

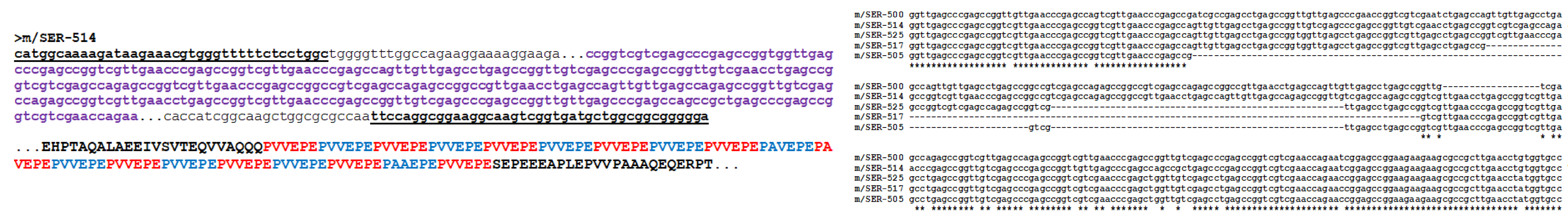
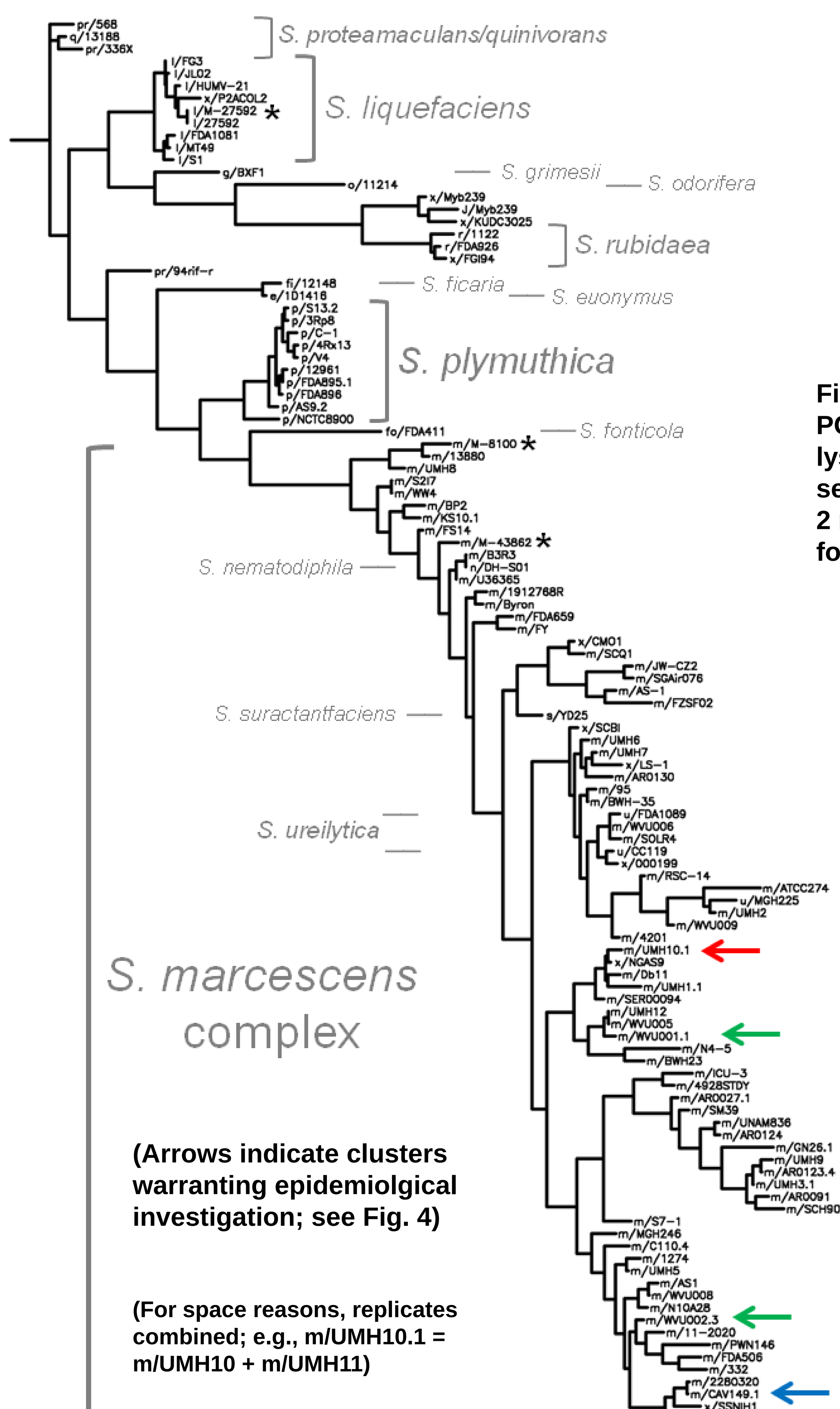


