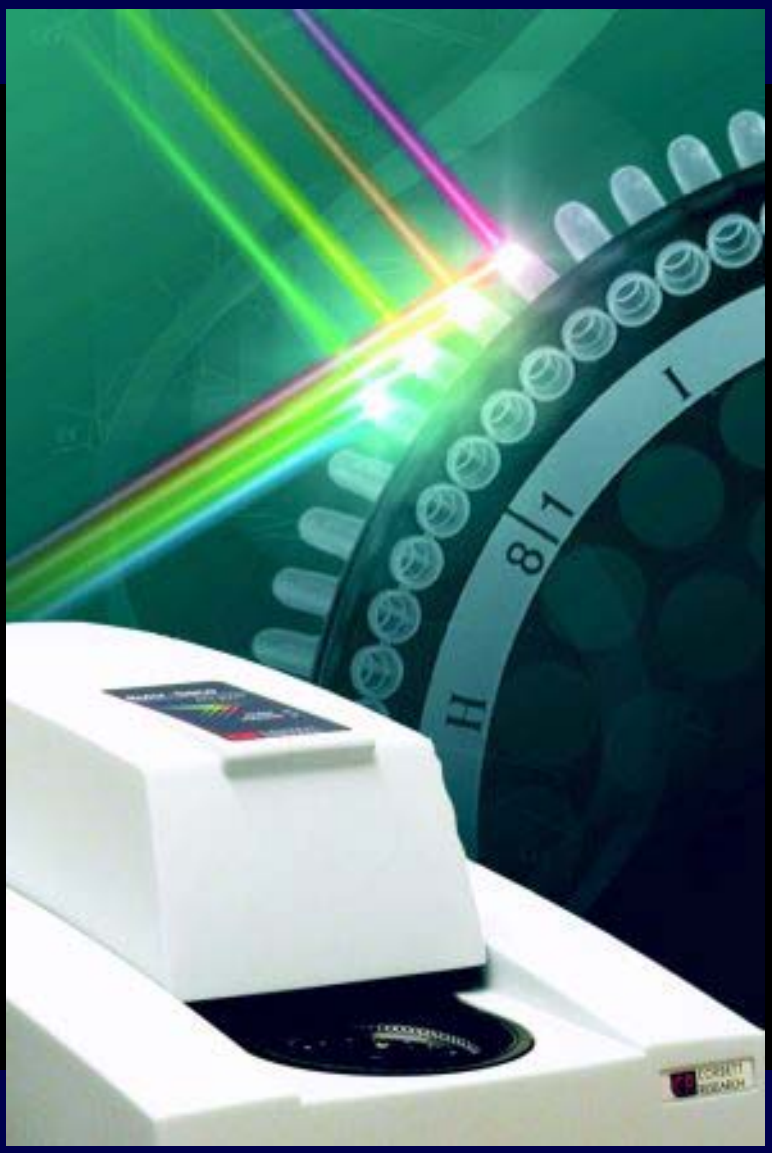
Rotor-Gene 3000™

Four-Channel Multiplexing System



CORBETT RESEARCH

Real-Time DNA Detection System

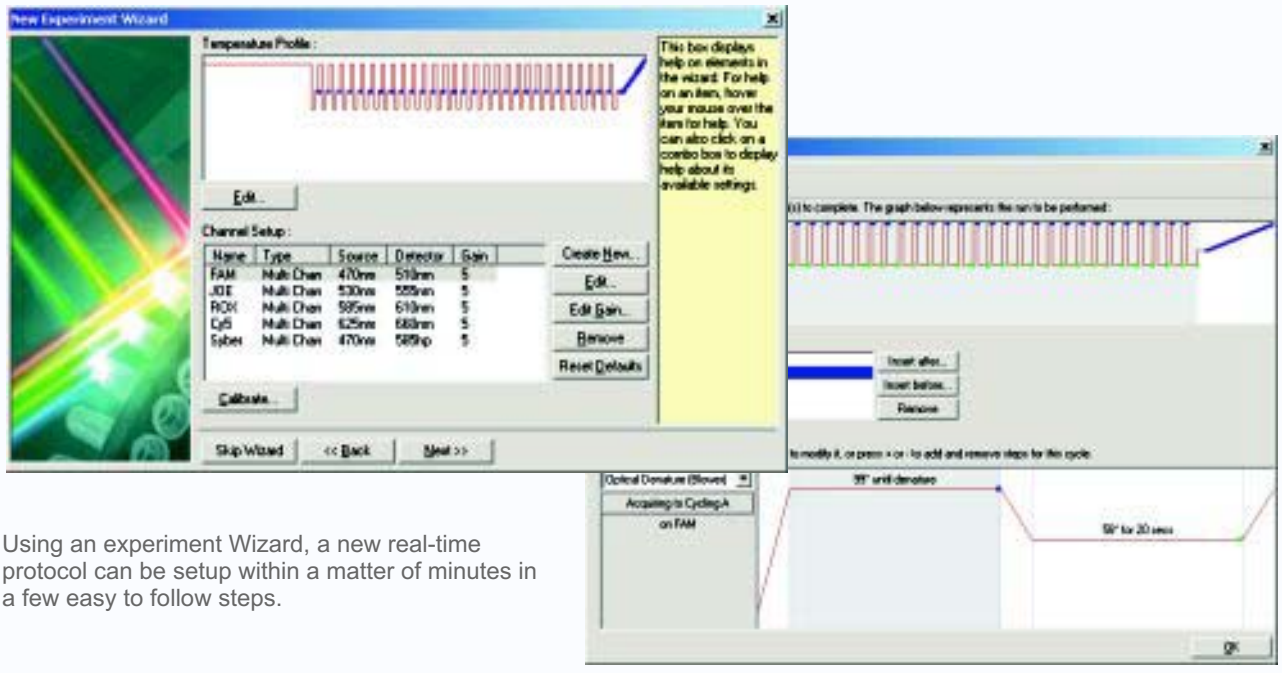
Open Chemistry Platform

The Rotor-Gene multi-filter system can detect all available real-time chemistries including Sybr-Green, dual-labelled and MGB probes, FRET and Molecular Beacons.

Most DNA amplification enzymes/ buffers can be used on the system to generate Quantitation/Melt data. It is not necessary to use expensive kits that are specific to the instrument.

User-Friendly Software Interface

The Rotor-Gene offers the most user-friendly real-time analysis software system currently available. Developed over the past three years based on customer feedback worldwide, the software has been refined to provide an intuitive, Wizard driven interface, enabling highest possible levels of flexibility and automation.



Using an experiment Wizard, a new real-time protocol can be setup within a matter of minutes in a few easy to follow steps.

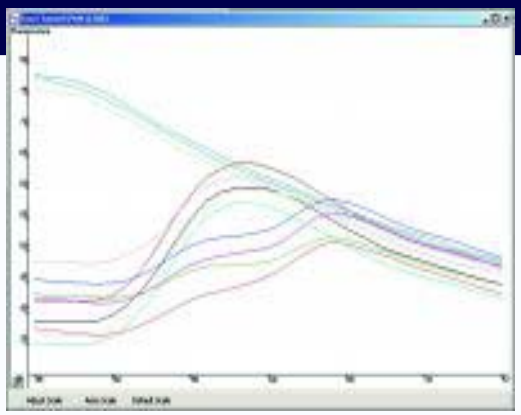
Download Software Upgrades

All Rotor-Gene users are given free access to software upgrades which can be downloaded from our website. There is also a registered users group where information can be shared and suggestions made to further improve or customise the software.

Mutation Detection

MeltCurve Analysis

After amplification, the samples are heated and the change in fluorescent energy is monitored to generate a melt curve. The differential of this curve reveals the melting temperature for each amplicon and allows automatic calling of the genotype.



