Application Note

Compilation and investigation of Australian Aboriginal X-STR haplotypes using the Investigator® Argus X-12 QS Kit

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Introduction

Analysis of X-chromosome short tandem repeat (X-STR) loci is a simple alternative to mitochondrial DNA analysis for operational forensic laboratories and supplements autosomal and Y-chromosome STR analysis. X-STR analysis is particularly useful in complex kinship analyses such as those in deficiency paternity cases, missing person investigations, and disaster victim identification (DVI) (1). X-STRs are also extremely useful for screening candidate lists in familial searches where female individuals are identified as potential relatives of the offender.

The Investigator® Argus X-12 QS Kit from QIAGEN® is the sole commercial X-STR system, and simultaneously amplifies the Amelogenin gender marker, autosomal D21S11 marker (to prevent sample mix-up), and 12 X-STR markers that belong to 4 different linkage groups (LG): LG1 (DXS8378, DXS10135, DXS10148); LG2 (DXS7132, DXS10074, DXS10079); LG3 (HPRTB, DXS10101, DXS10103); and LG4 (DXS7423, DXS10134, DXS10146) (Figure 1).

The utility of the Argus X-12 QS Kit for routine forensic casework requires access to X-STR haplotype datasets for relevant populations to enable statistical evaluation of a ▷

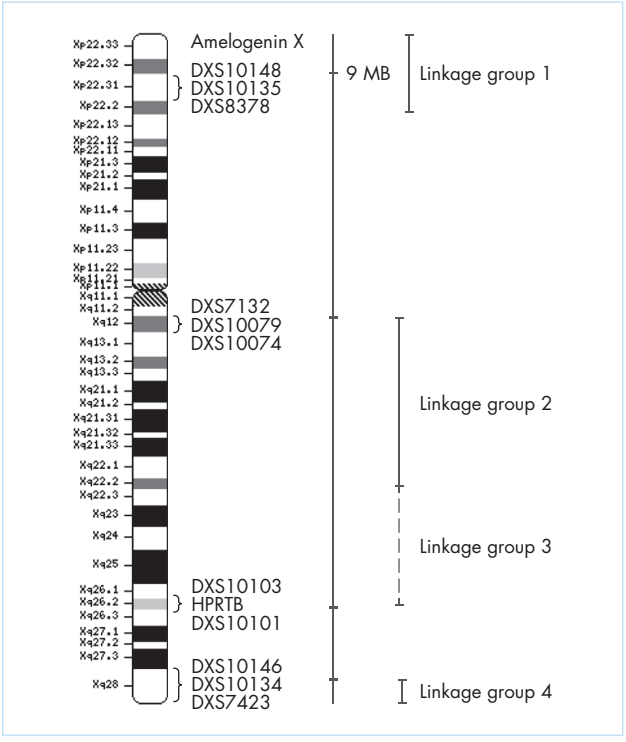

Figure 1. The four linkage groups of X-STR markers in the Investigator Argus X-12 QS Kit.

profile match. For Australian forensic laboratories, access to European, Asian, and Australian Aboriginal haplotype datasets are most critical as these sub-populations make up the majority of the overall Australian population. While appropriate and extensive X-STR haplotype data currently exists in the published literature for European and Asian populations (2–27), haplotype data for Australian Aborigines is not currently available.

To provide Australian forensic laboratories with access to X-STR haplotype data for Australian Aborigines, we generated Argus X-12 QS haplotypes and relevant forensic parameters for 298 male Australian Aboriginal individuals. We also investigated the genetic distance between Australian Aboriginal X-chromosomes from different tribal regions, and between the Aboriginal population and other global populations.

Materials and methods

Sample preparation

Included in this study were DNA samples from 298 self-declared Australian Aboriginal males from four tribal regions of Aboriginal Australia that fall within the state of South Australia (Desert, Eyre, Riverine and Spencer) (28), as well as an urban population from the capital city of Adelaide (Table 1). The samples were accessed under the Criminal Law (Forensic Procedures) Act (CLFPA) 2007 of South Australia. DNA was extracted from FTA paper using the DNA-IQ™ system (Promega Corporation, Madison, WI, USA) and the concentration was determined using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA).

