



### Investigator Argus X-12 QS: Kinship Analysis using X-STRs

Green Mountain DNA Conference  
August 2016

### Outline

- 1 Introduction to XChr STR
- 2 Kit concept and overview
- 3 Technical details
- 4 Integrated Quality Control
- 5 Summary



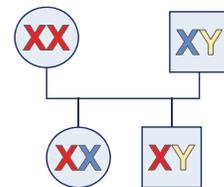
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### Introduction

#### X-chromosomal inheritance



- **Mothers:** to daughters and sons
- **Fathers:** only to daughters

• X-chromosomal testing allows the study of certain special cases using the analysis of the X chromosomal lineage

Set of X-STR markers used in Investigator Argus X-12 QS

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

- 12 X-chromosomal markers
- 4 linkage groups of 3 markers each
- One marker per linkage group can be used like an autosomal STR
- Each set of 3 markers is handled as a haplotype
- The Forensic ChrX Research Group initiated the online data base ChrX-STR.org (<http://www.chrx-str.org>) that provides population data and haplotype frequencies

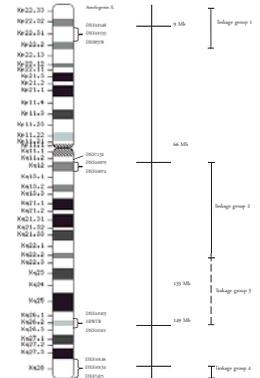
● Haplotypes occur with lower frequency than single STRs

Position of the 4 linkage groups

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

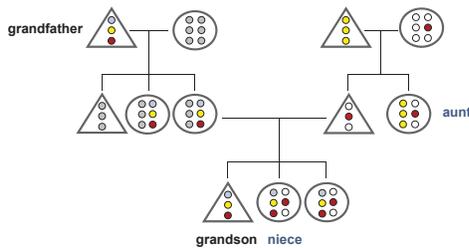
- Each set of 3 markers is handled as a haplotype
- Haplotype frequencies are already available
- Haplotypes occur with lower frequency than single STRs

Edelmann et al., FSIgen, X-chromosomal Haplotype frequencies of four linkage groups using the Investigator Argus X-12 Kit; FSI: Genetics – Letter to the Editor, DOI information: 10.1016/j.fsigen.2011.01.001



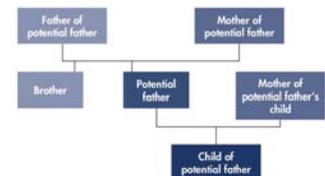
X-chromosomal haplotypes

- Color triplets symbolize one of 4 linkage groups per X-chromosome
- Haplotype frequencies are already available
- One color stands for a certain allele
- Haplotypes occur with lower frequency than single STRs



Applications for X-chromosomal analysis

- Autosomal STR markers belong to routine in paternity and kinship analysis since they have been introduced for human ID
- ChrX markers have a series of advantages with
  - Deficiency kinship cases – where the father cannot be typed
  - More complex deficiency paternity cases – when half-sisters and/or grand mothers are to be examined
  - Paternity testing of blood relatives
  - Maternity testing



● ChrX STRs can, however, only be used in paternity case disputes involving daughters, as there is no allele inherited by descent in a father-son relationship

