Paternity doubts erased

Our improved noninvasive paternity testing workflow delivers answers with greater confidence

Sample to Insight

Noninvasive prenatal paternity testing (NIPPT)

Prenatal paternity testing has evolved to become safe and noninvasive. Circulating cell-free DNA (ccfDNA) from the fetus can be isolated from maternal blood plasma and used to assess paternity. No longer is there a need for invasive amniocentesis or chorionic villus sampling and their associated risks. A simple blood draw is all that's required.

The amount of fetal ccfDNA compared to the maternal contribution is relatively low, especially in the very early weeks of gestation. The ccfDNA is also more fragmented than cellular DNA. This is why the traditional CE-based STR methods used for more routine paternity applications have not proved viable for NIPPT in the past.

Our new NIPPT workflow offers specific advantages for low level, degraded and mixed sample analysis. Combine efficient purification of ccfDNA, best in class DNA quantitation and powerful next-generation sequencing (NGS) kits to confidently detect STR and SNP markers.

Erase doubt with accurate information about maternal and paternal contributions.

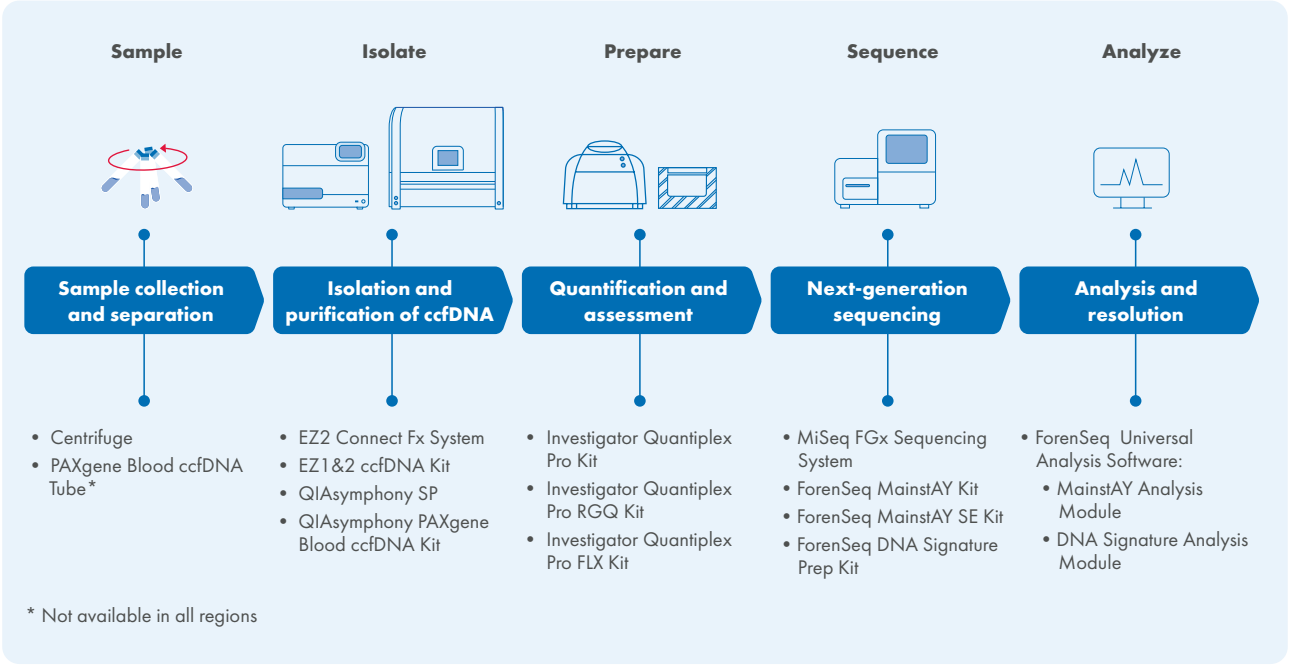
Confident, accurate and cost-effective NIPPT workflow

From sample collection to DNA sequencing and analysis

The QIAGEN HID workflow is an efficient and cost-effective option for NIPPT analysis.

This improved NIPPT workflow combines dedicated ccfDNA sample collection and isolation protocols with quantitative analysis and assessment. Add ForenSeq kits for NGS power to efficiently and cost-effectively sequence the most common autosomal and Y-STRs with the option to add SNPs based on the level of discrimination needed.

Sequence-level variation provides higher resolution capabilities to help distinguish between maternal and paternal contributions.



Maternal blood sampling, stabilization and separation

The PAXgene® Blood ccfDNA Tube is designed for the collection of human whole blood and the stabilization of ccfDNA.

