QIAGEN® Paternity and Kinship Testing Solutions

Human Identification and Forensics

Sample to Insight

Advanced automation and STR solutions for paternity and

Autosomal STRs are in worldwide use for kinship and forensic analysis, and the extended CODIS set has brought powerful upgrades to the battery of core markers available. However, there are still scenarios where supplementary STRs or NGS technology 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.

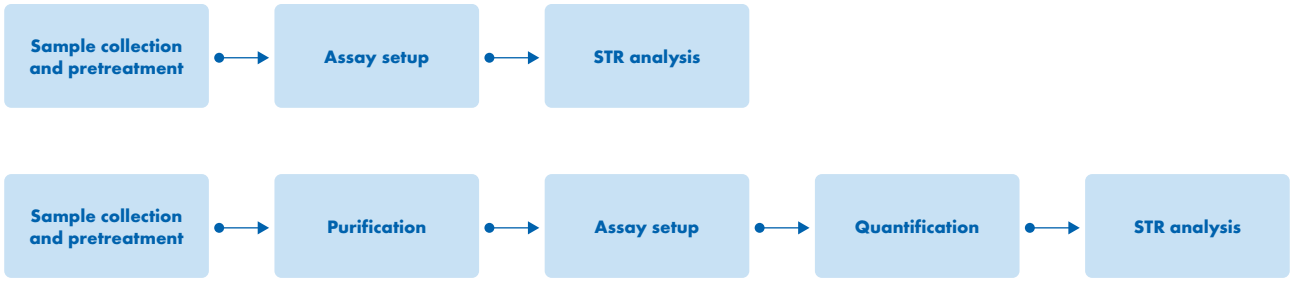


Figure 1. Standard workflows applied in paternity and kinship analyses.
The main solutions for paternity testing are direct amplification STR assays from paper or swabs (workflow 1). For more challenging samples or complex cases, the full workflow including DNA purification and quantification is recommended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

Sample collection

Responsibility from the start

Whether you're looking to simplify sample collection, reduce your storage costs, increase first pass success rates, or even have a specific, customized kit in mind to improve your workflow, we have a solution for you.

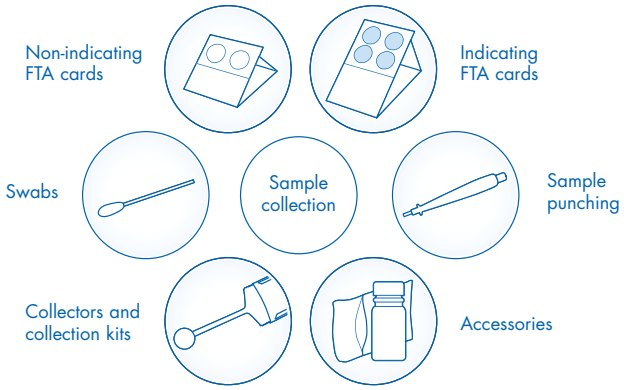


Figure 2. Sample collection solutions.

For more information and a full list of products, go to
www.qiagen.com/forensic-sample-collection

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 setup from low- to high-throughput scales
- **Investigator® 26plex QS Kit** for amplification of the CODIS core markers including Penta D, Penta E and D6S1043, with Quality Sensor
- **Investigator 24plex QS Kit** and Investigator 24plex GO! Kit for amplification of the CODIS core markers, with Quality Sensor
- **Investigator IDplex Plus** and for CODIS-15 markers
- **Investigator Argus Y-28 QS Kit** for Y-chromosomal analysis
- **Investigator Argus X-12 QS Kit** for X-chromosomal analysis in difficult kinship or deficiency cases
- **Investigator HDplex Kit** with complementary autosomal markers for more complex cases
- **QIAgility®** for integrated rapid, high-precision automation of PCR and CE-plate setup
- **QIAamplifier®** for fast and high performance end-point PCR in a 96-well format

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

Kit	Quality Sensor	Marker set	Number of STR markers	Direct amplification from paper or swabs	Amplification from purified DNA
Investigator 26plex QS Kit	●	Autosomal standard	24	Supplementary protocol	●
Investigator 24plex QS Kit	●	Autosomal standard	22	Supplementary protocol	●
Investigator 24plex GO! Kit	●	Autosomal standard	22	●	
Investigator IDplex Plus Kit		Autosomal standard	15		●
Investigator HDplex Kit		Autosomal supplementary	12		●
Investigator Argus X-12 QS Kit	●	XChr/autosomal	12/1	Supplementary protocol	●
Investigator Argus Y-28 QS Kit	●	YChr	27	Supplementary protocol	●

Simplified DNA purification in complex cases

For standard kinship analysis, you may choose to skip DNA purification and move directly to 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).