Fig. 3. Dendrogram of GenBank nr/nt SerMT1 sequences



(Arrows indicate clusters warranting epidemiolgical investigation; see Fig. 4)

(For space reasons, replicates combined; e.g., m/UMH10.1 = m/UMH10 + m/UMH11)

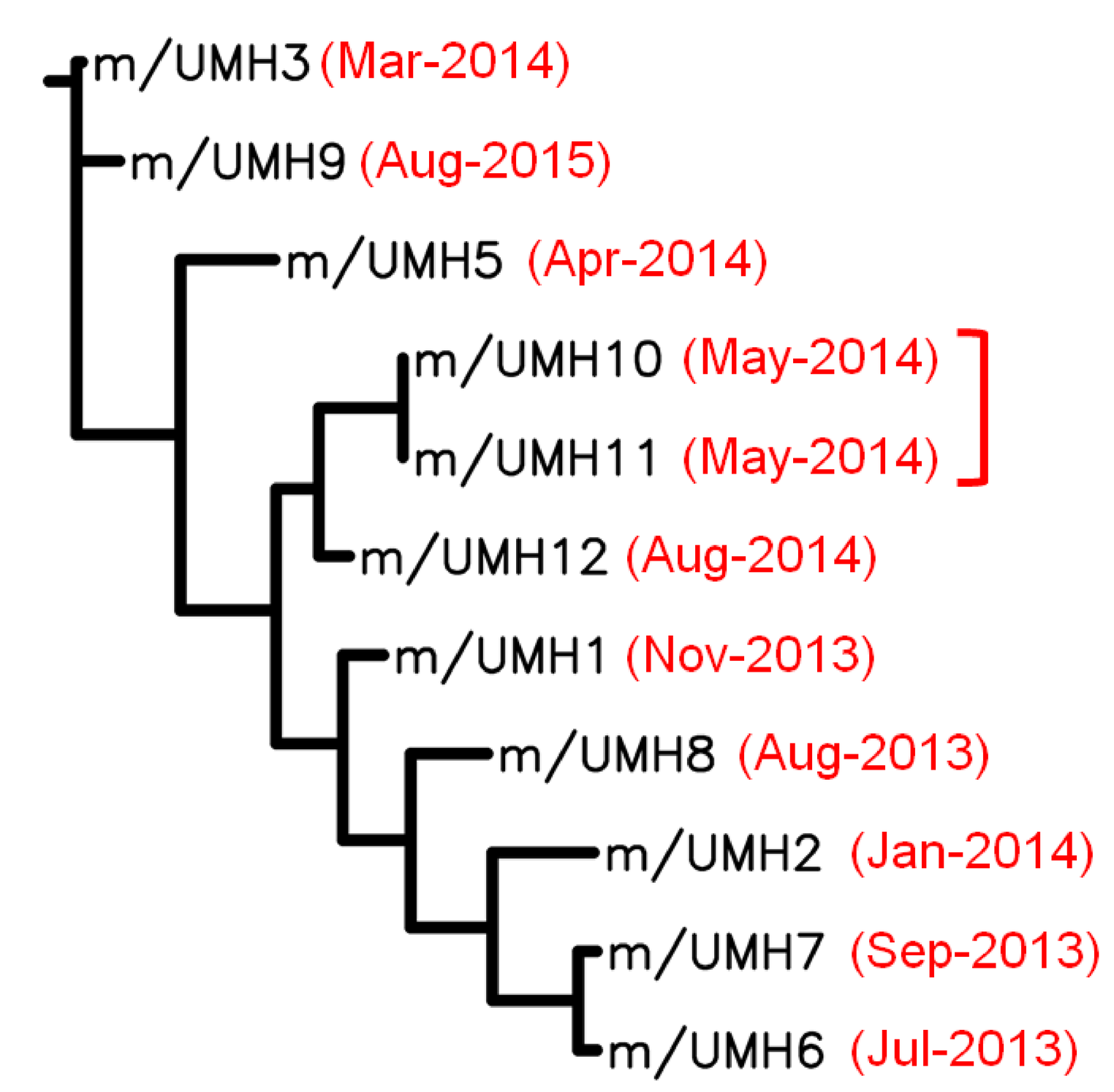


Fig. 4. Dendrograms of GenBank SerMT1 sequences from isolates collected at UMH (left) and WVU or CAV (right), including clusters (brackets) indicated by arrows in Fig. 3

