Brief Review

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PCR BY THERMAL CONVECTION

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The Polymerase Chain Reaction (PCR) allows for highly sensitive and specific amplification of DNA. It is the backbone of many genetic experiments and tests. Recently, three labs independently uncovered a novel and simple way to perform a PCR reaction. Instead of repetitive heating and cooling, a temperature gradient across the reaction vessel drives thermal convection. By convection, the reaction liquid circulates between hot and cold regions of the chamber. The convection triggers DNA amplification as the DNA melts into two single strands in the hot region and replicates into twice the amount in the cold region. The amplification progresses exponentially as the convection moves on. We review the characteristics of the different approaches and show the benefits and prospects of the method.

Keywords: Polymerase chain reaction; DNA; thermal convection; quantitative PCR; biotechnology; thermophoresis.

1. Introduction

Since the determination of the structure of DNA fifty years ago, methods to manipulate and detect DNA in a sequence dependent manner became the core of molecular biology. The field exploded with the invention of the polymerase chain reaction (PCR).[1,2] This reaction allowed to exponentially amplify a piece of DNA with a specific sequence to high amounts. In a way, the role of PCR in molecular biology is comparable to how transistors in electronics simplified the amplification of complicated analog and digital signals.

The polymerase chain reaction proceeds in three steps which are periodically repeated (Fig. 1): (i) heating a DNA double helix at 95°C to dissociate the double helix into two single strands (melting); (ii) decreasing the temperature to 50–65°C in the presence of a large excess of two short DNA fragments also called primers, which are complementary to the terminal target sequence and form short double helices at the ends (annealing); (iii) raising the temperature to 72°C in the presence of a thermostable polymerization enzyme called polymerase which elongates each of the two short double helices to generate twice the amount of the target DNA

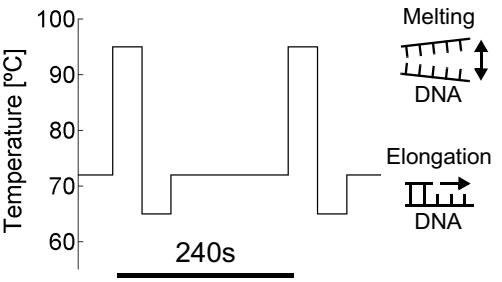

Fig. 1. Temperature oscillation for PCR. In a standard polymerase chain reaction (PCR), the reaction volume is heated periodically between temperatures of 95°C and 55°C–72°C. The target DNA is molten at high temperatures into two single strands, each of which are elongated at colder temperatures to twice the amount of double stranded DNA by a protein. This leads to a exceedingly sensitive exponential amplification of DNA molecules. Additionally, a more intricate mechanism of "priming" the elongation reaction with high amount of short single stranded DNA allows to pick the piece of DNA to be amplified based on their terminal sequences. The PCR reaction is central to a large variety of methods in molecular biology.

(elongation). The temperature cycling can be simplified in many cases to only two temperature levels.

The power of PCR is based on the combination of two main characteristics. PCR shows a tremendous sensitivity. It is an exponential chain reaction which at its best doubles the targeted DNA sequence in each temperature cycle and thus can detect DNA at minute quantities. PCR can be optimized to amplify a single DNA molecule that floats in a 25 μl volume into millions of copies. In comparison, surface based detection methods such as DNA chips are diffusion limited and less sensitive by several orders of magnitude. Since PCR is an exponential amplification, it allows to infer the initial DNA concentration as an exponential measure of the amplification time. This method, also called quantitative or real-time PCR, allows to detect DNA sequence specifically over a broad range of up to 9 orders of magnitude.

PCR is very specific. PCR amplifies only if its short DNA fragments (Primers) exactly match and bracket the sequence of the DNA to be amplified. Primers have a typical length of 20–30 base pairs and the permutation possibilities of $4^{20} = 1.1\times10^{12}$ are sufficient to pick a specific site on the human genome. PCR can amplify a short piece of DNA out of the billions of base pairs of a human genome if and only if the primers match to a single mutation in the genome. Both characteristics make it possible to use PCR as specific and sensitive tool to detect viruses such as HIV and SARS based on their sequence information.[3]

However, the PCR reaction has some drawbacks. Initially, the reaction needed tedious and precise pipetting of all the necessary components. This problem was recently resolved by compacting all the necessary molecules into dried beads.[4] Optimizing the annealing temperature and other fine tuning may also take some time. Moreover, the PCR cyclers that apply a temperature sequences to the reaction vessels are costly. Standard PCR cyclers also do not allow for very fast changes in the vessel temperature.

