Custom Publishing From:

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

Sponsored By:

Canon
CANON BIOMEDICAL

PCR: AMPLIFIED TO THE END POINT

INTRODUCING 2-MINUTE PCR

Since PCR was introduced, DNA replication has incrementally gotten easier and faster. Now, a revolution has happened. NEXTGENPCR heats and cools instantly — "ramp rates" no longer exist. Using one thermocycler, you can run more than ten plates per hour. The math adds up quickly — 8 hours of 10 plates in a 384-well format means more than 30,000 data points in a single day.

Ultrafast
100 base pairs and 30 cycles in 2 minutes

Versatile
Open chemistry

Flexible
96-well or 384-well format without changing blocks

Precise
Well uniformity of less than 0.1°C

GO FASTER

www.canon-biomedical.com/gofaster

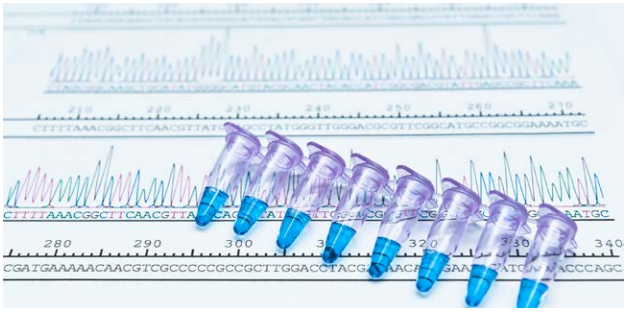
contactus@canon-biomedical.com | www.canon-biomedical.com | 844-CANONBIO

For research use only. Not for use in diagnostic procedures.
The NEXTGENPCR products are manufactured by Molecular Biology Systems, B.V. www.nextgenpcr.com

Canon
CANON BIOMEDICAL

A Series of Fortunate Events: The Polymerase Chain Reaction

"The idea first came to Mullis in 1983 while he was driving down a California highway: a pivotal moment that has since transformed life science."



PCR's Humble Beginnings

The polymerase chain reaction (PCR) was developed by Nobel Laureate Kary B. Mullis in 1985 while he was working as a molecular biologist at the Cetus Corporation (a biotechnology firm in Emeryville, California) to make millions of copies of small amounts of, or hard-to-come-by, DNA.[1] The idea first came to Mullis in 1983 while he was driving down a California highway: a pivotal moment that has since transformed life science. PCR was developed as a manual process initially, which meant it was slow and laborious.[2,3] Seeing its potential for many areas of research, scientists at the Cetus Corporation partnered with PerkinElmer and set about to automate the process, and thus, "Mr Cycle", the world's first thermocycler, was born. By 1989, Cetus and Hoffman-LaRoche had joined forces to develop and commercialize in vitro human diagnostic products using PCR technology.[4] Several commercial companies have since picked up the baton, resulting in an abundance of choices for life scientists.

PCR in the World

PCR is now a staple in biochemistry, biology, and chemistry labs worldwide. In research, it's primarily used as a starting point for various other assays. Examples include creating multiple copies of DNA before sequencing or before introducing DNA into a host organism.[5] However, PCR has become an essential assay in the real world and helped to advance research in areas as diverse as diagnosing Legionnaire's disease[6] and detecting HIV.[7] The technique is crucial for the diagnosis of genetic disease; it's used in preimplantation diagnosis prior to in vitro fertilization procedures, screening fetuses for genetic disease before birth, and diagnosing inherited diseases.[8] It can be used for precision medicine, such as in the treatment of cancer,[9] and it's also used to monitor the spread of infectious diseases in both animal and human populations.[10] PCR can even be used in archaeology to identify insects trapped in amber,[11] or to track human migration patterns.[12]

Steps to Extension: The Basic PCR Procedure

The fundamental steps of a PCR reaction are denaturation, annealing, and extension, and these steps are repeated for many cycles. A thin PCR tube or plate containing a DNA template, "free" nucleotides, and two short DNA primers are mixed together along with a DNA polymerase enzyme and magnesium-containing buffer. The tube is heated (typically 94 °C to 96 °C) to denature the DNA template into single strands, exposing the DNA segment of interest. Upon cooling (typically 45 °C to 60 °C), the DNA primers anneal to complementary DNA sections. The tube is then heated to the active temperature of the DNA polymerase (typically to 72 °C), extending the primers by incorporating the "free" nucleotides into the complementary strand of the DNA. In traditional end-point PCR, one cycle takes a few minutes and results in two copies of the DNA segment of interest. This cycle is repeated (25 to 45 cycles) to create millions of copies of the desired DNA fragment.[5]

