poultry litter and also made up a substantial proportion (high concentration) of the bacterial population in the litter. Fecal-contaminated poultry litter rather than feces was used to develop the biomarker to ensure that the MST target could survive deposition on poultry litter and subsequent spreading on fields. The method development can be divided into the following stages: (I) target identification; (II) validation of target sequence for poultry litter –specific marker; and (III) quantification.

45. *Target identification.* A preliminary screening effort searched for bacterial DNA sequences that were common and represented a substantial fraction of the microbial population in fecal-contaminated turkey and chicken litter, as well as in soils impacted by land application of poultry litter. Because of the uncertainty about which bacterial group would yield the best poultry-specific target, DNA from three different groups was analyzed: *E. coli, Bacteroidales* (a bacterial family to which the genus *Bacteroides* belongs) and total bacteria. The 16S rRNA gene was chosen as the target for PCR. This gene is used as a “molecular chronometer” because it tends to be very stable and mutate at a very low rate, so that the rate of change is proportional to evolutionary distance and changes occur over geological time periods (generally thousands to millions of years) (Woese, Kandler & Wheelis, 1990). The use of this gene as a target reduces errors related to sensitivity and specificity because it is not prone to change. The 16S rRNA genes of each bacterial group were amplified by PCR, and terminal restriction fragment length polymorphism (TRFLP) was used to create DNA fragments that allowed identification of potential targets. Cloning and DNA sequencing of potential targets from each pool (*E. coli, Bacteroidales* or total bacterial DNA) was carried out to determine the precise sequence of the gene fragment. Each DNA sequence was compared to the worldwide NCBI (National Center for Biotechnology Information) database, which is a repository for gene sequences from all organisms. The DNA sequence comparison was used to screen out (discard) non-useful targets that had been identified in habitats or animals/humans other than the gastrointestinal tracts or feces of poultry. The DNA sequence screening process yielded four sequences that were ubiquitous in poultry litter and contaminated soil, and also contained unique sequences that allowed development of target-specific PCR primers. Three of these sequences were from the total bacteria DNA pool and one was from the *E. coli* DNA pool. The sequence derived from the *E. coli* pool was identified as the closely-related bacterium *Pantoea ananatis.* Following comparison with the NCBI database, none of the candidate sequences were from the *Bacteroidales* pool because all were found in other habitats or animals.