Dna profiling using capillary gel electrophoresis

Everyone have unique DNA fingerprint except identical twin and it can be used to distinguish between individuals. DNA profiling uses the non-coding sequence of DNA strands to perform the analysis. It can be used in both health and judicial system. The application of DNA profiling in judicial system is discussed only in this paper.

The most common DNA profiling techniques are restriction fragment length polymorphism (RFLP) and polymerase chain reaction – short tandem repeats (PCR-STR). Nowadays, the PCR-STR has replaced the traditional RFLP method to perform the DNA analysis. The capillary gel electrophoresis is used for the separation of DNA molecules and the fluorescence primer is detected by CCD. The principle and methodology of DNA profiling by capillary gel electrophoresis using RFLP and PCR-STR are discussed in detail within this paper.

Although DNA profiling helps a lot in health and judicial system, public have great concern in privacy and security of DNA database. Also, DNA profiling faces a lot of challenge in handling some complex sample, such as bone or hair sample, mixture of human DNA.

INTRODUCTION

DNA profiling is one of the most analytical tools in forensic science. It can be applied in health system to diagnose the health diseases and judicial system to give the supporting evidence to the court to make a correct conviction. In the disaster, DNA profiling helps to identify the individuals. DNA profiling uses the non-coding DNA region which doesn’t contain any genetic information. Two most common methods in DNA profiling are restriction

fragment length polymorphism (RFLP) and polymerase chain reaction – short tandem repeats (PCR-STR). Capillary gel electrophoresis is used for the separation of DNA strands in according to its molecular size. The gel in the capillary acts as the molecular sieve to separate the DNA strands in the basis of molecular size.

PRINCIPLE AND METHODOLOGY

DNA consists of phosphate groups which bonded with deoxyribonucleotide by the formation of phosphodiester bonds with 3-hydroxyl of the deoxyribonucleotide and 5-hydroxyl of the next sugar. Four major bases available to attach with the sugar are adenine (A), guanine (G), thymine (T) and cytosine (C). Adenine (A) interacts with thymine (T) while guanine (G) interacts with cytosine (C) only due to the steric hindrance. With the exception of identical twin, everyone should have unique DNA sequence. Everyone inherits half of the DNA from their mother and father respectively. DNA can be found in every cell except red blood cells and located at the nucleus. The structure of DNA is shown as follows:

The nuclear DNA contains 23 pairs of long molecules called chromosome. The human nuclear genome consists of 50, 000 – 100, 000 genes and only minor part of DNA molecule contains such coding sequence that holds genetic information. Most of the regions of DNA molecules do not hold any genetic information and is the spacer DNA between the genes. These regions belong to non-coding sequences forming introns and are used in forensic science to distinguish between individuals. About 3 -5 % of DNA are in coding sequence and are used for protein expression. In the non-coding region, almost 99. 9% of DNA doesn’t have any difference between individuals and

only 0. 1% of DNA shows polymorphism. In the forensic DNA profiling, the regions of genome with a specific variable number of times of repeated sequence of base pairs are used for the analysis. On the other word, the region of genome with high length polymorphism is used for DNA profiling. These regions are called variable number of tandem repeats (VNTR) with 20 – 50 base pairs per repeat. Recently, short tandem repeats (STR) which have repeated sequence of 2 – 4 base pairs per repeat are used for modern advanced method in forensic DNA profiling. VNTR and STR regions are the locus with a number of alleles that constitute a genetic polymorphism. Most of the mutation takes place in these non-coding sequences.

From the criminal scene, blood, hair, saliva, semen or even bone samples can be collected. To ensure the samples collected from the scene is from the human being, the sample should be verified before performing the DNA profiling. For example, the red stain on the floor is suspected to be the human blood. The phenolphthalein test should be performed. The blood hemoglobin possesses peroxidase which is the enzyme to accelerate the decomposition of hydrogen peroxide and produces oxygen. The oxygen will oxidize the phenolphthalein to give the color change from colorless to pink. The chemical reaction is shown as follows:

Afterwards, the nucleus DNA should be isolated before performing the DNA analysis. The basic steps in the DNA extraction are shown as follows:

Cytolysis to disrupt the cell and expose the DNA by adding the lysis buffer. The common lysis buffers are tris-HCl, EDTA in sodium chloride solution.