Table 1. Regional origin of the 298 male Australian Aboriginal samples

Region	Number of samples
Desert	67
Eyre	3
Riverine	46
Spencer	79
Urban	103
Total	298

Investigator Argus X-12 QS analysis

Amplification was carried out at half the recommended volume (12.5 µl) with 250 pg of template DNA and 27 cycles of PCR on a ProFlex™ thermal cycler (Applied Biosystems®). The cycling conditions were as per the manufacturer's instructions (29). The resulting amplicons were electrophoresed on an Applied Biosystems 3500xL Genetic Analyser (Life Technologies Corporation). The data was analyzed using GeneMapper® ID-X v1.4 (Life Technologies Corporation) using a peak amplitude threshold of 100 relative fluorescence units (RFU). The sizing of micro-variant alleles was confirmed by re-amplification of the sample.

Data analysis

Statistical parameters of forensic interest (allele frequency, haplotype frequency (HF), gene diversity (GD), haplotype diversity (HD), polymorphism information content (PIC), power of discrimination (PD), mean exclusion chance (MEC), Kruger's MEC formula for deficiency cases, Kishida's MEC formula for standard trios involving daughters, Desmarais' formula for standard trios involving daughters, and Desmarais' Duo formula for father/daughter or mother/son duos) were calculated using StatsX v2.0 software (30). The pairwise exact test for linkage disequilibrium (LD) was performed using Arlequin v3.5.1.2 software (31), and Power of Exclusion (PE) was calculated by $1 - \sum(HF)^2$.

The genetic relationships between the different tribal regions within the Australian Aboriginal population, as well as between the whole Aboriginal population and ten global populations (6,7,15-18,20,24,26,32,33), were examined using two approaches. First, non-metric multidimensional scaling (MDS) plots were created using the R (34) add-on MASS (35) from pairwise F_{ST} genetic distances (applying a minimum of 0.0001) of the full Argus X-12 haplotypes in Arlequin v3.5.1.2. Second, histograms of genetic structure were created using the STRUCTURE v2.3.4 program (36) with the admixture model and correlated allele frequencies. STRUCTURE analyses were performed with 1000 burn-in and MCMC repeats for each of seven iterations of K.

The optimal K value was identified using STRUCTURE HARVESTER (37), and all iterations of K were combined into a single STRUCTURE plot using CLUMPAK (38).

Results and Discussion

Allele and haplotype parameters

Parameters of forensic interest are presented in Table 2. A total of 168 alleles were detected with a frequency span of 0.0033 to 0.5714. The most informative marker, DXS10135 (LG1) (PIC = 0.946), comprised 29 alleles, with the most common allele being 20 (frequency = 0.093). The least informative marker, DXS7423 (LG4) (PIC = 0.590), comprised five alleles, with the most common allele being 15 (frequency = 0.5714). These markers were also the most and least informative in other population studies (2, 15, 32).

A summary of haplotype sharing is presented in Table 3. When considering the entire Argus X-12 haplotype, 294 (98.7%) haplotypes were unique. The number of unique haplotypes for LG1, LG2, LG3 and LG4 were 201 (67.5%), 131 (43.9%), 111 (37.2%) and 141 (47.3%), respectively. While the complete 12-locus haplotype was only shared between two individuals, identical LG haplotypes were observed in up to 20 individuals. The most informative linkage group was LG1 (PIC = 0.993), followed by LG2 (PIC = 0.986), LG4 (PIC = 0.985) and finally LG3 (PIC = 0.980), with the most common haplotype frequencies equal to 0.0166, 0.0432, 0.0532 and 0.0698, respectively. Gene diversity values for all LGs were above 0.98, with LG1 (0.9967) being the most diverse. ▷

Table 3. Haplotype sharing for 298 Australian Aboriginal Argus X-12 QS haplotypes based on individual linkage groups and full 12-locus haplotypes

Haplotypes observed *n* times	LG1	LG2	LG3	LG4	Full haplotype
n=1	136	66	56	74	290
n=2	45	30	23	38	4
n=3	10	16	9	12	
n=4	8	5	4	7	
n=5	2	4	3	2	
n=6		3	7	3	
n=7		1	1	1	
n=8		1	2	1	
n=9		3	3	1	
n=11		1			
n=12			1		
n=13		1	1		
n=16				2	
n=20			1		
Number of unique haplotypes	201	131	111	141	294
Discrimination capacity (DC) %	67.4	44.0	37.2	47.3	98.7

