Human Identification and Forensics:

QIAGEN® Paternity Testing Solutions

Advanced automation and
STR solutions for kinship testing

Sample to Insight

QIAGEN

Advanced automation and STR solutions for paternity

Autosomal STRs are in worldwide use for kinship and forensic analysis, and the recently released extended CODIS set has brought powerful upgrades to the battery of core markers available. However, there are still scenarios when supplementary STRs could enhance the data necessary for interpreting complex kinship patterns. That's why QIAGEN offers a broad portfolio of preanalytical and analytical products for all types of kinship analyses.

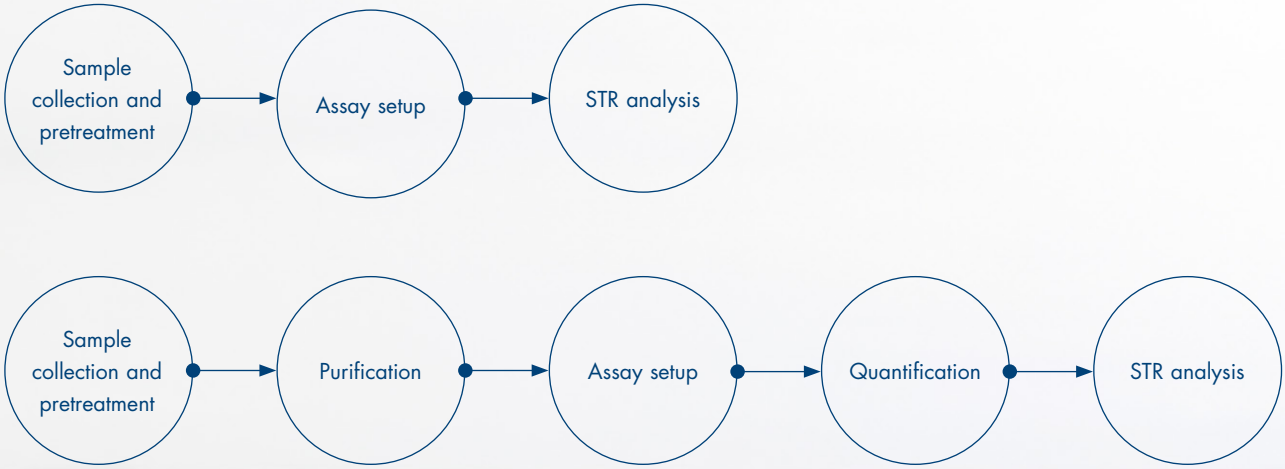


Figure 1. Standard workflows applied in paternity and kinship analysis. The main solutions for paternity testing are direct amplification STR assays from paper or swabs (workflow 1). For more challenging samples or complex cases, the full workflow including DNA purification and quantiifcation is recommended (workflow 2). For both setups, QIAGEN provides supportive automation for low, medium or high throughputs.

High-quality Investigator® solutions

Independently of the workflow you choose, you can trust in your results with QIAGEN Forensic Grade products. QIAGEN's entire Investigator product portfolio and corresponding manufacturing sites are compliant with the ISO18385 forensic standard to minimize the risk of human DNA contamination in products used to collect, store and analyze biological material for human identification.

ISO 18385
Forensic DNA Grade

For more Forensic Grade quality, see **www.qiagen.com/forensicgrade**.

Figure 2. ISO 18385 Forensic DNA Grade.

and kinship testing with a unique Quality Sensor

The main solutions for paternity testing are direct amplification STR assays from FTA® paper or swabs. For more difficult cases, we offer kits using standard or complementary autosomal STR markers to enhance the power of discrimination. In addition, we offer the only commercially available X-chromosomal kit for complex deficiency cases (Table 1). All assays have been validated and tested for their use in paternity testing, and have been studied thoroughly for allele frequencies. To complement all these kits, QIAGEN offers nucleic acid purification technology specifically developed for human identification testing as well as automated reaction setup.