Incubate the sample with lysis buffer for 30 minutes on ice and centrifuge for 10 minutes at 4oC to get the DNA pellet.

Removal of cell membrane by the addition of detergent, such as sodium dodecyl sulfate (SDS)

Removal of protein by the addition of protease K and incubate it at 37oC in the water bath overnight.

Perform the phenol-chloroform extraction by the addition of same volume of phenol: chloroform mixture and aqueous buffer solution and centrifuge the mixture at 4oC. After the phase separation, the aqueous layer is on the top while the organic layer is at the bottom. Most of the DNA is in the aqueous layer so that it can be isolated.

Precipitation of DNA in ethanol or iso-propanol and re-dissolve in Tris EDTA buffer at pH 8. 0.

Chelating agent can be used to bind with metal ions, such as magnesium and calcium ions that inhibit the Polymerase chain reaction (PCR).

Two common techniques are used for the DNA analysis. They are restriction fragment length polymorphism (RFLP) and polymerase chain reaction – short tandem repeats (PCR-STR). RFLP is the traditional DNA profiling technique which uses the variable number of tandem repeats (VNTR) in the non-coding regions. The restriction enzyme should be used which cut the long DNA strand into a fragment at a precise sequences of 4 – 8 base pairs called recognition sites.

The restriction enzyme cuts the DNA at the recognition site in the VNTR regions. The fragments will be separated by gel electrophoresis. The agarose gel which immersed in an alkaline buffer solution acts as a mesh to separate the mixture of fragments in according to its size of molecule. Under the alkaline medium, the DNA molecule carries negative charge. When a high potential is applied across the agarose gel, the negative charge DNA molecules will move toward positive electrode with different speed. DNA fragments with smaller size will move faster than ones with larger size. As a result, the mixture of DNA fragments can be separated by gel electrophoresis. The DNA fragments then transfer to a nylon membrane by placing it on top of the membrane and covering it with absorbent paper towels. Denature the DNA fragments by adding strong alkaline solution to the gel to break the hydrogen bond between the strands and bound to the nylon membrane with single strands. This method is called southern blotting. To visualize the DNA fragment in different position of the nylon membrane, the radioactive DNA probe is added which is complementary to part of the interesting locus in VNTR region. The probe hybridizes with single-strand complementary fragments. The unbounded probe is washed out and the DNA fragments can be detected by photographic film. The multiple loci can be identified by adding the mixture of single locus probes that identify multiple loci in VNTR region and give the multiple loci DNA fingerprint.

Polymerase chain reaction – short tandem repeats (PCR-STR) is the modern technique in DNA profiling. The DNA molecules amplify its number by the polymerase chain reaction. 1, 2 This method is applicable for trace amount of genetic materials in the sample. To initiate the synthesis of DNA, the

fluorescence primers, four deoxyribonucleoside triphosphates (dATP, dGTP, dTTP and dCTP), polymerase are needed. The primer is oligonucleotide which flanks the locus of STR region to be amplified in PCR. The reaction mixture is heated to 90 – 95oC to denature the target DNA and makes it single-stranded. The temperature is lowered to 50 – 60oC so that the fluorescence primers will bind with complementary sequences of target DNA. The temperature is raised up to 72oC so that the polymerase starts to synthesize the new DNA strand. 3 The PCR is performed for a suitable number of cycles in order to synthesize enough DNA strands. Normally, 25 – 30 cycles should be repeated to amplify enough DNA strands for the DNA analysis. After the first temperature cycle, the replicated DNA strands are produced which is shorter than the original template strands but still longer than the length of STR locus. This intermediate DNA strands will be amplified in each temperature cycle to produce precise-length DNA strands. 4 The DNA allele ladder that represents all possible alleles at the loci should be prepared so that to judge the size of the DNA fragments after the PCR. In the United States, the FBI has standardized a set of 13 STR loci on chromosomes for DNA profiling and organizes the CODIS database for the forensic identification in criminal cases.

In the capillary gel electrophoresis, a mixture of DNA can be separated base on the size of the molecule. For the DNA molecule, it is impossible to be separated base on the electrophoretic flow because the charge increases with the mass of the molecule.