Table 2. Forensic parameters for 298 male Australian Aboriginal X-STR haplotypes

Linkage Group	Locus	No. of Alleles	GD	PIC	PE	PD Male	PD Female	MEC Kruger	MEC Kishida	MEC Desmarais	MEC Desmarais Duo
LG1	DXS8378	6	0.688432	0.626243	0.686145	0.686145	0.841593	0.418497	0.626123	0.626243	0.479978
	DXS10135	29	0.945521	0.939339	0.94238	0.94238	0.993639	0.883155	0.939339	0.939339	0.888631
	DXS10148	25	0.904294	0.893559	0.90129	0.90129	0.982526	0.803369	0.893559	0.893559	0.815868
LG2	DXS7132	9	0.779197	0.742729	0.776609	0.776609	0.916216	0.568859	0.742729	0.742729	0.611339
	DXS10074	14	0.833709	0.810139	0.83094	0.83094	0.950618	0.667458	0.810139	0.810139	0.696786
	DXS10079	11	0.792296	0.763375	0.789664	0.789664	0.92947	0.603292	0.763375	0.763375	0.637138
LG3	HPRTB	7	0.730219	0.685252	0.727793	0.727793	0.883362	0.498883	0.685133	0.685252	0.545212
	DXS10101	18	0.895557	0.883372	0.892582	0.892582	0.979252	0.785263	0.883154	0.883372	0.800189
	DXS10103	7	0.656385	0.618138	0.654204	0.654204	0.844359	0.434814	0.618019	0.618138	0.470837
LG4	DXS7423	5	0.589634	0.529267	0.587675	0.587675	0.77158	0.334316	0.529267	0.529267	0.382037
	DXS10134	17	0.848233	0.829917	0.845415	0.845415	0.960606	0.701965	0.830029	0.829917	0.724216
	DXS10146	20	0.872576	0.856704	0.868279	0.869677	0.970043	0.741288	0.856372	0.856704	0.761282

Duplications were observed at marker DXS10146 in four samples, and all consisted of alleles 26 and 33. A null allele was observed at DXS10146 in one sample. A total of 11 micro-variants were seen at three loci (DXS10101, DXS10135 and DXS10148). All micro-variants were observed once, except for one micro-variant at DXS10148, observed six times, and another micro-variant at DXS10101, observed three times. Two of the observed micro-variants (allele 30.2 at DXS10135 and allele 23.2 at DXS10148) have not previously been recorded and may be unique to the Australian Aboriginal population.

Linkage disequilibrium analysis

Linkage disequilibrium (LD) was observed for all locus pairs within the same linkage group ($p<0.0008$ after Bonferroni correction), except for marker pair DXS10146-DXS7423 within LG4. LD was also observed between loci of different linkage groups, including DXS10101-DXS8387, DXS10135-DXS10079, DXS10135-DXS10101, DXS10135 DXS10134, DXS10074-HPRTB and HPRTB-DXS7423.

Intra-population comparison

Comparison of the full 12-locus X-STR Aboriginal haplotypes between individuals from the Desert, Eyre, Riverine and Spencer tribal regions and urban Adelaide using an MDS plot (Figure 2) showed a level of genetic divergence between all groups, with the exception of the Desert and Eyre regions, which clustered together. Genetic divergence between Aboriginal tribal regions has been previously described for other genetic markers (39–44) and is likely due to geographical, cultural and language differences, but also the degree of European admixture that has occurred. European admixture is known to occur most frequently in the more populous areas such as the Spencer, Riverine and urban Adelaide regions. As individuals from the Desert and Eyre regions are located in the Australian outback, it is expected that they will cluster together through the sharing of ancestral X-chromosomes caused by geographical isolation and an absence of European admixture.

Genetic divergence between the Aboriginal regions was also investigated using STRUCTURE. At the optimal K value (K=3), a small distinction could be observed between the Desert and Eyre groups and the remaining groups (Figure 3), which most likely reflects the absence of admixture in these remote regions. Overall, however, the STRUCTURE plot demonstrated that genetic structure of X-chromosomes within the Aboriginal population was minimal.