The thermal mass of traditional instruments makes it impossible to change the temperature fast enough to reach the speed limit of the PCR reaction. A PCR reaction is typically limited by the time of the polymerase to complement single stranded DNA into double stranded DNA in the elongation step. If PCR is used only to detect a DNA with a certain sequence, the length of the amplified DNA can be chosen to be only 50–100 base pairs short. Then the speed limit of PCR is around 15–30 s for a temperature cycle. This ultimate speed limit will probably be pushed in the future, for example with faster polymerases.

To reach such a fast temperature change, complicated and tedious PCR instrumentation is required. For example, thin capillaries are heated and cooled by air in a LightCycler.[3] Also, several microfluidic approaches of PCR have been pursued.[5,6] Cheap instrumentation operating at high speeds can now be realized by using convective PCR.

Convective PCR, although only in its infancy, was proved to work at reasonable to high speed in three very different and mostly simple geometries (Fig. 2). The idea is that a constant temperature gradient across a suitably chosen chamber geometry induces thermal convection between temperatures of about 65°C and 95°C. The reaction liquid rises in the hot regions of the chamber due to thermal expansion and contracts in the colder regions and moves downwards. The geometry is chosen such that the reaction liquid oscillates along laminar trajectories with speeds compatible to the times needed for a PCR reaction. A good thermal convection design will shuttle most of the volume across a temperature profile that can drive PCR.

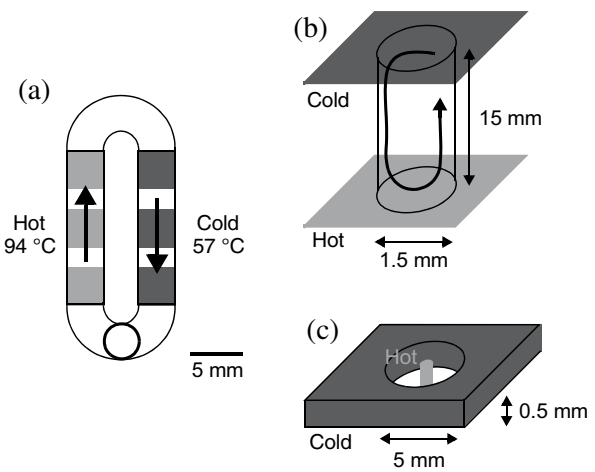

Fig. 2. Designs to perform PCR by thermal convection. Convective PCR utilizes the naturally occurring thermal convection between constant, but differentially applied heating of a chamber. Instead of cyclic heating and cooling a liquid, thermal convection naturally implements the temperature oscillation needed for PCR. Up to now, three convective PCR designs have been tested by different groups. (a) One side of the 0-shaped channel is heated and triggers a circular flow. (b) A Rayleigh–Bénard cell in a circular tubing is heated from below and results in a convective flow. (c) Central heating in a flat disk chamber results in a toroidal convective PCR.

The limitations of convective PCR need to be further explored. The size of possible reaction chambers is expected to range from 10 μm to some centimeters. The limits are determined by diffusion for small volumes on the one hand and costs and non-laminar mixing towards larger reaction volumes on the other hand. These limits set a wide range of possible volumes while the choice of chamber geometries allows to set the flow speeds necessary for PCR.

2. Three Designs

Benett, Richards, Wheeler, Milanovich *et al.*[7–9] designed a closed microfluidic heating pipe (Fig. 3(a)). A microfluidic tubing in the shape of a "0" heats the liquid on one side and cools it on the other side. The PCR solution is placed in a polypropylene bag between heating blocks (Fig. 3(b)). The advantage of such a design is the circulation of all the volume through both temperature zones. There is no dead volume which does not reach the hot or cold temperatures. However the parabolic flow profile results in a distribution of convection times. This leads to a variety of amplification times within the same volume. The first prototype[9] cycled 75 μl for 30 minutes with a return time of the convection flow of approximately 24 s. The resulting PCR yields a clear and bright band (Fig. 3(c)). The authors have used 3D models on a supercomputer to predict the fluid dynamics and to confirm a much more simple 1D calculation. Maximal flow speeds are measured to be 2.3 mm/s. Previously, the same group built fast but nonconvective PCR instrumentations.[5,6] They point out that the convective approach has a very low power consumption. They measured only 850 J per amplification, which should allow AA-batteries as power source.

