Polymerase Chain Reaction: The Past, Present and Future

The polymerase chain reaction (PCR), sometimes described as 'molecular photocopying', is a laboratory technique that allows researchers and technicians to amplify DNA or RNA from as little as a single strand. The technology was first created in 1983 by Biochemist Kary Mullis. Here we look at the development of this technique over the years, and how the technology behind the xxpress® thermal cycler has taken PCR into the future.

1. A Brief Introduction to PCR

PCR is now a standard tool in **research, analytical and diagnostics laboratories**. It can create millions or even billions of copies of the target DNA or RNA in just a few hours or less. Before PCR, this process involved cloning DNA or RNA into vectors for transfer and expression in bacteria, a laborious and time-consuming method that could take weeks (2).

The PCR process involves three key steps, all carried out in a thermal cycler at different temperatures and for different lengths of time:

- **Denaturation** at ~94-98°C for 20-30 seconds. *This 'melts' the hydrogen bonds between the two strands of DNA, causing them to separate.*
- **Annealing** at ~54-65 °C for 20-40 seconds. *This adds primers (short strands of DNA complimentary to the section of DNA to be copied). They mark the beginning and end of the DNA to be copied.*
- **Extension** at ~72 °C. *The separated DNA strands are used as templates for Taq DNA Polymerase to build two double-stranded DNA pieces.*

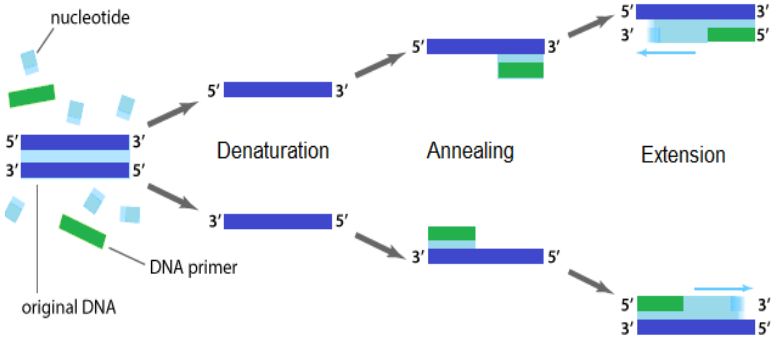

Figure 1. Diagram to show the 3 steps of a PCR cycle. Each cycle doubles the amount of DNA using strands from the previous cycle as templates.

When Mullis originally developed PCR, it required adding polymerase in after every cycle – in 1986 he refined it to use a **heat-stable polymerase** that only needed to be added once every run (1).

To ensure accuracy and consistency, the temperatures and dwell times are critical. It is therefore vital that PCR thermal cyclers can reach and maintain the required temperature quickly, evenly and accurately (3; 4; 5; 6; 7).

2. Facing the Challenges

Much of the development of PCR has focused on three key issues: getting the samples to the right temperature, keeping them at the right temperature for the right length of time, and maintaining this across all samples.

The earliest forms of PCR heated the samples using lamps, and cooled them using water (8). Most modern forms of PCR use Peltier-based devices. These rely on the temperature changes created when an electric current is run across the joint between two metals – one direction raises the temperature and the other direction lowers it.

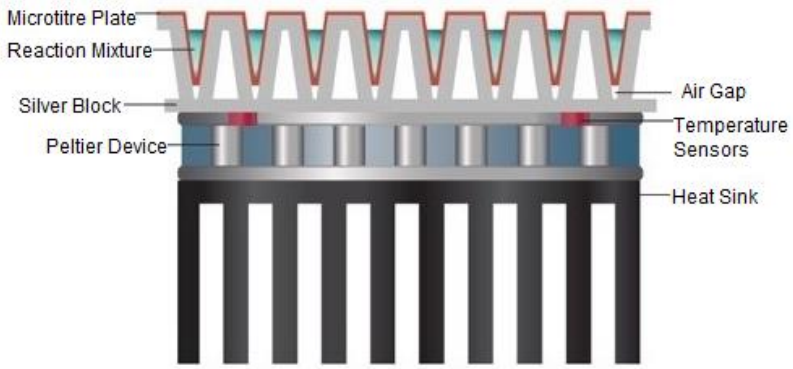

Figure 2. Diagram to show a Peltier device.

The reaction mixture is placed within plastic tubes or microtiter plates inside wells in heat exchange blocks. The block transmits heat from the Peltier device to the samples, meaning heating and cooling is passive, along temperature gradients. While the Peltier effect can be controlled precisely, the temperature change can be slow to transmit, leading to **under- and overshoots of temperature** in the sample. Overshoots can damage the sample and undershoots lead to incomplete reactions, whilst both increase the time taken to get to the right temperature.