Trust QIAGEN for your paternity testing applications and benefit from our sample and assay technologies expertise. Our product range includes:

- A broad portfolio for DNA purification and assay set up from low- to high-throughout scales
- **Investigator 24plex GO! Kits** for direct amplification of the new CODIS core markers including Quality Sensor
- **Investigator 24plex QS Kits** for amplification of the new CODIS core markers from extracted DNA, including Quality Sensor
- **Investigator IDplex Plus** and **IDplex GO! Kits** for CODIS 15 markers
- **Investigator Argus X-12 QS Kit** for X-chromosomal analysis in difficult kinship or deficiency cases
- **Investigator HDplex Kit** with complementary markers for more complex cases
- **QIAgility** for integrated rapid, high-precision automation of PCR and CE-plate setup

Table 1. Overview of QIAGEN's Investigator STR PCR portfolio for kinship analysis

Amplification	Marker	Number of STR markers	Quality Sensor	Recommended kit
Direct amplification	Autosomal standard	22	•	Investigator 24plex GO! Kit
		15		Investigator IDplex GO! Kit
	Gonosomal/autosomal	12/1	•	Investigator Argus X-12 QS Kit
Amplification from extracted DNA	Autosomal standard	22	•	Investigator 24plex QS Kit
		15		Investigator IDplex Plus Kit
	Supplementary autosomal	12		Investigator HDplex Kit
	Gonosomal/autosomal	12/1	•	Investigator Argus X-12 QS Kit

DNA purification in complex cases

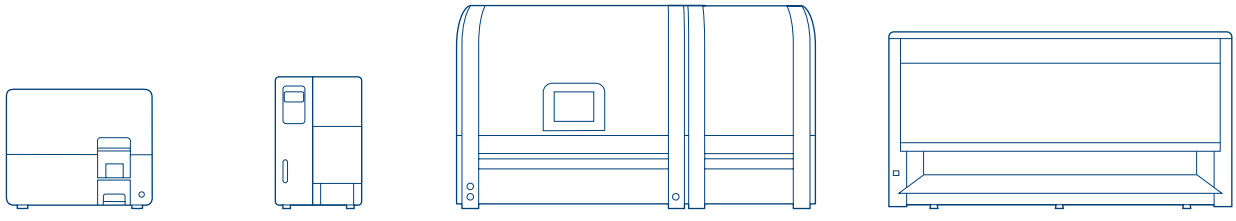
For standard kinship analysis, you may choose to skip DNA purification and move straight to **Investigator STR GO! Kits**. These assays permit direct amplification. However, for difficult cases, we recommend initial purification of DNA to attain the most reproducible results and run the widest selection of assays. In these cases, we offer a broad portfolio for all kind of sample throughput, including manual or automated processing, for trusted quality and performance (Table 2 and Figure 3).

Table 2. Overview of QIAGEN's DNA purification portfolio from low to high throughput

Instrument	Kit	Max. throughput* (samples/day)	Max. throughput* (samples/year)	Technology
QIAcube®	QIAamp® DNA Investigator Kit	48	12,000	Spin columns
EZ1® Advanced XL	EZ1 DNA Investigator Kit	56	14,000	Magnetic bead cartridge
QIAsymphony® SP/AS	QIAsymphony DNA Investigator Kit	120	29,000	Magnetic bead cartridge
STAR Q SP/AS	Investigator STAR Lyse&Prep Kit	192	46,000	Magnetic bead

*Calculations of throughput based on Good Practice Guidelines with pre- and post-PCR work separated by rooms and persons. Analysis done from sample to result including sample lysis (if necessary), hands-on time, PCR- and CE-run; data interpretation is not included.

While the **QIAamp DNA Investigator Kit**, used manually or automated on the **QIAcube**, offers great flexibility and performance for low-throughput labs and small sample batches, we also offer a variety of automated solutions for labs that need larger batches or high-throughput testing. These include the **EZ1 Advanced XL** (for sample preparation), our **QIAsymphony SP/AS** (for sample preparation and assay setup) and our latest high-throughput instrument, the **STAR Q SP/AS**.