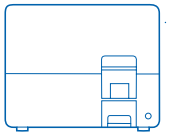
Table 2. Overview of QIAGEN's DNA purification portfolio from low- to high-throughput

Instrument	Kit	Max. samples/day/analyst*	Technology
QIAcube® Connect	QIAamp® DNA Investigator Kit	48	Spin columns
EZ2® Connect FX	EZ1&2™ DNA Investigator Kit	288	Magnetic bead cartridge
QIAsymphony® SP/AS	QIAsymphony DNA Investigator Kit	120	Magnetic bead cartridge
Open platforms	Investigator STAR Lyse&Prep Kit	up to 288**	Magnetic bead

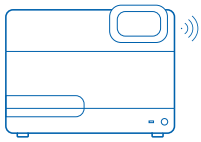
* Calculations of throughput based on Good Practice Guidelines with pre- and post-PCR work, separated by rooms and persons. Analysis done from sample-toresult including sample lysis (if necessary), hands-on time, PCR- and CE-run; data interpretation is not included.

** depending on instrument used.

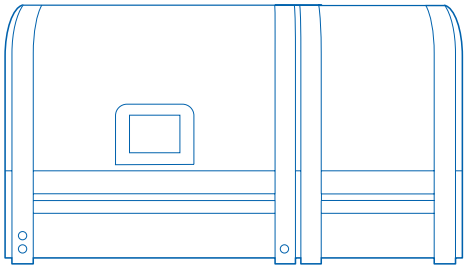
While the **QIAamp DNA Investigator Kit**, used manually or automated on the **QIAcube Connect**, offers great flexibility and performance for low-throughput labs and small sample batches, we also offer a variety of automated solutions for labs that need larger batches or high-throughput testing. These include the **EZ2 Connect Fx** (for sample preparation), our **QIAsymphony SP/AS** (for sample preparation and assay setup) and solutions of high-throughput open DNA extraction platforms from third party supplier, such as Hamilton, TECAN or others.



QIAcube Connect with QIAamp DNA Investigator Kit



EZ2 Connect Fx with EZ1&2 DNA Investigator Kit



QIAsymphony SP/AS with QIAsymphony DNA Investigator Kit

Like the QIAsymphony chemistry, but want to run it on a third party open platform? We offer the **Investigator STAR Lyse&Prep** as well as many scripts and protocols for this application.

Third party open DNA extraction platforms with Investigator STAR Lyse&Prep Kit

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

Automated low/medium to high-throughput assay setup for reference samples

Working in high-throughput paternity laboratories, you face unique challenges related to DNA sample processing every day. The demand for ever greater sample numbers, while maintaining the same high first-time success rates and cost per sample means a constant endeavor to maintain quality standards and sample continuity. As quality standards and cost must not be compromised, this usually means that throughput is. To address these challenges, and to enable you to realize the full potential of your laboratory, we collaborate with Hamilton. Their easyPunch STARlet automates STR assay setup from FTA® or other collection card samples and has been developed as part of laboratory workflows enabling hundreds of samples to be processed each day.

For more information and support on using QIAGEN chemistry on Hamilton instruments please contact our Tech Service department.

Table 3. Features of easyPunch STARlet

Feature	
Daily throughout (as part of a sample to capillary electrophoresis workflow)	Capacity of 384 samples a day in batches of 96 samples
STR assay setup	Pre-validated protocols for QIAGEN® Investigator STR GO! direct amplification assays
Validtated sample input types	• EasiCollect® FTA cards • Copan NUCLEICards
Pre-treatment	• E1.2 mm sample punch, into PCR reaction mix in PCR plate • Full image recording of punchinh step
Operation	Simple-to-use GUI and software for step-by-step setup and operation
Pipet tip attachment	Compressed O-Ring Expansion (CO-RE)
Liquid level detection	Capacitative LLD (c-LLD) ans pressure-based LLD (p-LLD)
Pipetting method	Air displacement pipetting
% CV when pipetting 1 µl (under defined conditions)	4%
Numer of pietting channels	4
Assay setup tim from sample loading	96 samples in under 90 minutes

Increased assay efficiency with Investigator STR QS Kits for

The basis of any paternity test is a full STR profile, typically using a CODIS marker set. **The Investigator 26plex QS Kit** provides convenient paternity testing with the highest mean exclusion chance due to the choice of markers. You can use this assay for direct amplification from reference samples or amplification from purified DNA — it is optimized for both, without any compromise.