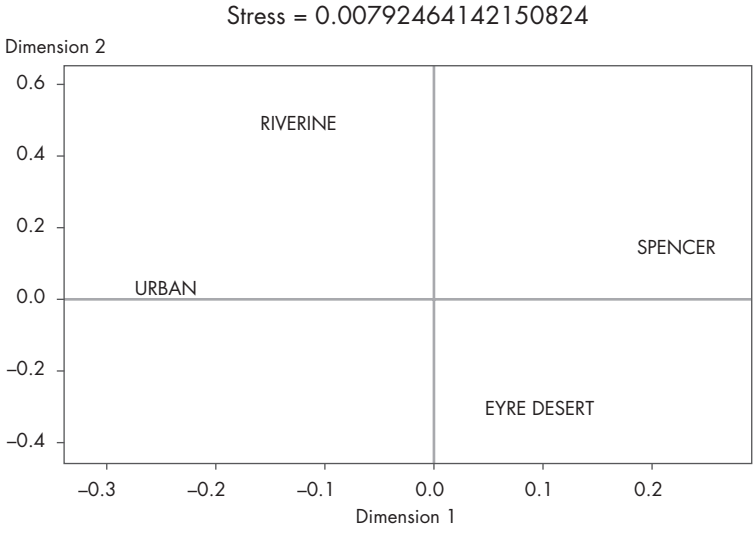

Figure 2. Multidimensional scaling plot displaying the genetic distance between the Desert, Eyre, Riverine, Spencer and urban Adelaide regions for the full 12-locus Argus X-12 QS haplotypes.

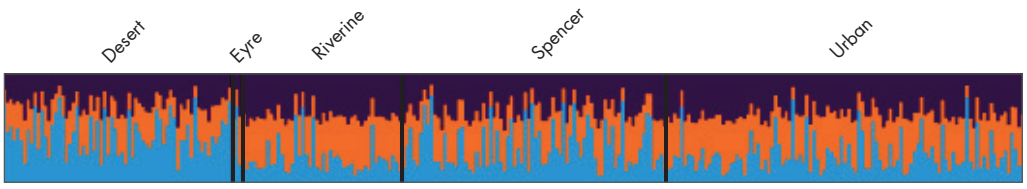

Figure 3. CLUMPAK plot displaying the genetic structure of the full 12-locus X-STR haplotypes for the Desert, Eyre, Riverine, Spencer and urban Adelaide regions as generated in STRUCTURE v2.3.4 at K = 3. The optimal K = 3 value was determined using Structure Harvester.

Inter-population comparison

Comparison of the full 12-locus X-STR haplotypes of Australian Aborigines to other global populations using an MDS plot (Figure 4) showed that the Aboriginal population was genetically distinct from other populations but most similar to Western Europeans. The genetic diversity of Australian Aboriginal X-STR haplotypes was further investigated using the STRUCTURE program. At the optimal K value (K = 5), two distinct clusters were observed (Figure 5). The Australian Aboriginal population, as well as the German, Swedish, Belarusian, Hungarian and Emirati populations, belonged to cluster 1, while the Argentinian, Chinese, Korean and Mongolian populations belonged to cluster 2. Some genetic similarity of Australian Aboriginals to Western Europeans is not unexpected due to high levels of inter-breeding between these two populations following British colonization in 1788. It is well understood that the majority of European admixture in the Australian Aboriginal population originates from the paternal side (45). Unlike mitochondrial DNA, which is strictly maternally inherited, the X-chromosomes within a population can be maternal or paternal in origin. Therefore, the similarity of Australian Aboriginal X-STR haplotypes with those of other European populations is unsurprising and supports the disruption of ancestral Aboriginal X-chromosomes via inter-breeding with Europeans. Australian Aborigines are also expected to have close genetic similarity to Oceanians due to co-migration via the ancient landmass of Sahul approximately 50,000 years ago. However, no X-STR haplotype data currently exists for Oceanic populations to enable this comparison.

