Polymerase Chain Reaction (PCR)

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Abstract:

Polymerase chain reaction (PCR) is popular widely used methods which makes millions to billion copies of a specific D N A molecules helping scientists to take small sample of D N A And amplify it to an enough amount to study it in details. This method was invented 1984 by the American biochemist Kary Mullis at Cetus corporation. Polymerase chain reaction is very sensitive and specific test used as diagnostic test for different purpose.it is highly specific and and rapid diagnostic test for different infectious disease such as mycobacterium tuberculosis, an aerobic bacteria, human immune deficiency virus (H I V), corona virus (COVID-19), Pertussis (whooping cough), rapid and accurate diagnosis of anthrax specially in case of bioterrorism, salmonella, Malaria, Helicobacter pylori infection. Polymerase chain reaction is also used for diagnosis of cancers specially lymphoma and leukemia. It is also used broadly in research and forensic medicines. According to the importance of polymerase chain reaction in diagnosis of infectious and noninfectious disease we have reviewed principle, procedure, Medical application, strong point (Advantages) and negative point (disadvantages) of polymerase chain reaction.

Keywords: P C R, Review, infectious disease.

Introduction

Polymerase chain reaction (PCR) is standard and widely accepted scientific method in molecular biology, genetic, and microbiology which is used to make rapidly million to billions of copies of specific D N A sample allowing scientist, researcher, and microbiologist to take a very small sample of D N A and modify it to large enough amount to study it in details. Polymerase chain reaction was developed in 1984 by the American biochemist Kary Mullis.I n these days polymerase chain reaction is he most useable, accurate and valid technique in medical and biological research labs and has different application. Polymerase chain reaction is more sensitive and specific technique which has rapidly become one of the most useable technique,

Because it is rapid, inexpensive, simple, and accurate. Polymerase chain reaction is a quick and easy method for generating unlimited copies of D N A fragment, from the Daily practicalities

of medical diagnosis to the theoretical framework of systemic, from court of law to field studies

Of animal behavior .polymerase chain reaction take analysis of tiny amount of genetic material to a new level precision and reliability. Furthermore, many important contribution to the Development and application of Polymerase chain reaction have been made (24).polymerase chain reaction .The Polymerase chain reaction is commonly carried out in a reaction volume of 10-200µl in a small reaction tubes of (0.2-0.5 ml) volume in a thermal cycler. The thermal cycle heat and cold the reaction tubes to achieve the temperature required at each step of reaction. Many modern thermal cycler make use of the peltier effect, which permit both heating and cooling of the block holding the polymerase chain reaction tubes simply by reversing electric current .Thin walled reaction tubes permit favorable thermal conductivity to allow for rapid thermal equilibration .Most thermal cycler heated lids to prevent condensation at the top of the reaction tubes. Older thermo cyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

Goals:

A: To know medical application of polymerase chain reaction.

B: Principle of Polymerase chain reaction.

C: Studying advantage and limitation of polymerase chain reaction.