Benefits of the Investigator 26plex QS Kit include:

- Best mean exclusion chance due to 24 STR markers including Penta D and Penta E
- A single kit for both purified DNA and reference samples simplifies the workflow
- Integrated performance control due to Quality Sensor optimized for ≥ 24 cycles
- 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 26plex QS Kit amplifies the 20 CODIS core loci, plus the highly informative Penta E, Penta D markers, D6S1043, DYS391 and Amelogenin (Table 4), 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, as well as from purified DNA. For laboratories that want to stay with the former CODIS-15 market set, we offer the IDplex Plus, or Investigator 24plex QS Kit and Investigator 24plex GO! for CODIS-20.

Table 4. Investigator 26plex QS Kit dyes and markers

Dye	Markers						
6-FAM™	Amelogenin	TH01	D3S1358	Penta D	D651043	D21S11	
BTG	TPOX	DYS391	D1S1656	D12S391	Penta E		
BTY	D10S1248	D22S1045	D19S433	D8S1179	D2S1338		
BTR2	D2S441	D18S51	vWA	FGA			
BTP	QS1	D16S539	CSF1PO	D13S317	D5S818	D7S820	QS2

Table 5. Investigator 26plex QS Kit technical specifications

	Purified DNA	Reference sample
Sample input	Up to 15 µl	1.2 mm FTA punch or 2 µl swab lysate
Volume per reaction	25 µl	20 µl
Matrix	BT6	
Fluorescence dyes	6-FAM, BTG, BTY, BTR2, BTP, BTO	
Genetic Analyzers	Applied Biosystems® 3500 Genetic Analyzers, Applied Biosystems 3130/3130xl Genetic Analyzer (upgraded to 6 dyes)	

direct amplification

Better quality control checks for your STR analyses

Our Investigator STR QS assays 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 5). Investigator STR 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.

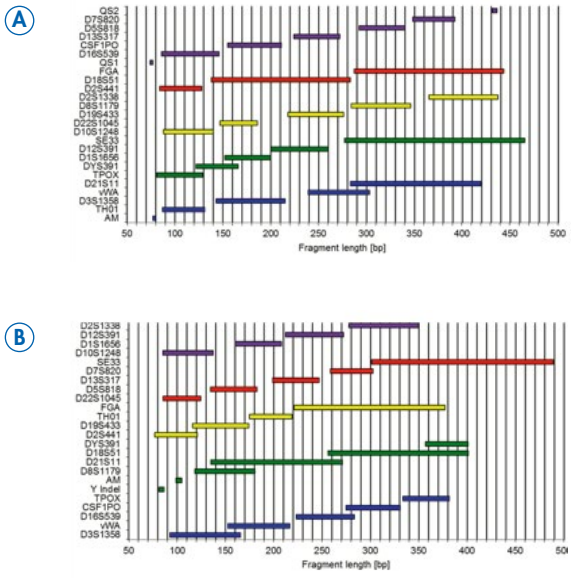


Figure 4. Allele distribution comparison.
A Primer design of the Investigator 24plex QS Kits minimizes allelic overlap to get unambiguous results and increased statistical relevance for rare alleles. **B** In contrast, the GlobalFiler™ Kits (Thermo Fisher Scientific) has various allelic overlaps, which 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.

