qPCR Efficiency Calculator

About Kapa Biosystems

KAPABIOSYSTEMS | next generation thinking in enzyme technology

Kapa Biosystems was founded in 2006 to develop next-generation PCR reagents. Our molecular evolution technology platform is currently being used to optimize enzymes for DNA amplification, next-generation DNA sequencing, and molecular diagnostic applications.

The majority of biomedical and research applications were designed around a small subset of native enzymes. The limitations of these applications are often the result of constraints due to enzyme structure or function. Recent advances in molecular evolution have allowed enzymes to be tailored to suit specific application requirements. Enzyme engineering improves existing methods and provides the foundation on which entirely novel applications can be built.

Kapa Biosystems conducts advanced protein engineering, taking advantage of recent developments in genomics, proteomics, and synthetic biology.

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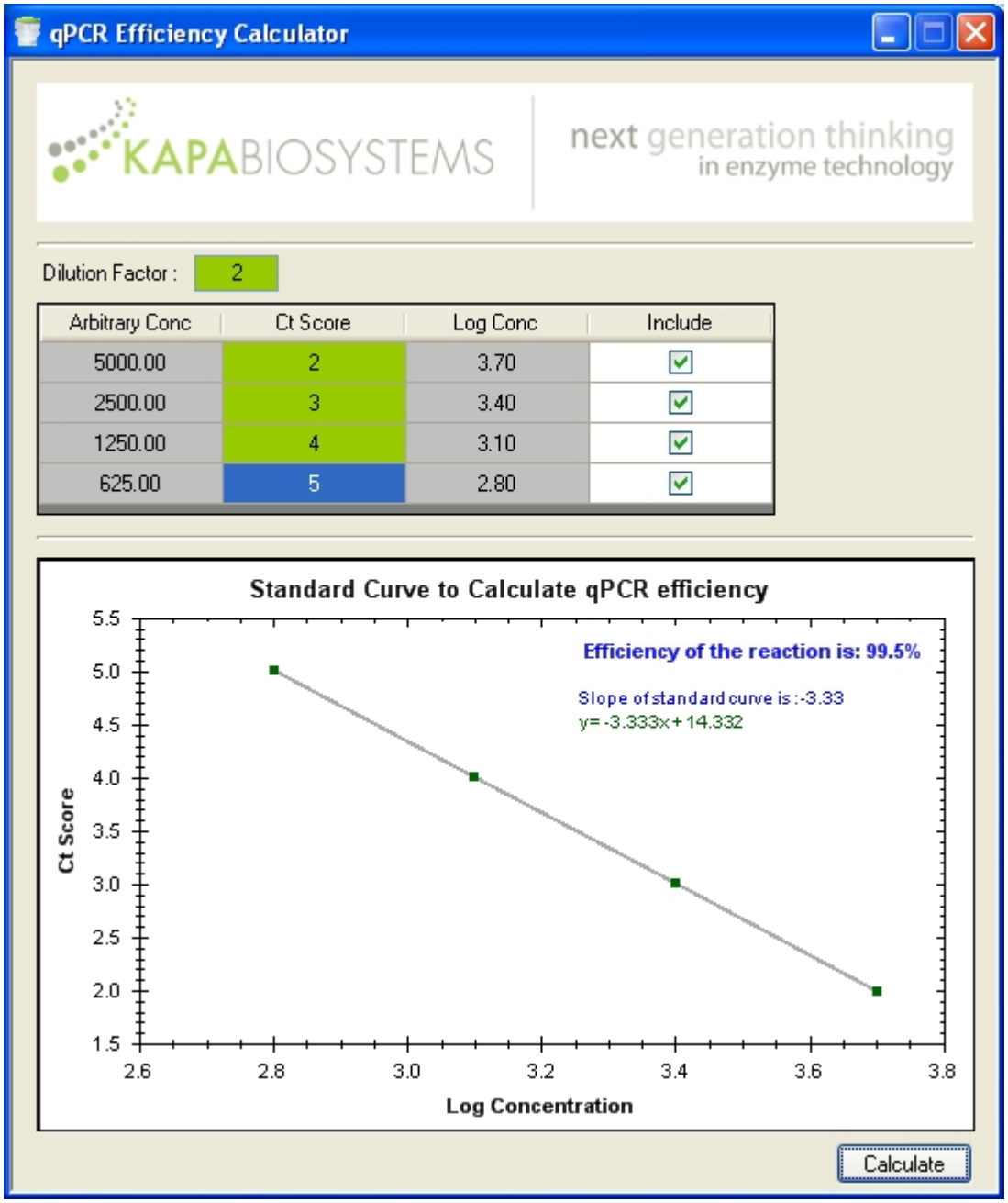
Introduction