QIAcube with QIAamp DNA Investigator Kit

EZ1 Advanced XL with EZ1 DNA Investigator Kit

QIAsymphony SP/AS with QIAsymphony DNA Investigator Kit

STAR Q SP/AS with Investigator STAR Lyse&Prep Kit

Figure 3. Overview of QIAGEN's sample purification portfolio including chemistry and supportive automation.

Automated high-throughput assay setup for reference samples

For laboratories specializing in centralized and high-throughput kinship analysis, time and money are even more crucial than for other setups and applications. To address this requirement, QIAGEN has developed two automated workflows for reference samples collected on buccal swabs or paper. These workflows use Investigator STR GO! Kits and automate sample pretreatment as well as PCR setup using QIAGEN's **STAR Q Swab AS** or **STAR Q Punch AS Instruments** (Figure 4). These instruments combine QIAGEN's expertise in performance and process safety with high-throughput liquid handling to enable processing of up to 360 samples per day with no compromise in quality.

STAR Q Swab AS Instrument: High-throughput lysis and assay setup for human identity swab samples

- Efficient and safe processing of epithelial and blood samples from swabs used in high-throughput reference sample laboratories
- Validated for most commonly used swab types, including GE Healthcare Omni swabs, Sarstedt cotton swabs, Copan flocked swabs and Puritan polyester swabs

STAR Q Punch AS Instrument: High-throughput punching and assay setup for human identity paper samples

- Designed for efficient and safe processing of epithelial and blood samples from cards used in high-throughput reference sample laboratories
- Validated for GE Healthcare EasiCollect® cards and Copan NUCLEIC-CARD™ system collection cards*

Benefits of the STAR Q Swab and STAR Q Punch AS Instruments include:

- Easy-to-use software ensuring minimal training requirements
- Fast setup and implementation with prevalidated assay setup protocols
- High first-pass success rates with Investigator STR GO! Kits

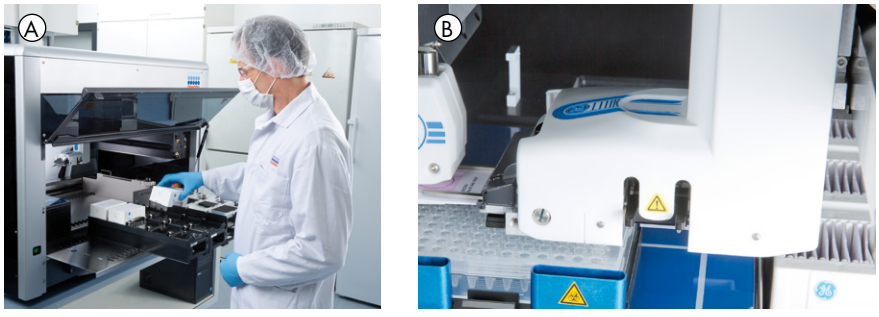


Figure 4. QIAGEN's STAR Q Instruments. **A**: STAR Q Swab AS Instrument (left) and **B**: STAR Q Punch AS Instrument (right) for high-throughput processing of reference samples.

Want to learn more? Go to **www.qiagen.com/forensicsHTP**

* Other card types can be processed: please contact your local QIAGEN representative for details.

Save money and begin your analysis with Investiga

The basis of any paternity test is a full STR profile using a CODIS marker set. The **Investigator STR 24plex GO! Kit** provides convenient paternity testing without the need for DNA purification.

Benefits of the Investigator 24plex GO! Kit include:

- Best mean exclusion chance due to 22 STR markers
- Ideal for reference samples from buccal cells or blood on FTA paper or swabs
- Integrated performance control due to the Quality Sensor
- Validated for use in human identification according to SWGDAM and ENFSI guidelines
- Minimized allelic overlap, reducing the risk of misinterpretation and enhancing result quality
- Optimized time-to-result attained as a consequence of fast process times

The Investigator 24plex GO! Kit amplifies the new 20 CODIS core loci, plus the highly informative SE33 marker, DYS391 and Amelogenin (Table 3), thereby providing the highest power of discrimination for routine work. Samples can be amplified directly from buccal cells or blood on FTA, or from swabs. The kit is also available in a format with optimized sensitivity and performance for purified DNA samples (Investigator 24plex QS Kit). For laboratories that want to stay with the former CODIS-15, we offer the IDplex Plus and IDplex GO! Kits.

