Diagnosis of the Mutation in Human beta globin gene using PCR

Abstract

PCR can be used as a quantification method to express a particular gene sequence. It is very beneficial do quantify the presence of a particular sequence in vast gene array along with its expression in the biological system. RFLP or restriction fragment length polymorphism is a technique which can be used to identify the mutation in a particular sequence of gene by identifying the change in sequence in one of the restriction sites presents in the gene towards our respective restriction enzyme (in this case Bsu361). This experiment would demonstrate how the PCR could be utilised to amplify a section of the beta globin gene of human containing sickle cell mutation. It can provide a screening of the mutation by restriction enzyme digestion which can be identified if the restoration site of the enzyme Bsu361.

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Introduction

Due to the mutation in the gene encoding the beta chain of haemoglobin in human body, a disease is generated in the red blood cells known as sickle cell anaemia. The sickle cell anaemia is characterised by the changing of the shape and morphology of the red blood cells from biconcave to sickle shape. This can reduce the surface volume and size of the red blood cells and decrease the oxygen carrying capacity of the blood. This is accompanied with tiredness, loss of appetite, stunted growth and also in proper functioning of several organs of the human body due to lack of sufficient oxygen in the blood. The experiment is designed to express the mutation in the beta globin gene of humans by manipulating the restriction site of Bsu361. Through the screening of the mutated gene using RFLP, the mutation in the genetic sequence of the beta globin gene can be identified as the mutation may lead to the change in the sequence recognised by the restriction enzyme Bsu361 and it would not be cleaved (Detemmerman et al., 2018). During the gel electrophoresis, the migration of the DNA band could reveal that whether the restriction enzyme had cut the DNA sequence or not. If the DNA sequence is not cut then it would mean That the restriction site of Bsu361 has been mutated and therefore the person from whom the sample is obtained is likely to have sickle cell anaemia. The PCR or polymerase chain reaction is a very efficient process to determine the presence of specific segment of the genome by but rapidly producing billions or millions of copies which can be later studied in great detail using gel electrophoresis or genome sequencing. The PCR involves short daily fragments which are denatured add are extended using specially designed primers add daily polymerase to amplify that particular segment of interest (Zhu et al., 2020).

Aim

To identify and amplify the region of mutation in the human beta globin gene and to understand the gene expression of sickle cell Anaemia using PCR which can help to screen the mutation by RFLP (Restriction fragment length polymorphism).

The mutation in the gene sequence for Human beta globin

ACTCCTGAGGAG

The above sequence is the region where the mutation in the gene takes place responsible for the sickle cell anaemia in a 5kbp long beta globin gene sequence. The sequence changes to which results in the production of Valine from codon GTG in place of GAG which codes for glutamic acid.

Gel electrophoresis

The gel electrophoresis is to technique to separate the DNA molecules based on their size. The technique is helpful to segregate the DNA molecules based on the do you need lens. The molecule having more weight is more expected to be retained at the starting point whereas the denim molecule having less molecular weight is expected to go through the gel. The delicate process is often used to visualise the results of the PCR. By calculating the migration of the DNA fragments in the gel matrix, the size of the DNA bands can be estimated which can give the possibility of the mutation in the human beta globin gene.

Materials required

The materials which are required for performing the PCR and gel electrophoresis for the detection and amplification of mutation in human beta haemoglobin gene are-

- *Taq* DNA polymerase

- Oligonucleotides
- Dedicated pipettes or mechanical pipettes
- Thermal cycler
- Centrifuge tubes
- DNA to be amplified
- Diluted primers
- PCR tubes or PCR plates
- dNTP mixture containing 10mM each of dGTP, dTTP, dATP, dCTP.
- Sterile filter tips
- Solution having restriction enzyme Bsu361
- Gel electrophoresis chamber
- Agarose gel (50mM Tris base, 30mM sodium acetate, EDTA mixed in deionized water, pH 7.8)
- TAE buffer with 2M sucrose, EDTA and 0.2 mM bromophenol blue.
- Gene probe with radioactive due acting as an indicator.

Methods

Nucleotide extraction

The nucleotide sequence was extracted from the provided sample and the human beta globin gene was demonstrated. The corresponding sequence of the site of mutation flanking the

restriction enzyme has been highlighted. The mutation in the genomic sequence changes the Adenine in the sequence to Thymine which leads to production of Valine, resulting in sickle shaped blood erythrocytes.

Primer selection (manual)

The selection of primers was done to explicitly highlight the mutated regions of the human beta globin genome. The possibility of mutation in the beta globin gene has been highlighted and viewed (Ghannam & Varacallo, 2020).

