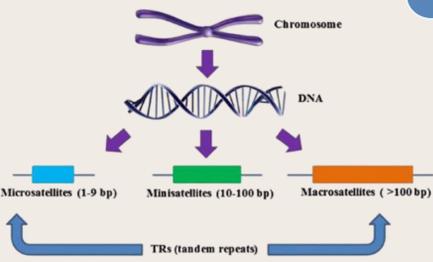


## Abstract

Microsatellites are repetitive sequences of nucleotides typically 2 to 6 base pairs long which were found throughout the genome of living organisms. Due to their high degree of variability compared to other regions of DNA, they contribute to significant genetic diversity and are well-suited for forensic applications, particularly in individual identification and paternity testing. This research focus on the characteristic analysis of microsatellites on the X-chromosome locus DXS6789 in a Thai male population and investigate the genetic variations that could be useful in forensic investigations. Buccal swab samples were collected from 50 Thai male volunteers aged between 20 and 60 years old. Genomic DNAs were extracted using the NucleoSpin® Tissue kit, resulting in a genomic DNA size of approximately 20 kb. Polymerase chain reaction (PCR) was employed to amplify the partially DXS6789 locus and gel electrophoresis revealed a PCR product size of about 300-400 bp on the DXS6789 locus. The amplified DNA was purified using the AccuPrep PCR/Gel Purification kit. An investigation on the microsatellite repeat patterns revealed two types of nucleotide repeats on the DXS6789 locus : TATC(TATG)<sub>n</sub>(TATC)<sub>n</sub> and TATG<sub>n</sub>TATC<sub>n</sub>. A study of DNA samples from a group of Thai males revealed nine distinct patterns of repeated nucleotide sequences : TATC(TATG)<sub>10</sub>(TATC)<sub>9</sub>, TATC(TATG)<sub>10</sub>(TATC)<sub>10</sub>, TATC(TATG)<sub>9</sub>(TATC)<sub>7</sub>, TATG<sub>10</sub>TATC<sub>6</sub>, TATG<sub>9</sub>TATC<sub>6</sub>, TATG<sub>11</sub>TATC<sub>6</sub>, TATG<sub>8</sub>TATC<sub>7</sub>, TATG<sub>10</sub>TATC<sub>5</sub>, TATG<sub>10</sub>TATC<sub>10</sub>, with allelic frequencies of 0.18, 0.06, 0.02, 0.34, 0.30, 0.04, 0.02, 0.02 and 0.02 respectively. Based on these frequencies, the Power of Discrimination (PD) and Polymorphic Information Content (PIC) were calculated as 0.755 and 0.718, respectively. These findings demonstrate the diversity of the DXS6789 locus in Thai males, which suggesting its potential for effective forensic applications in both individual identification and paternity testing.

## Introduction



Microsatellites, also known as Short Tandem Repeats (STRs), are highly polymorphic DNA sequences composed of repeating units of 2-6 base pairs. These sequences are widely used in forensic science for personal identification and kinship analysis due to their high variability.

New STR loci are the challenge for forensic science application. We are interested in investigation new STR loci in the Thai population to create a genetic database and apply it to forensic science.

This study focuses on the DXS6789 locus, which has been analyzed in various populations, including Germany, the Philippines, and Taiwan. This research examines the repeat patterns and genetic statistics of DXS6789. Previous studies have identified repeat patterns such as TATC(TATG)<sub>n</sub>(TATC)<sub>n</sub> and (TATG)<sub>n</sub>(TATC)<sub>n</sub> at this locus. Additionally, the power of discrimination (PD) and polymorphism information content (PIC) values indicate that DXS6789 is highly suitable for forensic applications. Studying the repeat patterns of this locus in the Thai population will enhance the accuracy and efficiency of personal identification in forensic science. Moreover, it can serve as a genetic database for Thai males and contribute to future research.

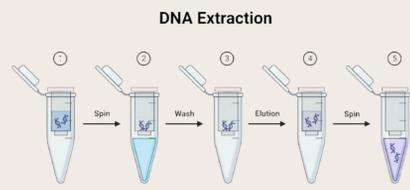
## Objectives

- To study the genetic polymorphism of DXS6789 locus in Thai male populations
- To investigate allele frequency distribution of chromosome x STR DXS6789 loci in Thai male populations

## Materials and methods



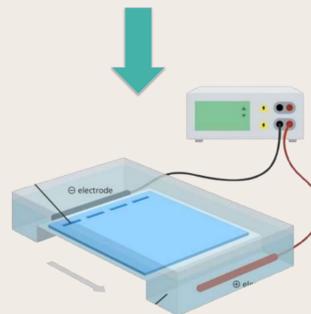
Collect samples from 50 Thai males aged between 20 - 60 years old using dental swab. Ethic approval from CMU (COA No. 021/63)



