Genetic Diversity Analysis of the STR Locus DXS6809 in the Thai Male Population for Forensic Applications

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Abstract

Short Tandem Repeats (STRs) or microsatellites on the X chromosome are valuable genetic markers in forensic analysis, particularly in relationship testing and human identification. However, genetic information on X-STR variation in the Thai population remains limited. DXS6809 locus is a highly polymorphic X-linked tetranucleotide repeat marker with high power of discrimination (PD) and exclusion rendered, making it a useful locus for human identification investigating complex kinship or deficiency cases. This study aimed to analyze the STR locus DXS6809 in Thai male population to evaluate its genetic variation and parental identification. Thirty-six unrelated Thai male samples were collected from buccal swabs and DNAs were extracted using the NucleoSpin® Tissue Kit. DNA quantity and quality were examined by agarose gel electrophoresis and a Biodrop Spectrophotometer. The extracted DNAs were amplified by Polymerase Chain Reaction (PCR), purified using the PrimeWay Gel Extraction/PCR Purification Kit, and DNA sequencing were performed using the Big Dye terminator method. Sequencing data were analyzed with BioEdit version 7.7. The PCR products were approximately 300–400 bp in length. Here, only male samples were analyzed, as males carry a single X chromosome that facilitates straightforward interpretation of allelic variation. The STR repeats of DXS6809 was identified as $(CTAT)_n$-$(ATCT)_n$-N_9-$(TATC)_n$-$(ATCT)_n$-N_{10}-$(ATCT)_n$, with eight observed allelic patterns. Among these, $(CTAT)_9$-$(ATCT)_3$-N_9-$(TATC)_3$-$(ATCT)_5$-N_{10}-$(ATCT)_{13}$ showed the highest allele frequency. Allele frequencies ranged from 0.03 to 0.22, with a Power of Discrimination (PD) of 0.861 and a Polymorphic Information Content (PIC) of 0.847, indicating that DXS6809 is an informative locus for forensic identification. However, as this study was analyzed only one X-STR locus in male samples, additional loci, larger sample sizes, and female participants should be included to enhance population representativeness for future work.

Introduction

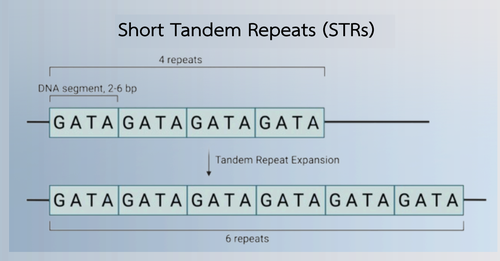


Figure 1: Structure of Short Tandem Repeats (STRs).

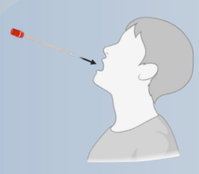
Microsatellites, also known as Short Tandem Repeats (STRs), are highly polymorphic DNA sequences composed of short repeating units of 2–6 base pairs distributed throughout the genome. Due to their high variability among individuals, STRs are widely used in forensic science, particularly for personal identification and kinship analysis.

The development of new STR loci remains a challenge in forensic applications, as increasing the number of informative markers can enhance the accuracy and discriminatory power of genetic identification systems. In addition, X-chromosomal STRs (X-STRs) have gained increasing attention in forensic genetics due to their unique inheritance patterns, which provide valuable information particularly in complex kinship analysis, such as deficiency paternity cases, half-sibling testing, and incest investigations. In this study, we focus on the DXS6809 locus, an X-chromosomal STR, which has been previously investigated in several populations, including German, Japan, and China populations. DXS6809 is a highly polymorphic locus characterized by complex repeat patterns and has demonstrated high values of power of discrimination (PD) and polymorphism information content (PIC), indicating its suitability for forensic applications. However, genetic data on DXS6809 in the Thai population remain limited. Therefore, this study aims to investigate the allelic diversity and repeat patterns of the DXS6809 locus in the Thai population, with the goal of evaluating its forensic applicability and contributing to the development of a Thai forensic genetic database.

Objectives

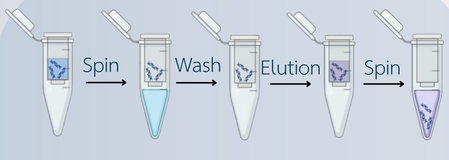
- To study the genetic polymorphism of the X-chromosomal STR locus DXS6809 in the Thai male population.
- To investigate allele frequency distribution of STR locus DXS6809 among Thai male population.

