B190 Bias in Quantitative Polymerase Chain Reaction (qPCR): Does It Matter for Forensic Applications?

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Learning Overview: After attending this presentation, attendees will understand the different sources of bias in quantitative PCR (qPCR) and the impact on downstream Short Tandem Repeat (STR) genotyping processes.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by bringing attention to the differences in quantitation standards as well as differences in commercial quantitation kits. It will aid the forensic community in better understanding bias and its effect on downstream processes such as STR genotyping.

Commercial STR assays used by the forensic human identity community require tight control of the amount of DNA template amplified by PCR. This requires the ability to accurately measure the concentration of human genomic DNA in a casework sample extract prior to PCR amplification. Commercially available qPCR kits are routinely relied upon to determine the concentration of casework extracts; however, these kits rely upon commercial DNA standards for relative quantitation estimates. qPCR measurements do not provide the absolute concentration of a DNA extract but rather the relative concentration compared to the standard curve. Variation in these quantitative measurements may lead a laboratory to possibly overestimate or underestimate the concentration of an unknown extract and that may lead to incorrect dilution into the PCR workflow.

Variation that may lead to bias in qPCR results originate from a variety of other sources such as, but not limited to, pipette errors, commercial kit targets, accessibility of the amplification target within the human genome, and variation in the concentration of commercial DNA standards present within qPCR kits.[1] Additionally, it has been shown that the source of commercial DNA standards (i.e., blood versus cell lines) can impact the reliable quantification of DNA extracts and prompted the need for an international standard reference DNA material.[2,3]

To investigate the sources of bias, a set of 80 human blood samples (spanning across three U.S. populations) were evaluated with three commercial qPCR kits. This set of samples allows for sample bias to be investigated along with commercial qPCR kit bias. Standard curves were generated with the commercial DNA standard provided within the qPCR kit and SRM 2372a to investigate the effect on the quantification on unknown extracts.[4]

To examine the impact (if any) of the downstream PCR process, STR genotyping was conducted on a subset of the samples. Additionally, droplet digital PCR (ddPCR) was used to determine the quantity of the unknown extracts examined. Digital PCR allows for absolute quantitation of an extract without the need of an external calibrant. Recently, the National Institute of Standards and Technology (NIST) developed ten ddPCR assays that span 8 chromosomes within the human genome for the certification of SRM 2372a.[5]

Reference(s):

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3. Kline M.C., Duewer D.L., Redman J.W., Butler J.M. NIST Mixed Stain Study #3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. (2003) *Anal Chem* 75(10):2463-2469.
4. Romsos, E.L., Kline, M.C., Farkas, N., Duewer, D.L., Toman, B.: Certification of Standard Reference Material 2372a Human DNA Quantitation Standard. (2018) *Special Publication* (NIST SP). 260-189.
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SRM 2372a, Quantitative PCR, qPCR