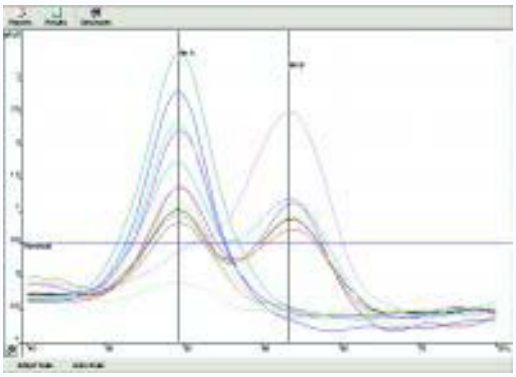
The raw data shows the signal of the donor probe increasing during the melt

Quenched FRET analysis

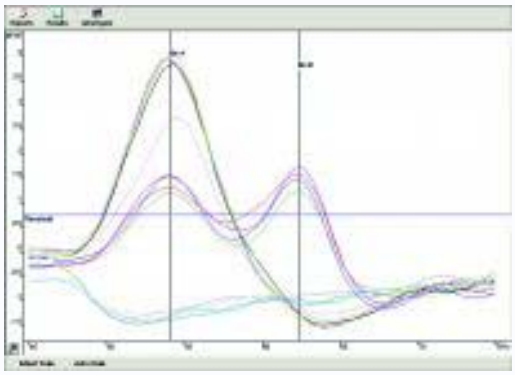
Traditionally FRET analysis has been performed by transferring energy from one probe to another and measuring the energy transfer. Quenched FRET looks at the increase in energy of the donor probe during a melt and has the advantage of using less spectral bandwidth per probe set.

Up to four mutations on different alleles can be multiplexed into one tube to generate genotypes across a range of loci using standard fluorophores and Black Hole (BH) quenchers. There is no need to use expensive proprietary dyes to minimize cross-talk for multiplex applications (ie: Lc640, Lc705).

Edit genotyping definitions for automated calling



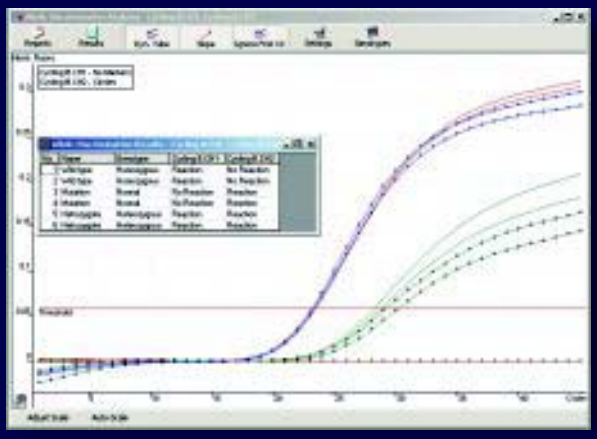
Haemochromatosis H63D
Channel 1, FAM/BH1 quenched probe set



Haemochromatosis C282Y
Channel 2, JOE/BH1 quenched probe set

Allelic Discrimination

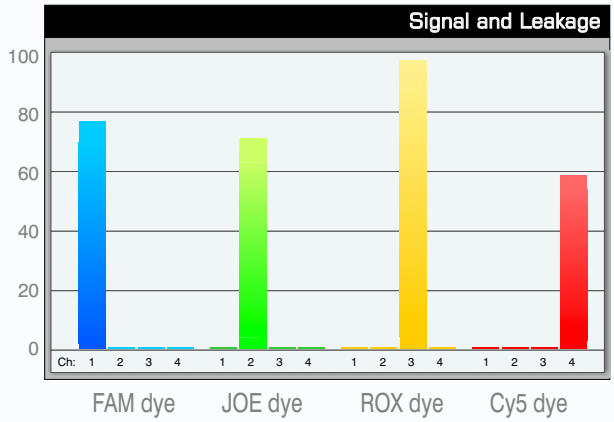
Using dual labelled probes in a multiplex reaction, genotype data can be generated across a range of loci and disease states. Probes are designed to hybridise specifically to wild type and mutation sequences. A threshold value is set to determine positive or negative amplification so genotypes can be called automatically.



4 Channel Multiplexing

No Spectral Overlap

The worlds first centrifugal, real-time DNA amplification system. The fluorescence of up to four different probes can be detected in a single tube. By exciting each dye at its peak wavelength, sensitivity is maximised and cross-talk between channels is minimised.



4 Separate Light Sources

Blue, green, orange and red are used to evenly span the visible spectrum. Six separate detection filters are selectable providing the standard four channels for multiplexing and an additional two filters for specialised applications.

