Development and Validation of the New Investigator® Argus X-12 QS Kit

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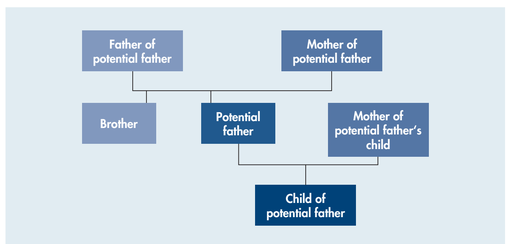
Introduction

Analysis of X-chromosomal short tandem repeats (STRs) provides a powerful tool to complement autosomal and Y-chromosomal STRs in solving complex kinship and deficiency cases. The Investigator Argus X-12 Kit has been widely used for this purpose. In order to improve robustness and speed, and to facilitate analysis further, we have recently updated the assay.

Updated kit at a glance:

- Addition of the Quality Sensor (QS) as an internal performance control, indicating whether the PCR reaction has performed properly.
- Inclusion of D21S11 as an autosomal alignment marker. By comparing the genotype of the alignment maker between X-chromosomal and autosomal STR analyses within a case, potential sample mixup can be detected.
- Inclusion of additional SNP primers to overcome null alleles due to primer binding site mutations of DXS10101, DXS10146 and DXS10148 found at elevated frequencies within African populations (1).
- Fast Reaction Mix 2.0, which allows short PCR cycling protocols and provides a high inhibitor tolerance. A standard 30-cycle PCR using 500 pg of template DNA now can be finished within 80 minutes.
- No changes to the 12 X-chromosomal STR markers.
- Minor modifications to the primers were made (e.g., to improve A-addition to the final PCR product and to obtain a cleaner fluorescent baseline).
- Addition of 14 alleles to the allelic ladder.



X-chromosomal analysis. Such analysis is useful in solving deficiency kinship cases, paternity testing of blood relatives, and maternity testing.

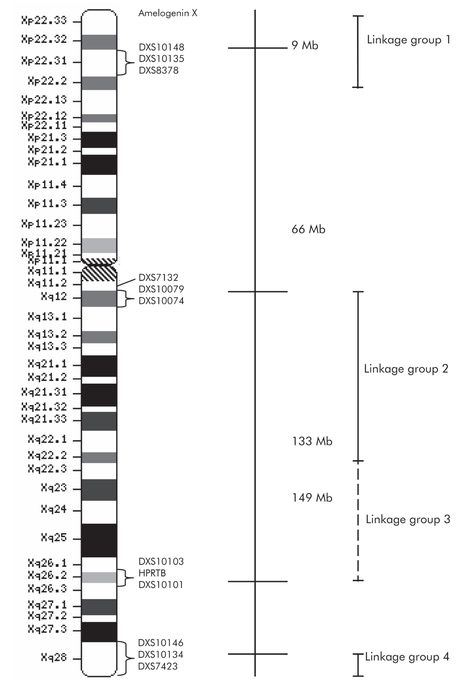
Kit Configurations

The features of the Investigator Argus X-12 QS Kit include:

- Co-amplification of 12 X-chromosomal markers from 4 linkage groups and D21S11
- Well-established 5-color setup (Matrix B5)
- 25 and 100 reaction kit size

Linkage groups

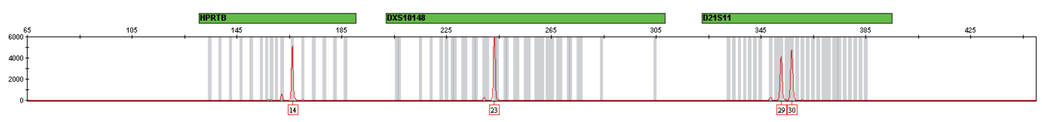
Linkage group	
1 (Xp22)	DXS8378 – DXS10135 – DXS10148
2 (Xp11)	DXS7132 – DXS10074 – DXS10079
3 (Xp26)	HPRTB – DXS10101 – DXS10103
4 (Xp28)	DXS7423 – DXS10134 – DXS10146



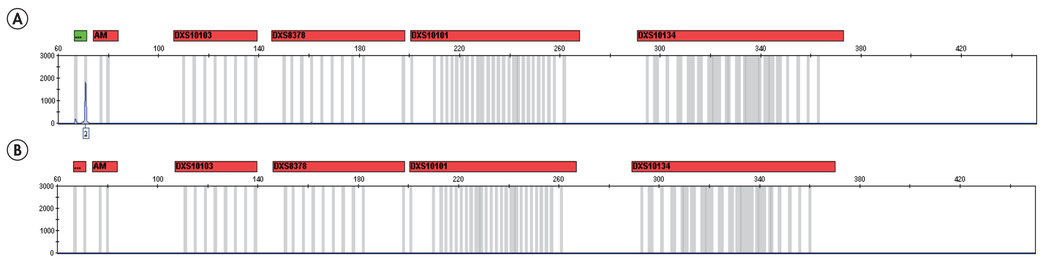
X-chromosomal markers used in the Argus X-12 QS Kit. Three markers each are combined as a linkage group and can be handled as haplotypes. Distances from the p-telomere are shown in Mb (**www.ncbi.nlm.nih.gov/genome/guide/human as of 10/2009**). The ChrX-STR.org 2.0 database provides valuable information on allele frequencies and tools to perform calculations.