This application has been designed to determine the qPCR efficiency of a serially diluted DNA Library sample using the Ct scores and dilution factor as the input. It generates a standard curve of the DNA library material that enables visualizing the precision of the serial dilutions. If there are any outliers due to dilution or pipetting inaccuracy they can be easily visualized and removed prior to linear regression analysis and subsequent slope calculation which is used in determining the qPCR efficiency. Although this application has been designed for use with the KAPA Library Quantification kits, it can also be used for calculating dilution-based qPCR efficiencies for all qPCR applications.



Dilution Factor

Insert a numerical value determined by the dilution factor of the library material. When performing the suggested library dilutions as set out in the KAPA Library Quantification Kit TDS, this value will be "*2*" as the library material is diluted two-fold. If using ten-fold dilutions, enter the value "*10*" into the dilution factor field.

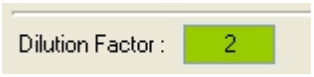


Image 2.1

Table Fields

The data grid (*see Image 3.1*) is used to enter values obtained from the qPCR instrument. The only value the user can enter is the Ct Score values. All values are used in calculating the final data points for the graph display.

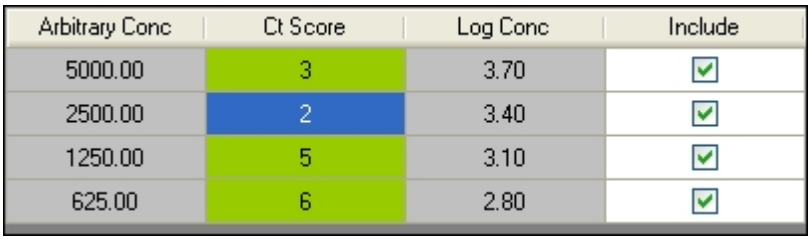


Image 3.1

Arbitrary Concentration

An arbitrary concentration (*see Image 3.2.1*) is automatically assigned based on the dilution factor. This is used to generate an arbitrary log concentration (this is used to generate the x-axis in the Standard Curve plot).

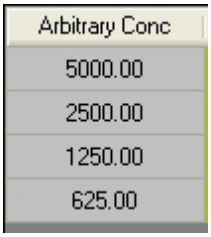


Image 3.2.1

Ct Score

Enter the Ct Score (*see Image 3.3.1*) for each of the library dilutions as determined by the qPCR instrument. If performing replicate reactions for each dilution then use the average Ct for each dilution set.

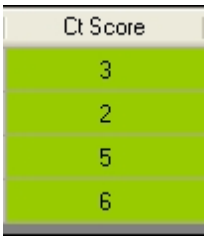


Image 3.3.1

Log Concentration

The log concentration (*see Image 3.4.1*) is obtained from the arbitrary concentration (automatically assigned based on the dilution factor). This is used to generate an arbitrary log concentration (this is used to generate the x-axis in the Standard Curve plot).

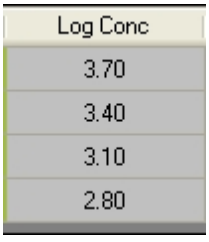


Image 3.4.1

Including/Excluding Outlier

To obtain accurate qPCR efficiency measurements, obvious outliers may be removed by de-selecting the outlier data point (*see Image 3.5.1*). Outliers can be visualized after entering **all** Ct scores (for all 4 library dilutions) and clicking the Calculate button. Ct scores are represented as **grey squares** on the Standard Curve plot. To remove an outlier, deselect the data point concerned and click on the Calculate button again. A best fit linear regression slope and data points (in **green squares**) is calculated.