Krishnan, Ugaz and Burns[10] designed a restricted Rayleigh–Bénard cell (Fig. 4(a)) where the bottom of a cylindrical chamber is heated to 97°C and the top is cooled to 61°C. The instrumentation is deceptively simple since the chambers

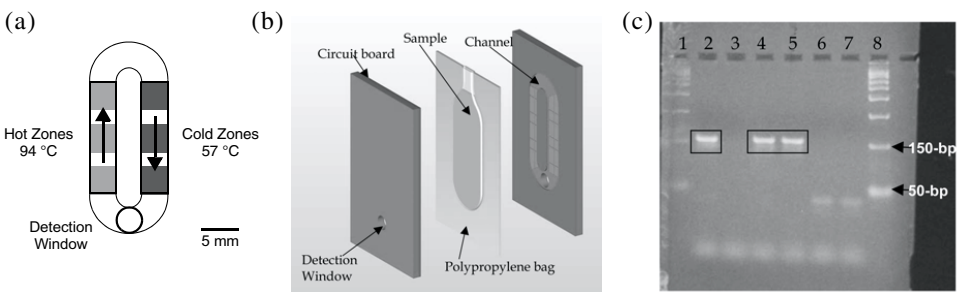

Fig. 3. Convective PCR in a circular pipe. (a) One side of the 0-shaped channel is heated to about 94°C, the other to 57°C. Thermal expansion automatically shuttles the reaction volume from hot to cold and back to the hot part of the piping.[7–9] (c) Gel electrophoresis of 160 bp convective PCR product after 30 minutes in lanes 4 and 5, compared to a standard benchtop MJ Research thermal block PCR in lane 2 and negative controls in lanes 3, 7 and 8. For details see Ref. 9. Figures (b) and (c) reprinted with permission from Ref. 9.

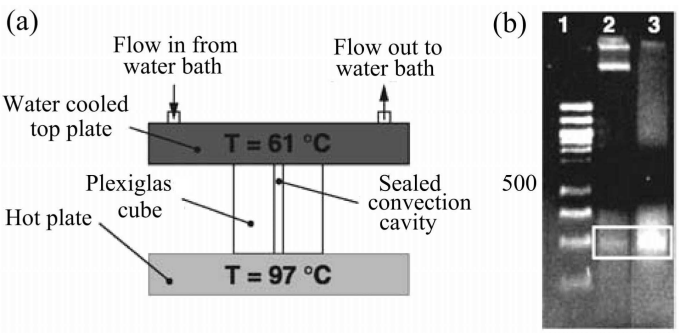

Fig. 4. Convective PCR in a Rayleigh–Bénard cell. PCR in a restricted Rayleigh–Bénard convection cell is induced by heating from below.[10] This leads to laminar convection that elegantly implements the temperature oscillation needed for PCR. (b) Analysis of the product with gel electrophoresis. A 295 base pair product is amplified from human DNA. The product of convective PCR after 1.5 h is shown in lane 2 and clearly yields product at the expected location. Lane 3 shows the product of a PCR performed in a standard PCR instrument. Figures reprinted with permission from Ref. 10.

are made by drilling holes in plexiglas tubes, heated by a hot plate and cooled by a water bath. Based on precise flow measurements with fluorescent beads, the chamber holes were chosen to be 15 mm deep and 1.5 mm wide. It is remarkable that such a simple approach leads to a PCR reaction. The system is optimized to be above the onset of Rayleigh–Bénard convection. Therefore, stationary convection patterns form between the hot bottom and the cooler top of the chamber. Rayleigh–Bénard convection is laminar since it is restricted by the walls of the chamber. From a 0.9 ng/μl sample of human DNA, a 295 bp fragment of the single copy b-Actin gene was amplified with a visible result after 1.5 h (Fig. 4(b)).

Braun *et al.* designed a toroidal convection PCR.[11,12] Here a chamber of 2.5 mm radius and 1 mm thickness is heated in the center by a focussed infrared beam (Figs. 5(a) and 5(b)). This leads to a hot center at about 95°C and a 60°C warm periphery of the chamber. Such a convective PCR is induced all-optically and allows for a cheap reaction chamber without contact to heating devices. The toroidal convection geometry creates only short heat pulses as the liquid passes the central spot with high speed (Fig. 5(c)). The temperature profile is much more compatible with the timing of a standard, non-convective PCR (Fig. 1). The flow is laminar with a Reynolds number of about 1. Fluid dynamic calculations can easily be performed in 2D on a simple PC. The calculations show that about half of the volume is cycled between 60°C and 95°C. The simulated return times reveal a low dispersion with an average value of 12 ± 4 s. Again the power needed to drive the convective PCR is low: 25 minutes of PCR adds up to 75 J of light power, meaning 900 J electrical power to drive the IR Laser.

