

# Teaching Molecular Microbiology: Next Generation Sequencing vs Multiplex PCR

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## ABSTRACT

Experience in Next Generation Sequencing (NGS) is a highly desirable skill for molecular technologists. Previously, our Molecular Pathology program partnered with a local laboratory affiliate to develop a hands-on NGS educational protocol. Recently, we expanded this instructional laboratory to include the BioFire FilmArray instrument, a sample-to-answer multiplex polymerase chain reaction (PCR) platform. We used these two methods to illustrate the strengths and weaknesses of each technology and to reinforce the principles of molecular microbiology. After several classroom lectures, 18 students were given a protocol to prepare an NGS DNA library from unknown bacterial isolates. Each student performed DNA isolation, PCR of 16S rRNA, and magnetic bead PCR purification. The samples were given to the affiliate laboratory for loading and analysis on the Life Technologies Ion Torrent NGS platform. Meanwhile, the students were

paired up to perform the FilmArray gastrointestinal assay on their pooled bacterial isolates. Students compared the quantitative results of NGS with the qualitative results of the FilmArray, and they were able to determine which bacteria were detected by each method as compared with the key. There was high correlation between the two methods, although some false negatives and false positives were observed for each assay. These observations were used for classroom discussions about differences in microbial DNA isolation efficiencies, test menus, analytical sensitivities, and procedural limitations of each method. In sum, the integration of the NGS and multiplex PCR methods into a unified laboratory unit provided valuable hands-on technical experience while simultaneously illustrating the concepts of molecular microbiology.

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