CHAPTER 1 INTRODUCTION

This chapter briefly introduces PCR technique and its applications, PCR thermal cyclers, and the characteristics of our Gene Q Thermal Cycler.

1 PCR Technique

PCR (Polymerase Chain Reaction), or cell-free molecule cloning, is a technique for amplifying nucleic acids in vitro, emulating natural DNA replication process. The PCR technique, using two DNA strands to be amplified as template, and a pair of synthetic oligonucleotides as primers, rapidly reproduces the specific DNA pieces under the catalysis of a thermostable DNA polymerase. Because of its simplicity, rapidity, specificity, and sensitivity, PCR has been widely applied to all fields of life sciences since its invention by Mullis in 1983 and the discovery of the thermostable DNA polymerase by Erlich in 1989. Great achievements have been obtained in PCR's applications in such areas as cytology, virology, oncology, genetics, forensics, and immunology. PCR technology is a milestone in the history of modern molecular biology.

2 PCR Applications

Research Areas	Gene cloning, DNA sequencing, mutation analysis, gene recombination and mutagenesis, identification and adjustment of DNA sequence of protein structure, detection of gene extension, construction of synthetic genes, construction of cloning and expression carrier, detection of polymorphism of a gene's inscribed enzyme;
Clinical Diagnoses	Bacteria(spirochaeta, rickettsia, diphtheria bacillus, colon bacillus, dysentery bacillus, and clostridium); Virus(HTLV, HIV, HBV, HCV, HPVS, EV, CMV, EBV, HSV, measles virus, rotiform virus, B19 virus, and Lhasa virus); Parasite(malaria); Human hereditary diseases(Lesh-Nyhan syndrome, hemophilia,BMD, and DMD);
Immunology	HLA locus typing, qualitative analysis of T-cell receptor or antibody diversification, immune body gene mapping, quantitative analysis of lymph genes;
Human Genome Project	Identification of DNA markers using discrete repetitive ; Sequence, construction of a genetic linkage map(detection of DNA, polymorphism, or semen mapping); Construction of a physical map, sequencing, and map expression;
Forensics	Specimen analysis in the venue, and HLA-Dq$_\alpha$locus typing;
Oncology	Pancreas cancer, rectum cancer, lung cancer, thyroid gland cancer, melanin cancer, and leukemia;
Social and Colony Biology	Genetic species research, evolution research, animal protection research, ecology, environmental sciences, and experimental genetics;
Paleontology	Specimen analysis in archeology and museum;
Biology	Diagnosis of animal hereditary diseases and detection of plant pathogeny.

3 PCR Thermal Cyclers

A PCR thermal cycler is a key device throughout the PCR experiment. Its performance determines the accuracy of the experimental results. Its parameters, such as temperature keeping and temperature ramp rate, play an extremely important role in DNA denaturation, annealing and extension.

Chief Specifications of a PCR Thermal Cycler:

Temperature display accuracy	The bias between display temperature and practical temperature, directly affects the quality of DNA annealing, elongation, and denaturation, and should be reduced to an accepted level;
Heating/Cooling Rate	Higher cooling/heating rate can greatly decrease the experimental time and maintain the enzyme's activity;
Temperature Uniformity	Excellent temperature uniformity guarantees consistent amplification conditions for the same batch of reaction samples and then avoids false positive or negative of the results caused by mismatched operating conditions;
Temperature Control Accuracy	Higher temperature control accuracy ensures temperature stability during the PCR amplification reaction, and therefore increase the reliability of the experimental results;
Intelligent Degree	More intelligent thermal cycler means less labor intensity for test personnel and ensures the whole PCR reaction to be accomplished successfully.

4 Common PCR Thermal Cyclers

a) Gradient Water Bath Thermal Cyclers
A gradient water bath thermal cycler consists of three water baths operating at different temperatures. An automatic mechanical manipulator soaks the sample tube containing reactants in the three water baths cyclically, and thus finishes the three processes of denaturation, annealing and extension.
This device has high temperature control accuracy, high heating/cooling rate, and excellent temperature uniformity. However, its intelligent level is very low, and it cannot accomplish some relatively complex PCR processes. There are other disadvantages associated with this type of thermal cycler, such as liquid evaporation in the bath, lack of the soak mode, inability of long-term operation without human interference, temperature fluctuation caused when the sample tube is put into the bath during heating/cooling process, and last but not least, the pollution issue. As a result, this kind of thermal cycler has gradually been abandoned in the market.

b) Compressor Cooling Thermal Cyclers
At the center of a compressor cooling thermal cycler is located a block made of good conduction material (aluminum or silver), over which several conical holes are distributed. Each of the conical holes has the same shape as a standard microcentrifugal tube to ensure good contact between the two. The resistance heater on the block's outside bottom is responsible for block heating, whereas the compressor takes charge of block cooling. Controlling the heater and compressor with a microcomputer completes the required heating, cooling, and temperature keeping processes.
This device has much higher intelligent level and smaller size compared to a gradient water bath thermal cycler. Nevertheless, the compressor's inertia not only limits the cooling rate (to about 1℃/sec), but also increases the temperature undershoot. Moreover, there exists temperature gradient over the block near the inlet and outlet of the compressor's evaporation pipe (lower temperature near the inlet area and higher temperature near the outlet area).

