DEVELOPMENTAL VALIDATION OF THE POWERQUANT™ SYSTEM

Margaret M. Ewing[1], Jonelle M. Thompson[1], Robert S. McLaren[1], Vincent M. Purpero[2], Kelli J. Thomas[2], Patricia A. Dobrowski[3], Gretchen A. DeGroot[3], Erica L. Romsos[4], Douglas R. Storts[1]

[1]Promega Corporation, 2800 Woods Hollow Rd., Madison, WI 53711
[2]Wisconsin State Crime Laboratory – Madison, 4626 University Ave., Madison, WI 53705
[3]Wisconsin State Crime Laboratory – Milwaukee, 1578 S. 11th St., Milwaukee, WI 53204
[4]National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8314

The PowerQuant™ System is a 4-target qPCR multiplex for quantification of DNA samples recovered from forensic evidence that includes a multicopy autosomal target (84bp) for quantification of total human autosomal DNA, two multicopy Y-chromosome specific targets (81bp and 136bp) to estimate the concentration of human male DNA, and a multicopy degradation target (294bp) to enable assessment of human DNA degradation. The longest target in the system is the 435bp internal PCR control (IPC) and its amplification performance may be used to gauge the presence of PCR inhibitors and help distinguish inhibition from degradation in a DNA sample. When DNA is degraded, the DNA concentration obtained with the autosomal target ([Auto]) is greater than the degradation target concentration ([D]) and the resulting [Auto]/[D] ratio provides information about DNA integrity. The ratio of the autosomal DNA concentration to the concentration of human male DNA ([Y]) may be used to determine whether the sample contains a mixture of female and male DNA ([Auto]/[Y]). Data provided by the different targets in the PowerQuant™ System may be used to identify a favorable workflow for a sample and normalize the DNA concentration prior to short tandem repeat (STR) analysis.

A developmental validation was completed following the *Scientific Working Group on DNA Analysis Methods* (SWGDAM) *Validation Guidelines for DNA Analysis Methods* with the PowerQuant™ System to examine the performance. The following selections from the developmental validation studies are presented: a population study to evaluate copy number variation of the autosomal, Y and degradation targets; quantification of samples with low concentrations of human DNA to establish the sensitivity of the assay; stability studies with samples containing PCR inhibitors or degraded DNA; and an investigation of the assay's ability to generate reliable [Auto]/[Y] ratios with samples containing mixtures of female and male DNA.

Key Words: qPCR, quantification, PowerQuant™, [Auto]/[D], [Auto]/[Y], inhibitors, degraded human DNA