An Overview of Real-Time PCR Platforms

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Abstract

Real-time PCR continues to have a major impact across many disciplines of the biological sciences and this has been a driver to develop and improve existing instruments. From the first two commercial platforms introduced in the mid 1990s, there is now a choice in excess of a dozen instruments, which continues to increase. Advances include faster thermocycling times, higher throughput, flexibility, expanded optical systems, increased multiplexing and more user-friendly software. In this chapter the main features of each instrument are compared and factors important to weigh up when deciding on a platform are highlighted.

History of Real-time PCR

Today it is clear that few techniques have had such a powerful impact on biology than the development of the polymerase chain reaction (PCR). More recently the PCR has become even more sophisticated with the introduction of real-time PCR. Initial work by Higuchi and

colleagues (Higuchi *et al*., 1992) first demonstrated the simultaneous amplification and detection of specific DNA sequences in real-time by simply adding ethidium bromide (EtBr) to the PCR reaction so that the accumulation of PCR product could be visualised at each cycle. When EtBr is bound to double-stranded DNA and excited by UV light it fluoresces, therefore an increase in fluorescence in such a PCR indicates positive amplification. Soon afterwards they introduced the idea of real-time PCR product quantitation or 'kinetic PCR', by continuously measuring the increase in EtBr intensity during amplification with a charge-coupled device camera (Higuchi *et al*., 1993). By creating amplification plots of fluorescence increase versus the cycle number, they demonstrated that the kinetics of EtBr fluorescence accumulation during thermocycling was directly related to the starting number of DNA copies. Fewer cycles are needed to produce a detectable signal, when a greater number of target molecules are present. Kinetic monitoring also provided a means whereby the efficiency of amplification under different conditions could be determined, providing for the first time insight into the fundamental PCR processes. Therefore, the principle underlying real-time PCR can simply be defined as the monitoring of fluorescent signal from one or more PCRs cycle-by-cycle to completion, where the amount of product produced during the exponential amplification phase can be used to determine the amount of starting material.

The approach described above was not ideal since EtBr binds non-specifically to DNA duplexes and non-specific amplification products, such as primer–dimers, can contribute to the fluorescent signal and result in quantification inaccuracies. Subsequent refinements, the most significant of which was the introduction of fluorogenic probes to monitor product accumulation, added a greater element of specificity to real-time PCR and provided greater quantitative precision and dynamic range than previous methods.

These significant advances to the basic PCR technique not surprisingly led to the development of a new generation of PCR platforms and reagents, which allowed simultaneous amplification and quantification of specific nucleic acid sequences cycle-by-cycle. Indeed a few years after Higuchi coined the term 'kinetic' or 'real-time PCR' the first

commercial platforms were released on the market. The first was the Applied Biosystems ABI Prism 7700 Sequence Detection System, followed by the Idaho Technology LightCycler (now manufactured and sold by Roche Diagnostics) (Wittwer *et al*., 1997) and its military field version, the Ruggedised Advanced Pathogen Identification Device (RAPID). Both of these platforms utilised fluorogenic chemistry and like any real-time PCR platform, they basically consist of a thermal cycler with an integrated optical detection system. New and improved models have now superseded these two instruments and several other manufacturers have introduced their own real-time PCR platforms. Choosing a suitable instrument is now a complex task. Real-time PCR offers many advantages that include:

- amplification and detection in an integrated system
- fluorescent dyes/probes allowing constant reaction monitoring
- rapid cycling times (20-40 mins for 35 cycles)
- high sample throughput (~200 –5000 samples/day)
- low contamination risk due to sealed reactions
- increased sensitivity (~ 3pg or 1 genome equivalent of DNA)
- detection across a broad dynamic range of 10 - 10^{10} copies
- reproducibility with a CV < 2.0 %
- allows for quantification of results
- software driven operation
- no more expensive than "in-house" PCR

Current disadvantages include:

- limited capacity for multiplexing using all chemistries
- development of protocols requires a high level of technical skill and/or support (research and development capacity and capital)
- high capital equipment costs
- analysis requires skill

This chapter is intended to provide an overview of the main features of real-time platforms, as well as highlighting aspects to consider when introducing real-time PCR to your laboratory. Information on the available fluorescent chemistries, their principles and methods is detailed in Chapter 3.

