Correlation of Spot Density with DNA Quantity Using AlphaQuant® Molecular Ladders

Application Note 104

Abstract

DNA analysis is a corner stone of molecular biology. Electrophoresis of DNA in agarose gels is among the most commonly used techniques for DNA analysis. Internal standards can be incorporated into DNA agarose gel experiments to maximize the quantitative information acquired in the experiment. DNA ladders are typically employed to determine the molecular size (number of base pairs) of double stranded DNA (dsDNA). The traditional method for quantifying the mass of DNA in agarose gels has been to extract the DNA from the gel in a series of clean up steps (usually involving chloroform), followed by a UV-VIS spectrophotometer reading. AlphaQuant Molecular Ladders provide DNA markers standardized for both molecular size and quantity of DNA. This eliminates the need for excision of DNA bands and extraction of DNA from agarose gels. AlphaQuant Molecular Ladders provide a convenient and fast method to determine size and quantity of unknown DNA samples in agarose gels.

Introduction

Size Determination

Typical molecular weight ladders are made of a set of linearized dsDNA fragments of different lengths. Molecules of linear dsDNA travel through agarose gels at a rate that is inversely proportional to the log of their molecular weight; the shorter the length of dsDNA the faster it will travel. The lengths of DNA chosen to create the ladder should have migration characteristics that result in a specific banding pattern in the agarose gel. A good quality ladder should meet the following criteria. It should consist of frequently occurring, evenly spaced DNA bands. The sizes of DNA fragments responsible for the banding pattern in the ladder should encompass the sizes of DNA fragments being analyzed (this allows for direct comparison of the bands in the ladder with the bands in the unknown). Reference bands for quick and easy orientation are very useful. Finally, there should be baseline resolution between the bands of the ladder.

Mass Determination

A key quality measure for a DNA mass standard is linearity. Linearity is the characterization of variation between a set of standards over a predetermined range of values. AlphaQuant Molecular Ladders quantify DNA from 15 ng–120 ng. Quantities of DNA within this range are measured as the intensity of the fluorescent signal generated by Ethidium Bromide dye associated with the DNA in an agarose matrix. Ethidium Bromide contains a planar group that intercalates between the stacked bases of DNA. The orientation and proximity of Ethidium Bromide with the stacked bases causes the dye to display an increased fluorescence compared to free dye (about a 20X increase in fluorescence). UV radiation at 302 nm is absorbed by the DNA and transmitted to the bound dye. The energy is re-emitted at 590 nm in the red-orange region of the spectrum. The FluorChem® SP is an imaging system designed and marketed by Cell Biosciences. One of the filters used by the Fl orChem SP is an interferencefilter (band pass filter) that blocks wavelengths less than595 nm. Light of appropriate wavelength passes throughthe filter and is detected by the silicone chip of the CCDcamera of the imaging system. The intensity of this light isregistered by pixels in the silicone chip. Integrated DensityValue (IDV) is the sum of all pixel values after background correction:

$$IDV = \sum (\text{each pixel value} - \text{background})$$

When AlphaQuant Molecular Ladders (stained with EtBr in an agarose gel) were analyzed on the FluorChem SP, each band in the ladder generated an Integrated Density Value. Correlation of the band IDV with the known mass of DNA in the band results in a standard (STD) curve. Samples with unknown amounts of DNA can be analyzed

and compared with the STD curve. The amount of DNA present in the unknown can be interpolated from the STD curve based on a measured IDV of the unknown.

Materials and Methods

AlphaQuant Molecular Ladder Number 1 (Cell Biosciences, Santa Clara, CA) was diluted with loading buffer and loaded in duplicate lanes on a 2% agarose gel containing ethidium bromide (Cambrex Bio Science, Rockland, ME). The following matrix describes the dilution scheme and the lane assignments for this experiment.

AlphaQuant #1 (µL)	Loading Buffe r (µL)	Lane Assignments
5	5	1, 2
4	6	3, 4
3	7	5, 6
2	8	7, 8
1	9	9, 10

Table 1. The gel was run for 45 minutes at 80 volts (Gibco BRL Life Technologies Model 250) in 1x TBE buffer. The gel was analyzed on the FluorChem SP gel documentation system (Cell Biosciences, Santa Clara, CA) using a 595 nm filter.

