Product survey: qPCR thermocyclers

From Silver to Silicon

Thermocyclers equipped with Peltier heated silver blocks or air heated carousels are dominating the qPCR cycler market. But new alternative thermal cycling concepts are gaining ground.

Thermocyclers (Thermal cyclers) for Real-Time quantitative PCR (qPCR) are basically "simple" instruments composed of a thermal system for temperature cycling, an optical system for amplicon detection and quantification, and a software to control the machine and analyse the data.

Choosing a suitable qPCR thermocycler out of approximately 50 different models currently on the market, however, can cause quite a headache. So, it's no wonder that life science technology forums and social networks for life scientists, such as ResearchGate, are overwhelmed with scientists begging for help on purchasing a suitable machine. Quite often the answer-posts suggest tried and true standard models, which have proven to perform well in daily qPCR routine work. Besides these classic instruments, which are usually equipped with Peltier heated solid blocks or air heated carousels, new interesting devices with modified or completely different heating concepts have entered the scene.

Solid silver blocks are still at the heart of many qPCR thermocyclers.

Solid but limited

Peltier heating of solid silver blocks is a robust and simple way to cycle the temperature in a qPCR machine. Silver blocks are, however, notorious for their inhomogeneous temperature distribution throughout the block, leading to slightly different temperatures in particular wells. Also, the ramp rates of solid blocks are physically limited and won't considerably exceed 2.5° C per second – despite all engineering tricks, such as a thin gold surface, to improve temperature conductibility.

But heating blocks must not necessarily be solid. In Roche's LightCycler 480 and 1536 models, engineers placed a thin, sealed vacuum vessel containing a working fluid, between the heating and cooling units. The temperature in this 'ThermoBase' is transferred quickly and very evenly to the wells of a microplate by evaporation and condensation processes inside the vessel. Both 480- and 1536-cyclers beat the ramp rates of ordinary block cyclers by a factor of about two.

Axel Scherer's group at the California Institute of Technology followed a similar idea. The Californian researchers hollowed out a silver block and filled the cavity with a thermally conductive liquid metal to considerably improve both block uniformity and ramp speed. The Scherer-type, hollow block cycler shines with a well-to-well uniformity of +/-0.1°C. It is now sold by next generation sequencing giant, Illumina, who snatched Scherer's start-up company Helixis in 2010.

i-cores

One of the first alternative temperature cycling concepts devoid of silver blocks or carousels was presented by the US company, Cepheid, in 2006 already. At the heart

of Cepheid's Smart Cycler are 16 (or up to 96) i-core modules, resembling slim, inkjet printer cartridges. Every i-core module contains a highly thermal conductive ceramic plate, a fan and two small optical blocks. The thin especially-formed reaction vessels made of polypropylene are pushed into a small slot to establish contact with the ceramic plate installed beneath the slot. The plate is heated by resistive heating elements deposited on the ceramic surface. A thermistor (thermal resistor) monitors the temperature while the fan produces an airstream to cool the reaction vessel. Because every module has its own control system, each of the 16 to 96 reaction sites can run a different protocol. Maximum heating rates of 10°C/sec and cooling rates of 2.5° C/sec allow cycling times for standard qPCR programmes of 20 to 40 minutes. That's pretty fast, however, not out of reach for many other qPCR cyclers, such as Roche's Light Cyclers or Qiagen's Rotorgene.

Who's the fastest?

Cycling times of less than ten minutes for a typical 40 cycle qPCR are promised by the UK company, BJS Biotechnologies, with their brand new Xxpress cycler. The impressive short cycling time is achieved by a smart resistive heating system. The essential part of the British cycler is a disposable metal sheet with a thin, integrated 24, 54 or 96-well plate that replaces the silver block and the usual microplates or reaction tubes of ordinary block cyclers. An electric current, flowing through the sheet at low voltage, heats the sheet, which acts as a direct resistive element in the electric circuit. Cooling of the reaction wells is simply done via forced air cooling by a fan.

Semiconductor experts at the Catania, Italy-based company, STMicroelectronics, went even one step further in downsizing the qPCR process by integrating it in a tiny silicon chip (S. Petralia *et al*., IMCS 2012 – The 14th International Meeting on Chemical Sensors, 341-3). The company's Incheck qPCR system is based on a lab-on-a-chip device composed of two fluidically--connected, silicon microreactors with a maximum volume of 12 µl each. To start the qPCR, the sample is loaded via a solution inlet into the tiny PCR reactor. The thermal cycling of the reaction is controlled by resistors and sensors, which are integrated into silicon layers beneath the little reaction chamber. The qPCR is run with a fluorescent dye, e.g., Cy5, as a hybridisation probe; the fluorescence signal of the amplicons, however, is not detected directly in the PCR reactor. The amplicons are transported through a fluidic bypass to a microarray chamber, where they may hybridise with appropriate capture probes on a microarray. The fluorescent intensities emitted from the microarray surface are detected by an external optical reader, which transfers the signal to a connected PC for data analysis.

Lab-on-a-chip cycler

Sounds like one of those countless lab-on-a-chip devices that have never made it beyond the proof-of-concept stage – but not in this case. STMicronics Singapore-based subsidiary, Veredus Laboratories, already offers In-check-based lab-on-a-chip devices for fast detection of flu, *Mycobacterium tuberculosis* and multiple tropical diseases. The Veredus system theoretically allows parallel detection of up to 100 analytes in one reaction. That's a big plus to standard qPCR systems that have only limited multiplexing abilities. But it still has a major drawback: it is rather a qualitative than a quantitative Real-Time PCR system, allowing only the qualitative identification of multiple DNA analytes.

But researchers are already working on quantitative Real-Time microarray PCR systems capable of multiplexing. Anke Pierik and her colleagues from Philips Research in Eindhoven, The Netherlands, recently presented an interesting concept for a quantitative Real-Time microarray PCR system (*Lab Chip*, 2012,12, 1897-1902).

Multiplexing qPCR

The Dutch team simply glued a cover glass with a cavity onto a printed microarray slide to get a PCR chamber and connected the chamber, with two needles serving as inlet and outlet channels. The micro reactor is heated by a thin film heater and cooled by three fans; fluorescence signals emitted from the array are scanned with a confocal fluorescence scanner. The trick to get a quantitative array PCR with the system is quite easy: the labelled amplicons hybridise during the annealing phase to the corresponding capture probes on the microarray. Monitoring the fluorescence at the end of the annealing phase of every cycle, leads to an S-shaped curve, from which the threshold values (Ct-values), necessary for amplicon quantification, may be obtained. That's the good news; the bad news is that the Dutch qPCR array cycler is still in the proof-of-concept stage.

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