Differential Pipetting

More accurate Pipetting **improves QPCR** and **galvanizes NGS,** aka Yes, Virginia, *of course* ***accuracy matters***! aka NGS/PCR 101 Up-Front Pipetting for Dummies

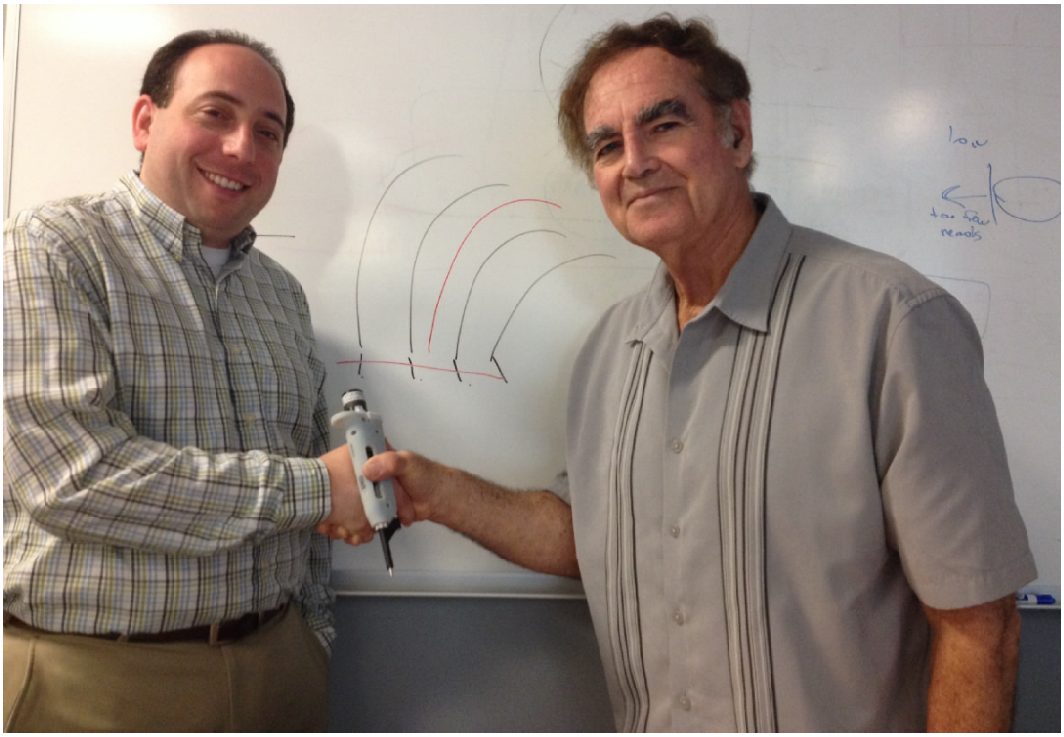
I have included things Hal Schneider said to me in “quotes” because I found them pleasingly clarifying and they stuck with me as pithy NGS pearls (neither of us claims linguistic originality).

“One strand of DNA is like a page in a book” in that it is the form or level that can be read by a sequencer like the Illumina“.

“The quantitative “Q“ in QPCR means that you need to know the QUANTITY of DNA in your sample”. “PCR is the technique for multiplying the DNA so you get up to a level where you can measure it, which must be done against the Standard Curve”.