- A non-crosslinking stabilization solution prevents the release of intracellular DNA
- The environment maintains constant ccfDNA levels during plasma storage

Maternal whole blood is separated via centrifugation. Plasma containing ccfDNA is transferred for ccfDNA isolation.

Note: The PAXgene Blood ccfDNA Tube is not available in all global regions.

Automated isolation and purification of ccfDNA

Automate processing of up to 24 ccfDNA samples at a time in 35 to 70 minutes with the EZ2® Connect Fx benchtop instrument and the EZ1&2® ccfDNA Kit.

Fetal DNA in maternal blood plasma is usually present as fragments of <200 bp[1]. The EZ1&2 ccfDNA Kit uses proven magnetic bead technology to efficiently purify these short circulating DNAs.

- Access dedicated human ID protocols easily
- Program flexible sample input (1–8 ml plasma)
- Reduce human error with prefilled cartridges
- Isolate high-quality DNA for downstream analysis
- Connect remotely to monitor and manage your runs from outside the lab

You can also generate good ccfDNA yields with a fully automated QIAsymphony® procedure and the QIAsymphony PAXgene Blood ccfDNA Kit. Select the STA protocol line to isolate predominantly small ccfDNA fragments.

[1] Chan KC, et al. Size distributions of maternal and fetal DNA in maternal plasma. Clin Chem. 2004; 50(1):88–92.

Quantification and assessment of ccfDNA

Accurate and comprehensive quantitation data is essential to ensure the correct amount of DNA is used for library preparation to maximize detection of the minor fetal contributor.

Investigator® Quantiplex® Pro Kits use quantitative real-time PCR to detect and quantify human and male DNA with a parallel assessment of DNA degradation.

- Sensitivity down to <1 pg/µl DNA
- High sensitivity for male DNA in a female background (up to 400,000:1)
- An Internal Control to flag the presence of potential PCR inhibitors

Choose the Investigator Quantiplex Pro Kit or the Investigator Quantiplex Pro RGQ Kit which includes a male DNA degradation assessment. For added sensitivity and convenience, choose the Investigator Quantiplex Pro FLX Kit with lyophilized chemistry in breakable, optical plates.

The Investigator Quantiplex Pro FLX Kit combines trusted multicopy markers for advanced assessment of quantity, degradation and inhibition with novel lyophilized chemistry in 96-well optical plates.

- Ready-to-use 96-well optical plates pre-filled with lyophilized qPCR Master Mix
- Trusted multicopy markers for assessment of quantity, degradation and inhibition
- Full flexibility with option to scale input volume from 1 µL to 18 µL for highest sensitivity
- Breakable plates and convenient seal removal to size for the throughput needed
- Sustainable room temperature storage to free-up freezer capacity

The capability for increased sample input up to 18 µL enables unprecedented higher sensitivity of approximately 10-fold compared to conventional quantification in human identification (see Figure 1).