Materials and methods



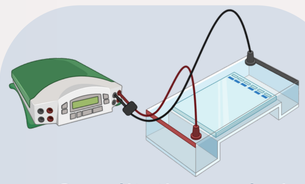
Collect Buccel cells samples

- 36 Thai male population
- Age: 20-60 years old
- Human Ethetic: CMU REC63/002



DNA Extraction

Genomic DNA extraction was performed using the NucleoSpin® Tissue Kit (Macherey-Nagel, Germany).



Quanlitative analysis

Genomic DNA was analyzed by 1.2% agarose gel electrophoresis.



Quantitative analysis

DNA concentration was measured using a BioDrop spectrophotometer (BioDrop Ltd., UK).



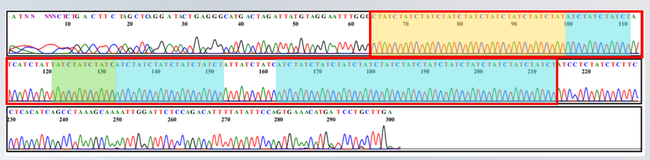
Amplification of DXS6809 by PCR

Forward primer: 5'GGCCTCACTTAATGGATGAAG3'
Reverse primer: 5'CAAGGAGGACCATGTTTCACTG3'



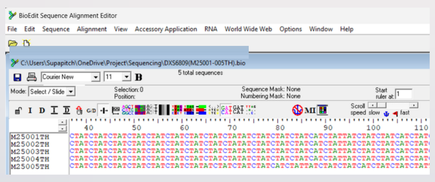
DNA purification

PCR products were purified using the PrimeWay PCR Purification Kit (1st BASE, Malaysia).



DNA squencing

DNA sequencing was performed using the BigDye Terminator method by Apical Scientific Sdn. Bhd.



BioEdit program analysis version 7.7

Results and discussion

Qualitative and Quantitative analysis of genomic DNA



Figure 2: 1.2% agarose gel electrophoresis,
Lane M :VC Lambda/*Eco*RI+*Hind*III and Genomic DNA 1-10.

After sample collection, genomic DNAs were extracted from 36 individuals and subsequently assessed for quality using agarose gel electrophoresis. The results showed that

- Genomic DNA revealed an approximate size of 20 kB when compared to the VC Lambda/*Eco*RI+*Hind*III marker.
- Smearing was observed, indicating partial DNA fragmentation during extraction.

For quantitative analysis, the genomic DNAs were measured at the absorbance 260 and 280 nm using Biodrop™ µLITE spectrophotometer.

- DNA concentrations ranged from 7.64 – 138.20 ng/µL.
- The absorbance ratio of A_{260}/ A_{280} ranged from 1.8 to 2.0, but some values were higher than 2.0, which indicated RNA contamination in the DNA samples.

DXS6809 PCR product analysis

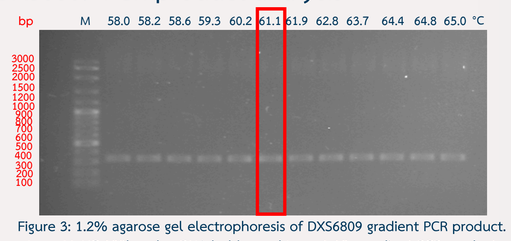


Figure 3: 1.2% agarose gel electrophoresis of DXS6809 gradient PCR product.
Lane M :VC 100bp plus DNA ladder and Lane 1-12 : gradient PCR product when using annealing temperature between 58.0°C-65.0°C.

After quality and quantity assessment, the genomic DNA samples were amplified using the polymerase chain reaction (PCR) technique. Optimization of the annealing temperature was performed, and **the optimal annealing temperature for DXS6809 PCR was 61.1 °C.**

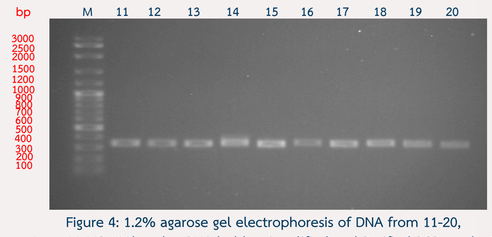


Figure 4: 1.2% agarose gel electrophoresis of DNA from 11-20,
Lane M :VC 100bp plus DNA ladder. Amplified and Purified PCR product.