The nature of passive heat exchange means the wells on the edge of the block take longer to get to the optimum temperature, and are likely to cool faster due to heat dissipation. This makes it harder to replicate identical conditions in all wells, very important in research and diagnostics.

Additionally, the plastic wall of the microtiter plate and the air gap both insulate the sample from the heat exchange block, introducing a **time lag** of up to 10 seconds.

These factors work together to increase variability and reduce the reliability of results. The time taken for Peltier-based thermal cyclers to run 40 cycles varies from 30 minutes to 2 hours (4; 3; 6).

3. Finding Solutions

PCR is a widely used technology, resulting in many different approaches. While this coverage is by no means exhaustive, it gives a feel for the different approaches:

Changing the construction of the Peltier blocks

Using thicker metal, including those made of silver which is highly heat conductive, improves the conductivity of the heat exchange block. However, this increases the thermal mass, meaning it takes longer to raise the entire block to the same temperature (3).

Another approach uses a hollow heat exchange block with a circulating conductive fluid e.g. the PCR Max Real-Time PCR system and the Roche Lightcycler 480. The PCR Max system takes ~40 minutes for 40 cycles of PCR, and the Roche system between 40 minutes and an hour (4; 9).

Moving away from Peltier-based technology

Other PCR thermal cyclers have moved away from Peltier-based heating altogether. The use of hot air increases the speed of heating and improves the variability – examples of this approach include Roche's Lightcycler 1.5 and 2.0, and QIAGEN's Rotor-Gene Q. Roche's thermal cycler takes ~30 minutes for 40 cycles, and Qiagen's about 40 minutes for 40 cycles. However, the thermal mass and conductivity of the air still cause some delay (10; 4).

Cepheid has used an I-CORE ceramic heating plate in its Smart Cycler, heating samples in disposable tubes and cooling using force-air cooling. This system takes 20-40 minutes for 40 cycles (5; 4)

Improving PCR technology has reduced the time taken for 40 PCR cycles over the years. However, there is still room for greater speed, improved consistency and accuracy, and simpler workflows for researchers and technicians.

Creating a new approach

By going back to first principles, rather than adapting existing technologies, BJS Biotechnologies has created an ultra-high speed thermal cycler, known as xxpress. This can run 40 cycles of qPCR in 15 minutes or less, making it the **fastest** thermal cycler in the world**.** Its innovative heating and cooling techniques also make it is the **most thermally accurate** technology as well.

Rather than a Peltier block and plastic sample tubes, it uses **resistive heating technology** in the sample plate itself – xxpress' flat-bottomed sample wells are lined with just 10 microns of polypropylene and mounted onto a highly conductive thin metal plate with a **very low thermal mass.** The plate has six electrical contact fingers allowing a range of resistive heating paths, with the amount of heat being directly proportional to the level of current. Finely-controlled and continuously-variable cooling air jets cool the zones.

Figure 3. The xxpress® qPCR thermal cycler.

Nine highly-accurate **infrared temperature sensors** sit below the plate and feed information to allow the system to adjust the heating and cooling constantly and maintain the target temperature. The low thermal mass of the plate means temperature changes can occur at 10 °C/second during heating and 8°C/second during cooling (3).

Peltier-based devices often need a changeover of entire heat exchange blocks *and* recalibration to use different size microtiter plates. The xxpress on the other hand, has interchangeable 24-, 54- and 96-well plates, cutting down the time between runs (10).

In the xxpress® system, samples sit closer to the heating element than in Peltier-based systems. This combined with the fast response time prevents under- and overshoot, leading to a **thermal uniformity between samples of ±0.3 °C** (3). This shortens each PCR cycle and improves accuracy, reliability and reproducibility.

The xxpress® plates use smaller reaction volumes, therefore reducing the use of costly reagents and saving users money.

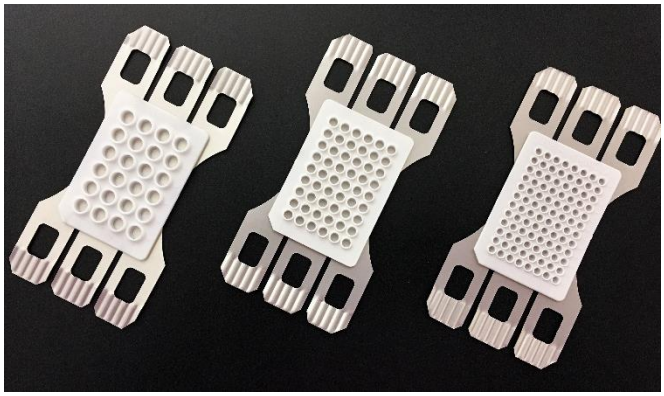

Figure 4. The 3 xxplate SBS sizes; 24, 54 and 96 well.

