

# Real Time PCR as a Method to Characterize DNA

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## ***Introduction***

Our current genotyping method includes measuring the optical density of each sample after the DNA is isolated. The final step of the Transnetyx genotyping method is a real time PCR reaction. Each sample is tested with a probe or a group of probes that was designed specifically for the strain. Each sample is also run with a positive control house-keeping gene. The house-keeping gene is used to characterize the DNA quantity and quality as well as be used mathematically to determine a “relative copy number” or RCN.

Optical density measurements are a common method for quantifying DNA yield. This method is popular for several reasons. Optical density measurements are passive; they do not affect the sample or react with it in any way. As a result, a concentration can be determined without losing any sample yield. It is quick; with the correct equipment, 384 samples can be measured in less than a minute. However, there are some drawbacks to using optical density as a means to determine DNA concentration. The method does not distinguish between types of nucleic acids. Single stranded DNA, double stranded DNA and RNA are all detected with an OD. Also, an OD is not sensitive to contaminants in the sample.

Real time PCR can also be used to quantify DNA yield, however sample is consumed during the process. Also, preparing a sample for real time PCR is both timely and expensive. However, real time PCR can be more informative than optical density measurements. PCR is very susceptible to contamination. If a sample yields good amplification, it is

very likely that there is little to no contamination in the sample. Also, both single stranded and double stranded DNA can be detected with real time PCR.

## ***Purpose***

We noticed through the course of testing, that samples that fall below the detectable limits of the optical density reading often have very good, and useful amplification results. As we began to look closely at OD data versus real time PCR data, we found that the OD data did not add any value to our process. Although OD readings are inexpensive and fast, our protocol already involves the downstream use of real time PCR.

## ***Experimental Design***

A serial dilution of whole genomic mouse DNA was performed, resulting in DNA samples ranging from 1.875 - 60 nanograms per microliter. The DNA was loaded into the wells of a 384 well optical plate and an OD260 measurement was taken on a Tecan Genios Instrument with the following settings:

<i>Meas. Mode:</i>	<i>Absorbance</i>
<i>Ref. Wavelength:</i>	<i>0 nm</i>
<i>Number of Flashes:</i>	<i>5</i>
<i>Temperature:</i>	<i>24.3C</i>
<i>Volume:</i>	<i>100µL</i>

The DNA was then used as template in a Real Time PCR run using two different real time reactions. The first probe is the standard, Transnetyx, house-keeping probe that is run with every sample, the second is a standard wild type probe that we developed for one of our customers. Universal PCR conditions were used with 20mL reaction volumes. Each sample was run in triplicate for each probe.

## Results

### OD Method

Sample Name	OD <sub>260</sub>	Conc. (ng/μL)
C1 - 60	1.641	60
E1 - 30	0.971	30
G1 - 15	0.520	15
I1- 7.5	0.278	7.5
K1 - 3.75	0.143	3.75
M1 - 1.825	0.077	1.875

Line Fit	
Slope	0.03
y-intercept	0.07
R <sup>2</sup>	0.99

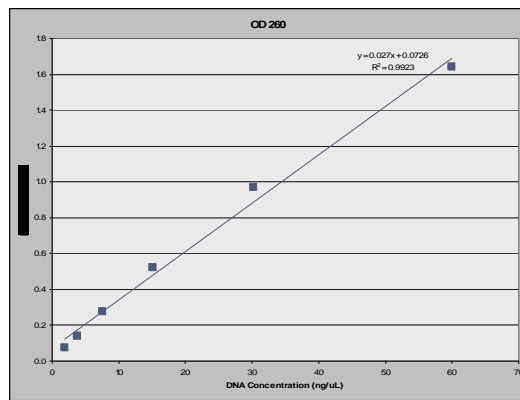


Figure 1. Standard Curve using OD Method

### PCR Method with Transnetx house-keeping probe

Sample Name	Average CT	Stdev CT	Conc. (ng/μL)
C1 - 60	21.28	0.17	60
E1 - 30	22.44	0.13	30
G1 - 15	22.98	0.04	15
I1- 7.5	23.95	0.07	7.5
K1 - 3.75	24.99	0.09	3.75
M1 - 1.825	25.87	0.08	1.875
O1 - water	Und	0	0