Quality Control Features

Two quality control features have been added to the Investigator Argus X-12 QS Kit. In addition to the gonosomal markers, D21S11 has been included in the red channel as an autosomal marker. It confirms consistency between X-chromosomal and autosomal profiles derived from the same sample in kinship analysis. D21S11 was chosen as it is included in all common STR marker sets in use globally. Furthermore, the assay now has a QS for general PCR performance control in the blue channel. QS distinguishes between negative results caused by the absence of amplifiable DNA and false-negative results caused by technical failures.



Alignment marker. D21S11 has been added in the red channel to allow cross-checks with autosomal profiles.



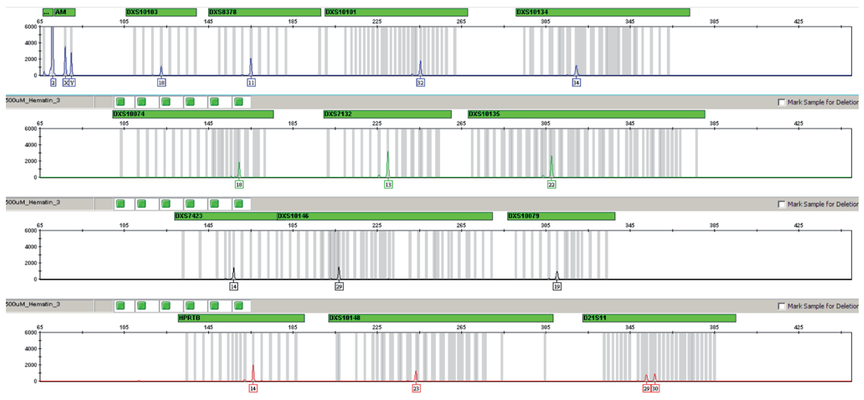
Quality Sensor. Ⓐ No template DNA added, but QS indicates successful PCR. Ⓑ False-negative result caused by PCR failure.

Improved Speed and Robustness

The Investigator Argus X-12 QS Kit utilizes the new Fast Reaction Mix 2.0 that combines fast PCR cycling, high inhibitor tolerance and convenient PCR setup. The cycling time of a standard 30-cycle amplification is about 80 minutes. The antibody mediated, hot-start *Taq* polymerase is included in the reaction mix to simplify the reaction setup. Up to 15 µl of sample can be added to the reaction.

Reaction setup

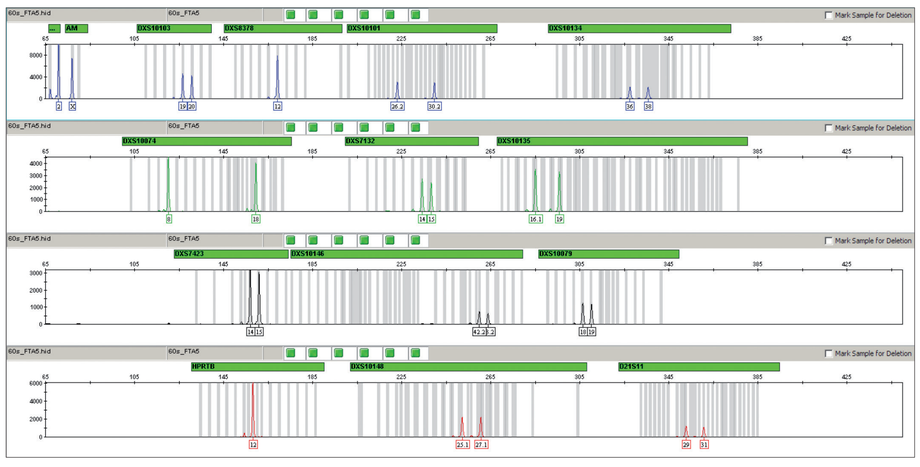
Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free water	Variable
Template DNA	Variable
Total volume	**25 µl**



Inhibitor tolerance. Control DNA 9948 (500 pg) was amplified in the presence of 500 µM hematin.

Direct Amplification

Although developed for use with purified DNA, The Argus X-12 QS Kit is capable of direct amplification of typical reference sample types, such as blood or buccal cells on Whatman™ FTA™ and other paper, or crude buccal swab lysates prepared using the Investigator STR Lysis Buffer. Punches of 1.2 mm diameter or 2 µl swab lysate can be added to the reaction. PCR cycle numbers required to obtain optimal results depend on the nature of the sample and should be evaluated in the laboratory using representative samples. Final protocols are currently under development.



Preliminary data for direct amplification. A 1.2 mm punch of a blood sample on FTA paper was amplified using 25 PCR cycles. Capillary electrophoresis was carried out on an Applied Biosystems® 3500 Genetic Analyzer as described in the kit handbook.

Conclusions

X-chromosomal analysis provides a very useful tool in complex paternity and kinship analysis supplementing common autosomal and Y-chromosomal analysis. The new Investigator Argus X-12 QS Kit makes use of 12 X-chromosomal markers arranged in 4 linkage groups. The marker set was kept identical to the previous kit version, in order to ensure that allele and haplotype frequency data acquired for many populations can still be used for analysis. However, various technical improvements have been made.

New and enhanced features:

- Quality Sensor and autosomal alignment marker added as valuable quality control features
- Improved typing results of samples carrying common mutations
- Faster and more robust amplification to further streamline the workflow
- More convenient reaction setup for manual and automated sample handling
- Optional direct amplification capability to complete existing workflows for reference samples

Reference

1. Elakkary, S., Hoffmeister-Ullerich, S., Schulze, C., et al. (2014) Genetic polymorphisms of twelve X-STRs of the Investigator Argus X-12 kit and additional six X-STR centromere region loci in an Egyptian population sample. Forensic Sci. Int. Genet. **11**, 26.

The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

1096088 08/2015

Sample to Insight