Image 3.5.1

Graph Display

The white graph area is used to display the calculated data points when the user click on the Calculate button (*see Image 4.1*).

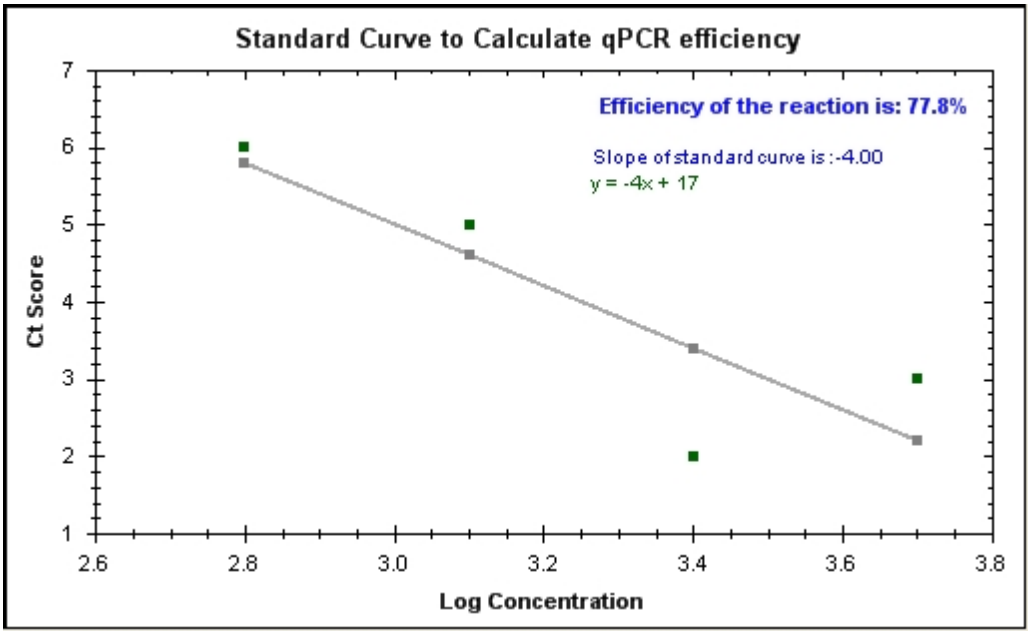


Image 4.1

The user can use a "right click" menu from anywhere within the white graph area (*see Image 4.2*). The most important functions will be covered in the following help section. The function "*Show Point Values*" will be ticked by default. Last mentioned function allows the user to move the mouse cursor over any data point, and view the relevant data point values.

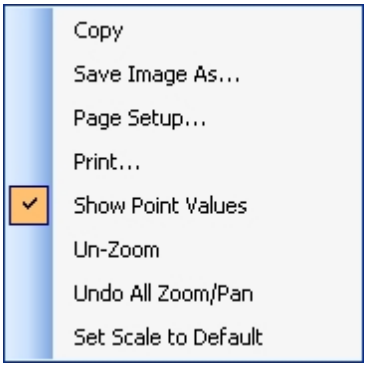


Image 4.2

Copy Graph

The copy graph function will place an image on the clipboard. The user can paste the image into any image or document manipulation software for editing. The copy function is handy when results must be noted and saved for later reference.

Save Graph

When selecting the "*Save as*" option the program will allow you to save a version of the graph into any location (*see Image 4.3.1*). This function is helpful when the developers must solve graphing problems (if any).