When multiplexing four channels, less than 1% cross-talk is seen between channels and no software algorithm is required to analyse data.

Ultimate Temperature Uniformity

As the samples are spinning at 500rpm there is absolutely no variation in the temperature from sample to sample, a key factor in the precision of this real-time cycling system. This allows for extremely short hold times since no temperature equilibration time is required as with a 96 well block system.

Variation in amplification efficiency due to temperature non-uniformity is therefore eliminated and there is no need to use a passive ROX reference dye in each sample.

Cost Effective Consumables

The Rotor-Gene can detect product at high sensitivity without the need for specialised reaction vessels (ie: optical clear caps or glass capillaries). Each unit also provides the flexibility of an interchangeable rotor system to allow for the throughput needs of the day. Provided standard are a 36 well rotor (0.2ml thin walled reaction vessels) and 72 well high throughput rotor (0.1ml strip tubes). The rotors can be interchanged for easy transition from moderate to high throughput applications.

Our custom 72 well loading block also allows for setup of samples using an 8 well multichannel pipette and, with tubes provided in strips of four, handling from block to rotor is fast and efficient.

Optical Denaturation™

Optical Denaturation™ is a patented method that has been developed to take full advantage of the unique features of the Rotor-Gene 3000.

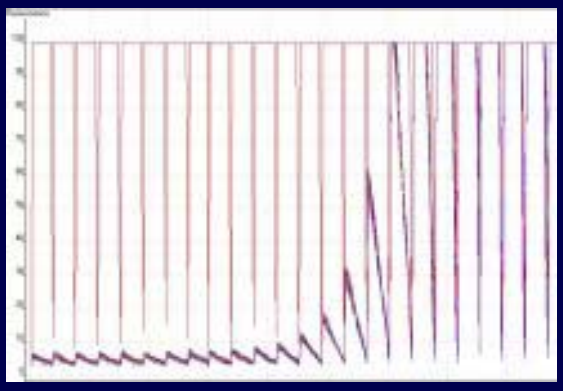
High Speed Data Acquisition

The unit is designed to take data at high speed. All 72 samples can be detected in one revolution, equivalent to 0.15 seconds.

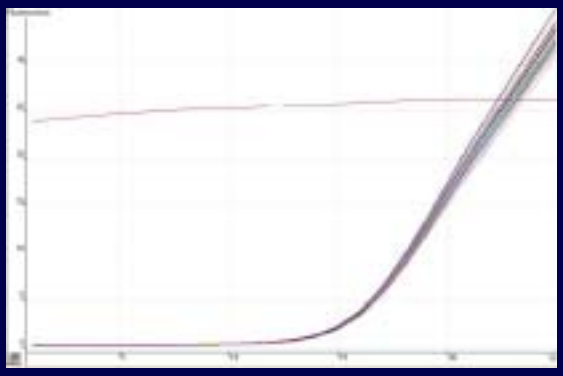
0.01°c Temperature Uniformity

Given that all samples cycle without any temperature difference, when one sample denatures, all samples denature.

Optical Denaturation™ actively monitors a DNA reference tube during amplification. A typical 2 step amplification requires only the annealing temperature to be defined. During optical denaturation the heating element is activated at full power and the reference sample is monitored at high speed; when the fluorescent level of the sample drops all the samples are denatured and the chamber is cooled back to the annealing temperature.



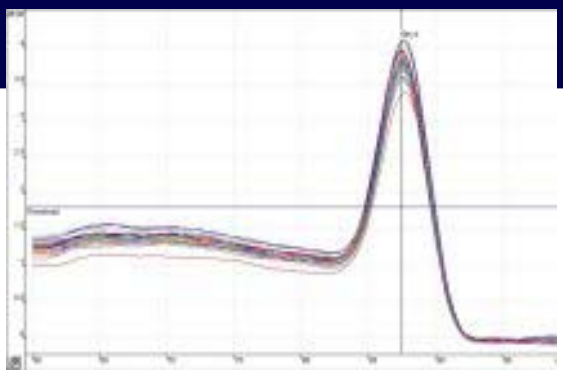
The trace shown in red is the DNA reference signal dropping during Optical Denaturation™



When data is acquired at the annealing temperature the DNA reference is seen as a constant level.