Real-Time PCR Platforms

A real-time PCR instrumentation platform consists of a thermal cycler, optics for both fluorescence excitation and emission collection, together with a computer and software for data acquisition and analysis. A wide range of systems are now available (see References) and these differ in their design and level of sophistication, providing users with several choices, which include: format, reaction vessels, emission and excitation wavelengths, throughput, level of control, chemistry, software, speed and applications. They all have in common the ability to measure the accumulation of PCR product during the exponential phase of the reaction using online fluorescence monitoring, whether specific or non-specific and hence provide accurate data on initial starting copy numbers. As amplification and detection are combined in a single step, the process can occur in a single closed reaction vessel, which eliminates any need for numerous post-PCR manual manipulations, as well as reducing the possibility of introducing contamination or variability. Additional technical advantages include both qualitative and quantitative PCR, mutation analysis, multiplexing and high throughput analysis. Although the fluorescence chemistries used in different platforms are similar, their mechanics and methodologies are wide ranging. A summary of the features of each platform is detailed in Table 1 and the reader is encouraged to refer to it throughout this chapter.

Real-Time PCR Thermocycling

The first component to consider in a PCR platform is the thermal engine. Successful thermal cycling is dependent on the accurate regulation of temperature in the sample vessels and the speed at which these target temperatures can be achieved. The majority of real-time platforms use advanced heating block technology based on the Peltier-effect, to actively transfer heat in and out of thin-walled plastic reaction vessels (*e.g.* ABI 7000, ABI 7900HT, DNA Engine Opticon2, Mx4000, Mx3000P, iCycler IQ). Peltier devices transfer heat from one side of a semiconductor to another. In general, blocks have significant mass and consequently a degree of thermal inertia.

Furthermore, the plastic insulating layer between the reaction vessel and the heater produces an additional thermal lag. As a consequence of this, the temperature transitions are relatively slow and blocks must be very carefully designed to minimise well-to-well variation. Other advances on the Peltier-based technology include its combination with Joule, resistive or convective technology to give improved temperature control and performance across the block. Three platforms employ alternative heat exchange technologies which permit more rapid thermal ramp rates than blocks, resulting in significantly increased thermocycling speeds. These include a stationary air-heated glass capillary format (LightCycler), a centrifugal air-heated plastic tube format (Rotor-Gene) and a high-thermal-conductivity ceramic heating plate plastic tube format (SmartCycler). For example, the time taken to equilibrate at 72°C using a Rotor-Gene is 0s compared to 15s with a standard 96-well block, resulting in run times that are on average 50% faster. Detailed information on the temperature specifications for each platform is shown in Table 2, which highlights that the LightCycler has the capacity to perform the fastest PCR and the Rotor-Gene has the smallest variation in temperature uniformity.

Real-Time PCR Optics

An integrated fluorimeter is required to detect and monitor the levels of fluorescence during the PCR process for real-time PCR and there is a range of options available for both the excitation light source and fluorescent emission detection. The light sources that cause fluorophore excitation can be classed as narrow- or broad-spectrum. If a broad-spectrum light source is employed (*e.g.* Mx4000, Mx3000P, iCycler IQ, ABI 7000) then filters can be used to provide light tuned to the excitation spectrum of a specific individual fluorophore. Such a system provides the user with a wider choice of available fluorophores, although it is best to select those with good separation of their emission spectra. A disadvantage of this optical system is that the light intensity passing through the filters can be limited and this could in theory limit the sensitivity of detection. There are currently two narrow-spectrum light sources used in real-time platforms, these can be light emitting diodes (LEDs) (*e.g.* LightCycler, DNA Engine