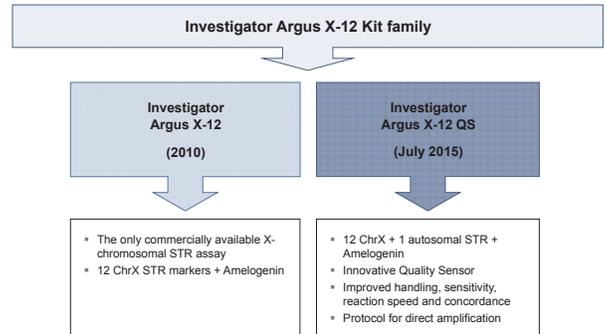


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## Kit concept and overview

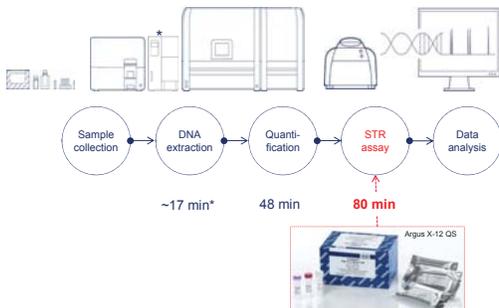


- Improved Xchr genotyping with integrated quality control steps



## Kit concept and overview

### Workflow optimization



- For purified DNA (casework protocol) the time to result for the Investigator Argus X-12 QS is ~125 min + CE/handling



## Kit concept and overview

### Summary

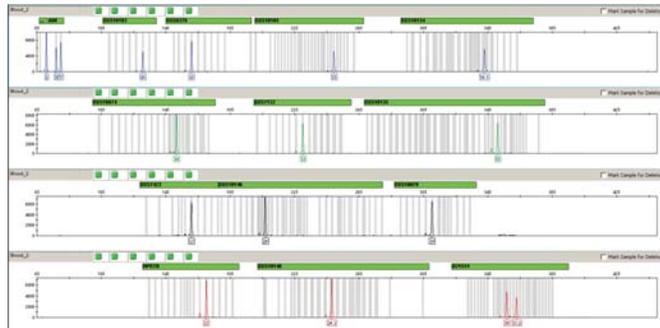
- 12 XChr markers plus Amelogenin and 1 autosomal STR
- Key features compared to former Argus X-12 Kit
  - New reaction chemistry (FRM 2.0)
    - Convenient manual and automated pipetting
    - Even higher stability
    - High sensitivity and inhibitor resistance
  - Integrated Quality Controls
    - New Quality Sensor for more information and workflow optimization
    - D21S11 as alignment marker to avoid sample mix up
  - Improved concordance rates (DXS10101, DXS10146, DXS10148)
  - Dye labels switched between DXS10101 (green → blue) and DXS7132 (blue → green)
  - New allelic ladder with 14 additional alleles

- A powerful assay for complex kinship and deficiency cases



## Kit concept and overview

DNA extracted from blood using EZ1 DNA Investigator Kit



Well balanced profiles for reliable typing

Sample to Insight

Investigator Argus X-12 QS

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## Kit concept and overview

Negative Control



Negative Control appears with clean baseline and amplification control (QC)

Sample to Insight

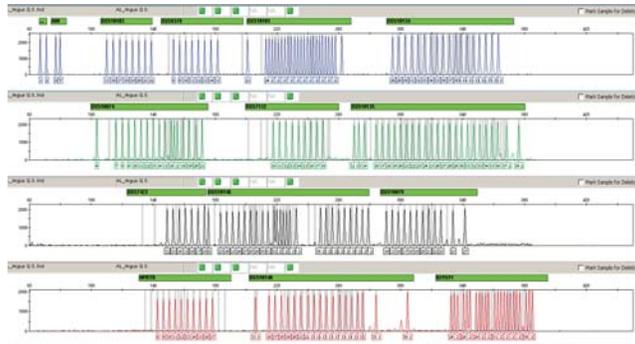
Investigator Argus X-12 QS

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## Kit concept and overview

Allelic Ladder



Improved allele calling due to additional alleles of the allelic ladder

Sample to Insight

Investigator Argus X-12 QS

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Sample to Insight

Investigator Argus X-12 QS

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## Technical details

### Kit content

Investigator Argus X-12 QS Kit	(25)	(100)
Catalog no.	383223	383225
Number of 25 µl reactions	25	100
Fast Reaction Mix 2.0*	750 µl	750 µl
Primer Mix Argus X-12 QS	63 µl	250 µl
Nuclease-free water	1.9 ml	1.9 ml
Control DNA 9947A	200 µl	200 µl
DNA size standard 550 (BTO)	13 µl	50 µl
Allelic ladder Argus X-12 QS	25 µl	25 µl
Quick Start Protocol	1	1

PCR

CE

\* Contains DNA Polymerase, dNTPs, MgCl<sub>2</sub> and bovine serum albumin (BSA).