Principle of Polymerase Chain Reaction:
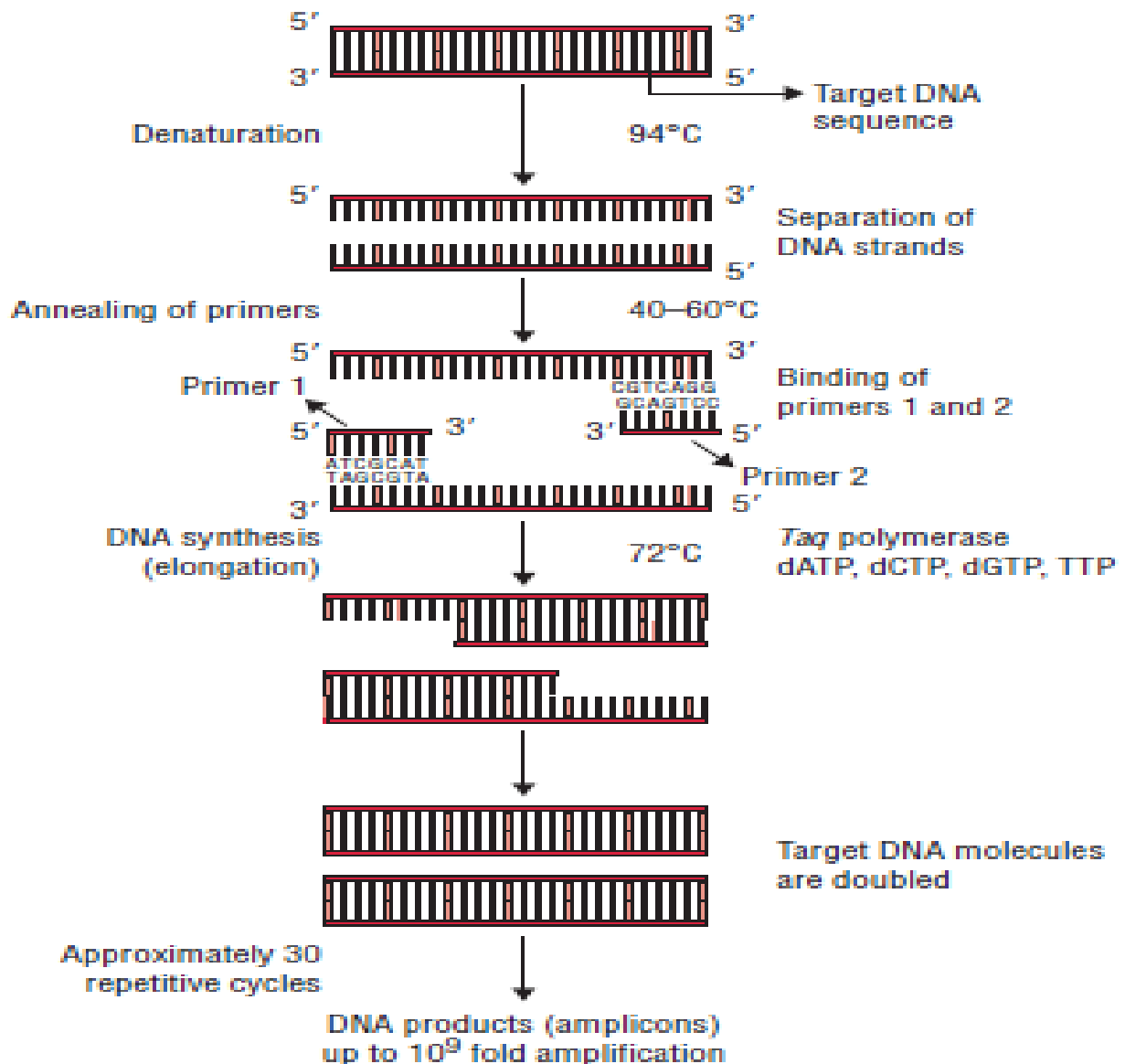
The reaction mixes for polymerase chain reaction contain:

A: The target D N A.

B: A very large excess of the desired primers.

C: Thermos table D N A polymerase.

D: four deoxyribonucleoside triphosphate.

Steps of Polymerase Chain Reaction

Step1: The target D N A containing the sequence to be amplified is heat denaturized to separate its complementary strands .Normally the target D N A is between 100-500 bp in length.

Step2: The temperature id lowered so that the primer can anneal to the D N A on both sides of the target sequence. Because the primers are present in excess, the targeted D N A strand normally anneal to the primers rather than to each other.

Step3: D N A polymerase extends the primers and synthesize copies of the target D N A sequence using the deoxyribonucleated triphosphate (23)

Schematic diagram of Polymerase chain reaction. Subhash Chandra Pariia Text book Microbiology and Immunology Second Edition ELSEVIER A division or Reed Elsevier India Pvt. Ltd ISBN: 978-81-312-2810-4

Medical Application of Polymerase Chain Reaction

1: Infectious Disease:

Polymerase chain reaction is gold standard, rapid highly specific diagnostic technique for diagnosis of infectious disease including, bacterial and viral (10).polymerase chain reaction also permit identification of those bacteria culture of which is difficult or growth is slow, such as mycobacterium tuberculosis and an aerobic bacteria, by using this technique scientist can identify virus from tissue culture (21).