After optimization of the annealing temperature, PCR amplification was performed, and the resulting **PCR products showed a fragment size about 300 – 400 bp on the DXS6809 locus when compared with VC 100bp Plus DNA ladder.**

DNA sequencing

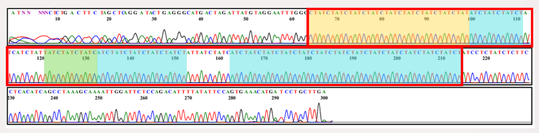


Figure 5: DXS6809 locus repetition patterns.

The PCR product were sequenced by Big Dye terminator DNA sequencing. Sequencing data were analyzed with BioEdit version 7.7.

Table 1: Repetition pattern and genetic parameter of DXS6809 locus in Thai males.

$(CTAT)_n$-$(ATCT)_n$-N_9-$(TATC)_n$-$(ATCT)_n$-N_{10}-$(ATCT)_n$	Number	Allele	Frequency	PD	PIC
$(CTAT)_7$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_3$ - N_{10} - $(ATCT)_{11}$	1	27	0.03		
$(CTAT)_7$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_3$ - N_{10} - $(ATCT)_{14}$	2	30	0.06		
$(CTAT)_8$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_3$ - N_{10} - $(ATCT)_{11}$	7	31	0.19		
$(CTAT)_9$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_3$ - N_{10} - $(ATCT)_{12}$	4	32	0.11		
$(CTAT)_9$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_5$ - N_{10} - $(ATCT)_{13}$	**8**	**33**	**0.22**		
$(CTAT)_9$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_5$ - N_{10} - $(ATCT)_{14}$	5	34	0.14		
$(CTAT)_9$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_5$ - N_{10} - $(ATCT)_{15}$	1	36	0.03	**0.861**	**0.847**
$(CTAT)_{10}$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_4$ - N_{10} - $(ATCT)_{11}$	1	31	0.03		
$(CTAT)_{10}$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_5$ - N_{10} - $(ATCT)_{13}$	4	34	0.11		
$(CTAT)_{10}$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_5$ - N_{10} - $(ATCT)_{14}$	1	35	0.03		
$(CTAT)_{11}$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_5$ - N_{10} - $(ATCT)_{12}$	1	34	0.03		
$(CTAT)_{11}$ - $(ATCT)_3$ - N_9 - $(TATC)_3$ - $(ATCT)_5$ - N_{10} - $(ATCT)_{14}$	1	36	0.03		
Total	36		1		

Table 2: Genetic parameters of DXS6809 in Thai males when were compared to other populations.

country	n	PD(male)	PIC	Reference
China(Han)	399	0.822	0.824	Qiu-Ling Liu et al. (2011)
Argentina	100	0.772	0.797	Bobillo et al. (2011)
Japan	339	0.804	0.78	Nakamura et al. (2010)
German	419	0.835	0.825	J. Edelmann et al. (2003)
Italy	100	0.809	0.812	Turrina et al. (2007)
Ecuador	200	0.805	0.789	Zambrano et al. (2015)
Thailand	**36**	**0.861**	**0.847**	**This study**

- DXS6809 locus in Thai males has one repetition pattern: $(CTAT)_n$-$(ATCT)_n$-N_9-$(TATC)_n$-$(ATCT)_n$-N_{10}-$(ATCT)_n$
- From sample 28 Thai males, there are 8 different repitition patterns.
- $(CTAT)_9$-$(ATCT)_3$-N_9-$(TATC)_3$-$(ATCT)_5$-N_{10}-$(ATCT)_{13}$ showed the highest allele frequency.
- When these results were compared with other countries, the genetic parameters in the Thai population were found to be slightly higher. This suggests that DXS6809 shows high variation in Thai male samples and may be useful for improving the accuracy of forensic databases in Thailand.

Conclusions

- The genomic DNA size was 20 kB compared with VC Lambda/*Eco*RI+*Hind*III marker.
- The concentrations of genomic DNA samples are ranged from 7.64 – 138.20 ng/µL.
- The optimize annealing temperature of DXS6809 locus is 61.1 ˚C.
- The PCR product sizes are about 300 – 400 bp when compared with VC 100bp Plus.
- $(CTAT)_9$-$(ATCT)_3$-N_9-$(TATC)_3$-$(ATCT)_5$-N_{10}-$(ATCT)_{13}$ revealed the most frequent repeat pattern with a frequency of 0.22.
- The value of power of discrimination (PD) is 0.861.
- The value of polymorphism information content (PIC) is 0.847.

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