Table 3. Investigator 24plex Kits dyes and markers

Dye	Markers						
6-FAM™	Amelogenin	TH01	D3S1358	vWA	D21S11		
BTG	TPOX	DYS391	D1S1656	D12S391	SE33		
BTY	D10S1248	D22S1045	D19S433	D8S1179	D2S1338		
BTR2	D2S441	D18S51	FGA				
BTP	QS1	D16S539	CSF1PO	D13S317	D5S818	D7S820	QS2

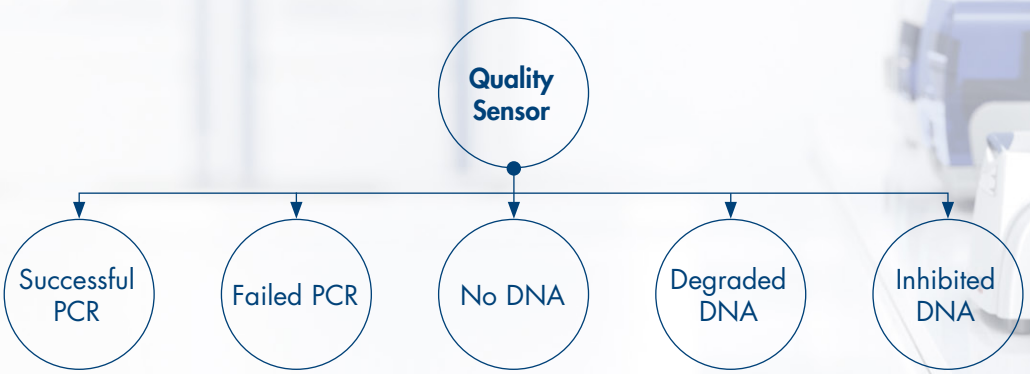
Table 4. Technical specifications

	Investigator 24plex QS Kit	Investigator 24plex GO! Kit
DNA input volume	Up to 15 µl	1.2 mm FTA punch or 2 µl swab lysate
Volume per reaction	25 µl	20 µl
Matrix	BT6	BT6
Fluorescence dyes	6-FAM, BTG, BTY, BTR2, BTP, BTO	6-FAM, BTG, BTY, BTR2, BTP, BTO
Genetic Analyzers	Applied Biosystems® 3500 Genetic Analyzers; Applied Biosystems 3130/3130xl Genetic Analyzer (upgraded to 6 dyes)	Applied Biosystems 3500 Genetic Analyzers; Applied Biosystems 3130/3130xl Genetic Analyzer (upgraded to 6 dyes)

tor STR GO! Kits for direct amplification

Better quality control checks for your STR analyses

Investigator 24plex Kits include an integrated quality control feature, the unique Quality Sensor, which allows the generation of additional, valuable data for performance checks. With Quality Sensor, you can confirm a successful PCR amplification and distinguish between the absence of DNA due to improper sampling and a failed PCR amplification, as well as differentiating between degradation and inhibition. This information can be used to choose the most appropriate rework strategy and streamline the overall workflow for direct amplification with higher first-success rates.



For better quality control in your STR analysis, see **www.qiagen.com/qualitysensor**.

Results you can trust

For kinship testing, like other human identity applications, accuracy of results as well as ease of data interpretation are key. That's why QIAGEN invests heavily in optimal primer design (Figure 6). Investigator 24plex Kits have been designed with reduced allelic overlap wherever possible. This ensures that for rare alleles, you get unambiguous results you can trust. Attaining results with better statistical relevance lowers costs by reducing reagent and material expense for reruns, saves lab time per result, and reduces time required for data analysis.