Table 1. Features of Real-Time PCR Platforms

Company	Applied Biosystems	Applied Biosystems
Model	ABI Prism 7000	ABI Prism 7900 HT with automation accessory
Laser/Lamp	Tungsten halogen lamp	Argon ion laser
Detector	CCD camera	CCD camera
Thermocycling	Peltier element 9700 block	Peltier element 9700 block
Excitation spectrum	350-750 nm	488 nm
Filters/detection channels	4-position fixed filter wheel: FAM/ SYBR Green I, VIC/JOE, TAMRA and ROX	500-660 nm continuous wavelength detection
Format	96-well plates or 0.2 ml tubes	96- and 384-well plates (interchangeable blocks)
Time (40 cycles)	1.75 hours	1.75 hours
Reaction volume	25-50 μl	5-20 μl
Fluorescence chemistry	Hydrolysis probes, SYBR Green I, other chemistries possible but not supported	Hydrolysis probes, SYBR Green I, other chemistries possible but not supported
Multiplexing supported	2-plex hydrolysis probes	2-plex hydrolysis probes
Passive reference	ROX	ROX
Dimensions (H x W x D)	39 x 51 x 53 cm	64 x 125 x 84 cm
Weight	34 Kg	114 Kg
Other features	Primer/probe design software included	Primer/probe design software included
	PCR mastermix kits	PCR mastermix kits
	Parameter Specific Kits	Parameter specific kits
	Assay by design and assay on demand services	Assay by design and assay on demand services
		Robotic plate un/loading for up to 84 plates
		High throughput >5000 wells 8-hour day (30,000 endpoint)

Abbreviations:
LED, light emitting diode
CCD, charge-coupled device camera
PMT, photomultiplier tube
FAM, carboxyfluorescein
JOE, carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein
TAMRA, carboxytetramethylrhodamine
ROX, carboxy-X-rhodamine

Company Model	Roche LightCycler	Roche LightCycler Ver. 2
Laser/Lamp	LED	LED
Detector	3 photodetection diodes	6 photodetection diodes
Thermocycling	Heated air	Heated air
Excitation spectrum	470 +/- 10 nm	470 +/- 10 nm
Filters/detection channels	3 channels: 530, 640, 710 nm	6 channels: 530, 560, 610, 640, 670, 710 nm
Format	32 glass capillaries	32 glass capillaries
Time (40 cycles)	30 mins	30 mins
Reaction volume	20 μl	20 μl or 100 μl
Fluorescence chemistry	Hybridisation probes, hydrolysis probes, molecular beacons, SYBR Green I	Hybridisation probes, hydrolysis probes, molecular beacons, SYBR Green I
Multiplexing supported	2-plex hybridisation probes	4-plex hybridisation and 2-plex hydrolysis probes
Passive reference	Not required	Not required
Dimensions (H x W x D)	45 x 30 x 40 cm	
Weight	20 Kg	
Other features	Primer/probe design software available	Primer/probe design software included
	PCR mastermix kits	PCR mastermix kits
	Parameter specific kits	Parameter specific kits
	Relative quantification software available	Relative quantification software included
		Nucleic acid quantitation

Abbreviations:
LED, light emitting diode
CCD, charge-coupled device camera
PMT, photomultiplier tube
FAM, carboxyfluorescein
JOE, carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein
TAMRA, carboxytetramethylrhodamine
ROX, carboxy-X-rhodamine

Company Model	Stratagene Mx4000	Stratagene Mx3000P
Laser/Lamp	Quartz tungsten halogen lamp	Quartz tungsten halogen lamp
Detector	4 PMTs	1 scanning PMT
Thermocycling	Solid-state resistive/convective peltier hybrid block	Solid-state peltier-based block
Excitation spectrum	4 customisable filter wheels in the range 350-750 nm	4 customisable filter wheels in the range 350-750 nm
Filters/detection channels	4 customisable filter wheels in the range 350-830 nm	4 customisable filter wheels in range 350-700 nm
Format	96-well plates, 0.2 ml tubes, 8 x 0.2 ml strips	96-well plates, 0.2 ml tubes, 8 x 0.2 ml strips
Time (40 cycles)	1.5 hours	
Reaction volume	10-50 μl	25 μl
Fluorescence chemistry	Hydrolysis probes, molecular beacons, scorpions, amplifluor, SYBR Green I	All chemistries
Multiplexing supported	4-plex	4-plex
Passive reference	Optional ROX	Optional ROX
Dimensions (H x W x D)	76 x 46 x 51cm	33 x 46 x 43 cm
Weight	50 Kg	20 Kg
Other features	PCR mastermix kits Integrated computer	PCR mastermix kits

