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“Optimizing Unbiased Degenerate PCR Primers for Comprehensive Detection of Human Papillomavirus Genotypes”

Abstract

Human papillomaviruses (HPV) are a family of small oncogenic DNA viruses comprised of more than 200 distinct genotypes that infect 14 million people per year in the US. Vaccination programs like Gardasil have been shown to reduce the rates of disease caused by HPV, but current vaccines only provide protection against infection with a limited subset of HPV genotypes. For these vaccinations to remain effective, it is essential that the most prevalent HPV genotypes are determined through continuing surveillance of infected populations. To date there are no population-based screening programs that comprehensively survey circulating HPV genotypes. We are developing a high throughput DNA sequencing (MiSeq) assay that is comprehensive of all HPV types and unbiased to the most common types. Preliminary tests using the high-throughput sequencing platform on banked clinical specimens demonstrated robust detection of some HPV genotypes but limited detection of others. We redesigned MY09/11 degenerate PCR primers to improve detection of more types of HPV. The primers have generated an appropriately sized band of 450 base pairs on gel electrophoresis. Application of this technology will give us a more accurate measure of the HPV genotypes currently circulating in the population and show the effectiveness of Gardasil by observing the rates of the HPV strains in the vaccine. It will also show the emergence of new strains as strains are eliminated by the vaccine.