### Reaction setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free water (added in step 4)	Variable
Template DNA (added in step 4)	Variable
<b>Total volume</b>	<b>25 µl</b>



## Technical details

### PCR program and cycling times

Component	Time	Number of cycles
98°C*	60 s	
61°C	100 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	100 s	27 cycles
72°C	5 s	
68°C	2 min	
10°C	∞	-

\* Hot-start to activate DNA polymerase.

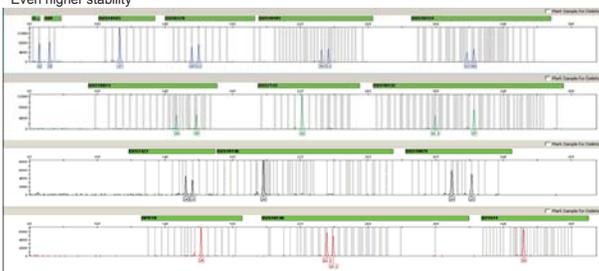
### Improved pipetting scheme and PCR protocol



## Fast Reaction Mix 2.0 - Advantages

### Improved PCR chemistry

- Convenient manual and automated pipetting
- Even higher stability



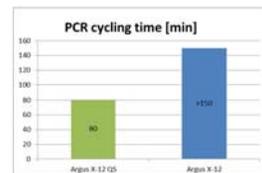
Investigator Argus X-12 QS provides enhanced specificity and efficiency of the multiplex PCR reaction



## Fast Reaction Mix 2.0 - Advantages

### Improved PCR chemistry

- Convenient manual and automated pipetting
- Even higher stability
- Even faster reaction speeds



\*Cycling time based on 30 cycles on a GenAmp PCR System

Investigator Argus X-12 QS Kit outperforms in PCR speed

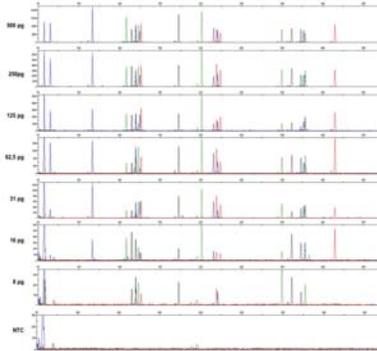


## Fast Reaction Mix 2.0 - Advantages

### Improved PCR chemistry

- Convenient manual and automated pipetting
- Even higher stability
- Even faster reaction speeds
- Highly inhibitor resistant and sensitive

Inhibitor	Concentration
Humic Acid	>250 ng/μL
Hematin	500 μM
Calcium	3 mM
Indigo carmine	> 12 mM
Tannic Acid	> 5.000 ng/μL
Collagen	> 250 ng/μL