Take Your Pick: Types of PCR

End-point PCR describes analysis after all cycles of PCR are complete (i.e., after amplification is complete). It is the name for the original standard amplification technique, but PCR now comes in many varieties. Real-time PCR (also known as quantitative PCR, qPCR, or RT-qPCR), measures the accumulation of amplified DNA in real time, which allows quantitative analysis of gene expression, removes the need to run PCR product on a gel after the reaction, and gives insight into how well a reaction worked. Reverse-transcription PCR (RT-PCR), uses RNA as a template prior to being converted to cDNA for the PCR reaction, which is a sensitive technique for genetic disease testing, characterizing gene expression, and detecting viruses. Nested PCR, is a modified PCR protocol whereby non-specific binding is reduced by using two pairs of PCR primers (one "outer" pair and one more precise "nested" pair) in two consecutive rounds of PCR to enhance the specificity and yield of the desired amplicon. Multiplex PCR amplifies multiple targets in a single PCR reaction, which saves time, and also makes comparison of multiple amplicons possible.[13]

For references, please see page 7.

PCR: World Applications

"PCR and its inherent sensitivity have revolutionized genetic testing"

PCR is the cornerstone of genetic diagnostic testing; however, it is also the foundation for various other applications, in scientific fields as diverse as forensics and agriculture.

PCR Proof: Genetic Diagnostics

Cystic fibrosis, sickle cell anemia, phenylketonuria, and muscular dystrophy are just a handful of the genetic diseases that use PCR as a test method.[1] Genetic testing is normally performed on cells, or sometimes just a single cell, taken from an embryo or fetus before birth. PCR and its inherent sensitivity have revolutionized genetic testing in this respect and have allowed individuals undergoing in vitro fertilization to test for certain genetic diseases before an embryo is even implanted.[2] Paternity testing is also carried out by PCR. A cheek swab is taken from the parents and child, and sections of DNA called loci are amplified to enable the comparison between each individual.[3]

Solving Crimes: Forensics

Genetic fingerprinting is a tool used for identifying or ruling out suspects in criminal cases based on their DNA profile. Minute amounts of DNA from samples as diverse as hair, touch DNA, or bodily fluids can be taken from a crime scene and amplified by PCR, identifying a single suspect from millions of others.[4] DNA databases allow the comparison of all known genetic profiles. PCR is the gold standard in genetic fingerprinting as its fast turnaround, small equipment footprint, ease of use, and high sensitivity allow for simple yet effective analysis of the smallest traces of DNA. PCR from mitochondrial genes has also gained popularity in recent years in forensic science due to mitochondria's inherent high copy number per cell, matrilineal inheritance, and lack of recombination of its DNA.[4]

A Tool in Agriculture

Agriculture makes use of PCR during product development: seed quality control, gene discovery and cloning, vector construction, transformant identification, screening and characterization are just a few uses. Third-party diagnostic testing services rely on PCR to test for the presence or quantity of genetically modified (GM) material in a product and to estimate GM copy number or zygosity in seeds and plants.[5]

In grain handling and processing, GM events are also tested for by PCR; for example, a country producing GM crops may export to a country that does not allow certain genetic modifications. In this case, the importing country will use qualitative PCR to test for GM events to make sure the imported product is compliant.[5]

Sequencing

Traditional (Sanger) sequencing and next-generation sequencing (NGS) both make full use of PCR to amplify DNA prior to DNA sequencing and to create templates for sequencing. Sanger sequencing involves using capillary electrophoresis in combination with automated base calls to sequence long DNA fragments, producing one forward and reverse read, whereas NGS allows millions of fragments to be sequenced in a single run. To avoid problems with DNA strand reassociation during sequencing reactions, single-stranded DNA (ssDNA) templates may be produced by PCR.[6]

All the Small Things: PCR in Microbiology

Rapid detection is essential for foodborne illnesses to enable the correct treatment protocol to be followed. PCR's sensitivity and specificity make it an ideal tool in this respect. Various PCR types are used for pathogen detection including traditional PCR and quantitative PCR, which can detect single bacterial species such as *E.coli* and *Shigella* spp., and multiplex PCR, which can detect multiple species at once through simultaneous amplification of multiple gene targets.[7]