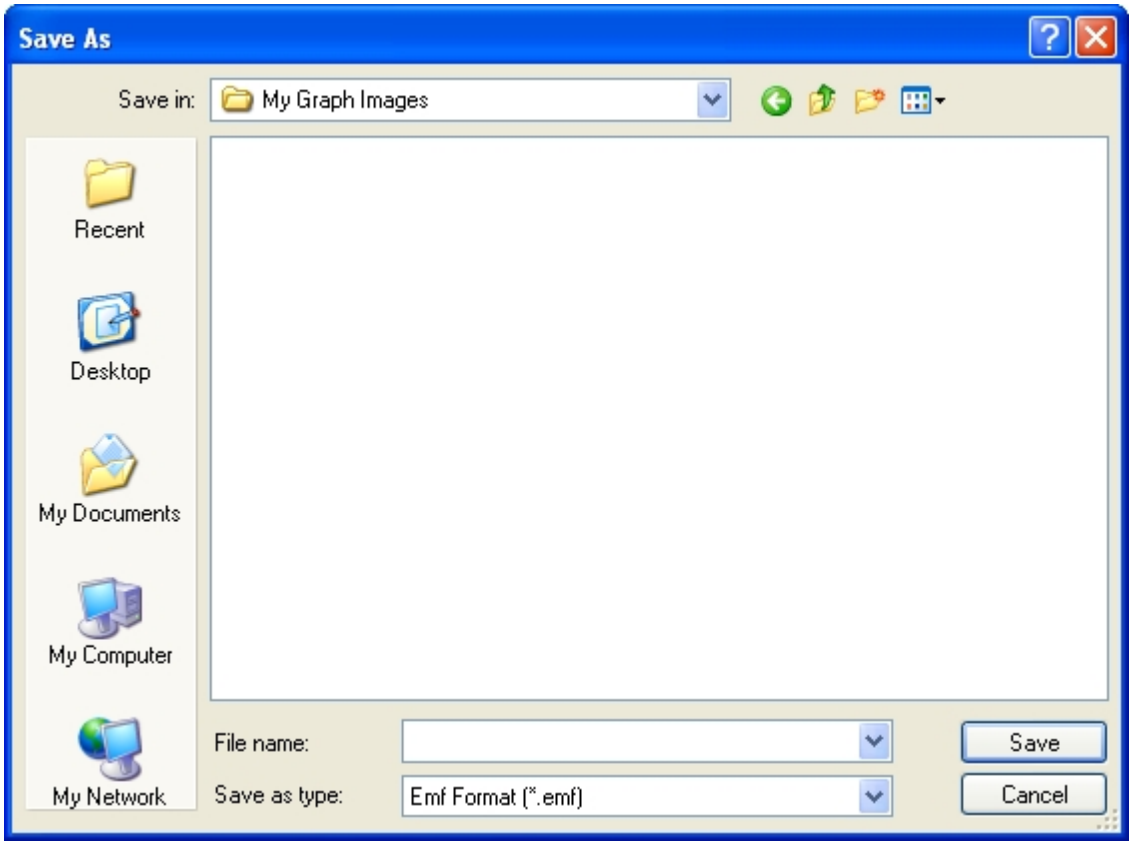


Image 4.3.1

Print Graph

The printing of graphs is useful when you want to refer back to results in a meeting or want to print the graph into PDF format. The page setup of the printout can also be done from the menu. The default Microsoft Windows printer selection box will be displayed on selection of the print function (*see Image 4.4.1*).