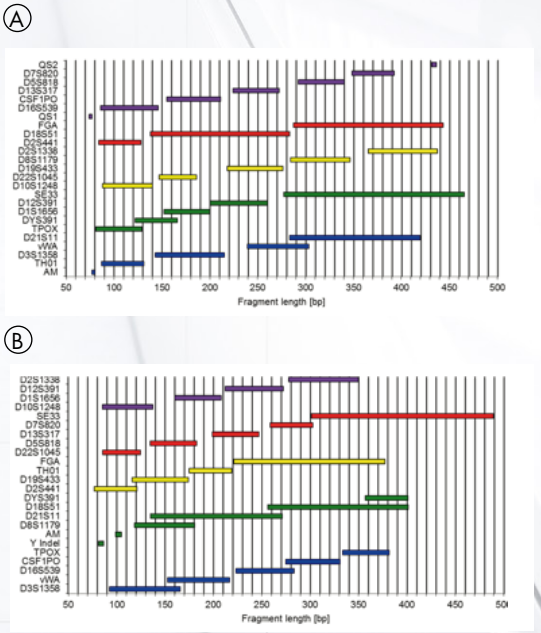


Figure 6. Allele distribution comparison. A: Primer design of the Investigator 24plex Kits minimizes allelic overlap to get unambiguous results and increased statistical relevance for rare alleles. **B**: In contrast, the GlobalFiler® Kits (Thermo Fisher Scientific) has various allelic overlaps, which lead to an increased risk of data misinterpretation, the need to retype samples, and as a result, increased overall cost at lower statistical relevance. Marker and allele distribution according to published alleles (February 2015) is shown.

Use the Investigator Argus X-12 QS Kit for complex

In some cases, normal autosomal STR analysis is not sufficient to solve a case. ChrX genotyping can complement the analysis of autosomal and ChrY markers very efficiently, especially in complex cases of kinship testing. The **Investigator Argus X-12 QS Kit** is the only commercially available kit that allows STR analysis using X-chromosomal analysis (Table 5). This is particularly powerful in special X-chromosomal lineage tracing.

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

- Co-amplification of 12 ChrX markers (clustered in 4 linkage groups) and D21S11 as autosomal alignment marker, to minimize the risk of sample mix-up (Figure 7)
- 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 approx. 80 minutes
- High sensitivity and inhibitor resistance
- Optional protocols for direct amplification

Table 5. Investigator Argus X-12 QS Kit dyes and markers

Dye	Markers					
6-FAM	QS1	Amelogenin	DXS10103	DXS8378	DXS10101	DXS10134
BTG	DXS10074	DXS7132	DXS10135			
BTY	DXS7423	DXS10146	DXS10079			
BTR	HPRTB	DXS10148	D21S11			

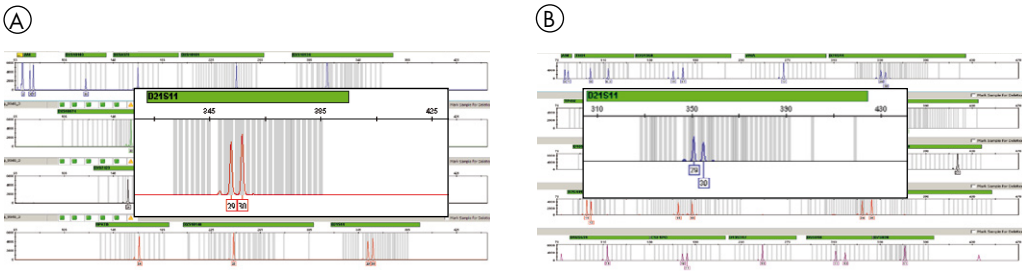


Figure 7. Alignment of D21S11. Sample profiles from the **A**: Investigator Argus X-12 QS Kit and the **B**: Investigator 24plex QS Kit, with highlighted D21S11 amplicons.