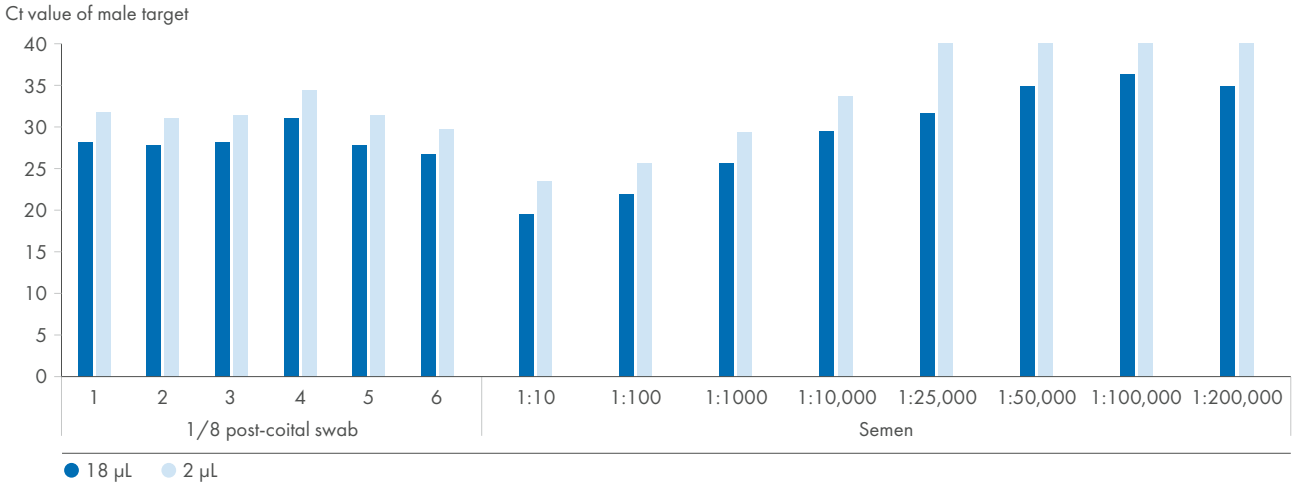


Figure 1. Quantification with a larger input sample volume is consistently approximately 10-fold higher. A sample of 1/8 swab was cut from postcoital swabs and processed with the Investigator Casework GO! Kit (A). Dilutions of a semen sample were spotted directly onto swabs and the whole swab was processed with the Investigator Casework GO! Kit (B). After lysis, 2 µL or 18 µL volumes were analyzed with the Investigator Quantiplex Pro FLX Kit.

Next-generation sequencing and analysis

MiSeq® FGx Sequencing System

The MiSeq FGx Sequencing System allows you to prepare and sequence libraries and analyze data in a single workflow. Dedicated reagent kits and matched analysis software combine for answers in all human identification investigations, including questions of paternity.

The ForenSeq® Universal Analysis Software (UAS) simplifies complex bioinformatics and data management.

The UAS includes analysis modules for all ForenSeq kits. The following are recommended for NIPPT analysis:

- ForenSeq MainstAY Kit
- ForenSeq MainstAY SE Kit
- ForenSeq DNA Signature Prep Kit

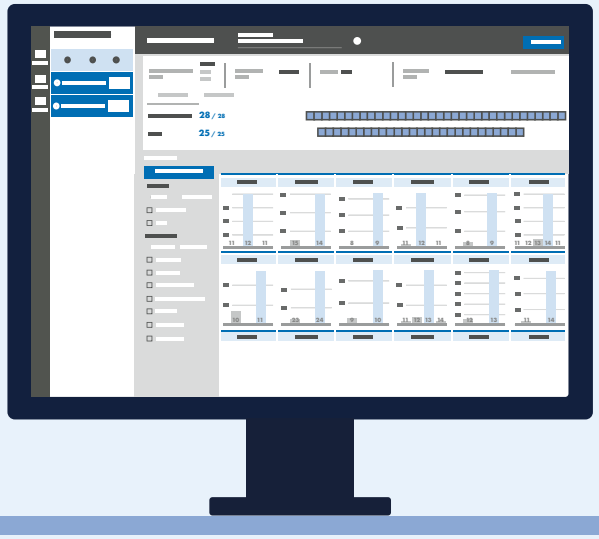
The UAS is accessible though a web browser and automated post-sequencing data analysis generates review-ready results within 1 hour of run completion.

ForenSeq MainstAY Product Line (with SE33)

Autosomal STRs (27/28)

Y-STRs (25)

UAS: MainstAY Analysis Module



ForenSeq MainstAY Product Line

The ForenSeq MainstAY Kit targets 27 core autosomal and 25 Y-STRs generating highly discriminating and cost-effective data.

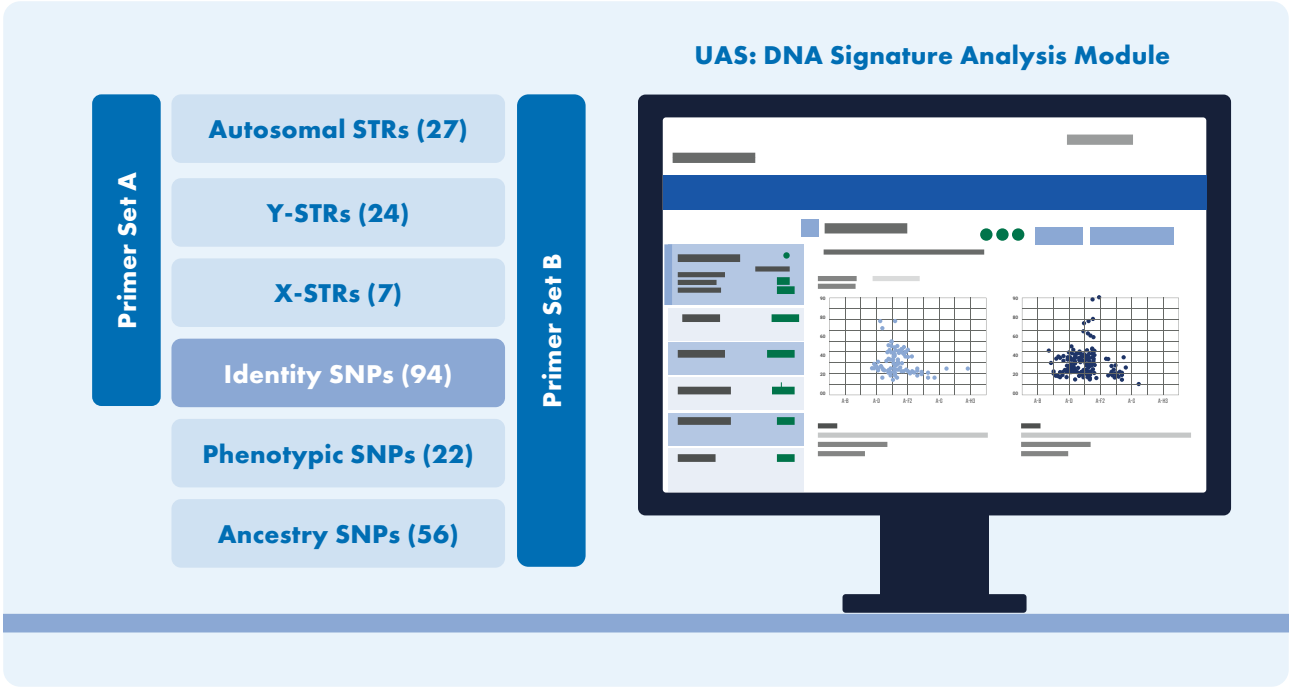
The ForenSeq MainstAY Kit SE includes the same markers as the ForenSeq MainstAY Kit plus SE33 to target 28 autosomal STRs.

