Microsatellite DNA Analysis on Locus DXS6789 in Thai Males for Forensic Science Application

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Abstract

Investigation of X-chromosomal microsatellite DNA at the DXS6789 locus and determination determine allele frequencies in the Thai male population were performed for forensic applications. Microsatellites or Short tandem repeats (STRs) are repetitive DNA sequences with high polymorphisms and are widely used for individual identification. Thirty-four unrelated Thai male volunteers aged between 20 and 60 years were investigated. Buccal epithelial cells were collected, and genomic DNAs were extracted using the NucleoSpin® Tissue kit, yielding DNA fragments of approximately 20 kb. The DXS6789 locus was amplified by PCR and the producing PCR products of approximately 300 kb in length. The amplified products were purified using the PrimWay PCR/Gel Purification kit and subsequently were sequenced using the Big Dye terminator method. Sequence data were analyzed using BioEdit software version 7.7. The results showed that two repeat structure patterns were observed at the DXS6789 locus in the Thai male population: (TATC)(TATG)$_n$(TATC)$_n$ and (TATG)$_n$(TATC)$_n$. The most frequent allele was (TATG)$_{10}$(TATC)$_6$, consisting of a total of 16 repeats, with the highest allele frequency of 0.4706. The power of discrimination (PD) value for this locus was 0.732. These findings indicate that the DXS6789 locus exhibits sufficient polymorphism and discriminatory power, demonstrating its potential applicability for individual identification in forensic investigations.

Introduction

Microsatellites or Short Tandem Repeats (STRs)

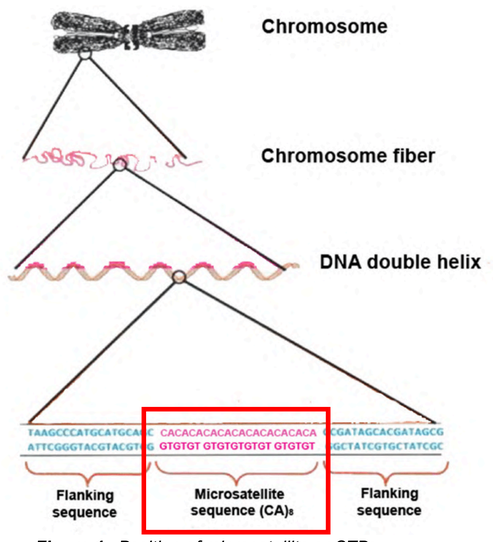


Figure 1 : *Position of microsatellite or STRs*

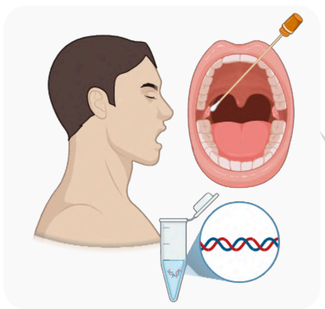
are short repetitive DNA sequences consisting of 2–6 base pairs distributed throughout the human genome. Due to their **high polymorphism**, STR markers are widely used in forensic identification and kinship analysis.

In addition to autosomal STR markers, X-chromosomal STRs provide valuable information in **complex kinship cases**, **particularly involving female offspring** or **missing parental information.**

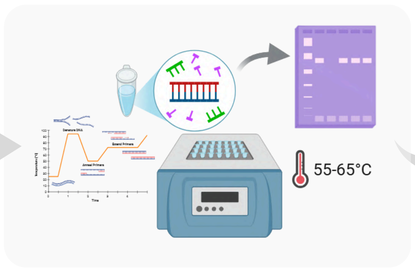
The DXS6789 locus is an X-STR marker located on the X chromosome that exhibits genetic variability in several populations. However, population data for Thai individuals remain limited. Therefore, this study aims to investigate the genetic polymorphism of the DXS6789 locus in bThai males for forensic science applications.