DNA Extraction using NucleoSpin® Tissue kit



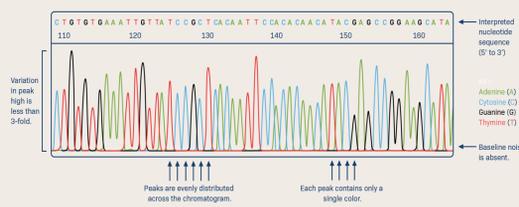
Quantitative analysis of genomic DNA using BioDrop spectrophotometer



Qualitative analysis of genomic DNA using 1.2% agarose gel electrophoresis



Amplification of DXS6789 by polymerase chain Reaction (PCR) technique



DNA sequence at Apical Scientific SND. BDH., Malaysia and Data analysis by BioEdit version 7.7.1

## Results

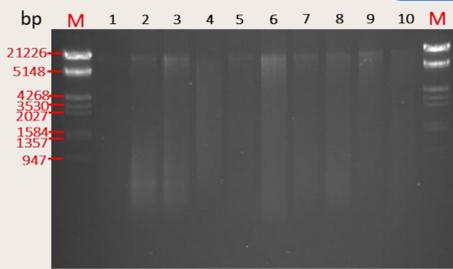


Figure 3: 1.2% agarose gel electrophoresis (120 v, 35 min). Lane M : VC Lambda/EcoR I HindIII. Genomic DNA M24001TH-M24010TH.

### Genomic DNA Extraction

- The genomic DNA size was 20 KB compared to the VC Lambda/EcoR I HindIII marker.
- The concentrations of genomic DNA sample are range from 13.56 – 98.62 ng/μL.

### DXS6789 PCR product analysis

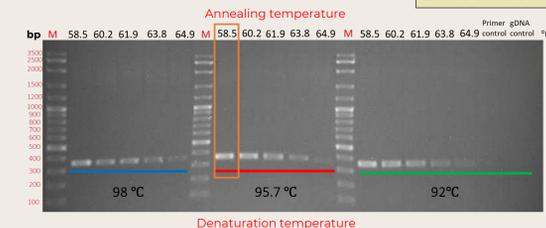


Figure 2 : 1.5% agarose gel electrophoresis of DXS6789 gradient PCR products from dental swab cells. Lane M : VC 100 bp plus DNA ladder and Lane 1-5 : DXS6789 locus gradient PCR product when using annealing temperature between 58.0 °C – 65.0 °C

- The optimize denaturation temperature is 95.7 °C and annealing temperature is 58.5 °C.

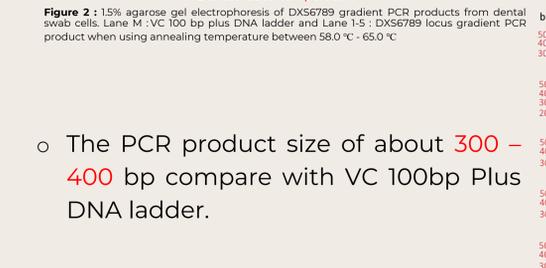
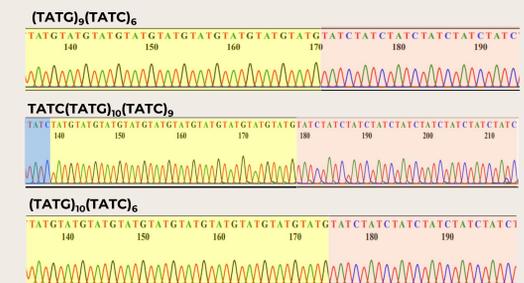


Figure 3 : 1.5% agarose gel electrophoresis(120 V, 35 min) of DNA from M24001TH-M24005TH. Lane M : VC 100 bp plus DNA ladder. Amplified and Purified PCR product

- The PCR product size of about 300 – 400 bp compare with VC 100bp Plus DNA ladder.

### DXS6789 DNA Sequencing



- DXS6789 locus has two repetition patterns : TATC(TATG)<sub>n</sub>(TATC)<sub>n</sub> and TATG<sub>n</sub>TATC<sub>n</sub>.
- From a sample of 50 Thai males, there are 9 different repetition patterns.
- TATG<sub>10</sub>TATC<sub>6</sub> had the most frequent repeat pattern.

## Conclusion

- The genomic DNA size was 20 KB compared with VC Lambda/EcoR I HindIII marker.
- The concentrations of genomic DNA sample are range from 13.56 – 98.62 ng/μL
- The optimize denaturation temperature is 95.7 °C and annealing temperature is 58.5 °C
- The PCR product size of about 300 – 400 bp compare with VC 100bp Plus DNA ladder.
- TATG<sub>10</sub>TATC<sub>6</sub> had the most frequent repeat pattern with a frequency of 0.34.
- The PD value was 0.755, and the PIC value was 0.718.
- Comparison with Other Populations: Higher PD & PIC than Germany (0.7394, 0.7039) and Portugal (0.7283, 0.6935), Similar values to Philippines (0.7478, 0.7120), Lower than Korea (0.8144, 0.7896) and Taiwan (0.7794, 0.7499)

## Acknowledgements

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- Members of FGFS Laboratory and Molecular Genetic Lab
- All of volunteers
- Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Chiang Mai University

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