Figure 6.1. Multiplex PCR with 16S rDNA primers for simultaneous detection of multiple bacterial targets. Primers select specific regions of DNA unique to organisms targeted. These are amplified using PCR for quantitation of bacterial populations present.
Figure 6.2. The Polymerase Chain Reaction (PCR). DNA template, primers A and B, and DNA polymerase (Taq) are combined in a reaction that cycles through denaturation, copying polymerization, annealing and extension temperatures, allowing the amplification of DNA between the primer pairs.

Figure 6.3. A restriction enzyme, or restriction endonuclease, is an enzyme that cuts double-stranded DNA when recognizing a specific short nucleotide sequence. The restriction enzyme Sau3A endonuclease cleaves DNA strands when it finds the sequence 5’ GATC and 3’ CTAG. These fragments can then be amplified using PCR.
Figure 6.4. Three restriction enzymes—HAEIII, Mbol and Sau3A—digest the same two samples. One sample comes from a culture and sensitivity (CS) vial containing a nutrient broth. The other sample vial contains formalin (F). Both vials have incubated for three days. The banding patterns are significantly different for the two vials, indicating the populations of microbiota present are significantly different. In addition, note that the CS vials have far fewer and heavier bands, indicating that the microbial populations have reduced diversity, due to the anaerobic die-off, and that the viable microbes have overgrown. Some bands present in the F vials are not detected in the CS vials, where whole groups have died off. L is a calibration of known DNA sizes.

Figure 6.5. Significant decreases in *Bifidobacteria* occur over 3 days in standard nutrient broth vials (CS) used in traditional culture methodologies. Formalin-fixed samples show more consistent levels.
**Figure 6.6.** Potentially pathogenic, the opportunistic bacteria *Staphylococcus aureus* shows significant overgrowth during a 3-day incubation in nutrient broth vial (CS). Formalin-fixed sample vial shows no growth. CFUs = colony forming units.

**Figure 6.7.** *Candida* species show significant overgrowth in nutrient broth vials (CS) over 3 days. Formalin-fixed sample vials do not exhibit growth, and are at a level that would not be reported as abnormal overgrowth. The CS samples at 1, 2, and 3 days would be reported as abnormal overgrowth.
REFERENCES