PCR also has use in the beer industry. The technique is used to detect bacterial contamination of wort, beer, and yeast slurries, or to detect the strain, health, and purity of yeast cultures. Real-time PCR quickly provides brewers with valuable information about the quality of their product that previously took days to obtain.[8]

It's Hereditary: Genealogy and PCR

A particularly useful aspect of PCR is its ability to amplify specific sections of the Y chromosome and mitochondrial DNA, which are used in genealogy testing. Both have been used extensively to study human lineages as they are both inherited in a haploid manner.[9]

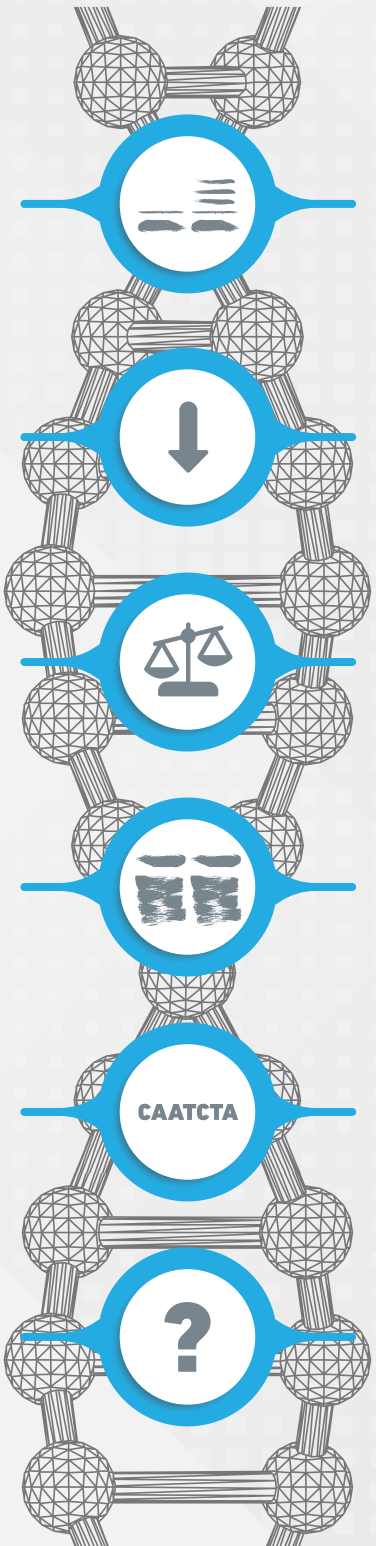
For references, please see page 7.

AMPLIFICATION ANGST

OVERCOMING COMMON PCR CHALLENGES

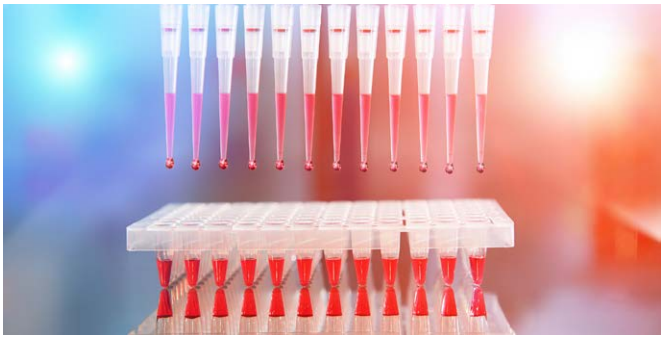
Ever had to deal with a petulant DNA template, shady PCR product smearing, or mysterious extra bands on your gels? PCR is full of challenges that can leave you feeling left in the dark. Our troubleshooting guide will help guide you back to the light!