Do you want to learn from other customers? See the interview at **www.qiagen.com/ChrXuser**

cases

Deficiency paternity cases

The major advantage of ChrX markers arises in deficiency paternity cases (i.e., when a biological sample from a putative father is not available and DNA from paternal relatives has to be analyzed instead). When female individuals have the same father, they also share the same paternal ChrX. An investigation of ChrX markers of two sisters or half-sisters can thus exclude paternity, namely through the presence of four different alleles or haplotypes, even when none of the parents is available for testing. Autosomal markers cannot provide such information.

Paternity cases involving blood relatives

In paternity cases involving close blood relatives, such as alternative putative fathers, the exclusion power of STRs is substantially decreased and ChrX STRs may be superior to autosomal markers. For example, if two alleged fathers are father and son, they would not share any X-chromosomal alleles identical by descent so that ChrX markers would be more efficient than autosomal markers.

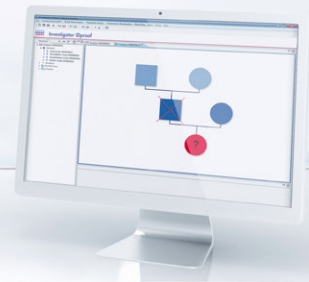
Paternity testing in rape and incest cases

After incest or criminal sexual assault, medically indicated abortion may terminate the pregnancy. By using ChrY markers, efficient paternity testing of such material is possible for male fetuses. For female fetuses in contrast, only autosomal and ChrX markers can be analyzed, the latter of which represent a more efficient means of paternity exclusion. Positive proof of paternity, however, relies mainly upon fetal alleles not shared with the mother.

Maternity testing

In some circumstances, mother/child testing may be necessary. Although mitochondrial DNA sequencing can resolve maternity, this technology is not available in all laboratories, is still expensive, and sometimes does not provide the level of certainty required in paternity and forensic science. For testing mother-daughter relationships, ChrX markers are equivalent to autosomal markers and do not provide any specific advantage. Testing mother-son kinship, however, is more efficiently performed using ChrX markers. The exclusion chance in such cases is identical to that of ChrX STRs in father/daughter tests (Table 8).

No.	Formula and explanation		Reference
I	$\sum_i f_i^3(1-f_i)^2+\sum_i f_i(1-f_i)^3+\sum_{i<j} f_i f_j(f_i+f_j)(1-f_i-f_j)^2$	MEC (mean exclusion chance) for AS markers in trios	1
II	$\sum_i f_i^3(1-f_i)+\sum_i f_i(1-f_i)^2+\sum_{i<j} f_i f_j(f_i+f_j)(1-f_i-f_j)$	MEC for ChrX markers in trios involving daughters	2
III	$1-\sum_i f_i^2+\sum_i f_i^4-\left(\sum_{i<j} f_i^2\right)^2$	MEC for ChrX markers in trios involving daughters (Desmarais)	3
IV	$1-2\sum_i f_i^2+\sum_i f_i^3$	MEC for ChrX markers in father/daughter duos	3



To increase discriminatory power, add the Investigator HDplex Kit

Despite the large number of established STR kits available on the market for paternity testing, there are still scenarios when supplementary STRs could enhance the data necessary for interpreting complex kinship and forensic patterns: making safer inferences about relatedness across distant relationships in deficient pedigrees, and improving the specificity of familial searching or expanding the points of reference to better interpret mixed profiles.

The **Investigator HDplex Kit** is suitable for purified DNA with 9 completely novel STRs, in addition to the ESS or CODIS expansion markers D12S39, D18S51 and SE33 (Table 9). The kit was developed specifically for maximized statistical relevance in paternity testing and can be used alongside other commercial kits to obtain maximum discriminatory power and minimum shared loci.