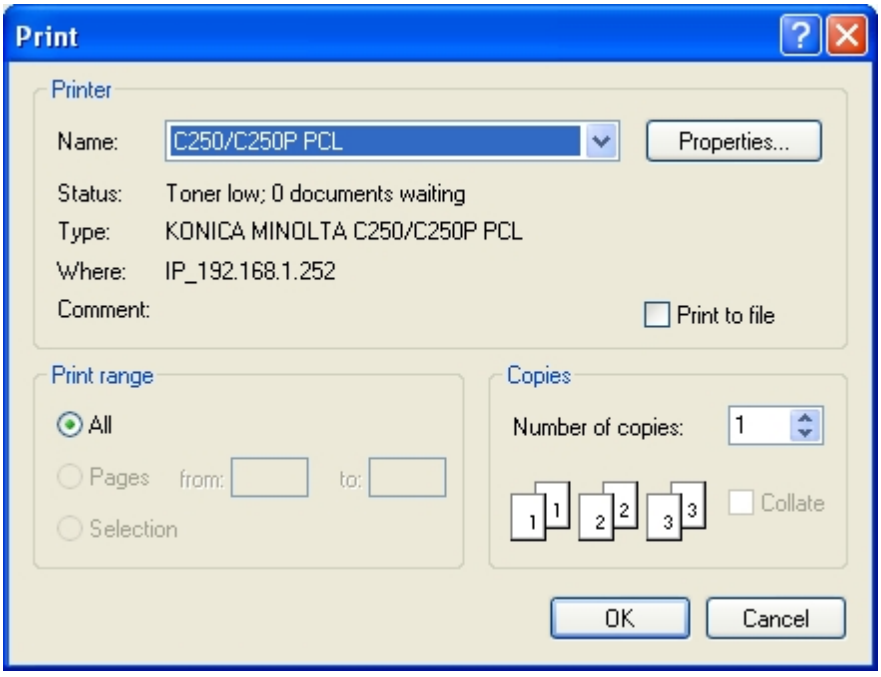


Image 4.4.1

Results

The graph is calculated and displayed every time the "*Calculate*" button is clicked. All data is re-calculated and a plot is generated on the graph area (*see Image 5.1*).

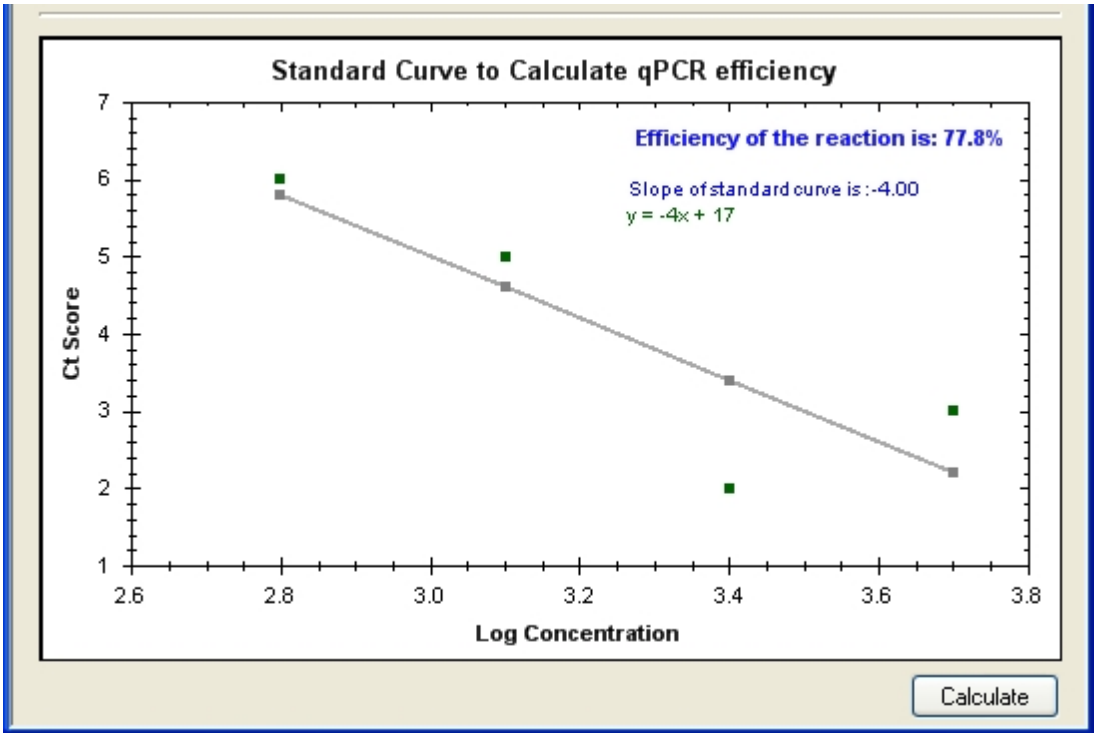


Image 5.1

Efficiency, slope and line formula is displayed on the plot area. The plot area scale is automatically calculated when the program receives the input in the data grid fields. Recalculation of plot area scale can be done from the graph "*right click*" menu.