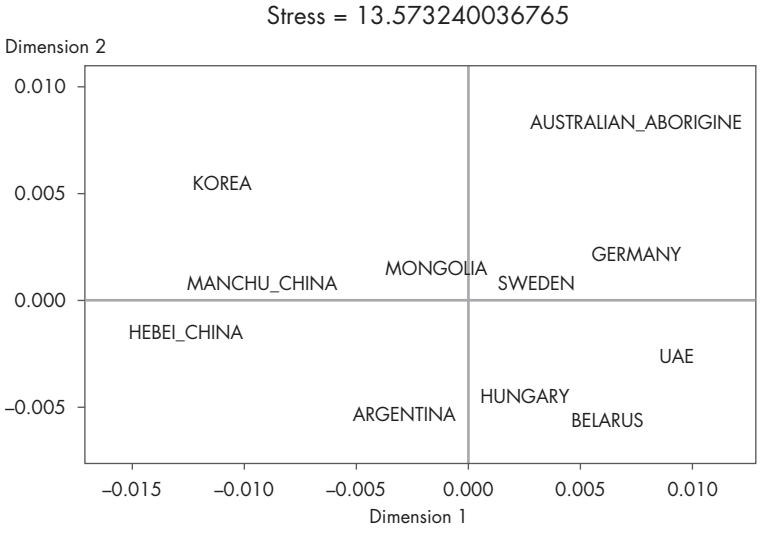

Figure 4. Multidimensional scaling plot displaying the genetic distance between Australian Aborigines and other global populations (6, 7, 15–18, 20, 24, 26, 32, 33) for the full 12-locus Argus X-12 QS haplotypes. MANCHU_CHINA = Manchu Province in China, HEBEI_CHINA = Hebei Province in China, UAE = United Arab Emirates.

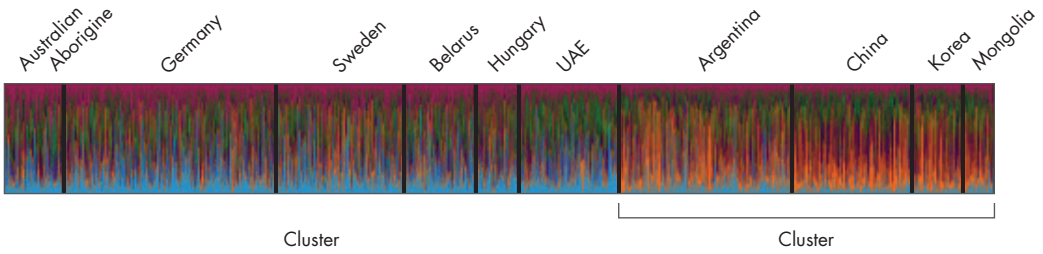

Figure 5. CLUMPAK plot displaying the genetic structure of the full 12-locus X-STR haplotypes for Australian Aborigines and other global populations (6, 7, 15–18, 20, 24, 26, 32, 33) as generated in STRUCTURE v2.3.4 at K = 5. Cluster 1 incorporates the Australian Aborigines and the European and UAE populations. Cluster 2 incorporates the Asian and South American populations. The optimal K=5 value was determined using Structure Harvester.

Conclusion

In this study, we successfully generated an Australian Aboriginal X-STR population database, consisting of 298 male haplotypes, using the Investigator Argus X-12 QS Kit. The creation of this dataset will enable Australian forensic laboratories to consider this population group, alongside European and Asian populations, when performing statistical evaluations of X-STR profile matches in casework. When considering the full 12-locus haplotype, the discrimination capacity of the Argus X-12 QS Kit for the Aboriginal population was high, at over 98%. Some genetic diversity of the X-chromosome within different Aboriginal tribal regions, and evidence of significant European admixture in the more urbanized regions, was consistent with previous findings for other genetic markers.

Summary

The Investigator Argus X-12 QS Kit ensures your X-STR analyses are of the highest quality and can be performed conveniently by providing:

- High discrimination power through the amplification of 12 X-chromosomal markers, allowing for robust X-STR population database creation
- QIAGEN's fast-cycling technology, enabling multiplex amplification in only ~80 minutes
- Integrated Quality Sensors that reveal helpful information about sample quality and PCR success

Acknowledgements

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Ordering Information

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Investigator Argus X-12 QS Kit (25)	Primer Mix, Fast Reaction Mix including *Taq* DNA Polymerase, Control DNA, allelic ladder Argus X-12 QS, DNA size standard 550 (BTO) and Nuclease-Free Water	383223
Investigator Argus X-12 QS Kit (100)	Primer Mix, Fast Reaction Mix including *Taq* DNA Polymerase, Control DNA, allelic ladder Argus X-12 QS, DNA size standard 550 (BTO) and Nuclease-Free Water	383225

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