A: polymerase chain reaction is the most acceptable and valid diagnostic technique .This technique can detect as little as one viral genome among the D N A of over 50 000 host cells (13). By using this technique Human immunodeficiency virus infection can detected earlier , blood donated blood can be screened directly, for Human immune deficiency virus, new born can be immediately tested after born tested for H I V virus, as we know in these days all countries of world are face to sever acute respirator syndrome caused by COV-2(SARS CO2) polymerase chain reaction is most useable sensitive and specific test for diagnosis of this disease, Polymerase chain reaction also can quantified effect of anti-viral drugs.

B: Bacterial cause of some disease such tuberculosis, are difficult to be sample from patients slow to be grown in the laboratory (doubling time of mycobacterium tuberculosis is 18hr and for result of culture 4-6 week is needed).Polymerase chain reaction can detect small amount of both dead and live in convenient sample. The overall sensitivity of polymerase chain reaction for diagnosis of tuberculosis is 55-90% with specifity of 99 %.

C: disease like Pertussis (whoopincough or hundred cough) are caused by bacteria bordetella pertussis .this bacteria cause serious acute respiratory infection that effect different animals and human and has led to death of money young children. Bordetella pertussis make a protein which has two part (Active sub unite and binding sub unite), this toxin cause lymphocytosis because prevent transport of lymphocyte to spleen and lymph node (4).polymerase chains reaction is the best technique that can detect gene for pertussis toxin. If we compare Polymerase chain reaction with culture it is more sensitive, specific, rapid and easy technique than culture (24).

D: Anthrax disease caused by large gram positive spore forming bacilli bacillus anthraces, which produce two storing exotoxin (edema factor and lethal factor), so it is used as a source for Weapons (bioterrorism).It is common in animals, but rarely cause disease in human .In human anthrax has three clinical types, cutaneous anthrax, pulmonary anthrax, gastrointestinal anthrax. Polymerase chain reaction in cause of bioterrorism is rapid and highly sensitive and specific diagnostic technique for anthrax (25).

E: polymerase chain reaction can be used for diagnosis of visceral leishmania (Kala –azar) disease with great accuracy, which is caused by leishmania donovani, this type of leishmania is most serious type of leishmania, and without treatment mortality is more than 90 percent(3)

2: Research Application of Polymerase Chain Reaction.

Nested polymerase chain reaction is a good and useable technique for many genetic and research laboratories, along with D N A finger print for forensics and other genetic cases (7)

Advantage & Disadvantage (Limitation) of Polymerase Chain Reaction:

1: Advantages

A: Polymerase chain reaction can be used for variety of experiment and analysis, this technique is also used for diagnosis of money human disease.

B: polymerase chain reaction very useful and confirmatory technique for money infectious disease, like tuberculosis, syphilis, Hepatitis, Mycoplasma, cytomegallo virus infection, Human immune deficiency virus, Cancers especially leukemia and lymphoma, Malaria, Toxoplasma Gondi , fungal infection, staphylococcal bacteremia, (5, 1).

C: Polymerase chain reaction is powerful research and practical research tool. The sequence of of unknown etiology of many disease are being figured out by polymerase chain reaction (12

D: polymerase chain reaction has high sensitivity (90-100%) and high specify (100%).

2: Disadvantages (Limitation) of Polymerase chain reaction

A: One big limitation of polymerase chain reaction is prior information about the target sequence is necessary in order to generate the primer that will allow its selective amplification (11.).

B: Another limitation of polymerase chain reaction is that even the smallest amount of contaminating D N A can be amplified, resulting in miss leading (12).

C: Require trained, experienced, man power and technology.

D: Costly and not all people can afford to do that.

E: Adequate space with air-condition, dehumidifier, laminar flow facilities.