Calculate Button

The slope of the linear regression line through the selected data points is used to calculate the efficiency of the qPCR reaction. Sub-optimal efficiencies may be the result of either sequential pipetting error during serial dilutions (including sub-optimal mixing of each successive dilution before the subsequent dilution) or sub-optimal qPCR efficiency due to the nature of the library template (this may be solved by increasing the combined annealing/extension time of the qPCR reaction from 45 sec to 75 sec).

Efficiencies of 95% – 105% are considered optimal for quantitative purposes although 90% – 110% may also be used for library quantification.

Linear Regression Calculation

The linear regression calculations are based on the following formula:

Regression Equation used is $y = a + bx$

where (b) is the slope of the equation and calculated

$$(N\Sigma XY - (\Sigma X)(\Sigma Y)) / (N\Sigma X^2 - (\Sigma X)^2)$$

where (a) is the intercept and calculated using

$$(\Sigma Y - b(\Sigma X)) / N$$

Note: N = the number of data points.

Point Values

Data points can be categorized into two different groups:

1.) Original Data

The above mentioned data category is entered by the user into the data grid (*see Image 5.4.1*).

Arbitrary Conc	Ct Score	Log Conc	Include
5000.00	3	3.70	☑
2500.00	2	3.40	☑
1250.00	5	3.10	☑
625.00	6	2.80	☑

Image 5.4.1

The user completes the **green** column with the values returned from the qPCR instrument.

2.) Calculated Data

The data grid (*see Image 5.4.1*) is used with the calculation method to determine the new new values displayed on the resulting graph.

Original Data

Original data points are represented on the graph by **green** data markings (*see Image 5.4.2*). The **original** data points are used in the linear regression calculation to determine the **calculated** data points on the graph. Data point values can be vied by moving the mouse cursor over the required position.

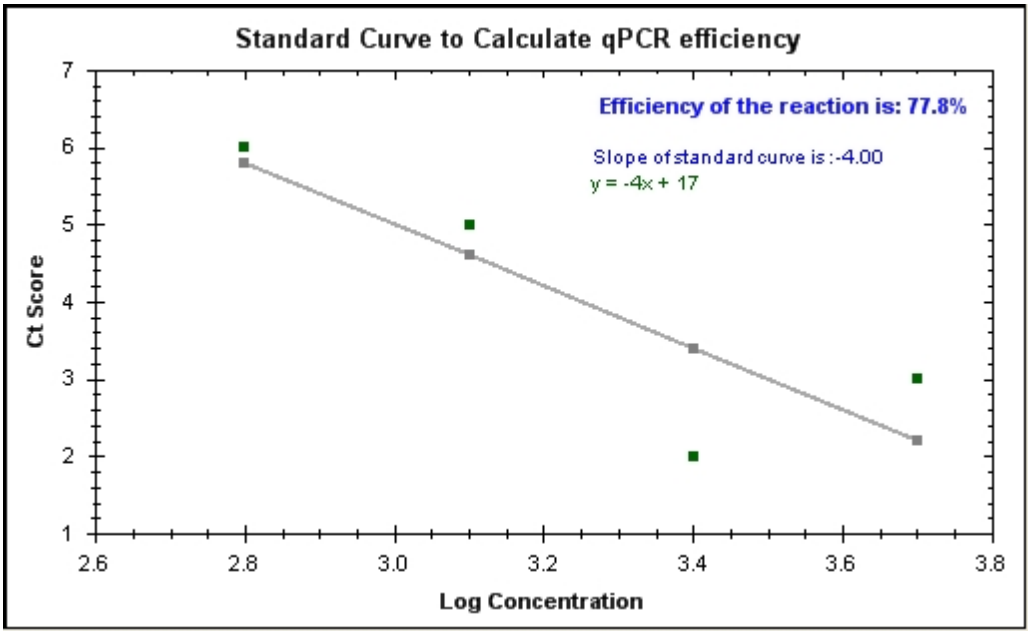


Image 5.4.2

Calculated Data

Calculated data points are represented on the graph by **grey** data markings (*see Image 5.4.3*). The **original** data points used in the linear regression calculation to determine the **calculated** data points on the graph. Data point values can be vied by moving the mouse cursor over the required position.

