Validation Report:

LEP Transgene

Support: 1-877-66MOUSE
orders@mendelworks.com
www.mousegenotyping.com

MOUSE INFORMATION

Transgene name:	LEP Transgene
Gene location:	Unknown
Type of modification:	Random insertion of a construct containing coding sequence of the human leptin (LEP) gene.
Mouse Line:	LEP 893C0

INVESTIGATOR INFORMATION

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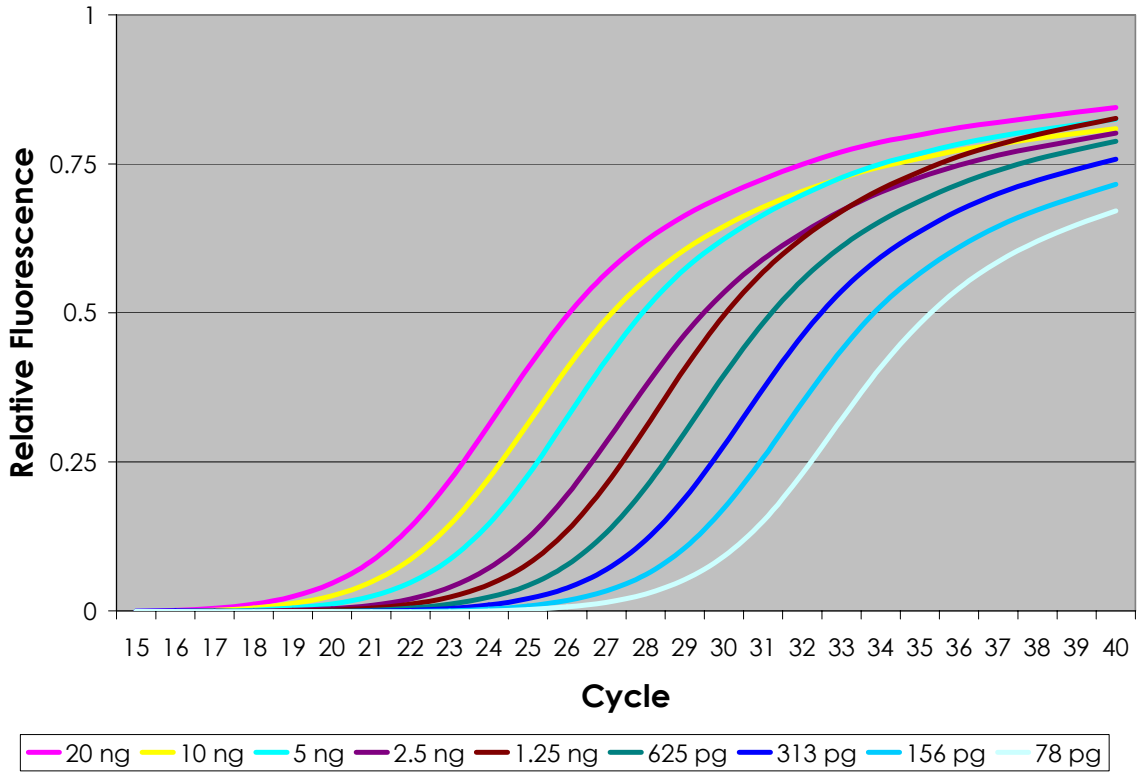
qPCR Assay – Sensitivity



Standard curve:	20ng, 10ng, 5ng, 2.5ng, 1.25ng, 625pg, 313pg, 156pg, 78pg, 39pg
Sensitivity:	<80 pg

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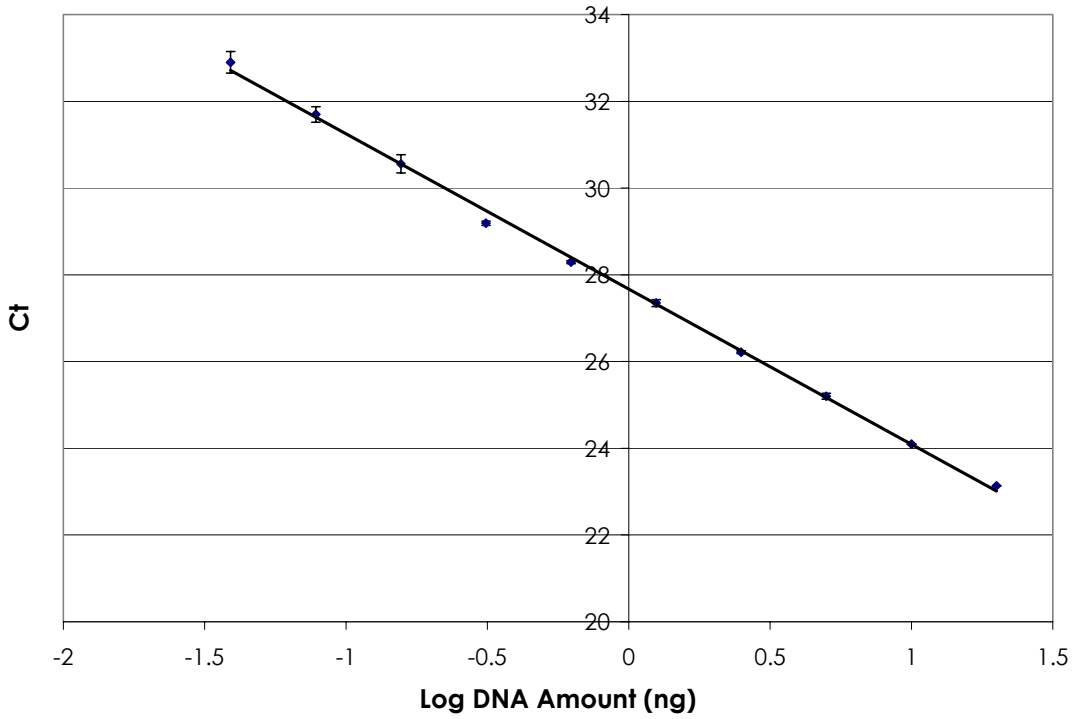
qPCR Assay – Efficiency