Abbreviations:
LED, light emitting diode
CCD, charge-coupled device camera
PMT, photomultiplier tube
FAM, carboxyfluorescein
JOE, carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein
TAMRA, carboxytetramethylrhodamine
ROX, carboxy-X-rhodamine

Company Model	Cepheid SmartCycler	Corbett Rotor-Gene
Laser/Lamp	4 high intensity LEDs	4 high power LEDs
Detector	Silicon photodetectors	PMT
Thermocycling	Resistive heating of ceramic plates with forced-air cooling	Resistive heater with air cooling and centrifugation
Excitation spectrum	4 channels (450-495, 500-550, 565-590, 630-650 nm)	470, 530, 585, 625 nm
Filters/detection channels	4 channels (510-527, 565-590, 606-650, 670-750 nm)	510, 555, 610 580 hp, 610 hp, 660 hp nm (high pass)
Format	16 proprietary tubes	36 0.2 ml or 72 0.1 ml plastic tubes
Time (40 cycles)	40 mins	30 mins
Reaction volume	25 or 100 μl	10-100 μl
Fluorescence chemistry	Hydrolysis probes, molecular beacons, amplifluor and scorpion primers, SYBR Green I	Hydrolysis probes, molecular beacons, hybridisation probes, SYBR Green I
Multiplexing supported	4-plex	4-plex
Passive reference	Not required	Not required
Dimensions (H x W x D)	30 x 30 x 25 cm	31.5 x 38 x 48 cm
Weight	10 Kg	17 Kg
Other features	PCR mastermix kits Random access (16 independent sites)	

Abbreviations:
LED, light emitting diode
CCD, charge-coupled device camera
PMT, photomultiplier tube
FAM, carboxyfluorescein
JOE, carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein
TAMRA, carboxytetramethylrhodamine
ROX, carboxy-X-rhodamine

Company Model	BioRad iCycler IQ	MJ Research DNA Engine Opticon2
Laser/Lamp	Tungsten halogen lamp	96 LEDs
Detector	CCD with intensifier technology	Dual PMTs
Thermocycling	Peltier-based	Peltier-based
Excitation spectrum	5 filter positions in range 400-700 nm	470-505 nm
Filters/detection channels	5 filter positions available, 2 provided	523-543, 540-700 nm
Format	96- or 384-well plate, 8 x 0.2 ml strip tubes	96-well plate, 8 x 0.2 ml strip tubes
Time (40 cycles)	2 hours	
Reaction volume	10-100 μl	10-50 μl
Fluorescence chemistry	Hydrolysis probes, molecular beacons, hybridisation probes, SYBR Green I	Hydrolysis probes, molecular beacons, hybridisation probes, SYBR Green I
Multiplexing supported	4-plex	2-plex
Passive reference	Fluorescein	Not required
Dimensions (H x W x D)	36 x 33 x 62 cm	60 x 34 x 47 cm
Weight	17.6 Kg	29 Kg
Other features	Primer/probe design software available	
	PCR mastermix kits	PCR mastermix kits
	Thermal gradient block	Thermal gradient block

Abbreviations:
LED, light emitting diode
CCD, charge-coupled device camera
PMT, photomultiplier tube
FAM, carboxyfluorescein
JOE, carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein
TAMRA, carboxytetramethylrhodamine
ROX, carboxy-X-rhodamine

Table 2. Temperature Specifications of Real-time PCR platforms

Platform	Max. heating/ cooling rate (°C/sec)	Temperature accuracy (°C)	Temperature uniformity (°C)
ABI 7000/7900HT	1.5/1.5	+/- 0.25	+/- 0.5
iCycler IQ	3.3/2.0	+/- 0.3	+/- 0.4
LightCycler	20.0/20.0	+/- 0.4	+/- 0.2
Mx4000	2.2/2.2	+/- 0.25	+/- 0.25
Mx3000P	2.5/2.5	+/- 0.25	+/- 0.25
DNA Engine Opticon2	3.0/2.0	+/- 0.4	+/- 0.4
Rotor-Gene	2.5/2.5	+/- 0.5	+/- 0.01
SmartCycler	10.0/2.5	+/- 0.5	+/- 0.5

Opticon2, SmartCycler, Rotor-Gene) or laser (ABI 7900HT). The SmartCycler and Rotor-Gene each have four LEDs that excite at different wavelengths, providing a greater selection of fluorophores and giving these instruments capabilities similar to those of the broad-spectrum platforms above. The LightCycler, DNA Engine Opticon2 and ABI 7900HT have single light source excitation, which ultimately limits the choice of fluorophores.

