Genotyping Primers for Fully Automated Multilocus Variable-Number Tandem Repeat Analysis of Escherichia coli O157:H7

We recently developed a multilocus variable-number tandem repeat (TR) analysis (MLVA) assay for Escherichia coli O157:H7 (2). In this assay, we identified seven loci that, when used in combination, were able to identify E. coli O157:H7 outbreaks, discriminate among genetically diverse isolates, and discriminate among isolates that were found to be highly related by pulsed-field gel electrophoresis but not known to be associated with an outbreak.

In our paper, we supplied primers for the seven loci (2). These primers successfully amplified the appropriate tandem repeat loci, and by sequencing each locus and counting the number of repeats we were able to assign an MLVA type for each isolate. We now have automated the process with new primers, using the approach described for Bacillus anthracis (1). Our fluorescently labeled primers have been designed to give each locus a discrete range on the sequencer, when possible, and color (Table 1).

The PCRs were multiplexed to reduce the overall number of reactions needed: TR1, TR5, and TR6 in the first reaction; TR3, TR4, and TR7 in a second reaction; and TR2 alone in a third reaction. All three PCRs were based on a 30-μl reaction volume with 3 μl of 10× PCR buffer, 1.5 mM MgCl₂, 25 μM each deoxynucleotide, 1.5 U of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, Calif.), and 1.0 μl of DNA. After primers were added to the reaction mixtures, water was added to a final volume of 30 μl. The first multiplex reaction mixture contained the following primer concentrations: 0.27 μM each TR1 and TR6 and 0.13 μM TR5. The second multiplex reaction mixture contained 0.17 μM each TR3 and TR4 and 0.2 μM TR7. The single PCR contained 0.33 μM TR2. The samples were placed on a GeneAmp PCR System 9700 (Applied Biosystems), and the temperature was raised to 94°C for 4 min followed by 32 cycles of 94°C for 45 s, 53 to 58°C for 45 s, and 72°C for 1 min. The final hold was for 5 min at 72°C. The three PCR products were pooled so that each isolate had its seven loci analyzed on one lane on the ABI PRISM 3700 Genetic Analyzer (Applied Biosystems).

Our genotyping results correlated with our previous results, with one exception (2). PHIDL 18 (1 out of 80 isolates, 1.25%) could not be genotyped after repeated attempts, even though the PCR was successful. Moreover, sequencing of eight isolates demonstrated that the genotyping primers were amplifying the tandem repeats of interest.

Due to migration differences of the ladder and products, the genotyping size differed from the expected size based on the length of the flanking region and TR. For all isolates, the observed size for each locus was reproducible from at least four independent runs. With these new primers, our MLVA protocol provides an automated, reproducible, highly discriminatory method for detecting outbreaks caused by E. coli O157:H7.

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REFERENCES


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