Methodology

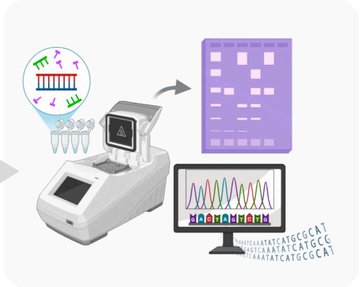
Part 1 : Investigation the repeating patterns of DXS6789 locus



- Collect buccal cell
- Human Ethic approval : CMU REC (63/002)

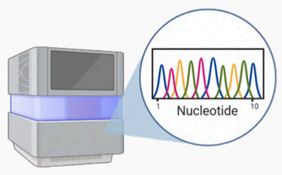


- Extract genomic DNA using NucleoSpin® Tissue Kit
- Optimize annealing temperature
- Amplify of DXS6789 locus



- DXS6789 PCR product analysis
- Purify PCR product using from agarose gel electrophoresis using Primeway Gel Extraction Kit

Part 2 : Analysis the genetic parameters to apply for forensic application



The DNA sequencing submitted by **Apical Scientific SND. BDH., Malaysia**

Analyze DNA sequencing results using **BioEdit (version7.7.1)** application

Calculate genetic statistics

Power of Discrimination (PD)

$$PD = 1 - \sum (p_i)^2$$

*The closer **PD is to 1.0, the better** the locus is for human identification*

Pi = frequency of the i allele in the population

Polymorphism Information Content (PIC)

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \left(\sum_{i=1}^{n} P_i^2\right)^2 + \sum_{i=1}^{n} P_i^4$$

***High PIC** : good marker for kinship and paternity testing.*

Pi = frequency of the i allele in the population

n = total number of alleles observed in the population

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All of volunteers

References

- *Kelkar et al., Genome Biology and Evolution, 2010v*
- *Hwa et al., International Journal of Legal Medicine, 2009*
- *Tabbada et al., Forensic Science International, 2005*
- *Kunjae, A. (2016). Analysis of the microsatellite DXS8377 polymorphism in Northern Thai males. Master's thesis, Chiang Mai University.*

Results and Discussion

Genomic DNA Extraction

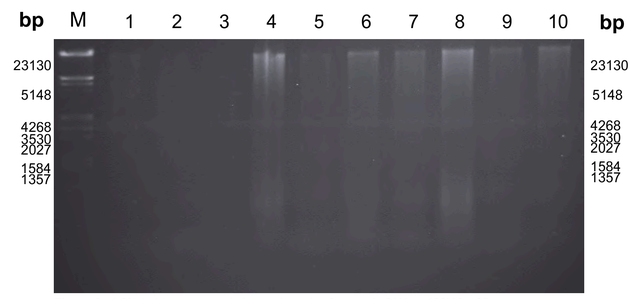


Figure 2 : *1.2% (w/v) agarose gel electrophoresis of genomic DNA at 120V for 35 min*
Lane M: VC Lambda/EcoR I + Hind III marker and Lane 1-10: Genomic DNA M25001TH-M25010

- The genomic DNA size was. **20 KB** when compared to the VC lambda/*EcoR* I + *Hind* III marker.
- The concentrations of genomic DNA sample are range from **7.64 - 138.20 ng/µL.**

DXS6789 PCR product analysis

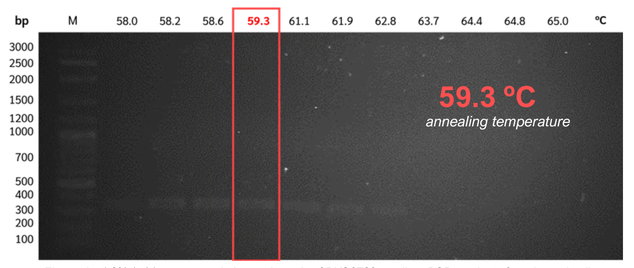


Figure 3 : *1.2% (w/v) agarose gel electrophoresis of DXS6789 gradient PCR products from buccal cells.*
Lane M: VC 100 bp plus DNA ladder and Lane 1-12: DXS6789 locus gradient PCR products