Results and Discussion

In this study, AlphaQuant 1 Molecular Ladder was used as the standard. This ladder is composed of 2 sets of bands that increase in molecular size (number of base pair (bp)) and mass (ng DNA) from bottom to top in the lane. The lower set of bands starts with a band containing 20 ng DNA and increases to 100 ng DNA. The upper set of bands is of particular interest because these are the bands that were analyzed in this experiment. Specifically, bands containing 15, 20, 25, 30, 40, 50 and 60 ng DNA were analyzed (Figure 1).

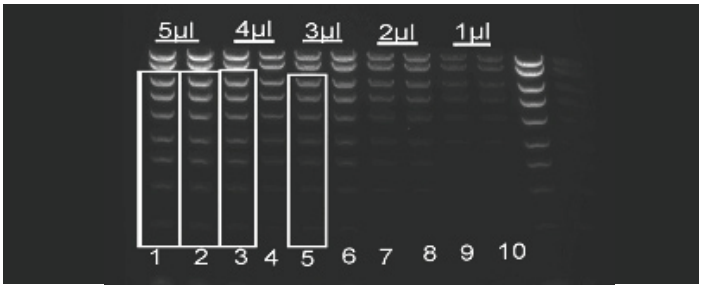

Figure 1. Lanes 1 and 2 are duplicate runs of a 5µl sample of the AlphaQuant 1 Molecular Ladder. In these lanes the bands from 15, 20, 25, 30, 40, 50 and 60 ng of DNA were analyzed and their IDV determined (Figure 1).

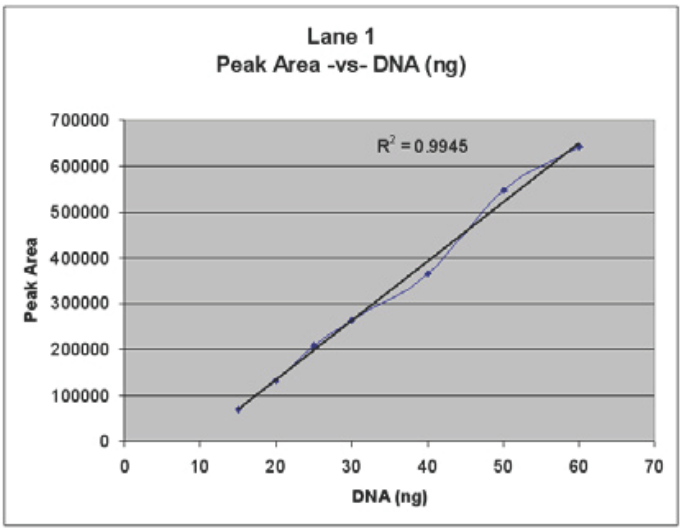

Figure 2A. Regression analysis of the quantity of DNA versus IDV for lane 1.

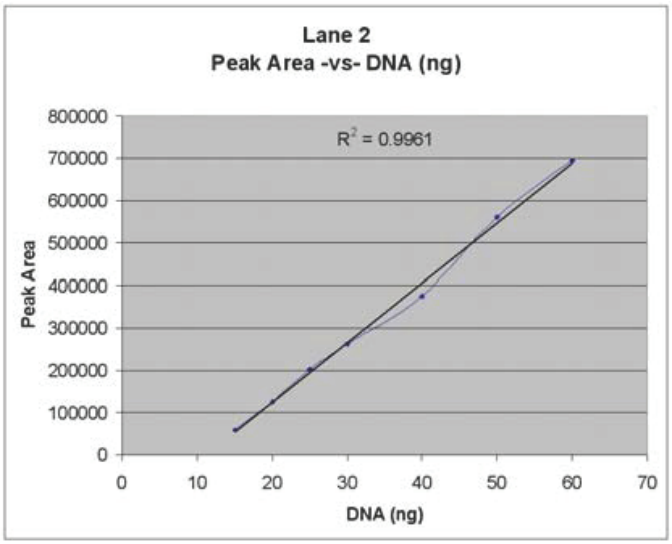

Figure 2B. Regression analysis of the quantity of DNA versus IDV for lane 2.