The described toroidal convective PCR circulates a volume of 20 μl and achieves an at least 100,000-fold DNA amplification within 25 minutes. The PCR was tested by amplifying 96 base pairs from λ-DNA which is a 50,000 base pair long viral DNA.

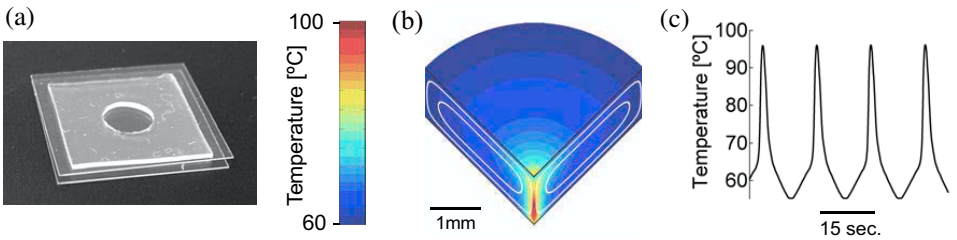

Fig. 5. Toroidal Convective PCR. (a) A cylindrical chamber is centrally heated by infrared radiation.[11,12] (b) This triggers a toroidal convection flow. (c) The calculated temperature along the paths of the flow shows for about 50% of the volume short and high temperature peaks that directly mimic the temperature pattern of standard PCR. The flow speed dispersion is quite low due to the lack of internal boundaries. Figures (b) and (c) reprinted with permission from Refs. 11 and 12.

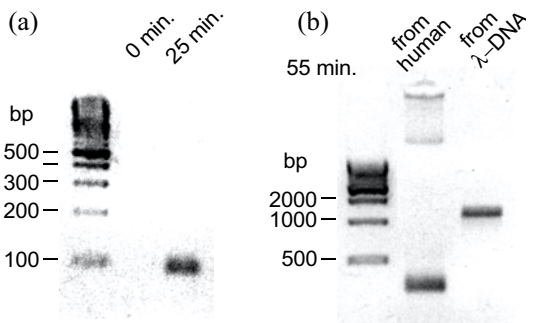

Fig. 6. Results of toroidal convective PCR. (a) A 96 base pair product from λ-DNA shows at least 100,000 $\times$ amplification after 25 minutes.[11] (b) A chamber with half the thickness and twice the radius allows to amplify a 295 base pair sequence from human DNA and can amplify even 1,100 base pair products. Figure (a) reprinted with permission from Ref. 11.

Analyzed by agarose gel electrophoresis, a clear band is found (Fig. 6(a)). The speed is comparable to the fastest PCR reactions available. Reducing the thickness of the chamber to 0.5 mm and doubling the diameter result in slower convection and allow the amplification of 1,100 base pairs after 60 minutes (Fig. 6(b)). The PCR of Fig. 4(b) could be reproduced by toroidal convective PCR with a clear band (Fig. 6(b)).

A standard method in PCR is the visualization of the amplified DNA by fluorescent dyes that are bright only if bound to double stranded DNA. Such imaging confirms the melting of double stranded DNA in the heated center of the convection by showing a dark spot (Fig. 7(a)). Fluorescence imaging of PCR is the basis for the important method of quantitative PCR,[13] also called real-time PCR. As described in the introduction, the time to reach a certain level of end product is a logarithmic function of the initial DNA concentration. For the toroidal convective PCR, quantitative PCR was demonstrated.[11] With increasing initial concentration of DNA, the fluorescence increases more early (Fig. 7(b)) and shows