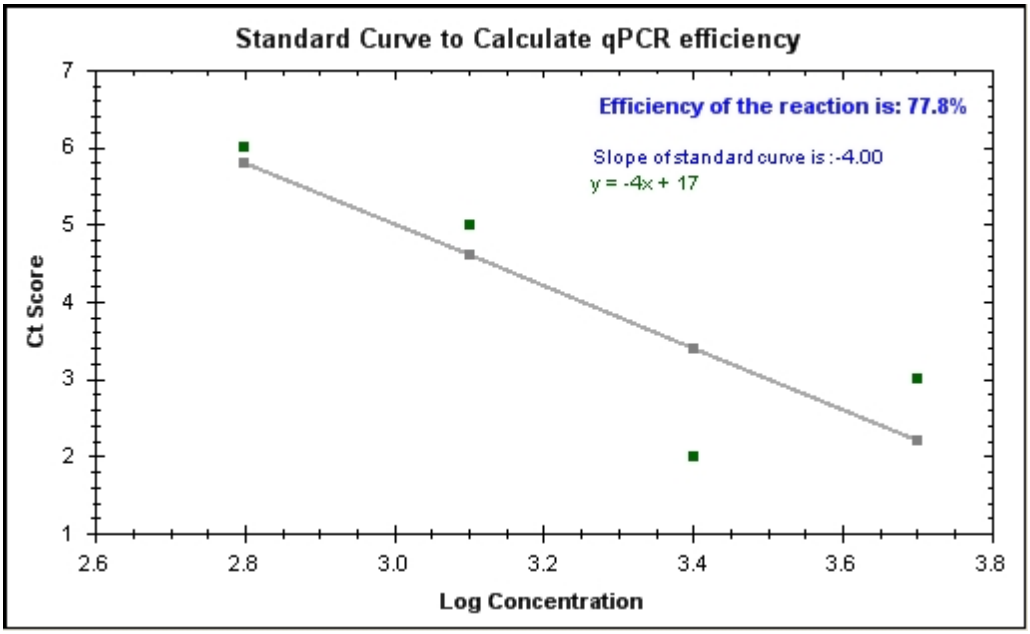


Image 5.4.3

Kapa Library Quantification Kits

Code	Description	Contents	
KK4820	Library Quantification Kit - Roche 454 FLX - Universal	5 ml	KAPA SYBR FAST Universal qPCR Master Mix (2X)
		200 ul	KAPA SYBR FAST ROX High (50X)
		200 ul	KAPA SYBR FAST ROX Low (50X)
		1 ml	Primer premix - Roche 454 FLX
		6 x 80 ul	DNA standards - Roche 454 FLX
KK4821	Library Quantification Kit - Roche 454 Titanium - Universal	5 ml	KAPA SYBR FAST Universal qPCR Master Mix (2X)
		200 ul	KAPA SYBR FAST ROX High (50X)
		200 ul	KAPA SYBR FAST ROX Low (50X)
		1 ml	Primer premix - Roche 454 Titanium
		6 x 80 ul	DNA standards - Roche 454 Titanium
KK4822	Library Quantification Kit - Illumina Genome Analyzer - Universal	5 ml	KAPA SYBR FAST Universal qPCR Master Mix (2X)
		200 ul	KAPA SYBR FAST ROX High (50X)
		200 ul	KAPA SYBR FAST ROX Low (50X)
		1 ml	Primer premix - Illumina Genome Analyzer
		6 x 80 ul	DNA standards - Illumina Genome Analyzer
KK4830	Library Quantification Kit - Roche 454 FLX - ABI Prism	5 ml	KAPA SYBR FAST ABI Prism qPCR Master Mix (2X)
		1 ml	Primer premix - Roche 454 FLX
		6 x 80 ul	DNA standards - Roche 454 FLX
KK4831	Library Quantification Kit - Roche 454 Titanium - ABI Prism	5 ml	KAPA SYBR FAST ABI Prism qPCR Master Mix (2X)
		1 ml	Primer premix - Roche 454 Titanium
		6 x 80 ul	DNA standards - Roche 454 Titanium
KK4832	Library Quantification Kit - Illumina Genome Analyzer - ABI Prism	5 ml	KAPA SYBR FAST ABI Prism qPCR Master Mix (2X)
		1 ml	Primer premix - Illumina Genome Analyzer
		6 x 80 ul	DNA standards - Illumina Genome Analyzer
KK4840	Library Quantification Kit - Roche 454 FLX - Bio-Rad iCycler	5 ml	KAPA SYBR FAST Bio-Rad iCycler qPCR Master Mix (2X)
		1 ml	Primer premix - Roche 454 FLX
		6 x 80 ul	DNA standards - Roche 454 FLX
KK4841	Library Quantification Kit - Roche 454 Titanium - Bio-Rad iCycler	5 ml	KAPA SYBR FAST Bio-Rad iCycler qPCR Master Mix (2X)
		1 ml	Primer premix - Roche 454 Titanium
		6 x 80 ul	DNA standards - Roche 454 Titanium
KK4842	Library Quantification Kit - Illumina Genome Analyzer - Bio-Rad iCycler	5 ml	KAPA SYBR FAST Bio-Rad iCycler qPCR Master Mix (2X)
		1 ml	Primer premix - Illumina Genome Analyzer