PREDICAMENT	SOLUTIONS
NON-SPECIFIC AMPLIFICATION Potential causes: Premature replication, annealing temperatures too low, contamination, poor primer design, incorrect magnesium concentration	Set up the PCR experiment on ice, use a hot start polymerase, increase the annealing temperature, use a dedicated clean work area and pipette, use a longer primer, avoid GC-rich ends, check for complementary sequences, adjust the magnesium concentration in small increments
LOW YIELD Potential causes: Poor primer design, incorrect annealing temperature, thick-walled PCR tubes, incorrect primer concentration, not enough cycles, low starting DNA concentration	Optimize the primer using software, optimize the annealing temperature by 1–2 °C, use thin-walled PCR tubes, increase the primer concentration in increments up to 0.5 µM, increase cycling up to 45 cycles, start with a DNA sample in the ng/ul range
INCORRECT PRODUCT SIZE Potential causes: Nuclease contamination, poor primer design, incorrect annealing temperature	Use fresh solutions, optimize the primer, optimize the annealing temperature by 1–2 °C
SMEARING OF PCR PRODUCT Potential causes: DNA starting template concentration too high, contamination, DNA polymerase concentration too high, too many cycles, poor primer design, incorrect magnesium concentration	Determine the best starting concentration using serial dilutions, use fresh solutions and a clean work area, lower the DNA polymerase concentration, reduce the cycle number in 3-cycle increments, optimize the primer, optimize the magnesium concentration
SEQUENCE ERRORS Potential causes: Incorrect nucleotide concentration, damaged DNA template, sequence toxicity to the host, problems with reaction conditions and components	Use a fresh nucleotide mixture or DNA template, limit UV exposure, clone into a different vector, decrease the magnesium concentration, extension time, and number of cycles, try using a high-fidelity polymerase
NO PRODUCT Potential causes: Almost anything! Primer problems, missing reaction components, incorrect temperatures during PCR, suboptimal template, contamination	Change the PCR conditions one component at a time to troubleshoot (optimize primer, fresh reagents, optimize DNA template, optimize magnesium concentration, increase cycles, check thermocycler program)



Fastest to the Finish Line: Increasing Throughput

"New technology allows the use of microplates that can handle up to 384 samples while eliminating temperature ramping requirements, enabling PCR reaction times of 2 to 10 minutes."



An avalanche of publications since the 1980s exists that details improvements to PCR protocols and components. Despite these improvements, a typical PCR reaction still takes hours to complete. However, the tides are changing, and things look set to speed up.

Time is of the Essence: PCR Problems

It's an unfortunate fact that any type of laboratory analysis comes with its fair share of challenges, and PCR is no exception. Potential issues include contamination, sequence errors, thermocycler problems, DNA template and primer issues, low yields, or no bands whatsoever - the source of many a researcher's hair pulling.[1] Many of these issues can be overcome by consecutively changing one reaction component at a time. Often, magnesium or DNA polymerase concentrations need adjustment, a fresh batch of reagents is required, or thermocycler conditions necessitate modification.[2] Troubleshooting PCR problems takes time, and when a single PCR reaction takes hours to complete, time is of the essence. However, all of these problems can be overcome, and with recent advancements in PCR technology, the troubleshooting process is quicker than ever before.

The Collaboration Game: PCR Progression

Scientists from commercial, government, and university research laboratories are to thank for advancements in PCR technology over the past 35 years. One of the first major PCR advancements, in 1986, was the isolation of the thermostable *Taq* polymerase from *Thermus aquaticus*,[3] a bacterium that resides in hot springs, which removed the need for human involvement during PCR (DNA polymerase from *E. coli* was used prior to this discovery). *Taq* has remained the DNA polymerase of choice for PCR ever since, and further enzyme modifications enable amplification of very long strands of DNA or copying with very high fidelity.

Computer software revolutionized PCR further, making once difficult-to-solve DNA primer and template problems much easier to deconvolute.[4] Furthermore, the creation of and continual addition to genetic libraries such as GenBank[5] and EMBL[6] have simplified the development and validation of molecular-based diagnostic procedures using PCR.

Equipment advances since the first automated thermocycler include new robotic elements, microfluidics, chip technology, thin-walled tubes, and nanotechnology. These technological breakthroughs have made PCR faster, workflows more efficient, and allowed the development of different types of PCR such as quantitative and reverse-transcription PCR. Other technical advances include "hot start" systems and the miniaturization of PCR assays.[7] These advances would not have happened if it weren't for multiple successful collaborations between industry and academia.

Full Speed Ahead: Ultrafast PCR

Traditionally, researchers using PCR were from academic laboratories. But more recently, the industry is seeing a trend where biopharmaceutical and other commercial ventures are also requesting PCR equipment. And with the increasing demand for PCR comes the need for highly efficient thermocyclers able to perform PCR in the shortest amount of time possible, able to handle multiple samples at once, and able to do all of this without compromising assay integrity.

Ultrafast PCR is the one of most recent advancements in PCR. New technology allows the use of microplates that can handle up to 384 samples while eliminating temperature ramping requirements, enabling PCR reaction times of 2 to 10 minutes.[8] This increased speed provides the flexibility to do high-throughput work or simply get to the result much faster to move research forward.