, where = electrophoretic mobility, q = charge, r = radius and

= viscosity

vep = E , where E = electric field

If the capillary packed with gel, such as polyacrylamide, it is possible to separate the DNA molecules base on the size. Under the low field strength, the DNA strands coil into the spherical particle. The electrophoretic mobility is proportional to the volume fractions of the pores of gel that the DNA can enter. As a result, the electrophoretic mobility decreases with increasing the molecular size. DNA with smaller molecular size moves faster because it can move more freely through the network of the gel.

The polyacrylamide gel will be injected into the capillary and it will be ejected out after the analysis. The gel will be used once only so that to reduce the chance of contamination by the previous sample. To synthesize the polyacrylamide gel, the dimethyl acrylamide is distilled to remove the stabilizers. It is then added into the 1 : 3 mixture of methanol and deionized water. The solution is purged with nitrogen for 1 hour to remove oxygen. The ammonium persullfate is added to form the sulfate free radical to initiate the vinyl addition reaction. The solvent is evaporated to collect the polyarylamide.

The electro-osmotic flow is created by the negative charge of the silanol group on the capillary wall and the bulk solution will move toward the negative electrode. The electro-osmotic flow creates the problem of reproducible DNA separation because the speed of DNA molecules will change from run to run. The polymer gel which adsorbed on the capillary wall will mask the charged sites and minimize this effect. The buffer solution

should be used to stabilize and solubilize the DNA and provide the charge carriers for the electrophoretic current. The common buffer used in capillary gel electrophoresis is N-tris-(hydroxymethyl) methyl-3-aminopropane-sulfonic acid and EDTA at pH 8. The additive such as formamide, urea and 2-pyrrolidinone are added to keep the DNA remains denatured. 5 The DNA fragments should be heated to denature at 95oC for 2 minutes and cool to 4 oC in thermocycler. Sample will remain denatured for at least 3 days. It will be injected into the filled gel capillary by electrokinetic or hydrodynamic injection method. Most of the capillary gel electrophoresis use electrokinetic injection, where a high positive voltage is applied in a defined time so that to move the negative charge DNA fragments into the capillary. When a high voltage is applied, the DNA fragments which carry negative charge will move toward the positive electrode and the mixture will be separated base on the size of molecule. The argon laser emits the electromagnetic wave at specific wavelength (488 nm and 514. 5 nm) to excite the fluorescence primers. The emission of light from the primer will be dispersed by the prism and finally detected by CCD. Four reactive dyes used in labeling DNA are shown as follows:

FAM (blue) TAMRA (yellow)

JOE (green) ROX (red)

APPLICATION OF DNA PROFILING

DNA profiling can be used in diagnosis of hereditary disease and chromosome aberrations. Also, it is used in judicial system to identify the suspects in the criminal cases, such as murder, rape, violent assaults. Every

contact leaves a trace. In the criminal scene, we can get some genetic materials, such as hair, bone, saliva, blood, epithelium cell under the victim fingernail and semen. In the rape case, we can collect the semen, saliva and hair samples from the scene. In the assault case, blood sample can be collected. DNA profiling helps to include or exclude the suspect in the criminal case and provide supporting evidence to the court. In the paternity and immigration cases, DNA profiling helps us established or disproved the request of citizenship on the basis of family relationship. For example, a child requests to get the Hong Kong citizenship because of his father is Hong Kong people. Then we need to work out the DNA profiles of his mother, child and father to decide if the man is the child father or not. DNA profiling can also be used for the identification of bodies by the comparison with possible relatives. For example in the air accidents when the victims or dead bodies cannot be recognized, then the DNA profile of the victims or dead bodies can be compared with the DNA profiles of possible relatives. 6 For the identification of long dead body, all nucleuses DNA have been degraded. As a result, the mitochondria DNA (mtDNA) in the bone or teeth can be used for the identification by comparing with the possible relatives. DNA profiling can be used in the study of virus, bacteria, plants and animals. It can be used to diagnose the infectious diseases and illegal transportation of animal.

PROS AND CONS OF DNA PROFILING

For the traditional RFLP technique, it uses high polymorphism VNTR regions for the analysis. There are a large number of alleles for each locus so that it is very rare to have identical DNA profiles from two unrelated individual. The polymorphism of RFLP is higher than STR. However, the restriction fragments

are large and show a continuous distribution. As a result, it is very difficult to distinguish between fragments with similar mass. Also, it is very difficult to automate the sample preparation and need a lot of manpower to perform the analysis. The reproducibility is not as good as short tandem repeats.