The coefficient of variation (R) for the two regression curves indicates a strong linear relationship between Peak Intensity (IDV) and DNA (ng) (Figures 2A and 2B). The R value for the duplicate samples in lanes 1 and 2 is 0.9945 and 0.9961 respectively.

For the DNA ladder tested in this experiment, the mass of DNA and the IDV yielded a strong linear correlation. This ladder contained a band with only 15 ng (the minimum required quantity for this ladder) of DNA. This was the lowest data point in the regression for lanes 1 and 2. The amount of AlphaQuant Molecular Ladder applied to subsequent lanes was decreased by serial dilution. The objective was to evaluate the strength of the correlation and linearity as the limit of detection for the system was approached.

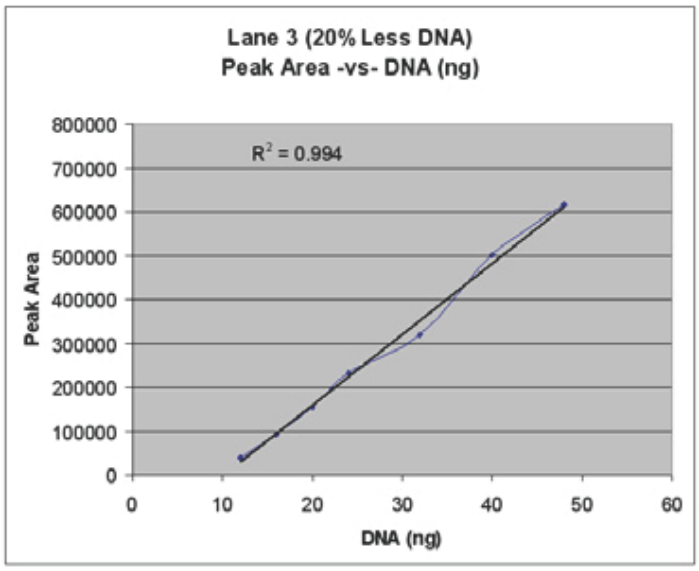

Figure 3. Regression analysis of the quantity of DNA versus IDV for lane 3.

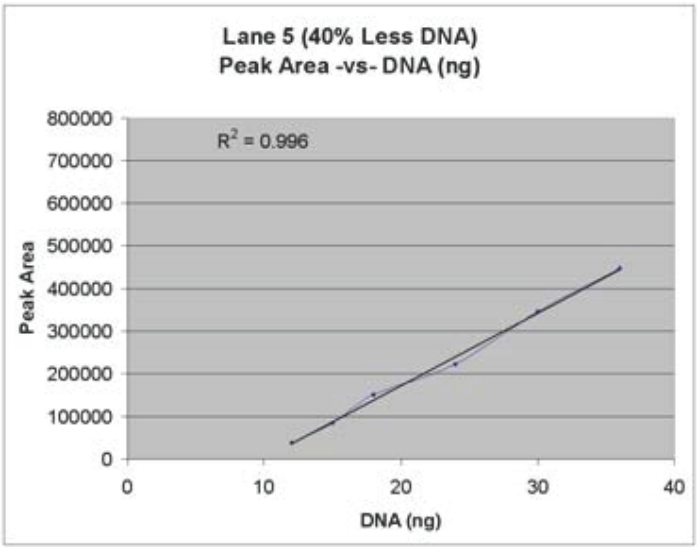

Figure 4. Regression analysis of the quantity of DNA versus IDV for lane 5.

For lane 3 the DNA level is 20% less than the minimum recommended amount. The system however has not reached the limit of detection and the correlation and linearity still remain strong (R=0.994) (Figure 3). Lane 4 was a duplicate of lane 3. The quantity of DNA is decreased again for lane 5. In lane 5 there was 40% less than the recommended minimum quantity of DNA. In lane 5, the band with the least amount of DNA faint to detect. This curve therefore has 6 data points (as opposed to 7 data points for previous lanes). Regression analysis of these data points shows a strong correlation and linearity (R=0.996) for the values that were within the detection limits (Figure 4).

This data clearly demonstrates that AlphaQuant Molecular Ladders are quantitative and exhibit excellent linearity. These results were achieved by using recommended amounts of AlphaQuant and the FluorChem SP's lower limit of detection. AlphaQuant Molecular Ladders are a high quality STD for the determination of molecular size and quantity of DNA.

Acknowledgements

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