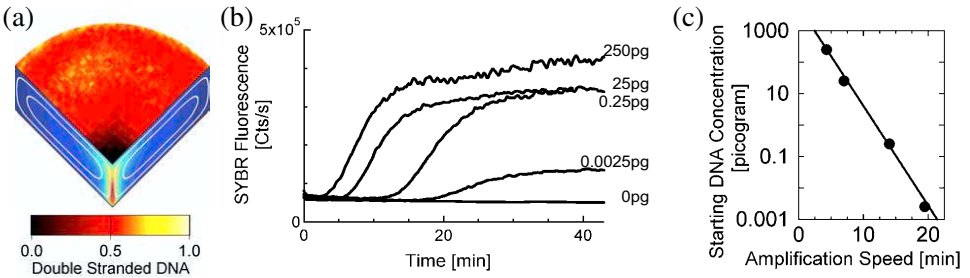

Fig. 7. Quantitative convective PCR. Quantitative PCR can be performed in a toroidal convection.[11] (a) Convective PCR can be monitored by fluorescent intercalating DNA probes. (b) Fluorescence of DNA probes show the amplified DNA concentration over time. Four reactions start with 250 pg, 25 pg, 0.25 pg, 0.0025 pg (= 50,000 copies) of a 86 base pair starting template. (c) Analog to standard quantitative PCR, the time to reach 10% of final product concentration is a logarithmic function of initial DNA concentration. Therefore, convective PCR amplifies DNA exponentially and can be used to perform quantitative PCR to infer initial DNA concentrations over many orders of magnitude. Figure (a) reprinted with permission from Ref. 12, Figs. (b) and (c) from Ref. 11.

the logarithmic dependence expected for exponentially amplifying real-time PCR (Fig. 7(c)). This confirms that the convection really hosts an exponentially amplifying chain reaction of DNA replication.

3. Outlook

Despite the short time by which convective PCR techniques were established, it has reached the performance of fast PCR instrumentations. Further understanding and optimizing the convection flow will lead to smaller, faster, simpler and cheaper designs. It has to be seen whether convective PCR can compete against parallel PCR in multiwell plates. Its major application probably lies in the field of either high speed PCR or low-cost point of care designs with minimal infrastructure requirements.

The simplicity by which the DNA replicating PCR reaction can be implemented in mesoscopic chambers is surprising. The combination of laminar convection and thermal expansion yields an elegant boundary condition for cyclic temperature change of diffusively separated laminar flow lines. One might speculate that similar conditions were crucial for the evolution of the first living molecules.[12] Such a claim is backed by comparable temperature conditions in porous rock near hydrothermal vents on the ocean sea floor[17–22] (Fig. 8) and by other experiments and arguments.[12,14–16] Highly interesting is that toroidal convection (Fig. 5) can accumulate 5,000 base pair DNA more than thousand-fold by an interplay of convection and heat-repulsion of DNA.[23] The latter effect is called thermophoresis, Soret effect or thermal diffusion[24–33] and is well-known for dust particles or polymers in nonpolar solvents. Therefore, two important prerequisites for autonomous molecular evolution of life would be combined in the identical setting: convective thermal

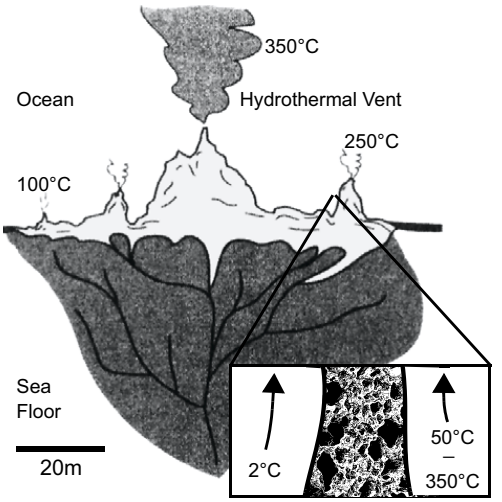

Fig. 8. Convective hydrothermal scenario for molecular evolution of life. Hydrothermal vents volcanically heated water at various temperatures and in various geometries at the deep-ocean sea floor. Reprinted by permission from Hannington *et al.*[19] (inset) Artistic rendering of pores subjected to the steep temperature gradients created between large scale flow of 2°C cold ocean water and hot water ejected by hydrothermal vents. Close proximity of both circulations have been observed.[17–22] In such a geometry, both convective replication and thermophoretic trapping could have driven the molecular evolution of life.

cycling to drive replication of genetic materials in a chain reaction and their thermophoretic accumulation.[23]

To conclude, thermal convection shows a very elegant way to perform the biologically highly relevant polymerase chain reaction (PCR). Although the technique is in its infancy, its low cost, simplicity, high speed and low power consumption already show great potential.

Acknowledgments

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