For the modern STR technique, polymerase chain reaction helps to amplify the loci of DNA strands so that to increase the sensitivity of detection. Also, the genetic materials collected from the scene normally are in trace amount. This technique is suitable for the trace amount analysis. The sample preparation can be automated and the contamination and labeling mistake can be reduced. However, contamination with DNA from other individuals may cause false result or wrong conclusion because of its high sensitivity. The polymorphism of STR is lower because the number of alleles in each locus of STR region is smaller.

DNA profiling helps us to diagnose some health diseases and viruses, identify the suspects in the crime and diagnosis of family relationship. If DNA profiling technique is used in proper way, it can help to reduce the number of wrongful convictions and protect the innocent. However, some people think that the request of DNA sample violates the individual’s right to privacy and their civil liberties. Also, the security of DNA and the access right are highly concerned. For example, if a person who have a certain genetic risk for certain diseases, the health insurer will deny his claims. Also, inappropriate use of DNA profiling and the sway of it over other evidence on juries and judges can create a system of wrongful convictions. From my point of view, if the DNA profiling only uses the non-coding sequence of DNA, it should not

involve any privacy or security because that are not related to protein expression.

DISCUSSION

One of the advantages of capillary gel electrophoresis over the traditional gel electrophoresis is the samples loaded into the separation medium in an automated in manner. Traditional gel electrophoresis techniques need to load the sample manually before initiating the separation process. Also, only small quantity of DNA sample is needed in each injection. As a result, it may be injected for several times for the retesting purpose. Most of the capillary electrophoresis uses electrokinetic injection method, where a high voltage is applied to draw the negatively charge DNA fragment into the capillary. The quantity of DNA injected into the capillary is related to the electric field (E), injection time (t), concentration of DNA in sample ([DNA]), the area of the capillary opening (πr2) and the ionic strength of the sample over the buffer. It can be describe by the following equation:

[DNA inj] = Et (πr2) (μep +μEOF) [DNA] (λbuffer /λsample)

Where [DNA inj] is the quantity of DNA injected into the capillary, E is the electric field, t is the time, r is the radius of the capillary, μep is the electrophoretic mobility, μEOF is the electro-osmostic mobility which is almost zero in coated capillary, [DNA] is the concentration of DNA in sample andλbuffer /λsample is the ionic strength ratio between buffer and sample solution. However, the negative charge ions such as chloride ions in the sample solution that will compete with the negatively charge DNA fragment so that to reduce the total amount of DNA injected because of the increase

of the ionic strength in sample solution (λsample). Also, smaller DNA molecules will travel quicker into the capillary opening than the larger ones because of higher electrophoretic mobility. To reduce the problem of the interfering ions, the sample can be diluted with deionized water to reduce the sample ionic strength. 7, 8

Formamide is a strong denaturant and it is commonly used in the preparation of single strand DNA samples. Formamide will be decomposed to produce the ionic products such as formic acid which is negatively charged and injected into the capillary. Its decomposed ionic products can cause the problems in sensitivity and resolution. As a result, the ultrapure formamide should be used as the denaturant and the sample solution should be frozen immediately before waiting for the analysis.

Sample stacking technique can be used to minimize the band broadening effect in the injection process. As the voltage is applied, the sample with low ionic strength will produce a high electric field that ends at the interface between the sample zone and buffer inside the capillary. The negatively charged DNA fragments will move quickly into the sample zone and stop moving in low electric field at the zone interface. The DNA fragments will be pre-concentrated at the interface and minimize the band broadening effect of the peak separation. To produce a good stacking interaction, the front end of the capillary should have a low conductivity. This can be done by dipping the capillary in water prior to sample injection.

In the past, cross-linked polyacrylamide gel is used as size separation in capillary electrophoresis. However, the air-bubble formation during the filling

of the capillary and hydrolysis degradation of the acrylamide at alkaline medium causes the problem in separation of biological sample. Nowadays, the uncross-linked polyacrylamide is used as capillary gel. It can be replaced after each run to reduce the chance of contamination. The polymer length and concentration affect the separation characteristics. Increasing the gel concentration and the polymer length improve the resolution but decrease the molecular weight range.