This increase in PCR speed can not only quicken the now-routine technique that has become a staple method in life science labs throughout the globe but also make the tedious task of PCR troubleshooting a little less, let's say, traumatic.

From industry to agriculture, diagnostics to microbiology, genealogy to forensics, ultrafast PCR is a simple solution to increase productivity. With advancements in PCR happening at a lightening-fast rate, it will be interesting to see where this California-dreamed-up invention takes us next.

For references, please see page 7.

Article 1 - A Series of Fortunate Events: The Polymerase Chain Reaction

References

1. "Kary B. Mullis – Facts" Nobelprize.org, Nobel Media AB, 2014. Accessed on June 11, 2018.
2. R.K. Saiki et al., "A Novel Method for the Prenatal Diagnosis of Sickle Cell Anemia," Amer Soc Human Genetics, 1985.
3. R.K. Saiki et al., "Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia," *Science,* 230:1350-1354, 1985.
4. M. Fakruddin et al., "Competitiveness of polymerase chain reaction to alternate amplification methods," *Am J Mol Biol,* 3:71-80, 2013.
5. L. Garibyan and N. Avashia, "Research techniques made simple: Polymerase chain reaction (PCR)" *J Invest Dermatol,* 133(3):e6, 2013.
6. B.M.W. Diederen et al., "Utility of real-time PCR for diagnosis of Legionnaires' disease in routine clinical practice," *J Clin Microbiol,* 46(2):671-677, 2008.
7. M. Holodniy et al., "Detection and quantification of Human Immunodeficiency Virus RNA in patient serum by use of the polymerase chain reaction," *J Infect Dis,* 163(4):862-866, 1991.
8. W. Navidi and N. Arnheim, "Using PCR in preimplantation genetic disease diagnosis," *Hum Reprod,* 6(6):836-849, 1991.
9. L. Chin et al., "Cancer genomics: from discovery science to personalized medicine," *Nat Med,* 17:297-303, 2011.
10. N.D. Wolfe et al., "Origins of major human infectious disease," *Nature,* 447:279-283,2007.
11. R.J. Cano et al., "Amplification and sequencing of DNA from a 120-135-million-year-old weevil," *Nature,* 363:536-538, 1993.
12. M.C. Fisher et al., "Biogeographic range expansion into South America by Coccidioides immitis mirrors New World patterns of human migration," *PNAS,* 98(8):4558-4562, 2001.
13. J. Singh et al., "A critical review on PCR, its types and applications," *Int J Adv Res Biol Sci,* 1:65-80, 2014.

Article 2 - PCR: World Applications

References

1. N. Mahdieh and B. Rabbani, "An overview of mutation detection methods in genetic disorders," *Iran J Pediatr*, 23(4):375-388, 2013.
2. Y.-S. Yang et al., "Preimplantation genetic screening of blastocysts by multiplex qPCR followed by fresh embryo transfer: validation and verification," *Mol Cytogenet*, 8:49-55, 2015.
3. M.A. Jobling et al., "The Y chromosome in forensic analysis and paternity testing," *Int J Legal Med*, 110(3):118-124, 1997.
4. A. Alonso et al., "Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies," *Forensic Sci Int*, 139(2-3):141-149, 2004.
5. M. Lipp et al., "Polymerase chain reaction technology as analytical tool in agricultural biotechnology," *J AOAC Int,* 88(1): 136-155, 2005.
6. U.B. Gyllensten, "PCR and DNA sequencing," *Biotechniques,* 7(7): 700-708, 1989.
7. J.W.-F. Law et al., "Rapid ethods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations," *Front Microbiol*, 5(770):1-19, 2014.
8. K. Sakamoto and W.N. Konings, "Beer spoilage bacteria and hop resistance," *Int J Food Microbiol*, 89(2-3):105-124, 2003.
9. J. Cummins, "Mitochondrial DNA and the Y chromosome: parallels and paradoxes," *Reprod Fertil Dev*, 13(7-8)533-542, 2001.