Benefits of the Investigator HDplex Kit include:

- Developed for difficult paternity, forensic and immigration
- Highest sensitivity and discriminatory power for kinship analyses
- Reliable differentiation of samples from related individuals
- Alignment markers enable sample confirmation

Table 9. Investigator HDplex Kit dyes and markers

Dye	Markers						
6-FAM	Amelogenin	D7S1517	D3S1744	D12S391	D2S1360	D6S474	D4S2366
BTG	D8S1132	D5S2500	D18S51	D21S2055			
BTY	D10S2325	SE33					

Do you want to learn more about the HDplex marker sets?

Watch the webinar *QIAGEN's HDplex STRs: Their Application to Forensic Analyses, Discrimination Power, and Patterns of Global Variation* at **www.qiagen.com/forensics-webinar**. This webinar outlines the completed studies on worldwide patterns of variability in the 12 novel HDplex STRs and their ability to enhance the power of paternity analyses when combined with existing markers.

Ordering Information

Product	Contents	Cat. no.
QIAcube (230 V)/QIAcube (110 V)	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits: includes 1-year warranty on parts and labor	9001293/ 9001882
QIAamp DNA Investigator Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56504
EZ1 Advanced XL	Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, 1-year warranty	9001874
EZ1 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridges (DNA Investigator), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K, Carrier RNA	952034
QIAsymphony SP	QIAsymphony sample prep module, 1-year warranty on parts and labor	9001297
QIAsymphony AS	QIAsymphony assay setup module, 1-year warranty on parts and labor	9001301
QIAsymphony DNA Investigator Kit (192)	For 192 preps of 200 µl each from casework and reference samples: Includes 2 reagent cartridges and enzyme racks and accessories	931436
STAR Q SP/AS Instrument	Includes installation and training, 1-year warranty on parts and labor and 1 year maintenance	9002650
Investigator STAR Lyse&Prep Kit (400)	For 400 preps of 300 µl each from casework and reference samples: Buffer ATL, Buffer QSL3, Buffer QSW1, Buffer QSW2, Bead Suspension G, Buffer ATE, Proteinase K, Carrier RNA	931447
STAR Q Punch AS Instrument	Instrument with 1-year warranty on parts. For use with GE Healthcare EasiCollect cards and Copan NUCLEIC-CARD system collection cards.	9002651
STAR Q Swab AS Instrument	Instrument with 1-year warranty on parts. For use with all commonly used swab types for human identity reference samples	9002652
Investigator 24plex GO! Kit (200)*†	Primer Mix, Fast Reaction Mix 2.0, Control DNA, Allelic Ladder, DNA Size Standard	382426
Investigator 24plex QS Kit (100)*†	Primer Mix, Fast Reaction Mix 2.0, Control DNA, Allelic Ladder, DNA Size Standard, Nuclease-Free Water	382415

* Larger kit sizes available; please inquire.

† Not available in all countries; please inquire.

Ordering Information

Product	Contents	Cat. no.
Investigator IDplex GO! (200)*†	Primer Mix, Fast Reaction Mix, Control DNA, Allelic Ladder, DNA Size Standard, Nuclease-Free Water	381636
Investigator IDplex Plus (100)*†	Primer Mix, Fast Reaction Mix, Control DNA, Allelic Ladder, DNA Size Standard	381625
Investigator Argus X-12 QS Kit (25)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, Allelic Ladder, DNA Size Standard, Nuclease-Free Water	383223
Investigator HDplex Kit (100)†	Primer Mix, Reaction Mix, DNA Polymerase, Control DNA, Allelic Ladder, DNA Size Standard, Nuclease-Free Water	381215

* Larger kit sizes available; please inquire.

† Not available in all countries; please inquire.

References

1. Krüger, J. Fuhrmann, W., Lichte, K.-H. and Steffens, C. (1968) Zur Verwendung der sauren Erythrocytenphosphatase bei der Vaterschaftsbegutachtung. Dtsch. Z. Gerichtl. Med. **64**,127–46.
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3. Desmarais, D., Zhong, Y., Chakraborty, R., Perreault, C. and Busque, L. (1998) Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA). J. Forensic Sci. **43**, 1046–9.

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.

Learn more about our human identity and forensic testing solutions for confidence in your evidence at **www.qiagen.com/forensics**.