- Demonstrated performance on low input and degraded samples
- Access sequence-level STR variation for maximum resolution of maternal and fetal DNA contributions
- Generate both autosomal and Y-STR results for added discrimination power with male fetuses
- Sequence up to 96 samples simultaneously

ForenSeq DNA Signature Prep Kit

Primer Set A of the ForenSeq DNA Signature Prep Kit combines 58 STRs (made up of autosomal, X and Y) with 94 identity SNPs for greater discrimination power than the ForenSeq MainstAY or ForenSeq MainstAY SE kits.

ForenSeq DNA Signature Prep Kit



Ordering Information

Product	Contents	Cat. no.
EZ1&2 ccfDNA Kit (48)	For 48 preps (2, 4 or 8 mL sample input volume each): 48 reagent cartridges (EZ1&2 ccfDNA), Magnetic Bead Suspension, Elution Buffer, Large-Volume Tubes (7 mL), Disposable Tip Holders, Disposable Filter-Tips, Elution Tubes (1.5 mL)	954854
QIAsymphony PAXgene Blood ccfDNA Kit (192)	Reagent cartridges, accessories and proteinase K vials for 192 preps	768536
PAXgene Blood ccfDNA Tube (100)*	100 blood collection tubes (10 mL). To be used in conjunction with the QIAamp® MinElute® ccfDNA Kit, the QIAamp Circulating Nucleic Acid Kit (50) or the QIAsymphony PAXgene Blood ccfDNA Kit (192)	768115
EZ2 Connect Fx System	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 2x EZ2 Connect racks (EZ2 Connect Fx Tip Rack and the EZ2 Connect Fx Tip Rack – Flip Cap Tubes), EZ2 Connect Fx Cartridge Rack and 1-year warranty on parts and labor	9003220
QIAsymphony SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
Investigator Quantiplex Pro Kit (200)	For use on Applied Biosystems® Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387216
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN Rotor-Gene® Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
Investigator Quantiplex Pro FLX Kit (576)	For use on Applied Biosystems Real-Time Systems: 6x single blistered 96-well optical plates with Master Mix, Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387516
MiSeq FGx Sequencing System	Desktop instrument with two run modes for a range of forensic genomics applications within a validated NGS workflow	15048976
Universal Analysis Software	Software pre-installed as a dedicated server specific for forensic genomics for run setup, sample management, analysis and report generation	9003364
ForenSeq MainstAY Kit (96)	Includes all the required reagents for 96 reactions to prepare sequencing libraries	V16000142
ForenSeq MainstAY Kit (384)	Includes all the required reagents for 384 reactions to prepare sequencing libraries	V16000128
ForenSeq MainstAY Kit SE (96)	Includes all the required reagents for 96 reactions to prepare sequencing libraries including the same markers as in ForenSeq MainstAY Kit + SE33	V16000183
ForenSeq DNA Signature Prep Kit (96)	Includes all the required reagents for 96 reactions to prepare sequencing libraries	V16000023
ForenSeq DNA Signature Prep Kit (384)	Includes all primary reagents necessary for 384 reactions to prepare sequencing libraries	15066151

* Not available in all regions

Learn more about applying NGS to NIPPT. Visit **qiagen.com/HIDandNGS**

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Technical Support **www.support.qiagen.com**
Website **www.qiagen.com**