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## **Bacteroidales terminal restriction fragment length polymorphism (TRFLP) for fecal source differentiation in comparison to and in combination with universal bacteria TRFLP**

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### **ABSTRACT**

Terminal restriction fragment length polymorphism (TRFLP) is an attractive community analysis method for microbial source tracking (MST) because it is accessible, relatively inexpensive, and can discern multiple fecal sources simultaneously. A new Bacteroidales TRFLP (Bac-TRFLP) method was developed and its source identification performance was evaluated by itself, in comparison to, and in combination with an existing universal bacterial TRFLP method in two laboratories. Sixty-four blind samples from 12 fecal sources (sewage, septage, human, dog, horse, cow, deer, pig, chicken, goose, pigeon, and gull) were used for evaluation. Bac- and Univ-TRFLP exhibited similarly high overall correct identification (>88% and >89%, respectively), excellent specificity regardless of fecal sources, variable sensitivity depending on the source, and stable performance across two laboratories. Compared to Univ-TRFLP, Bac-TRFLP had better sensitivity and specificity with horse, cow, and pig fecal sources but was not suited for certain avian sources such as goose, gull, and pigeon. Combining the general and more targeted TRFLP methods (Univ&Bac-TRFLP) achieved higher overall correct identification (>92%), higher sensitivity and specificity metrics, and higher reproducibility between laboratories. Our results suggest that the Bac-TRFLP and Univ&Bac-TRFLP methods are promising additions to the MST toolbox and warrant further evaluation and utilization in field MST applications.

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