Article 3 - Fastest to the Finish Line: Increasing Throughput

References

1. S.A. Bustin et al., "Quantitative real-time RT-PCR – a perspective," *J Mol Endocrinol*, 34:597-601, 2005.
2. K.H. Roux, "Optimization and troubleshooting in PCR," Adapted from PCR Primer: A Laboratory Manual, 2nd edition (eds. Dieffenbach and Dveksler), CSHL Press, Cold Spring Harbor, NY, USA, 2003.
3. R.K. Saiki, et al., "Primer-directed enzymatic amplification of DNA," *Science* 239:487-491, 1988.
4. K.A. Abd-Elsalam, "Bioinformatic tools and guideline for PCR primer design," *Afr J Biotechnol*, 2(5):91-95, 2003.
5. D.A. Benson, "GenBank," *Nucleic Acids Res*, 36:D25-D30, 2008.
6. M. Goujon et al., "A new bioinformatics analysis tool framework at EMBL-EBI," *Nucleic Acids Res*, 38(2):W695-W699 , 2010.
7. L.H. Lauerman, "Advances in PCR technology," *Anim Health Res Rev* 5(2):247-248, 2004.
8. S.A. Bustin, "How to speed up the polymerase chain reaction," *Biomolecular Detection and Quantification*, 12:10-14, 2017.

PCR AMPLIFICATION OF A 100 BP DNA FRAGMENT IN LESS THAN 2 MINUTES

100 BASE PAIRS

Anneal
Denature 3 Extend
STEPS

30 CYCLES

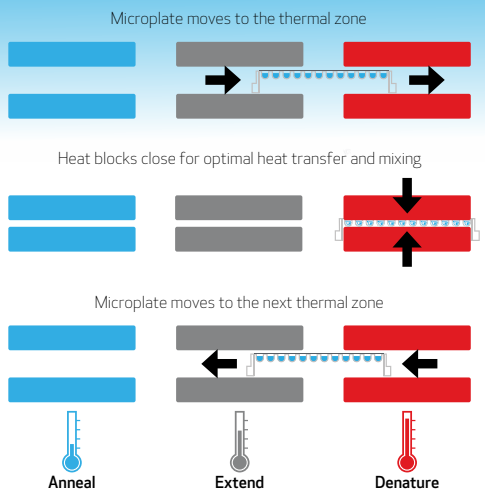
= 2 MINUTES

ULTRAFAST PRECISE FLEXIBLE UNCOMPLICATED VERSATILE

NEXTGENPCR, a novel thermocycler from Molecular Biology Systems

NEXTGENPCR virtually eliminates PCR ramp times by using unique thermal zones. As illustrated in Figure 1, a microplate moves between thermal zones maintaining the temperatures to denature, anneal, and extend DNA. The wells of the microplate are made from 40 micron polypropylene, and the samples are heat sealed into individual wells. Compression of the ultrathin microplate wells by the thermal zone blocks instantly transfers the applicable temperature and mixes the sample. By optimizing each step of the PCR, DNA amplification can be as quick as 1 minute and 59 seconds as demonstrated by the amplification of a 100 bp fragment of the *NQ01* gene shown in Figure 2.

Figure 1: Diagram of NEXTGENPCR thermal zones.

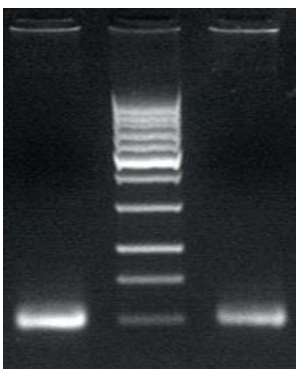


Amplification of a 100 bp fragment of the *NQ01* gene

Using the advanced mode function of NEXTGENPCR and reagents from KAPA Biosystems, the 100 bp fragment was amplified then visualized by running on a 2% agarose gel next to a 100 bp DNA ladder.

Reaction mix	Cycling profile
1X PCR buffer 0.3 mM of each dNTP 4 mM $MgCl_2$ 2.5 ng template DNA 1 M of each primer 0.5 Units of KAPA2G Fast HotStart DNA Polymerase	Initial Denaturation: 98°C, 10 sec 5 cycles Denaturation: 98°C, 2 sec Annealing: 60°C, 2 sec Extension: 75°C, 2 sec 25 cycles Denaturation: 98°C, 0.5 sec Annealing: 60°C, 0.5 sec Extension: 75°C, 0.5 sec

Figure 2: *NQ01* amplification product.



LEARN MORE

www.canon-biomedical.com/gofaster

contactus@canon-biomedical.com | 844-CANONBIO

For research use only. Not for use in diagnostic procedures.
The NEXTGENPCR products are manufactured by Molecular Biology Systems, B.V.
www.nextgenpcr.com

Canon
CANON BIOMEDICAL