4. The System Workflow

The xxpress® user interface was designed by Biotechnologists to be comprehensive enough to handle complex DNA analysis, yet **intuitive** enough not to require intensive training. The system operates using a touch-screen that guides users through the process step by step.

- Identify the type of PCR and the reagents used.
- Choose plate size (24, 54 or 96 wells).
- Set up the experiment i.e. select genes of interest and reference genes, adjust the thermal profile and measurement information.

The system remembers the user's preferences and choices, and includes data files on reagents, shortening the setup process.

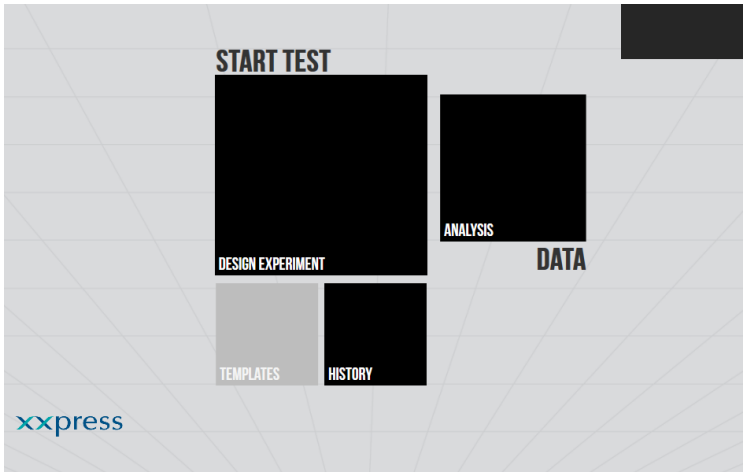

Figure 5. The xxpress® user interface home page.

The xxpress automatically recognizes the test plate format entered and crosschecks with the information inputted by the user.

5. The Need for Speed

As PCR technologies have evolved, so have their speed and thermal accuracy. This opens the possibility of PCR as a **'while you wait' test**, allowing doctors to perform diagnostic tests and prescribe the most appropriate medication all in one visit, particularly important in remote areas. Rapid testing also has a role in manufacturing, picking up suspected contamination quickly and reducing the time that production lines are halted, or the amount of spoiled product that must be destroyed.

Rapid, simple and reliable PCR tests can also have an important role in research, as shorter PCR cycle time, lower batch cost and **quicker turnaround** between batches allows researchers to follow trains of thought much more intuitively.

6. References

1. Elvidge, Suzanne. Who invented PCR? xxpress blog. [Online] 8 June 2012. [Cited: 20 September 2013.] http://xxpresspcr.com/who-invented-pcr/.

2. Stöppler, Melissa Conrad. PCR (Polymerase Chain Reaction). MedicineNet. [Online] 30 January 2009. [Cited: 2013 September 2012.] http://medicinenet.com/pcr_polymerase_chain_reaction/article.htm.

3. Burroughs, Nick and Karteris, Emmanouil. Ultra High Speed PCR Instrument Development. [book auth.] Tania Nolan and Stephen A Bustin. PCR Technology: Current Innovations. Third. 2012.

4. Jogan, J M J and Edwards, K J. An Overview of PCR Platforms. [ed.] Julie Logan, Kirstin Edwards and Nick Saunders. Real-Time PCR: Current Technology and Applications. s.l. : Caister Academic Press, 2009, 2.

5. Jain, Tarun. Cepheid's SmartCycler. Biocompare. [Online] 11 April 2006. [Cited: 2 September 2012.] http://biocompare.com/Product-Reviews/41185-Cepheid-s-Smart-Cycler/.

6. Elvidge, Suzanne. PCR – past, present and future. Laboratory News. 19 February 2013.

7. —. PCR 101: An introduction to PCR. xxpress blog. [Online] 4 July 2012. [Cited: 20 September 2013.] http://xxpresspcr.com/pcr-101-an-introduction-to-pcr/.

8. Ultrafast DNA amplification with a rapid PCR system. Hermann, Hanno, Knippschild, Claus and Berka, Alexander. November 2010, BTi.

9. Illumina. Eco Real-Time PCR System User Guide. 2012.

10. Burroughs, Nick and Karteris, Emmanouil. Chapter 10: Ultra High Speed PCR Instrument Development. [book auth.] Tania Nolan and Stephen A Bustin. PCR Technology: Current Innovations. Third edition. 2012.

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