Minimal Stress to Enzyme

From the run shown here, HBV was amplified using Optical Denaturation™ with a HBV amplicon used as the DNA reference. The melt point for this product is only 87.5°C, so there is no need to heat the reaction to 94°C to denature the DNA product. By using an optical feedback loop the denaturation conditions are determined by the system and not the user.



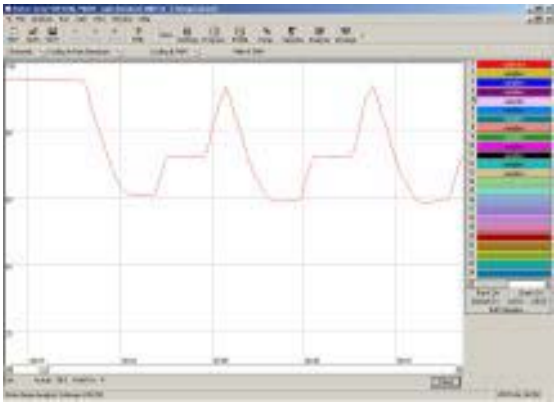
The melt curve for the HBV amplicon shows only a temperature of 87.5°C is required to denature the DNA

Faster Run Times

By heating the samples only the required amount to initiate denaturation, time is not wasted holding unnecessarily high temperatures. Run times can be reduced by up to 25%.

Volume Compensation

Optical Denaturation™, by virtue of optical feedback, automatically compensates for any volume being run.

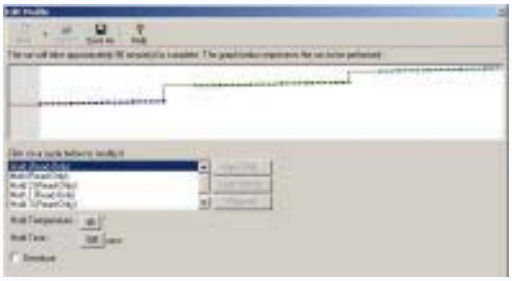


A typical temperature profile for Optical Denaturation™

Automatic Temperature Calibration

The Rotor-Gene 3000 uses a patented calibration rotor to define absolute temperature inside the tubes as the rotor is spinning. Temperature is measured at three different points across the operating range, any drift is compensated to within +/-0.2°C.

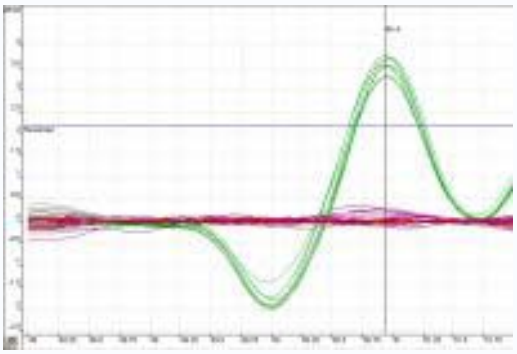
The Rotor-Gene 3000 is the only real-time system available that offers an automated temperature calibration feature.



The melt template to run the calibration rotor

Calibration Report

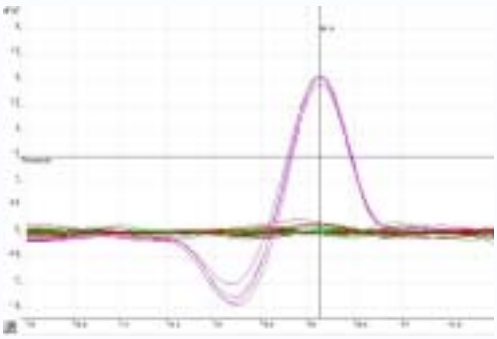
A report is automatically generated which is e-mailed to our service support centre for validation. If temperature calibration is required, a file is returned that automatically re-calibrates the unit.