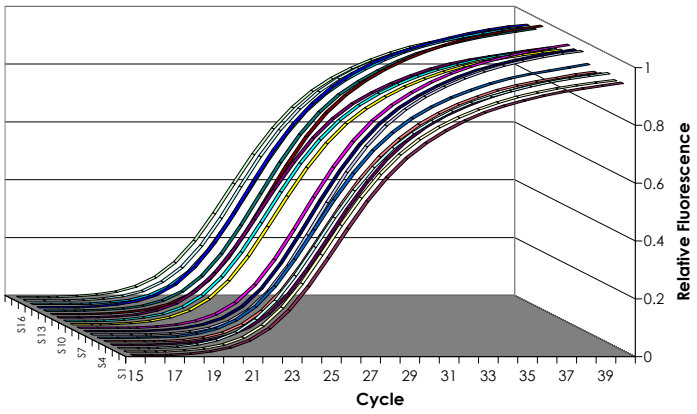


Standard curve: 20ng, 10ng, 5ng, 2.5ng, 1.25ng, 625 pg, 313pg, 156pg, 78pg, 39pg

$y = mx + b$

b = 27.671 m = -3.5793 R^2 = 0.9984 Efficiency = 90.3%

qPCR Assay - QC check (Amplification)



Relative fluorescence: 0.87 – 0.99 **Threshold:** 0.185 **Ct:** 21-25

Quantitative Zygosity Testing

Specificity: Based on the information provided by our clients, we design the best possible qPCR assay to the region(s) of interest. In addition, we will perform homology checks to make sure that the target domain for the assay is truly specific to the region in question and does not detect any other regions of the genome, where applicable.

qPCR Assay - Sensitivity: Using a serial dilution experiment, the threshold cycle (Ct) value for every dilution is determined. In principle, the sensitivity of the reaction is determined by the lowest dilution that is co-linear with all the other standard dilutions resulting in a high R^2 value (see "**qPCR Assay – Efficiency"** section). Typically, Ct scores greater than 35 are deemed too high for reliable genotyping.

qPCR Assay - **Efficiency:** The accuracy of real-time PCR is highly dependent on PCR efficiency. We determine qPCR amplification efficiency using serial dilutions. The template DNA is serially diluted and qPCR reactions are run for each dilution. Subsequently, the Ct values for each reaction are plotted against the log of the known amount of input DNA, for each dilution. From the slope of the linear regression line obtained, the amplification efficiency is calculated. The equation, y = mx + b, provides a measure of the reaction's efficiency across all pertinent DNA dilutions based on the slope of the curve (m). Efficiency (E) is calculated by the following equation: $E = 10^{-1/m} - 1$. Therefore, if the slope is near -3.322, then E = 1, which means that amplicon quantity doubles with every cycle. Yet, for example, if E = 0.8, the amplicon quantity is only duplicated every 1.25 cycles, which may be too low an efficiency to be reliable. Typically, slopes between -3.1 and -3.7 are acceptable. The y-intercept (b) is the number of PCR cycles (Ct) before a signal is detected when 1 ng of target DNA is present. Finally, R^2 is a statistical measure of how well the regression line approximates the data points. An R^2 of 1.0 (100%) indicates a perfect fit.

qPCR Assay - QC check (Amplification): Our qPCR assays employ dual-labeled fluorogenic probes designed to incorporate a 5' reporter dye and a 3' quencher. The derivation of a sigmoidal curve on a plot of relative fluorescence (RF) versus cycle number is indicative of successful qPCR amplification. The sigmoidal curve is a representation of the kinetic characteristics of the qPCR assay. Typically, during the initial cycles (cycles 1 - 15), the amount of amplicon present is too low for detection. Around cycle 15 or thereafter, enough PCR product has been produced for the instrument to detect a signal. It is during this exponential phase of amplification, that every cycle yields a detectable doubling of the amplicon. It is also during this phase that a point on the curve can be established and threshold can be determined. Threshold is the cycle number (Ct) at which the fluorescence signal is significantly above baseline. Our Amplification QC check is a measure of how well the qPCR assay behaves across multiple samples. Using the same threshold (typically between 0.05 and 0.2) across independent qPCR assays of the same reaction type, we determine each assay's amplification kinetics and Ct values. If all assays yield comparable sigmoidal curves and similar Ct values, the assay is validated for genotyping.