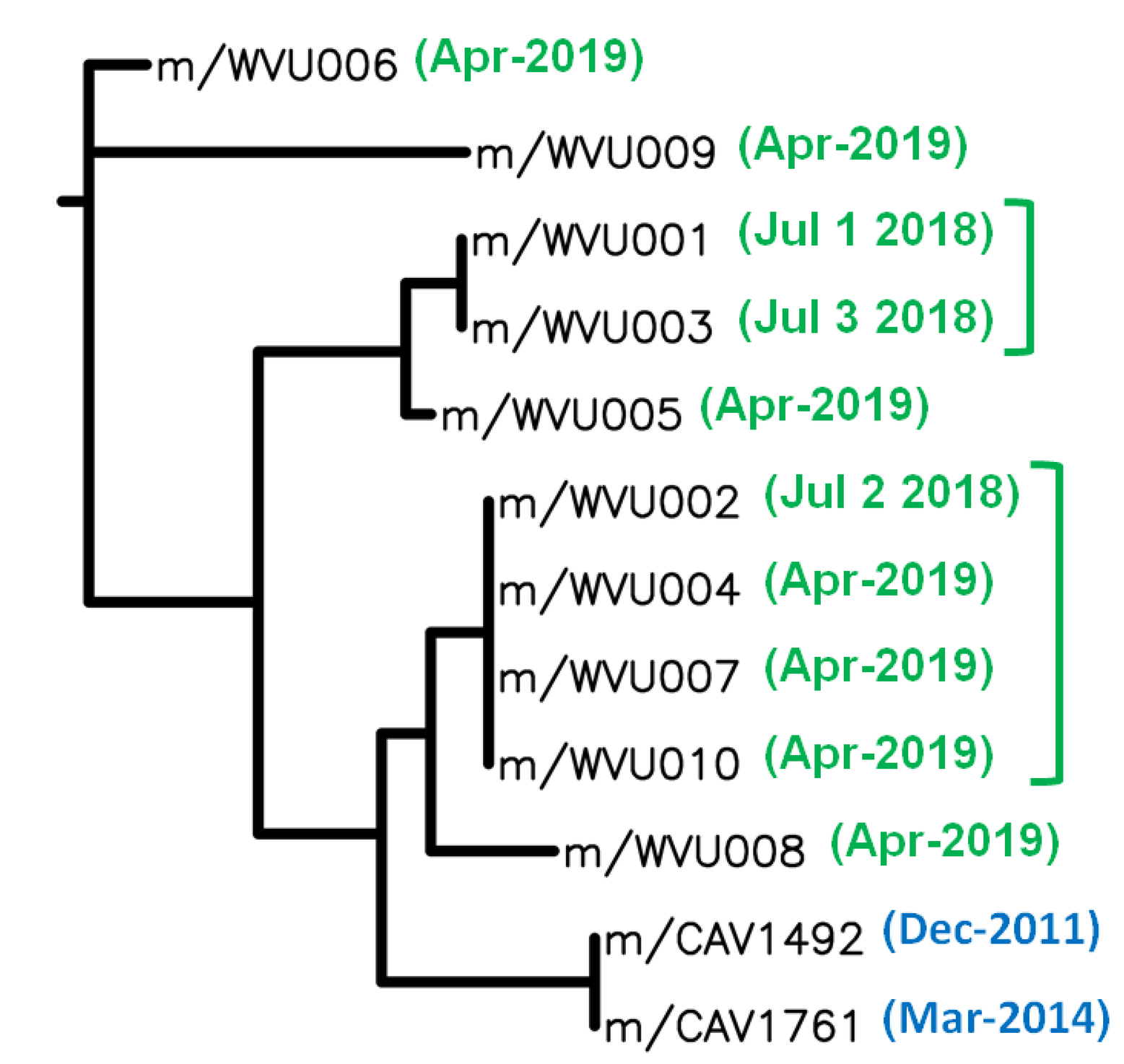


Fig. 5. Gel confirmation of SerMT1 PCR products from 3 *Serratia* colony lysates, and representative Sanger sequencing chromatogram including 2 repeat units (see Fig. 1, asterisks for dendrogram analysis)