c) Thermoelectric Cooling Thermal Cyclers

These are the most advanced thermal cyclers by far. A thermoelectric cooling thermal cycler also has a metal block at its center (like a compressor cooling thermal cycler). A thermoelectric module closely attached to the block's outside bottom accomplishes Block heating and cooling. Not only does this device inherit such features as high intelligent level and small size from a compressor cooling thermal cycler, but it also has higher temperature control accuracy and heating/cooling rate, thanks to the module's excellent temperature response characteristics. And the temperature uniformity across the block is much better compared to a compressor cooling thermal cycler because the modules are distributed over the block's outside bottom.

During its early development, a thermoelectric module was not able to withstand rapid and frequent heating or cooling for long. With improved workmanship, this difficulty has been overcome. Current long service-life thermoelectric modules have the capability of enduring such harsh conditions in a thermal cycler. As result, thermoelectric cooling thermal cyclers have gradually dominated the market.

5 Features **of the Gene Q Thermal Cycler**

The Gene Q Thermal Cycler is a thermoelectric cooling thermal cycler. It features:

a) Reliable and stable operation, because the thermoelectric module is manufactured via the U.S. ITI's thermoelectric cooling technique and the Japanese quality management model;
b) High heating/cooling (heating rate ≥5.0℃/sec., and cooling rate ≥4.0℃/sec.), which greatly reduces experimental time;
c) Automatic temperature ramp control, which allows different heating/cooling ramp settings so that the device can emulate other thermal cycler's temperature control process;
d) Automatic temperature and time increments/decrements during cycling program execution,
e) optimizing the PCR amplification conditions;
f) Automatic link among files, allowing a complex PCR amplification program to be executed;
g) The soak mode at a temperature up to 4℃ without human interference, after normal cycling program execution;
h) Instant inspection of total execution time and remaining execution time;
i) Optional heated lid, preventing the sample in a centrifugal tube from evaporation, which makes paraffin oil unnecessary and operation more convenient, and reduces the sample processing time;
j) Auto-restart in case of power failure, restoring the data before interrupting and continuing to perform the previously interrupted program.

CHAPTER 2 SPECIFICATIONS

This chapter describes the Thermal Cycler's operation, transportation and storage conditions, basic parameters, performance and functions.

1 Normal Operating Conditions

Ambient temperature: 10℃~30℃
Relative humidity: ≤70%
Power supply: ~220-240V, 50/60Hz ,200W
100-120V~, 50/60Hz, 200W

Note:	Before power-on, please check if the above operating conditions are satisfied. Pay special attention to the power line's reliable grounding.

2 Transportation and Storage Conditions

Ambient temperature: -20℃～+55℃
Relative humidity: ≤80%

3 Basic Parameters

Model / Parameters	TC-18/H(b)	TC-24/H(b)
Sample Number	18	24
Tube Volume (ml)	0.5	0.2
Size (mm)	297×212×200（L×W×H）	
Display Screen	320×240 LCD	
Weight (kg)	3.2	
Fuse	250V 2.5A (Φ5×20mm)	
	125V 5A (Φ5×20mm)	
Computer Interface	RS232	

4 Performance

Temperature range:	4℃～99℃
Heating rate:	≥5.0℃/sec(max)
Cooling rate:	≥4.0℃/sec(max)
Temperature control accuracy:	≤±0.3℃
Temperature uncertainty:	≤±0.2℃
Temperature display accuracy:	≤±0.5℃
Block temperature uniformity:	≤±0.5℃
Hotlid work temperature range:	105℃±5℃

Note: ①*Testing conditions for performance above:*
Ambient temperature:23±5℃,humidity≤70%
②*Testing temperature for temperature control accuracy and block temperature uniformity:55℃，72℃，95℃*

Max. number of cycles:	99
Max. procedures within a cycle:	9
Max. temperature keeping time:	5~~9~~9 min. 59 sec.
Max. incubation and in situ time:	99 hour 59 min.
Programs storage:	100

5 Software Functions

File editing and saving
File accessing, modifying and deleting
Automatic file link
Heating/cooling ramp setting
Automatic temperature and time increments/decrements during cycling program execution
Instantly displaying the data at each phase of program execution
Pause of program execution
Stop of program execution
Auto-restart in case of power failure
Sound alarm
Estimating total program execution time and remaining program execution time
Date and time (year, month, day, hour, minute, second) display and calibration
Protecting the device from further damage and alarming in case of failure

Note:	The above software functions are listed just as a reference. The Bioer Co. reserves the right to modify the software functions without notice.