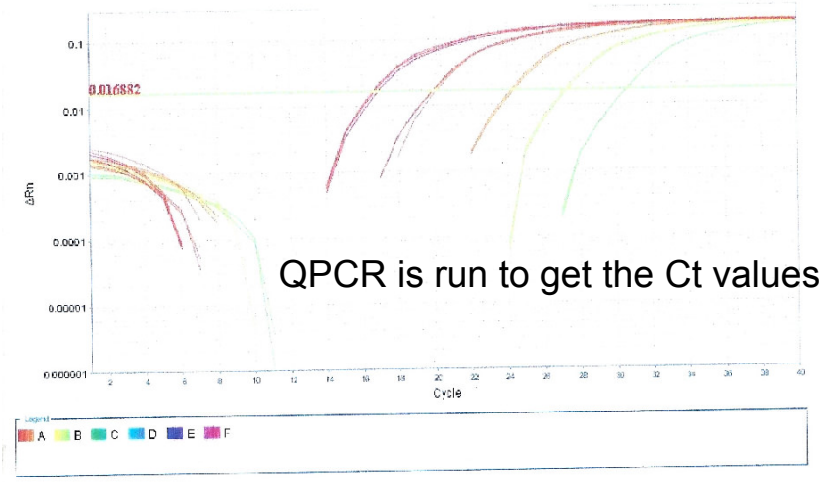
“The most important liquid-handling step for NGS is to try to pipette the accurate amount of material into the Standard Curves for the QPCR. Because this is so critical but often demands minimum sample it is often entrusted to manual pipetting by a highly skilled technologist, typically taking 2-3 hours for what mounts up to a few hundred pipettings (driven by triplicates because of occasional misses by even the best traditional pipettors). Only one tech does this because you don’t want more than one person’s hands on it”.

“ Efficient sequencing requires that the pooled genetic material you feed the sequencer is in a “happy zone” which lets the sequencer make the correct number of reads of clusters that are clean and free of overlapping fragments. And every sequencing run has a price tag“.



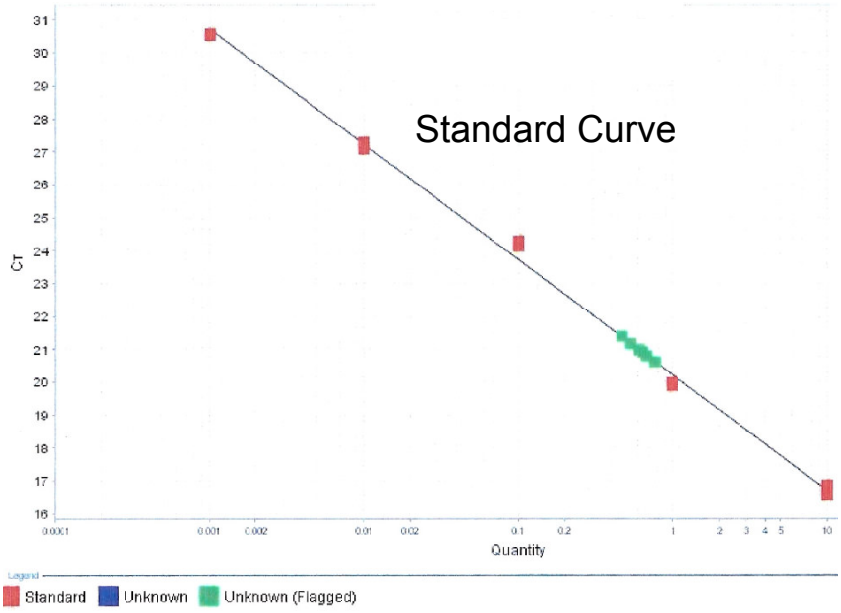
Hal Schneider of Claritas Genomics was formerly
Clinical Laboratory Manager of the Genetics
Diagnostic Laboratory at Boston Children’s Hospital

One does dilutions of the DNA Standard and of patient library samples that cover an extremely wide concentration range.



QPCR is run to get the Ct values

QPCR is run on both the DNA Standards and the patient Samples to determine the CT values. This is an example of a random rather than optimized set of curves.



This critical Standard Curve is then generated from the above data and used to give the DNA concentration in the patient samples.

"So the more accurate the pipetting early on [in the NGS process] the more accurate is the Calibration Curve and hence the most accurate quantification -- the most accurate concentration of the DNA in your sample". Better up front pipetting saves huge $ amounts in the sequencing that follows.

Any comments or critique on this are welcomed.

Donald Schwartz, President
Differential Pipetting, Inc.