Unparalleled resolution with Investigator Argus X-12 QS

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 6)
- 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 6. 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			

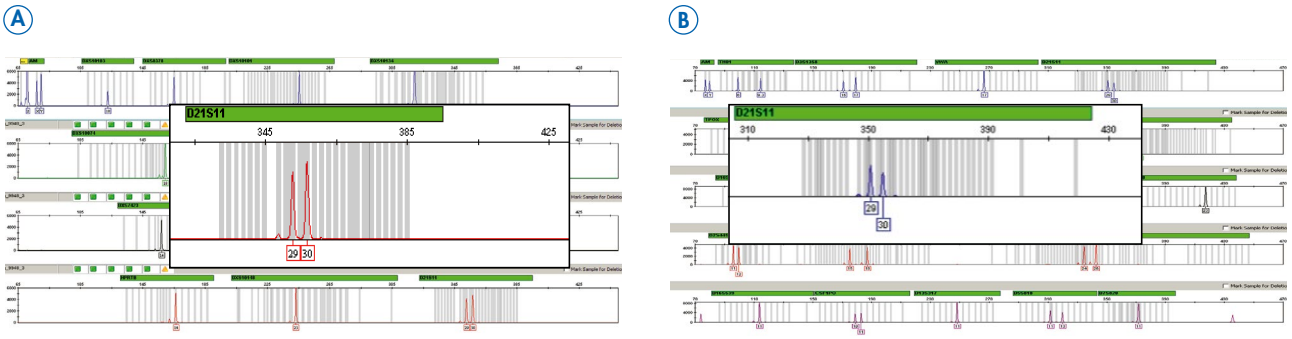


Figure 5. Alignment of D21S11.
Sample profiles from the **A** Investigator Argus X-12 QS Kit and the **B** Investigator 24plex QS Kit, with highlighted D21S11 amplicons to minimize the risk of sample mix-up.

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

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 neither parent 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 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 is possible for male fetuses. However, for female fetuses, 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 7).

Table 7. Mean exclusion chance for autosomal and ChrX markers

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

Best-in-class performance and powerful discrimination for Y-chromosomal analysis

Y-STR haplotype analysis is well established in resolving paternity disputes of male offspring and other types of paternal kinship testing, including historical cases. The new **Investigator Argus Y-28 QS Kit** was designed for optimal performance and unparalleled discrimination power, helping you obtain trusted results for challenging samples in less time. In particular, the inclusion of six rapidly mutating Y-STRs supports the resolution of paternal lineages and discriminate between closely related males.

The features of Investigator Argus Y-28 QS Kit include:

- Maximal discrimination capacity through 27 highly discriminating markers of which 6 are rapidly mutating (RM)
- A single kit for both purified DNA and reference samples simplifies the workflow
- Integrated performance control due to Quality Sensor optimized for ≥ 24 cycles
- Optimized Fast Reaction Mix 3.0 for best in class sensitivity and robustness
- Optimized time-to-result attained as a consequence of fast process times

Table 8. Investigator Argus Y-28 QS Kit dyes and markers

Dye	Markers					
6-FAM	YS389I	DYS391	DYS389II	DYS533	DYS390	DYS627
BTG	DYS458	DYS393	DYS19	DYS437	DYS449	
BTY	DYS460	DYS576	YGATAH4	DYS481	DYS448	DYS518
BTR2	DYS439	DYS549	DYS438	DYS456	DYS643	
BTP	QS1	DYS570	DYS635	DYS385	DYS392	QS2

Boosted discrimination with Investigator HDplex

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:

- 12 autosomal STRs to be used for more complex paternity, forensic and immigration cases
- High sensitivity and discriminatory power for kinship analyses
- Reliable differentiation of samples from related individuals
- Alignment markers, enabling 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: **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.

Unprecedented discrimination in complex cases with NGS

Although CE-based STR analysis for DNA profiling has advanced steadily, established methods have their limits, and sometimes cannot provide the level of discrimination required, particularly in complex kinship cases. Developed with leading paternity scientists, the QIAseq® Investigator ID SNP panel is designed to provide additional discrimination where STRs fall short. This panel consists of 277 primers, targeting 139 individual identity SNPs, selected for their utility in kinship testing.

