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 paternity cases. The Investigator 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 marker between X-chromosomal and autosomal STR analyses within a case, potential sample mix-up can be detected.
• Inclusion of additional SNPs 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 inhibition tolerance. A standard 30-cycle PCR using 500 pg of template DNA can be finished within 90 minutes.

Quality Control Features
Two quality control features have been added to the Investigator 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 STR analyses.

By comparing the genotype of the alignment marker between X-chromosomal and autosomal STR analyses within a case, potential sample mix-up can be detected. 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 STR analyses.

Improved Speed and Robustness
The Investigator 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 90 minutes. The unstably remodeled, homestyryl polynucleotide is included in the reaction mix to simplify the reaction setup. Up to 15 µl of sample can be added to the reaction.

X-chromosomal markers used in the Argus X-12 QS Kit. New markers marked are combined in a linkage group and can be handled as haplotypes.

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 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 frequencies can still be used for analysis. However, various technical improvements have been realized.

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

References

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.

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