The primer selected for PCR manually is

GACACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCC

It was made sure that the primer followed the terms and conditions often ideal primer.

Primer selection (computer generated)

The primers are selected based on a website study two analyse the primers. The sequence was copy to the website entry and the primers were interested. The primers were created according to the computerised mathematical equations which were already fed during the development of the website.

The sequence was pasted in a provided website named primer 23 and all the possible primers were outlined. From the list of primers, the primer

AACAGACACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGG GG starting from 5041 could be selected as the primer for the targeted mutation gene in PCR to study the mutation effectively.

Comparison of the Tm value between the primers

Both the primers selected by the two methods had higher GC content and the 3’ end in G or C. The primer selected through computerized method was greater in size, more than 20

nucleotides in length and had higher GC percentage (Sheppard et al., 2020). Therefore, the primer designed through computational methods had higher Tm value (58 C) than that from the manual method (51 C) to the presence of higher GC bonds.

Running gel electrophoresis

In 1% agarose gel, heat the solution then add 4 µl of EtBr into the agarose and mix carefully. Using a gel plate & comb, create 2 dams in the slots on gel plates. Pour the molten agarose into the gel plate of the electrophoresis chamber. Place the comb and cool the gel to room temperature. Pour electrophoresis buffer (1X TBE) to submerge the gel completely and remove the comb carefully. Prepare DNA sample mixture by mixing around 300 ng of DNA sample (after PCR) with 4 µl of gel loading dye. Add 3 µl DNA ladder into the first well by using a micropipette. Carefully place the prepared samples into adjacent wells. Electrophorese the samples for 45 minutes. Carefully remove the gel, place it onto the UV light box and take a picture for the gel.

Results

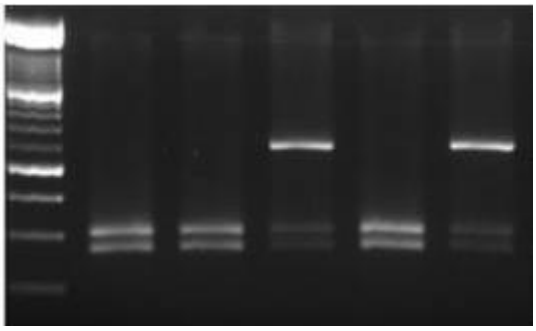

Fig: Gel diagram of human beta globin gene.

(Source: self-clicked)

The first column depicts the reference markers whereas the columns afterwards contain the samples. In the 1st, 2nd, and 4th well, the DNA sample has been digested with the restriction

enzyme Bsu361 as the DNA fragments have migrated farther distance with respect to the size depicted by the markers. This signifies that the gene sequence of the DNA samples in these wells have not been mutated signifying no occurrence of sickle cell anaemia. Whereas, in the 3rd and 5th well, the DNA fragments has not been digested by the respective enzyme which means that the sequence has been mutated at the restriction site of Bsu361 and therefore, has increased prevalence of sickle cell anaemia as the mutation in the beta globin gene is evident in these DNA sequences.

Discussion

The restriction the direction of BS36I enzyme would cleave it and convert it into smaller fragments. Therefore, the amplified DNA sequences should get shorter in length due to the action of the restriction enzyme upon them. If the bands become shorter, they would move further away from the negative electrode while running a gel electrophoresis (Suza et al., 2021). The bands obtained in the gel electrophoresis maybe compared with the original non mutated genomic sequence band and the distance of migration of the digested or fragmented bands should be observed. If the restriction endonuclease sequence is not digested then the bands obtained would be longer than the normal sequence and would form bands toward the negative electrode as they would find it difficult to move in the gel matrix due to their size (Ghani et al., 2021).

Conclusion

The mutation in the genomic sequence of the human beta globin gene has resulted in the change in the restriction site of BS36I restriction enzyme due to which the concerned enzyme cannot digest the dinner in that sequence and therefore the length of the daily is greater than that of the normal genomic sequence. The designing of the primer was done in both

manually and through computational methods out of which, the computational methods had higher GC content and therefore, higher Tm value. The primer up obtained through computerized method was used in the PCR as it had an ideal melting temperature value an was optimally selected for further PCR process.

By comparing the DNA bands obtained in the gel electrophoresis with that of the non mutated genomic sequence bands, it can be concluded that the changes in the nucleotide sequence ACTCCTGAGGAG in the beta globin gene can inhibit the restriction site of BS36I restriction enzyme (CCTGAGG) which can inhibit the action of the respective restriction enzyme.

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