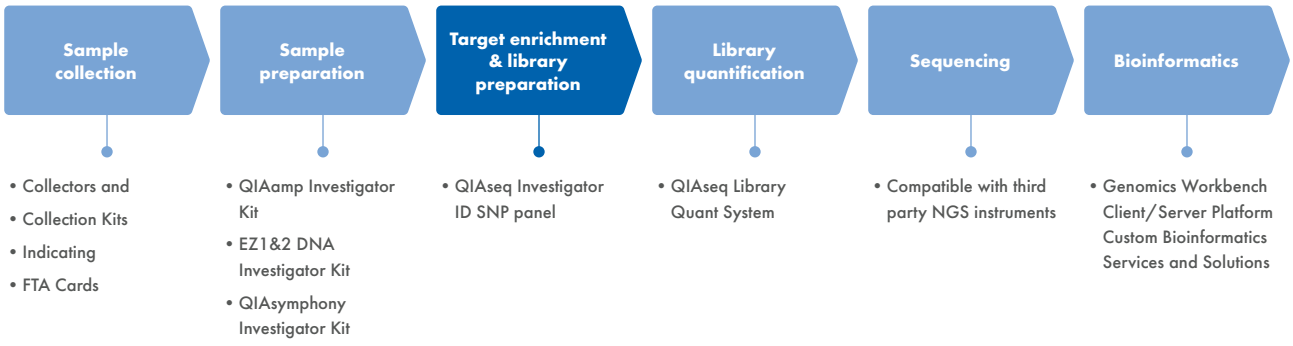


Figure 6. QIAGEN's NGS workflow solutions in kinship and paternity testing.

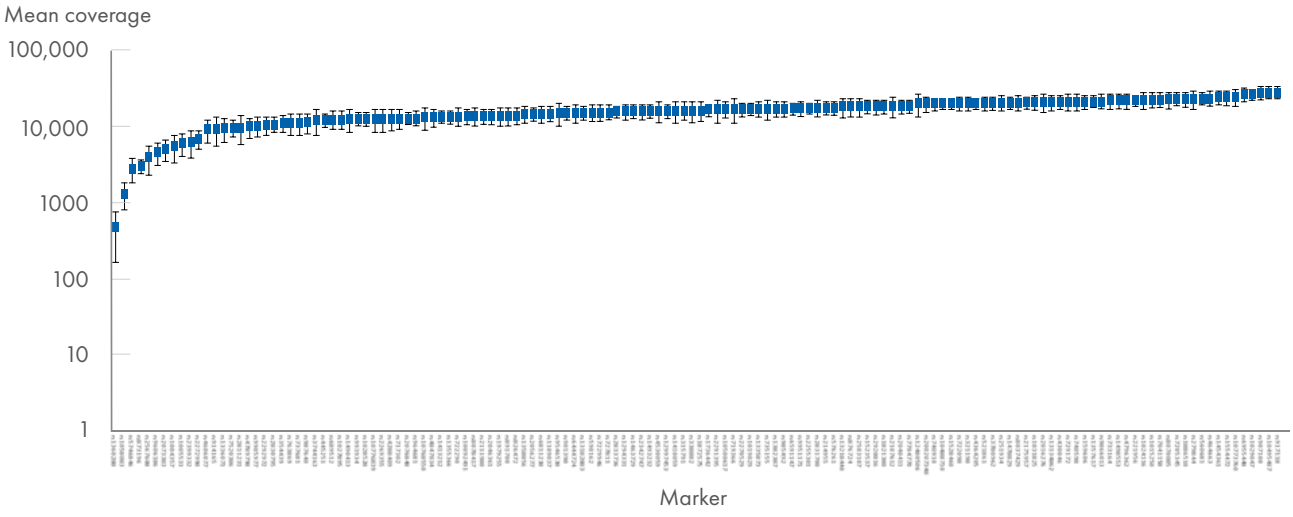


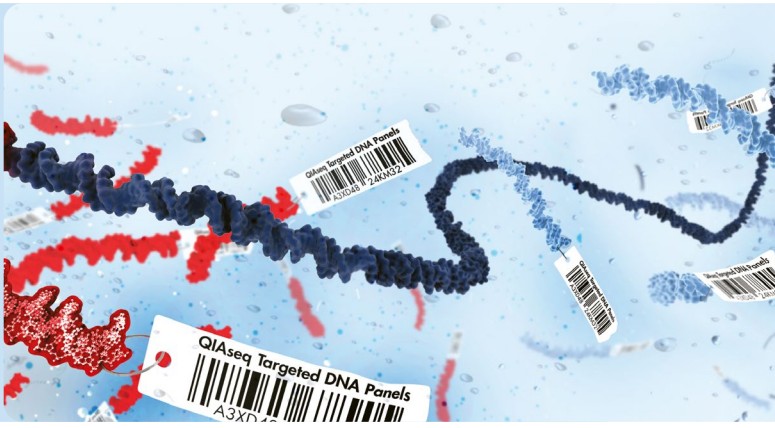
Figure 7. Alignment of D21S11.
Uniformity of coverage, for the 49 blood samples, for the 139 SNPs in the target enrichment panel sequenced on Illumina® MiSeq® All 139 SNPs are shown along the x-axis and a logarithmic scale of coverage on the y-axis. Only 1 SNP was below 1000X coverage (the coverage of this SNP was enough to make accurate calls).