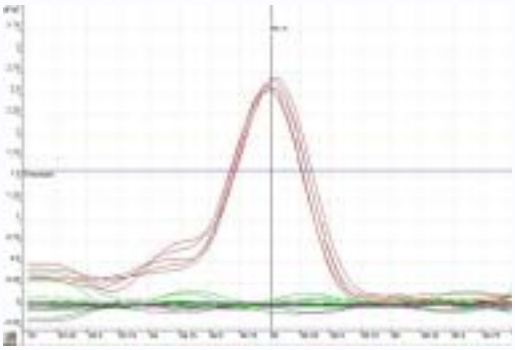
Low-range calibration at 50.9°C

Scheduled Validation

Regular temperature checks of the Rotor-Gene can be performed by anyone in the lab using this simple method. This is the only real-time system that can be validated remotely.



High range calibration at 95.0°C



Mid-range calibration at 76.2°C

Calibration Rotor

The calibration rotor is supplied pre-loaded with 0.1mL tubes that are fixed and cannot be removed.
The rotor is placed into the Rotor-Gene and a melt template is run to produce three distinct melt curves. These curves are analyzed to generate a temperature calibration report.

The only way this level of assurance can be attained on a 96 well block based cycler is to measure all 96 wells with a thermal data logger; a difficult and expensive procedure.

Quantification

Reproducibility

Due to the optic and thermal design of the system, there is no need to use an internal passive ROX reference. Even without a reference, standard deviations between replicates are typically 0.05.

Sensitivity

The system uses a photo-multiplier (PMT) that can detect a single photon of light. This gives excellent sensitivity even when amplifying a single copy of DNA.

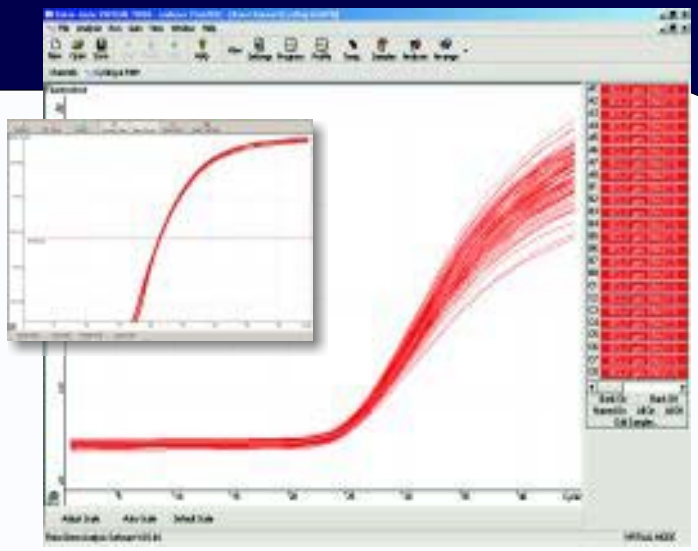
Linearity

The system uses a 16 bit analog to digital converter which has a broad dynamic range. This results in linear quantitation over a wide range of sample concentrations, typically 12 orders of magnitude.

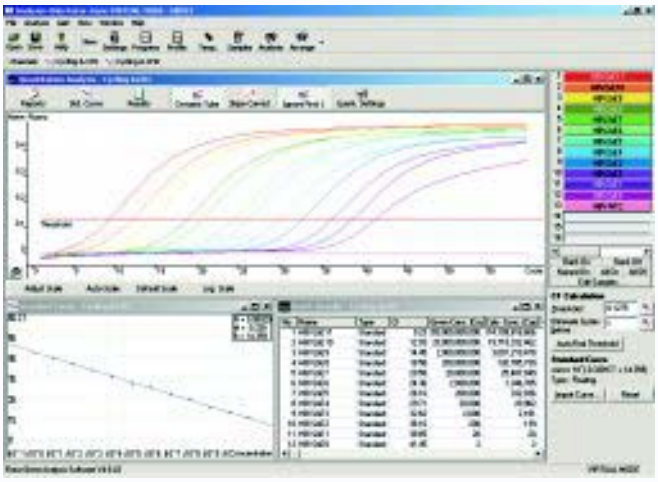
Precision

Simple optic design, together with ultimate temperature uniformity (due to centrifugal design) results in a highly accurate and reproducible system.