- The optimize annealing temperature is **59.3 degree celcius.**

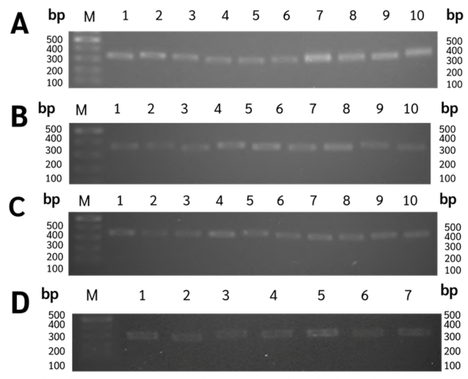
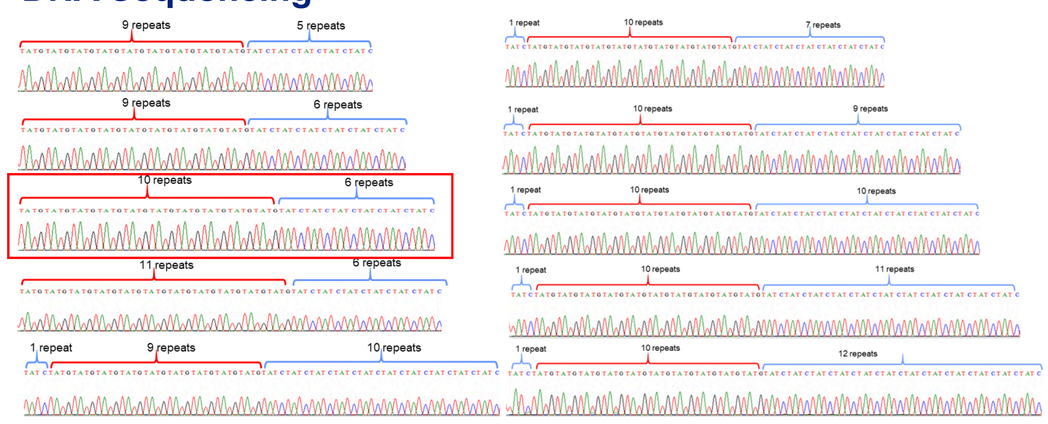


Figure 4 : *1.2% (w/v) agarose gel electrophoresis of gDNA at 120V for 35 min*
Lane M: VC 100 bp plus DNA ladder Lane 1-10: Genomic DNA M25001TH-M250034

- The PCR product size of about **350 base pair** compare with VC 100bp Plus DNA laddder.

DNA Sequencing



DXS6789 locus has two repititon pattern : **(TATG)n (TATC)n** and **(TATC)n (TATG)n (TATC)n** From a sample of 34 Thai males, there are **10 different repititon patterns. TATG$_{10}$TATC$_6$** had the most frequent repeat pattern.

Conclusions

Genomic DNA was 20 KB size
Compared with VC Lambda EcoR I + Hind III marker

***Concentrations of genomic DNA sample** are range from*
7.64 - 138.20 ng/µL

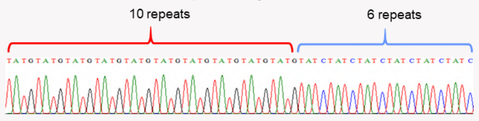
PCR conditions

98 °C
Denaturation temperature

Annealing temperature
59.3 °C

PCR product size
≈ 350 bp
Compared with VC 100 bp Plus ladder

TATG$_{10}$TATC$_6$
Most frequent repeat pattern
with frequency of 0.4706



PD = 0.732
ⓘ *Power of Discrimination*

PIC = 0.709
ⓘ *Polymorphism information content*

Table 1 : Genetic parameter of DXS6789 locus in Thai and other population

Population	References	n	Genetic parameters	
			PD	PIC
China	Han W. et al., (2008)	105	0.947	0.795
Korea	Shin SH, et al.,(2005).	401	0.814	0.789
Taiwan	Chen, M.-Y., & Pu, C.-E. (2004)	447	0.779	0.749
Philippine	Tabbada KA & de Ungria MCA, (2005)	115	0.747	0.712
Germany	Hering et al., (2001)	105	0.739	0.703
Portugal	Pereira R,et al.,(2007)	347	0.728	0.693
Thailand	Pawinee Chaiwong,et al., (2025)	50	0.755	0.718
	this study	34	0.732	0.709

Comparison with other populations

≈ *Similar to* 

⩔ *lower than* 