- Investigator Argus X-12 QS excels in and inhibitor robustness and sensitivity



## Argus X-12 QS inhibitor resistance

### Full testing scheme of inhibitors

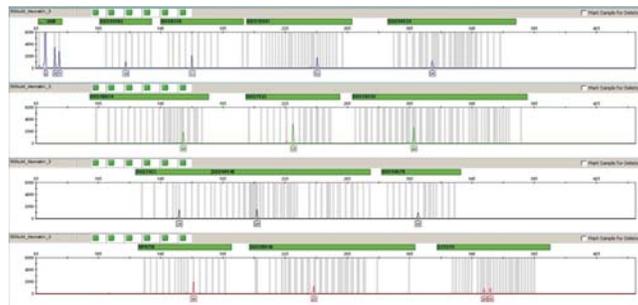
	Humic Acid				Hematin porcine				Calcium				Indigo carmine				Tannic Acid		Collagen								
	0 ng/μL	100 ng/μL	150 ng/μL	200 ng/μL	250 μM	500 μM	750 μM	1000 μM	0 mM	2 mM	3 mM	4 mM	0 mM	4 mM	6 mM	8 mM	10 mM	12 mM	0 ng/μL	3000ng/μL	4000ng/μL	5000ng/μL	0 ng/μL	150ng/μL	200ng/μL	250ng/μL	
QS	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
AM	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10103	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS8378	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10101	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10134	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10074	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS7132	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10135	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS7423	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10146	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10079	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
HPRT1B	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DXS10148	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
DZ1S11	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green

Green: Consistently full profile. Yellow: 75% of expected PCR products detected. Orange: 50% of expected PCR products detected. Red: Less than 50% of expected PCR products detected. Light Green: Consistently full profile with split peaks.



## Argus X-12 QS inhibitor resistance

### 500 pg control DNA 9948 in the presence of 500 μM hematin

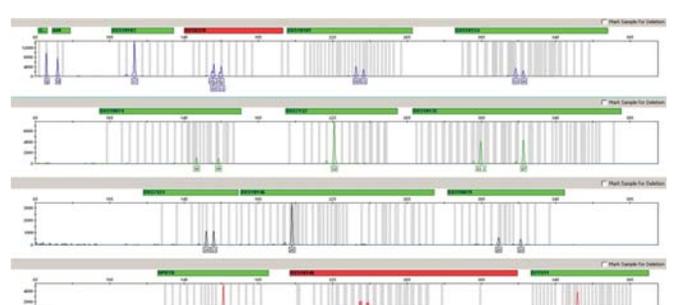


- Full profile in presence of 500 μM hematin



## Argus X-12 QS inhibitor resistance

### 500 pg Control DNA 9947 amplified in the presence of 3,000 ng/μl tannic acid



- Full profile in presence of 3,000 ng/μl tannic acid, but split peaks in DXS8378 and DXS10148



## Direct amplification of reference samples

Supplementary protocols available from product webpage

- Investigator Argus X-12 QS Kit allows direct amplification of typical reference samples
  - Blood or buccal cells on FTA
    - One 1.2 mm punch recommended
  - Buccal swabs
    - prepare a crude lysate preparation using the Investigator STR Lysis Buffer is recommended
    - 2 µl lysate can be applied as template for amplification.
- Optimize cycle numbers based on a representative batch of samples
  - Note: The Quality Sensor is optimized for 30 cycle amplifications and thus will show reduced signal heights at lower cycle numbers

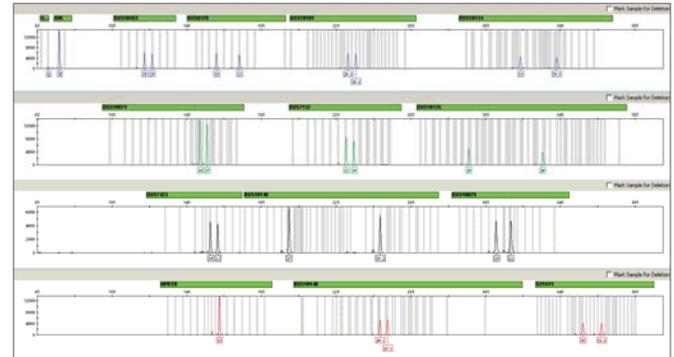


- The chemistry allows direct amplification of reference samples



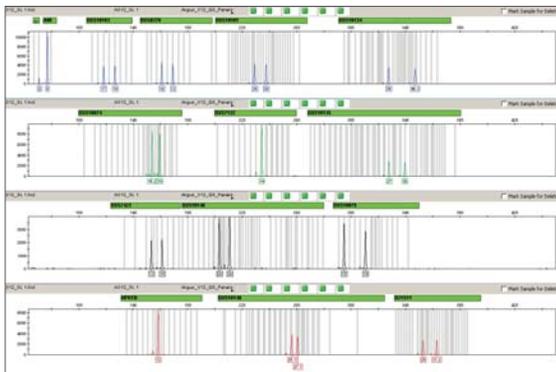
## Direct amplification of reference samples