In general, the detectors used in real-time platforms are set to measure narrow bands of the spectrum, although filter sets that can be customised by the user are available for the iCycler IQ, Mx4000 and Mx3000P. The number of detection channels that can be effective is dependent on the available range of excitation wavelengths. For example, if a single narrow range excitation source is available, one approach is to use fluorophores that are all excited to some extent in the same range and then to rely on software correction to deconvolute the light emitted from a given area of the spectrum, as was employed successfully with the now discontinued ABI 7700. Another approach is demonstrated with the LightCycler, where a narrow-spectrum light source excites the fluorophores SYBR or fluorescein and emitted light is collected via three discrete optical detectors. Two of these detect

long wavelength light emissions from fluorophores which are only minimally excited by the blue LED light source, but which are instead excited using FRET technology (see Chapter 3).

Real-Time PCR Chemistries

As the technology has advanced rapidly, second and third generation real-time PCR platforms have been developed with improvements seen in multiplexing and increased throughput capabilities. The optical characteristics of a given platform clearly have an impact on the ability to multiplex and also determine which probe systems are compatible. In addition, the analysis software may also predetermine the appropriate chemistries. The ability to mulitiplex the available fluorophores is fully discussed in Chapter 3. However, it is important to point out that the platform and choice of fluorescent chemistry are strongly linked. Indeed some platforms are biased towards a particular probe system and whilst the optics permit different probe chemistries to be excited and detected, often the analysis software does not and the user is required to export the data to a spreadsheet program for detailed user analysis. For example, the Applied Biosystems platforms do not officially support any chemistries other than hydrolysis probes. Therefore, the reporting chemistry required for an application should be strongly considered before a choice of platform is made.

Additional Platform Features

Several of the platforms employ a standard 96-well block format or interchangeable 384-well block and offer a medium to high throughput. An advantage of employing a block format is that standard PCR plates and tubes can be used and these tend to be cheaper than instrument-specific plastics (SmartCycler, Rotor-Gene) or glass capillaries (LightCycler). Also, the LightCycler and SmartCycler require centrifugation to move sample into the reaction vessel and these alternative designs may not be suitable for all applications. For the highest throughput, the ABI 7900HT combines a 384-well plate format with an automation accessory, which allows for up to 84 plates

to be loaded and unloaded, providing a throughput of >5000 wells per 8-hour day or 30,000 wells for end-point analysis only. These high throughput instruments are ideal for dedicated laboratories where large batches of samples are run, with few different cycling parameters. An additional specification of a few platforms is the ability to perform gradient thermocycling, which can be very useful at the assay optimisation stage.

Some platforms offer a low to medium throughput but are more flexible. Although the format may allow only 32 or even 16 samples, thermocycling times are faster and multiple runs can be performed thereby increasing the potential throughput. Performing multiple runs rapidly may be an advantage when several applications are employed which require different cycling parameters. The SmartCycler offers a new concept to real-time PCR of random access, which means independent programming of cycling parameters for 16 different assays. Each reaction vessel has its own element so that runs in available slots may be started any time, whilst other reaction sites are already in use.

Ideally, the analysis software supplied with the platform should be as user-friendly as possible but it is also important to check that the software can fully analyse results of the chosen probe chemistry. As already mentioned, some platforms and analysis software suites are biased towards certain chemistries. Some real-time instruments also have specific primer and probe design software that is either supplied with the hardware or available at extra cost. Such software can help simplify and speed up the assay design process and is optimised for that system and reagents. The LightCycler also has specific relative quantification software that is designed to determine the exact relative nucleic acid concentration normalized to a calibrator sample. This software speeds up and greatly simplifies this method of quantification.