Confidence in your sequencing data

QIAseq chemistry also includes Unique Molecular Indices (UMIs). Following sequencing, these UMIs enable raw reads to be mapped to individual DNA molecules, instead of just individual loci. This enables sequencing and PCR errors to be removed from the data and gives higher confidence in the sequencing data – even for very low-level sequences. This innovative feature of QIAseq enables, for example, confident sequencing of low-level fetal DNA in maternal blood in pre-natal paternity cases (4).

The inclusion of UMIs in the QIAseq panels and the increased confidence this gives enables much smaller SNP panels to be used compared with more traditional circulating fetal cell free DNA approaches. Paternity can confidently be assigned from safe, cost-effective prenatal testing.

Interested in an alternative to invasive prenatal testing? Read the application note at: **www.qiagen.com/paternity-UMIs**



To discover the full portfolio offering, please go to: **www.qiagen.com/NGS-in-HID**

Ordering Information

Product	Contents	Cat. no.
Sample collection		
OmniSwab Sterile (100)	100 sterile collection swabs with ejectable brush-like head used for buccal and saliva samples	WB100035
EasiCollect Plus (50)	50 buccal sample collection devices: Includes a foam applicator for direct sample transfer to an integral QIAcard® FTA Indicating	WB120472
EasiCollect Buccal Collection Kit	50 buccal sample collection kits: Includes EasiCollect (incl. barcode), return mailing envelop, multi-barrier pouch, nitrile gloves, tamper evident tape and desiccant	WB120237
QIAcard FTA Blood Collection Kit (50)	50 blood sample collection kits: Includes Indicating QIAcard FTA Micro (incl. barcode), disposable lancet, alcohol wipe, band aid, return mailing envelop, multi-barrier pouch, nitrile gloves, tamper evident tape and desiccant	WB120238
Uni-Core Punch 1.2 mm	Manual punch for precise sample disc removal from FTA cards	WB100028
DNA purification and assay setup		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Reagent bottle racks (3); 200 µl filter-tips (1024); 1000 µl filtertips (1024); 30 ml reagent bottles (12); rotor adapters (240); rotor adapter holder	990395
QIAamp DNA Investigator Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56504
EZ2 Connect Fx	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples	9003220
EZ1&2 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

* Larger kit sizes available; please inquire.

Ordering Information

Product	Contents	Cat. no.
Investigator STAR Lyse&Prep Kit (400)	For 400 preps from casework and reference samples: Buffer ATL, Buffer QSL3, Buffer QSW1, Buffer QSW2, Bead Suspension G, Buffer ATE, Proteinase K, Carrier RNA	931447
Investigator human identification PCR kits		
Investigator 26plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, Allelic Ladder, Nuclease-Free Water	382615
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
Investigator IDplex Plus (100)*	Primer Mix, Fast Reaction Mix, Control DNA, Allelic Ladder, DNA Size Standard, Nuclease-Free Water	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 Argus Y-28 QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, Allelic Ladder, DNA Size Standard, Nuclease-Free Water	383625
Investigator HDplex Kit (100)	Primer Mix, Reaction Mix, DNA Polymerase, Control DNA, Allelic Ladder, DNA Size Standard, Nuclease-Free Water	381215
NGS in human identification		
QIAseq Investigator ID SNP panel	CDHS-11454Z-318	333525
QIAseq Targeted DNA Custom Panel (96)	Kit containing ALL reagents (except indexes) for targeted DNA sequencing; Custom panel for 96 samples	333525

* Larger kit sizes available; please inquire.

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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.

Investigator Kits meet ISO18385 requirements. For more Forensic Grade quality, see **www.qiagen.com/forensicgrade**

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

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Website	**www.qiagen.com**