

BIOGRAPHY

Dr. Harrison Leong earned a B.A. in physics from Claremont McKenna College in 1980 and a Ph.D. in Bioinformation from the California Institute of Technology in 1986. He worked at SAM Technology (a sister company of EEG Systems Laboratory in San Francisco) investigating working memory in human subjects, inventing electrophysiological indices of attention, and managing the development of FDA cleared software for projecting spatially deconvolved electrophysiological signals measured at the scalp onto MRI reconstructions of the brain. In 1999 he went to work at Applied Biosystems (now the Life Sciences Group under Thermo Fisher) inventing algorithms to extract genetic information from quantitative polymerase chain reaction instruments and capillary electrophoresis sequencing instruments and managing teams of software engineers to deliver tools to provide these and other capabilities to obtain and visualize genetic information associated with infectious organisms and human beings. Examples include HIV genotyping, HLA genotyping, HBV quantification, CF genotyping by fragment analysis, copy number variation, molecular karyotyping, PCR-based protein quantification, protein stability assessment by melting, probe-based genotyping, digital PCR, and rare somatic mutation detection using capillary electrophoresis sequencing.

TITLE:

Modeling Immuno-qPCR to Estimate Protein Quantity in a Biological Sample

ABSTRACT:

Immuno-qPCR combines antibody techniques for identifying protein targets with quantitative polymerase chain reaction (qPCR) methods to provide a technology for quantifying a specific protein target in one biological sample relative to a reference sample. Referred to as the proximity ligation assay (PLA), the assay has been observed to achieve 10,000x increase in sensitivity compared to the enzyme-linked immunosorbent assay (ELISA), the current standard for detecting and measuring targeted protein content. An overview of PLA and qPCR will be given followed by a more detailed discussion of a mathematical model of the PLA reaction that can estimate protein quantity despite non-linear characteristics in the data that invalidate standard qPCR methods of analysis. Though standard qPCR methods of analysis do not require calibration for a relative quantification, the model makes it clear that calibration is necessary in the case where the proteins of interest exhibit non-linear characteristics and suggests alternative calibration approaches. Experiments have been devised and executed to generate data with “known” underlying relative protein quantities. A calibration and quantification approach are cross-validated against these data. Results suggest that the method can determine a difference in quantity of the targeted protein between an unknown and reference sample within a half log.