The majority of instrument manufacturers supply optimised real-time PCR mastermixes, these reagents benefit from being quality controlled, are easy to use, and usually offer reproducible and reliable results. However, in certain laboratories cost can be prohibitive, although

with other companies (*e.g.* Epicentre, Eurogentec, Invitrogen, TaKaRa, Qiagen) now supplying real-time reagents, competition in this market should lead to reduced costs. Target specific kits for a range of applications are available from some manufacturers and other companies (*e.g.* Applied Biosystems, Artus Biotech, AME Bioscience, Idaho Technologies, Minerva Biolabs, Roche, TaKaRa). Additionally, Applied Biosystems offer Assays-on-Demand and Assays-by-Design services for SNP genotyping by real-time PCR.

In the current world climate there has been a drive in the real-time PCR market towards portability, sensitivity and rapid response capabilities. Currently the RAPID and a portable version of the SmartCycler are available as field deployable real-time PCR machines, together with a range of freeze-dried PCR reagents and specific detection kits. They also provide simple 'push button' software which permits use by personnel with minimal training. A range of other handheld PCR biodetection devices have also been developed with the threat of bioterrorism in mind, (see also Chapter 11) such as: the Miniature Analytical Thermal Cycling Instrument (Northrup *et al*. 1998); the Advanced Nucleic Acid Analyser (Belgrader *et al*., 1998); the Handheld Advanced Nucleic Acid Analyser (Lawrence Livermore National Laboratories) and its commercial counterpart the Smiths Detection Bio-Seeq; and the Idaho Technology RAZOR instrument. Clearly, these instruments are designed with specific application requirements and their evaluation and implementation will require careful consideration.

Finally, a new introduction to the marketplace is the BioGene InSyte real-time platform, which uses a novel electrically conducting polymer technology (ECP). The ECP is formed into a 96-well moulded thermal plate, which allows ultra rapid thermocycling due to the low thermal mass combined with a high surface:area ratio. As there is temperature measurement of each tube, well-to-well uniformity issues have been eliminated. This platform also has significant flexibility as each tube is the heating element and can be thermally addressed for individual thermal cycling. The optics consists of a 473nm laser and a 32-channel spectrometer (520-720nm) with no filters, where each channel is a photomultiplier tube (PMT). This instrument should allow the majority of chemistries to be utilised. Since this is a relatively new

instrument experience and applications with this platform are currently unavailable.

Final Considerations

In weighing up the pros and cons of the different platforms for your laboratory, factors to consider include: supported chemistries; multiplex capability for that chemistry; throughput; flexibility; format; easy-to-use and robust software package; reproducibility; speed; size; technical support; customer support and not least the cost, not only of the initial equipment outlay and servicing but also the associated cost of consumables and reagents. It is also useful to 'try before you buy', most companies tend to provide a loan machine and if possible try to test a few of these once you have narrowed down your choice. User experiences should not be overlooked and there are now a number of useful websites and news groups where you can address you questions and queries (see References).

Clearly real-time PCR has undergone significant developments over the last ten years, which has resulted in a wide range of different platforms becoming available. The drive to improve current technology is likely to continue for the foreseeable future as real-time PCR finds it way into even more laboratories and specialist niches such as bioweapon sensing. As competition intensifies, users should benefit from more sophisticated high-throughput platforms and portable devices, with increasing levels of automation and cost-effectiveness, although making a choice of platform will continue to be difficult.

References

Papers

Belgrader, P., Benett, W., Hadley, D., Long, G., Mariella, R. Jr., Milanovich, F., Nasarabadi, S., Nelson, W., Richards, J., and Stratton, P. 1998. Rapid pathogen detection using a microchip PCR array instrument. Clin. Chem. 44: 2191-4.

Higuchi, R., Dollinger, G., Walsh, P.S., and Griffith, R. Simultaneous amplification and detection of specific DNA sequences. 1992. Biotechnology (N Y). 10: 413-417.

Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. 1993. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology (N Y). 11: 1026-1030.

Northrup, M. A., Benett, B., Hadley, D., Landre, P., Lehew, S., Richards, J., and Stratton, P. A. 1998. A miniature analytical instrument for nucleic acid acids based on micromachined silicon reaction chambers. Anal. Chem. 70: 918-22.

Wittwer, C. T., Ririe, K. M., Andrew, R. V., David, D. A., Gundry, R. A. and Balis, U. J. 1997. The LightCycler: a microvolume multisample fuorimeter with rapid temperature control. Biotechniques. 22: 176-181.