		6 x 80 ul	DNA standards - Illumina Genome Analyzer
KK4850	Library Quantification Kit - Roche 454 FLX - Roche LightCycler 480	5 ml	KAPA SYBR FAST Roche LightCycler 480 qPCR Master Mix (2X)
		1 ml	Primer premix - Roche 454 FLX
		6 x 80 ul	DNA standards - Roche 454 FLX
KK4851	Library Quantification Kit - Roche 454 Titanium - Roche LightCycler 480	5 ml	KAPA SYBR FAST Roche LightCycler 480 qPCR Master Mix (2X)
		1 ml	Primer premix - Roche 454 Titanium
		6 x 80 ul	DNA standards - Roche 454 Titanium
KK4852	Library Quantification Kit - Illumina Genome Analyzer - Roche LightCycler 480	5 ml	KAPA SYBR FAST Roche LightCycler 480 qPCR Master Mix (2X)
		1 ml	Primer premix - Illumina Genome Analyzer

System Requirements

The program is developed to make use of Microsoft .Net Framework technologies. The application can be installed on any Microsoft Windows operating systems (including and newer than Microsoft Windows XP). The qPCR Efficiency Calculator installer will check the target system for the required software (see list below). If the software is not found on the target system, the installer will attempt to download the software from the internet and install it onto your system.

Any Microsoft Windows XP installation with the latest fixes and service packs will already have the required software installed, and the qPCR Efficiency Calculator can install without any problems.

The following software must be installed on your system before you attempt to run the qPCR Efficiency Calculator:
(the installer will try to download and install the software if it is not detected on your system)

- Microsoft Windows Installer 3.1
  for manual download click here.

- Microsoft .Net Framework 3.5
  for manual download click here.

Hardware requirements is similar to the minimum requirements for the operating system:

* Pentium 300-megahertz (MHz) processor or faster.
* At least 128 megabytes (MB) of RAM.
* At least 5 megabyte (MB) of available space on the hard disk.
* CD-ROM or DVD-ROM drive (if installation is going to be done from CD-ROM).
* Video adapter and monitor with Super VGA (1024 x 768) or higher resolution (1280 x 1024 recommended).

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