The concentration and conductivity of the buffer solution in the electrophoresis will affect the resolution. Too high concentration and the conductivity will produce a large joule heating and cause overheating of the column and loss of the resolution. The buffer solution dissolves and stabilizes the DNA fragment, provides charge carrier for the electrophoretic current and can enhance injection. To avoid the secondary structure of the DNA molecules, the buffer addictive such as formamide or urea is added into the buffer solution. Also, the capillary temperature should be kept at 60oC. Since the electrophoretic mobility of the DNA molecules are affected by its conformation. As a result, it is very important to keep a stable temperature. The internal size standard such as ABI GS500 which is sensitive to temperature variation should be used to ensure the stability of the capillary electrophoresis. 9

For the gel-filled capillary, the viscous polymer solution will mask the charged sites on the capillary along the wall. As a result, the electro-osmostic flow is almost zero. However, the contaminants trapped inside the capillary after a series of injection will produce the active site along the capillary wall and induce the electro-osmostic flow. This causes the peak

broadening and shift the elution time. Also, the contaminants may interact with the DNA fragments and cause the loss of resolution. The best way to avoid the problems is to replace the capillary before the resolution failure. Normally, the capillary should be replaced at around 100 injections. By lowering the field strength, increasing polymer concentration and polymer length and using longer capillary can increase the resolution. However, longer analytical time is needed to achieve higher resolution.

In the PCR-STR analysis, at least 13 loci of different chromosomes should be analyzed to ensure the DNA profile of the suspect is very rare to occur in the population. The probability of occurrence can be calculated by the product rule. The frequencies of all individual alleles are multiplied for the homozygous locus (p2). For the heterozygous locus, an addition of factor 2 should be multiplied (2pq). The probability of occurrence of identical DNA profile at 13 STR loci is very rare in the population except identical twin.

CONCLUSION

Polymerase chain reaction-short tandem repeats (PCR-STR) is the modern technique in DNA profiling and can be used to diagnose the health diseases and judicial system. The DNA fragments can be separated in accordance with molecular size using the capillary gel electrophoresis. Although PCR-STR is a good forensic tool in DNA profiling, privacy and security are highly concerned and worried in the public.

NEW TREND AND CHALLENGES

The genetic materials collected from the scene are limited. As a result, it is very important to have high efficiency of DNA extraction. For some of the

hard tissue, such as bone and teeth, the DNA extraction is difficult. In the past, the bone or teeth should be pulverized to achieve higher extraction efficiency. Pressure cycling technology (PCT) helps to extract the DNA from hard tissue without pulverization. PCT uses alternating cycles of high and low pressures to induce the cell lysis. The sample and lysis buffer in the pulse tube is subjected to alternating cycle of high (up to 35, 000 PSI) and ambient pressure for 5 – 1 0 cycles to induce the cell cytolysis. The use of PCT for DNA extraction helps to increase the extraction efficiency and shorter the extraction time.

Mitochondria DNA (mtDNA) is used for DNA profiling when all nucleuses DNA are degraded or the amount is very limited. A cell has hundreds to thousands of mitochondria. Each mitochondrion contains 5 – 10 mtDNA. Such large amount of mtDNA is very useful if nucleus DNA is not available. The mtDNA is located outside the cell nucleus, it is inherited through the female. This means that the family members linked through an unbroken female line. As a result, the polymorphism of mtDNA is lesser than nucleus DNA.

The DNA can be degraded by enzymatic reaction of microbial or under high temperature. Analyze the degraded sample using STR technique result in dropout of larger STR loci, only a partial DNA profile can be obtained. Partial DNA profile does not provide enough power of discrimination. 10 Mini-STR technique uses the primers which target for the larger STR loci. Standard STR primers target longer sequences than STR locus. Mini-STR primers zoom in on the STR locus so that the resulting DNA strands are smaller with base pair less than 100. This increases the chance for the successful amplification of larger loci.

In the future, the capillary gel electrophoresis will be further optimized to increase productivity and reduce the manpower. Also, the size-range that maintains single-base resolution should be extended from 50 base pair to 250 or even 500 base pair. The temperature control should be enhanced to enable a high degree of precision from run to run.