Real Time PCR Quality Control

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1 Executive Summary

James Shira, Arizona Research Laboratories, came to the Stats Consulting Lab free student session to present and evaluate their lab's current real time PCR (polymerase chain reaction) quality control procedure. James desired validation of the current method or alternative solutions. The group explored the method and found it to be satisfactory. Further validation of the method is suggested through more data exploration and researching regulatory guidelines.

2 Detailed Summary

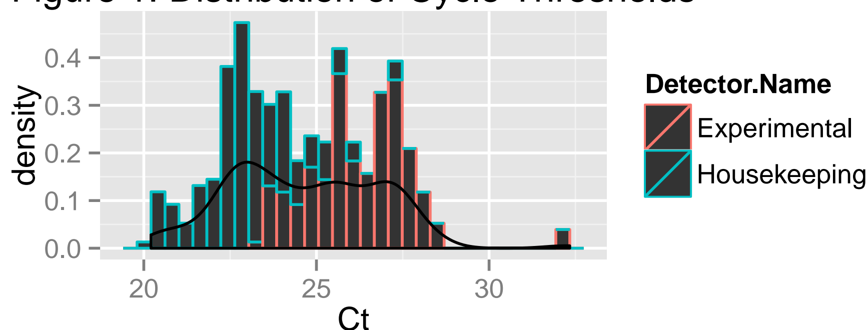
2.1 Current Method

In their lab protocol, all biological samples are repeated in triplicate to assess repeatability of the PCR process. One measure from each replicate is the Cycle Threshold, C_t, which is the number of PCR samples required to meet a critical threshold of light intensity. The current method examines the within sample variability through the standard deviation of the C_t values. Any technical replicate with a SD_{Ct} greater than 0.5 is flagged as failing the QC.

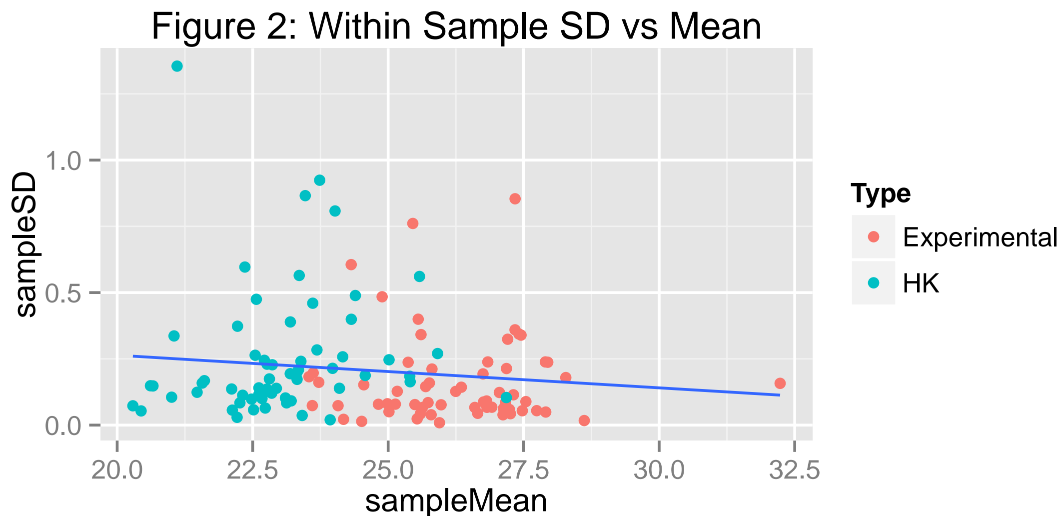
2.2 Exploratory Data Analysis

The data provided by James are 389 observations of real time PCR measurements. The most vital features of the data are C_t, $Sample.Name$, and $Detector.Name$. $Sample.Name$ is the biological sample label and detector name (from here on referred to as $Type$) is whether the gene is experimental or housekeeping in nature. Half of the observations are from the experimental type and half from housekeeping with a triplicate of the biology sample for both types. See the last page for details.

