ENZYME OLYMPICS- PERFORMANCE EVALUATION OF 18 COMMERCIALLY AVAILABLE DNA POLYMERASES IN AN OPTIMIZED MULTIPLEX PCR REACTION

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Current molecular biological techniques utilize the Polymerase Chain Reaction (PCR) as the primary mechanism for DNA amplification. In a high-throughput genotyping laboratory the PCR reagents account for a large consumable cost, the most expensive being the DNA polymerase. The Human Origins Genotyping Laboratory performs multiple custom assays for large genotyping projects. In a laboratory of our size, selection of a DNA polymerase can have a cumulative effect on the overall cost of operations. Thus, selection of a DNA polymerase must balance the performance with the cost-per-unit of enzyme. I compared 18 different commercially available DNA polymerases for performance consistency in a previously optimized multiplex PCR. My goal was to identify the polymerases with the greatest cross-brand consistency, in our pre-determined reaction conditions. This enables our laboratory to maintain the reaction performance while changing the source vendor.

I performed a Y-Chromosome STR multiplex assay, on a panel of 8 human samples using each of the 18 polymerase brands. I tested each polymerase at three different concentrations starting at the previously optimized concentration of .5 units per 10µL reaction, and decreasing to .35 and .25 units per 10µL reaction. I assessed the activity of each enzyme first by visualization on a 2% agarose gel and later by fragment analysis on an Applied Biosystems 3730 Genetic Analyzer, for quality and conformity of peak height as well as amplification of all loci in a STR multiplex reaction. Six of the 18 DNA polymerase samples performed equally well at the lowest concentration, .25units per 10µL reaction, evaluated by visualization in an agarose gel and analysis of electropherogram data. Next, these 6 polymerase brands were subjected to further reductions in the polymerase concentration. This series of reactions was designed to approach the threshold of activity, where I could identify the minimum concentration needed for each polymerase to remain effective. The minimum polymerase concentration provides additional insight into the functional enzyme activity. This is an important metric in a polymerase comparison seeking to identify comparable activity, rather than greater or lesser. To further evaluate performance, I also assessed the 6 remaining polymerases under variable concentrations in a second pre-optimized Y-STR multiplex assay. From this final test, 3 DNA polymerases displayed equal activity, even at our lowest tested concentration of .0625 units per 10µL. Results of this comparison allowed our laboratory to select a DNA polymerase based on its activity in our assays as well as the relative cost per unit of enzyme.