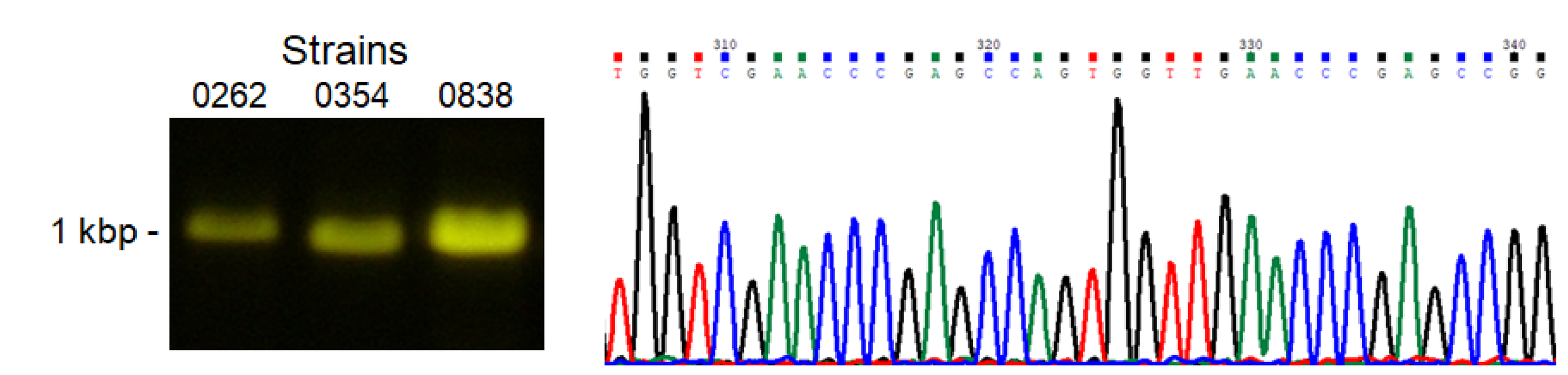
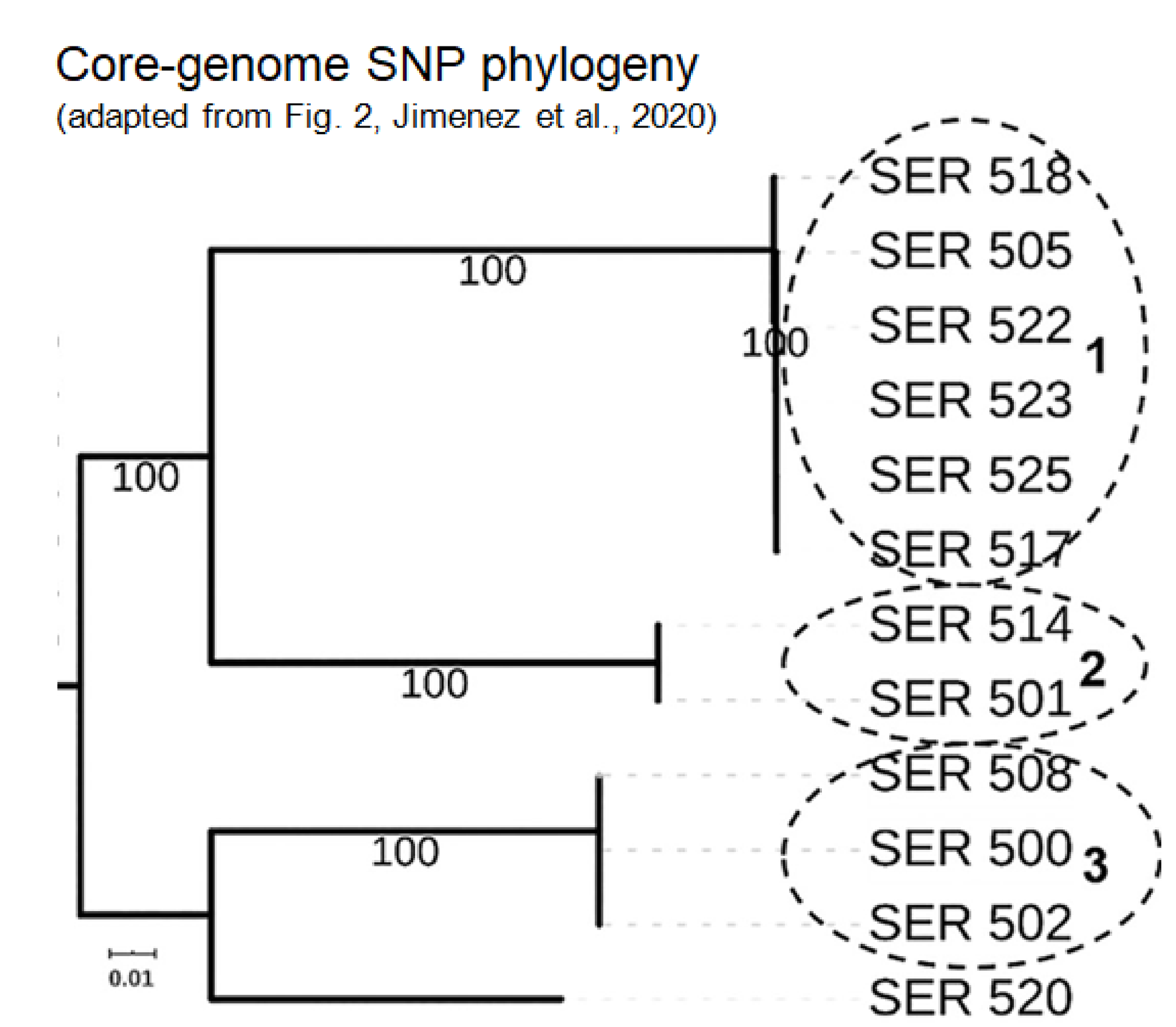
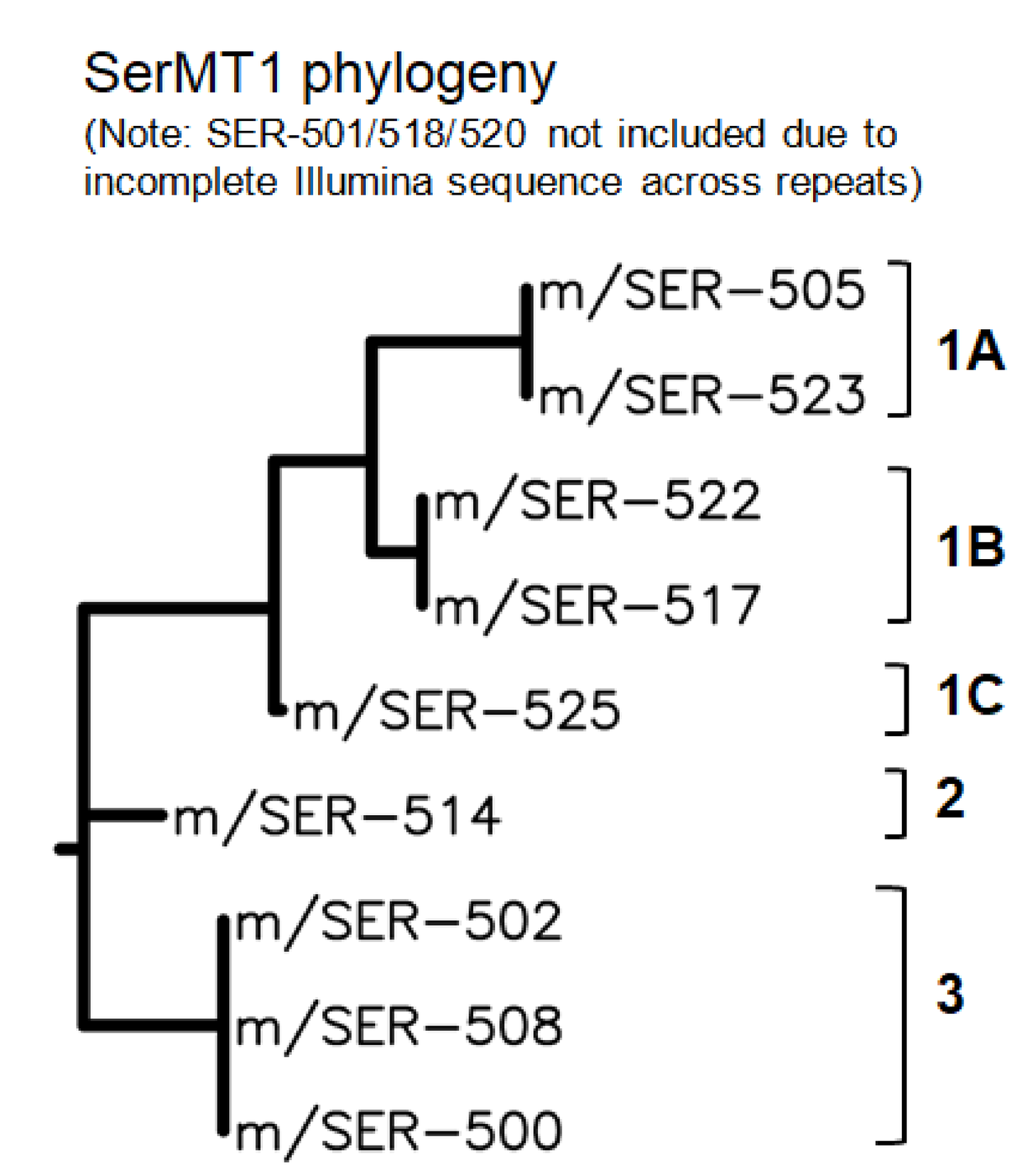


Fig. 6. Comparison of typing results for *S. marcescens* outbreak isolates from Miami, FL: (left) WGS-SNP-based typing which resolved 3 clusters (Jimenez et al., 2020); (right) SerMT1-based typing which further resolved cluster 1





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