Direct amplification of buccal cells on FTA with 27 cycles



## Direct amplification of reference samples

Direct amplification of buccal swabs with 28 cycles



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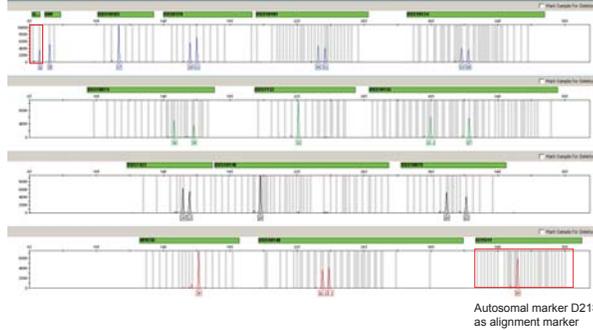




## Integrated Quality Control

There are two features that serves as integrated Quality Control parameters

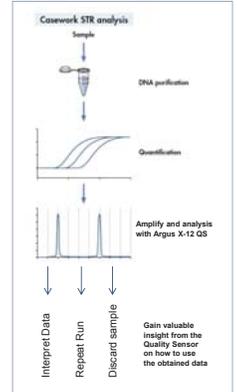
### Quality Sensor as internal PCR performance control



## Integrated Quality Control – The Quality Sensor

Use the Quality Sensor (QS) to understand the story behind the data

- The kit includes a Quality Sensor that will be able to give information about a successful or failed PCR run
- The Quality Sensor is enclosed in the Primer Mix and is amplified simultaneously with the markers
- The QS is labeled with 6-FAM, giving a small (71 bp) amplicon
- In combination with the actual profile the data allows interpretation of the state of the sample:
  - Detect PCR failure
  - Detect absence of DNA
  - Detect successful run

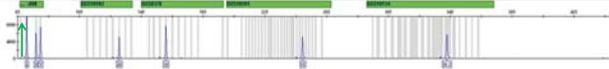


- The Quality Sensor provides additional information to improve the quality of results and reduce costs



## Quality Sensor (QS) explained

### Successful PCR run



### Absence of DNA in your sample



A peak is observed for QS, but no sample peaks appear across the profile. Repeating the PCR is not necessary.

### Failed PCR Amplification



Absence of QS and sample amplicon peaks across the profile. The PCR run needs to be repeated.



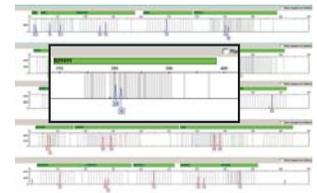
## Integrated Quality Control - The alignment marker D21S11

Usually, ChrX are analysed alongside with autosomal STR marker

### Sample profile using Investigator Argus X-12 QS



### Sample profile using Investigator 24plex QS



- The alignment marker D21S11 allows you to be confident a full autosomal profile and an X-chromosomal profile are from the same sample, minimizing risk of mix-ups



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## Summary

### Investigator Argus X-12 QS Kit

- Co-amplification of 12 X-chromosomal and D21S11 as an autosomal alignment marker, to minimize the risk of sample mix-up
- An integrated Quality Sensor for better decision making and data interpretation
- Faster results using FRM 2.0 PCR chemistry that allows for a PCR speed of approximately 80 minutes
- High sensitivity and inhibitor resistance
- Possibility of direct amplification of reference samples



- Argus X-12 QS: trusted sample analysis for complex kinship cases



# Thank you



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