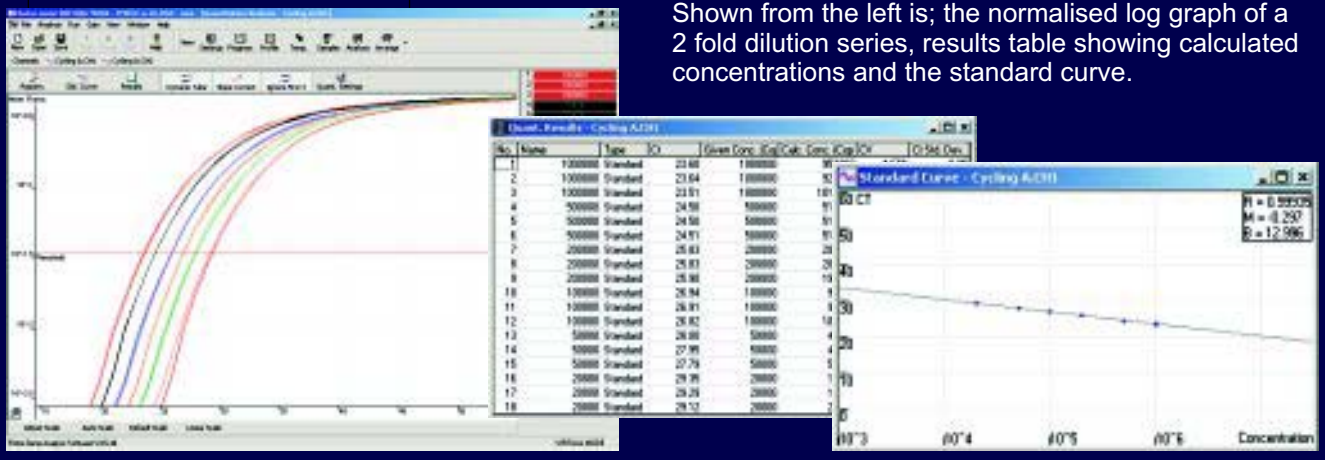
This can be best demonstrated when looking at a 2 fold dilution series where each sample concentration is run in triplicate, as shown below. No internal passive ROX reference is required to generate this data. Note the R value for the standard curve of 0.99935.



Shown above is the raw fluorescent data for 72 replicate samples, the insert shows the normalised data on a log scale. The standard deviation is 0.05 and the variation in Ct value across the 72 replicates



Data shows a 10 fold serial dilution, over 12 orders of magnitude, down to 2 copies of b-actin cDNA. Note the excellent linearity with an R value of 0.99930.



Shown from the left is; the normalised log graph of a 2 fold dilution series, results table showing calculated concentrations and the standard curve.

Excitation Source:	470nm, 530nm, 585nm, 625nm LED high power diodes
Detection Filters:	510nm, 555nm, 610nm bandpass 660nm, 580nm, 610nm high-pass
Fluorophores Detected:	Sybr-Green I, Fam, Tet, Joe, Vic, Max Rox, Tamra, Cy3, Cy5, Cy5.5, Tex Red
Temperature Range:	25-99^{o} C
Dimensions:	380(W) x 480(D) x 315(H)
Weight:	17 Kg
Electrical Requirements:	100-12-Vac @ 5 Amps 200-240Vac @ 3 Amps (50/60Hz)
Sample Capacity:	36 x 0.2ml Standard Micro-tubes 72 x 0.1ml Strip tubes
Heating/Cooling Rate:	2.5^{o} C/second (tube temperature) 5.0^{o} C/second (air temperature)
Temperature Uniformity (Sample to Sample):	+/-0.01^{o} C
Temperature Accuracy:	+/-0.5^{o} C
Temperature Precision:	+/-0.1^{o} C
Accessories:	36 well rotor, 72 well rotor 72 well loading block 0.2ml tubes, 0.1ml strip tubes 36 and 72 well locking rings
Computer System:	Desktop or Laptop
Minimum Requirement:	Pentium III, 600Mhz, 32 Meg Ram, 10G HDD, Serial Port, 14”monitor.

Designed and manufactured
in Australia by

CORBETT RESEARCH

A.B.N 73 003 634 973

1/14 Hilly Street, Mortlake 2137, Sydney, Australia
Phone: 61-2-9736-1320 Fax: 61-2-9736-1364
www.corbettresearch.com