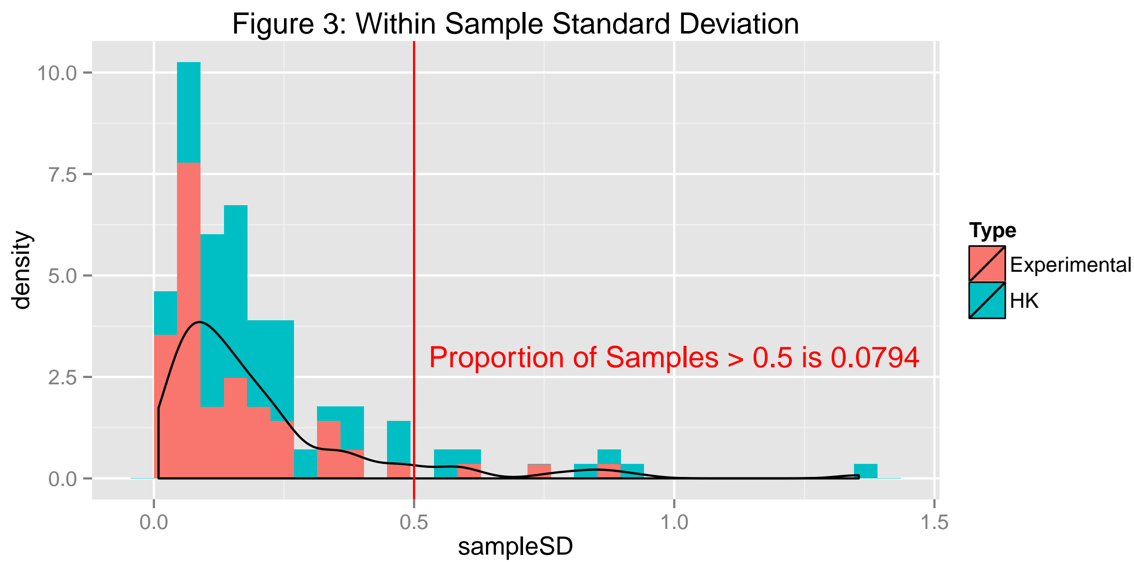
First the distribution of C_T values for all 389 observations is visualized in Figure 1. Note that the shape is bimodal with the experimental samples displaying the same shape, but with a higher center and outlying samples.



The difference in means between detector types suggests that a cutoff for standard deviation may need to account for any variation inflation due to a greater mean. The bivariate relationship between within sample means and within sample standard deviation will provide insight into whether variation inflation is occurring. No apparent relationship between variance and mean indicates that standard deviation will be appropriate to compare within sample variability. If variation inflation had been a factor then a transformation or another measure such as coefficient of variation would be options.



Lastly, the distribution of within sample standard deviations was explored. The main consideration here is the validity of the 0.5 cutoff as a QC criteria. The cutoff removed 8% of the observations. The distribution is right skewed and 0.5 appears to be an appropriate cutoff. No apparent bias as far as type of detector is suggested.



2.3 Results & Discussion

Based on the empirical distribution of within sample standard deviation, the 0.5 QC cutoff works well for these data. More data should be explored to fine tune and critique this cutoff. The cutoff could be relaxed or strengthened based on particular AFL client needs. Regulatory guidelines should be researched in order to further increase credibility and receive certification.

	Experimental	Housekeeping
ALPHA.1	3	3
ALPHA.2	3	3
ALPHA.3	3	3
BETA.1	3	3
BETA.2	3	3
BETA.3	3	3
Control 1	3	3
Control 1.1	3	3
Control 1.2	3	3
Control 2	3	3
Control 2.1	3	3
Control 2.2	3	3
Control 3	3	3
Control 3.1	3	3
Control 3.2	3	3
Control 4	3	3
Control 4.1	3	3
Control 4.2	3	3
Control 5	3	3
Control 5.1	3	3
Control 5.2	3	3
DELTA.1	3	3
DELTA.2	3	3
DELTA.3	3	3
EPSILON.1	3	3
EPSILON.2	3	3
EPSILON.3	3	3
KAPPA.1	3	3
KAPPA.2	3	3
KAPPA.3	3	3
OMEGA.1	3	3
OMEGA.2	3	3
OMEGA.3	3	3
PI.1	3	3
PI.2	3	3
PI.3	3	3
PSI.1	3	3
PSI.2	3	3
PSI.3	3	3
RNA 12.1H	3	3
RNA 12.1N	3	3
RNA 12.2H	3	3
RNA 12.2N	3	3
RNA 12.3H	3	3
RNA 12.3N	3	3
RNA 24.1H	3	3
RNA 24.1N	3	3
RNA 24.2H	3	3
RNA 24.2N	3	3
RNA 24.3H	3	3
RNA 24.3N	3	3
RNA 3.1H	3	3
RNA 3.1N	3	3
RNA 3.2H	3	3
RNA 3.2N	3	3
RNA 3.3H	3	3
RNA 3.3N	3	3
RNA 48.1H	3	3
RNA 48.1N	3	3
RNA 48.2H	3	3
RNA 48.2N	3	3
RNA 48.3H	3	3
RNA 48.3N	3	3