Review of Literature on Polymerase Chain Reaction Application and Diagnosis of Disease

Detectiion of Mycobacterium tuberculosis from 155 suspected cases of tuberculosis attendig Queen Mary tertiary hospital from 1 July 2000 to 30 June 2002 .for diagnosis of pulmonaty and extra pumonary tuberculosis withi over all polymerase chain reaction showed a sensitivit of 78.3% and specifity of 100%.Another study carried out in Mymensing Medical College Hospital Polymerase chain reaction was found to be 94.7 % sensitive and 100% specific(22).

For detection of brucella D N A In serum sample .This technique was found to be 91.9 % sensitive and 95.4 % specific whe tested with 65 negative control samples and 62 serum sample from patients with active brucellosis(20).

Polymerase chain reaction offers an attractive option for direct detection of Trepomema pallidum(a spirochets which caus syphilis).It is specifity for pathogenic trepmeme is 95-97% and sesitivity is 91-95%(15 .24).If we compre it with dark field microscope that has sensitivity of 79-97% and specefity of 77-1oo%(8).

Polymerase chain reacion(PCR) is good technique for detection and differentiaion Salmoneella typhi and paratyphi .It show 100% sensitivite for detection of salmonella typhi and para typhi(7).

Anthrax is zoonotic disease caused by bacillus anthrce (large grampositve bacilli),threaten human and animal in differrent part of world. A study was caried out in the Ngorongoro Conservationn Area on 152 patients.Polymerase chain reaction of D N A extraction from skin showe high sensitivity and specifity.sesitivity was (90-98%) and specifity was (87 -99%J).from anthrax (17).

Polymerase chain reaction and other nucleic acid amplification tests are the most sensitive metnods to diagnose pertusis. Primer for both B pertusis and Bordetella parapertusis should be included(9).

Neisseia gonorrhoeae is the most common sexually transmitted disease causing bacterium world wide .An an hoour polymerase chain reactionn targeting the carbamoyl –phosphate synthase suunit A(car A).gene was developed for the secific detection of Neisseriae gonorrhoeae in clinical specimens .Samples from 605 patients were cultured on selective medium and assaye by polymerase chain reacion in a double blind fashion .Of 605 rehthral /cervical sample analysed, 13 were Polymerase chianin positive , of which 11 were culture positve .The polymerse chain reaction showed 100% specifity and Sensitivity(14).

For diagnosis of Hepatitis A polymerase chain reaction is a specific and sesnsitive diagnostic technique .A study was carried out in Asan Medical Hospital on patients with acute seveer hepatitisfrom June 20 10 to July 2010.polymerase chain reaction for detection of HAV RNA showed sensitivity and specifity of 81.4% and 100% respectively(17)

Polymerase chair reaction is diagnostic confirmatory test and considered as the golden test for the diagnosis and follow up of hepatitis C virus infction.A study was carried out in Irag from Aprill 2014 to Dcember 2014, attending Ramidi teaching hospital, Ramidi children teaching hospital , and private cilinics. In diagnosis of Hepatitis C virus showed 60% Sensitivity and 100% Specifity(16).

Polymeras chain reaction assay are the preferred method to detect corona virus nucleic acid in redpiratory secretion and in stool sample. Viremia with SARS and MERS coronaviruses is detectable in the plasma by polymerase chain reaction(9)

Conclusion

For accurate and valid diagnosis of different disease (from infectious disease to genetic disease and cancers especially lymphoma and leukemia) and forensic research Polymerase chain reactionis highly sensitive, specific, more widely accepted, useable and rapid technique. Polymerase chain reaction has important and key role in diagnosis of disease with Nonspecific and atypical clinical presentations, and mixed infections, which help clinicians to start early treatment, manage better treatment plan and follow up for patients .This lead to reduce social and economic effect of disease on patients and their families. Some polymerase chain reaction finger print methods have high discriminative power and can be used to identify genetic relationship between individuals, such as parent –child or between siblings, and are used in paternity testing.

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