Line Fit	
Slope	-3.00
y-intercept	26.66
R <sup>2</sup>	0.99

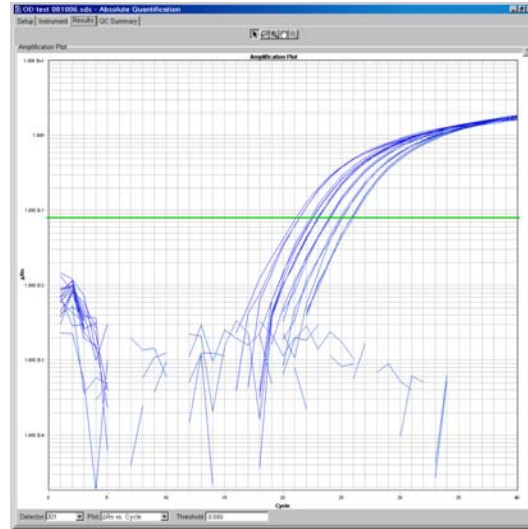


Figure 2. PCR amplification with house keeping probe

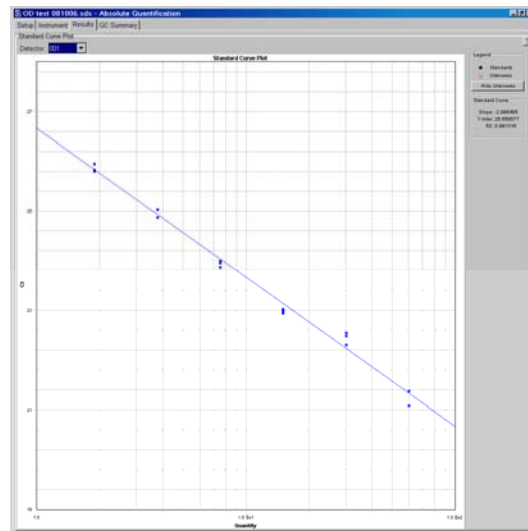
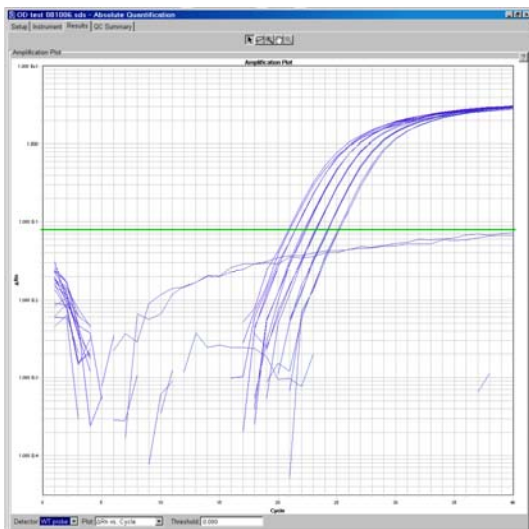


Figure 3. Standard curve with house keeping probe

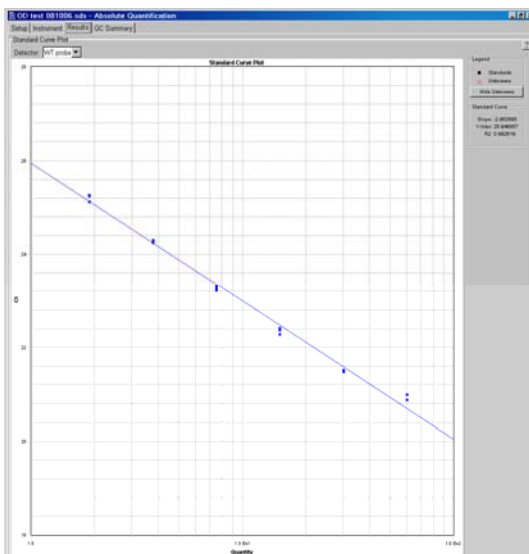
### PCR Method with Transnetx wild type probe

Sample Name	Average CT	Stdev CT	Conc. (ng/μL)
C1 - 60	20.94	0.08	60
E1 - 30	21.51	0.02	30
G1 - 15	22.36	0.07	15
I1- 7.5	23.27	0.04	7.5
K1 - 3.75	24.30	0.04	3.75
M1 - 1.825	25.22	0.08	1.875
O1 - water	Und	0	0

Line Fit	
Slope	-2.95
y-intercept	25.95
R <sup>2</sup>	0.99



**Figure 4. PCR amplification with wild type probe**



**Figure 5. Standard curve with wild type probe**

### ***Conclusion***

The results indicate that PCR is just as sensitive as an optical density reading in determining DNA quantity. We also have data that shows that a real time PCR measurement is more sensitive at low concentrations than optical density readings (data not shown). The accuracy of the Transnetyx genotyping protocol will not be affected by the removal of the optical density measurement.