

# Increased DNA Quantification Sensitivity: Evaluation of Investigator® Quantiplex® Pro FLX

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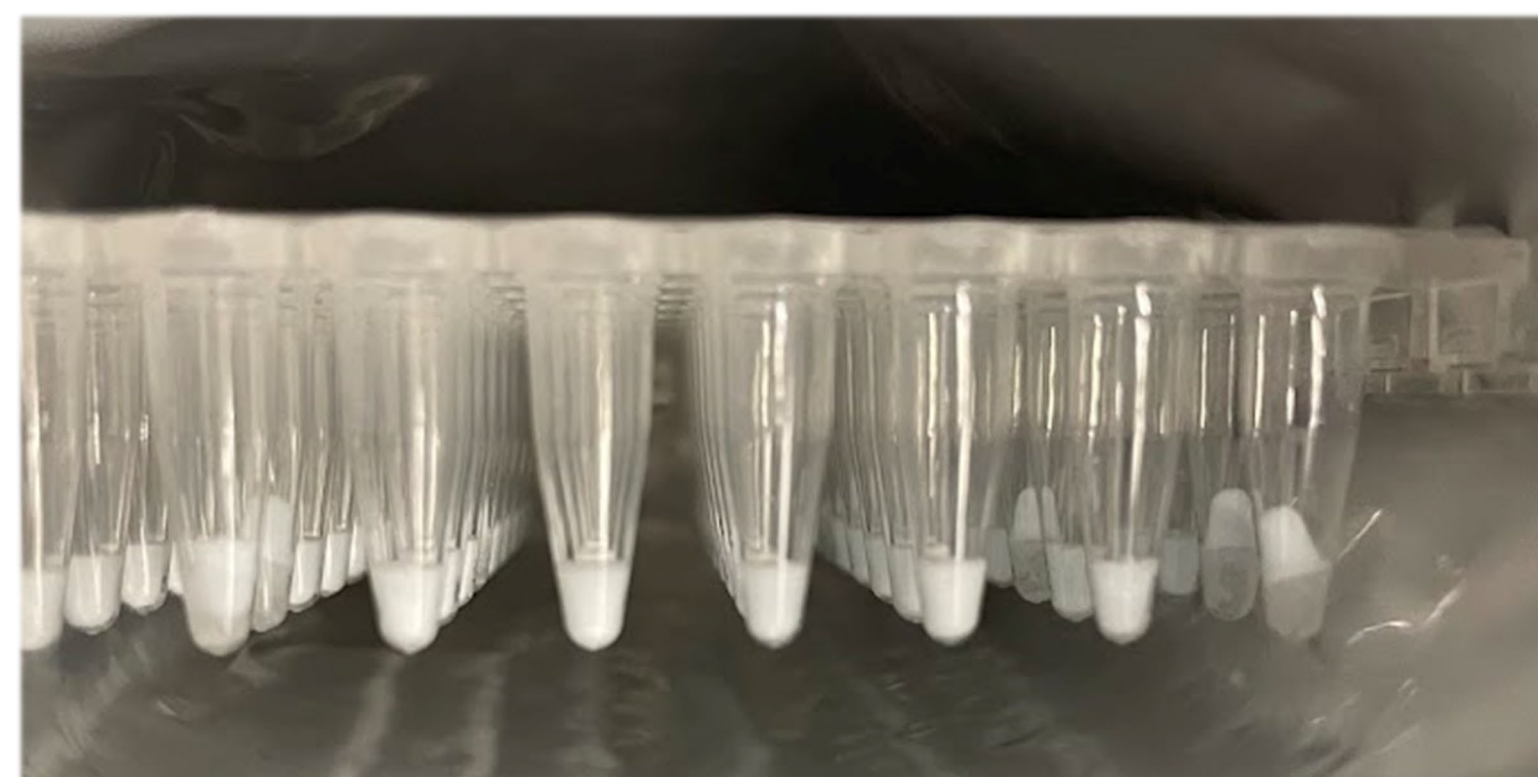
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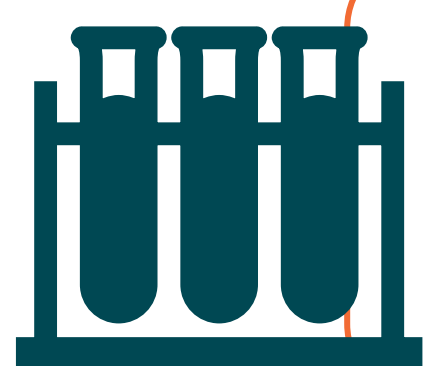
## INTRODUCTION

Accurate DNA quantification is essential for successful DNA profiling and forensic evidence analysis. Advancements in quantification chemistries have enabled routine detection of degradation, mixtures, and inhibition with minimal sample input, typically requiring only 2 µL<sup>1</sup>. However, as forensic science increasingly addresses complex challenges, such as low-template DNA and extreme female-to-male mixtures, the demand for greater sensitivity in quantification methods has grown. The Investigator® Quantiplex® Pro FLX kit introduces a lyophilized chemistry (Figure 1) that expands the sample input range from 2 µL to 18 µL, offering enhanced sensitivity and flexibility<sup>2</sup>. This study explores the kit's ability to detect DNA at extremely low concentrations, handle high female-to-male DNA ratios, and assess its compatibility with Y-screening workflows. These features aim to address the unique challenges posed by forensic samples, particularly in sexual assault evidence processing.



**Figure 1.** Lyophilized master mix in the Investigator® Quantiplex® Pro FLX kit are pre-aliquoted into PCR plate.

## MATERIALS & METHODS

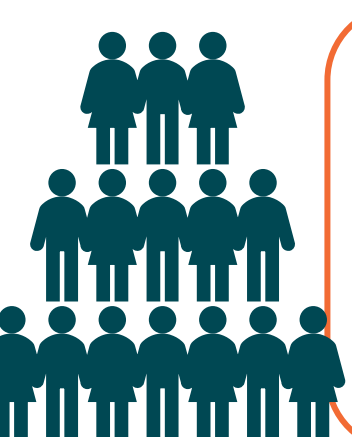


**Sensitivity**  
Control DNA M1



**DNA Mixtures (Male:Female)**

- 1:10,000
- 1:400,000
- 1:1,000,000



**Y-Screening Lysates with Investigator® Casework GO!**  
1/8 Swab Cuttings from Vaginal Swabs Spiked with Semen

## RESULTS & DISCUSSION

Expected Concentration	Human Target Concentration (pg/µL)	
	2 µL	18 µL
1000	1084	1175
500	525	636
100	128	130
50	70	66
10	13.4	13.9
5	8.3	6.4
1	1.4	1.2
0.5	0.6	0.5
0.25	0.36	0.28
0.125	0.310	0.087
0.0625	0.1803	0.0701

**Table 1. Sensitivity of Investigator Quantiplex Pro FLX.** Mean DNA concentrations (N = 3) detected from both sample input volumes.

- Higher sample input (18 µL) improved detection accuracy of human target at low concentrations of 5 pg/µL and lower.
- In the most extreme male-to-female mixtures, male DNA was detected in all six replicates using 18 µL sample input, while one replicate failed detection with 2 µL input.
- Increased sample input volume enhanced sensitivity in Y-screening, demonstrating that 2 µL of screening lysate may be insufficient for consistent male DNA detection, particularly in samples with low amounts of male DNA.

### Male Target Detection in DNA Mixtures

Male:Female Mixture	Sample Input	
	2 µL	18 µL
1:10,000	Green	Green
1:400,000	Green	Green
1:1,000,000	Orange (X)	Green

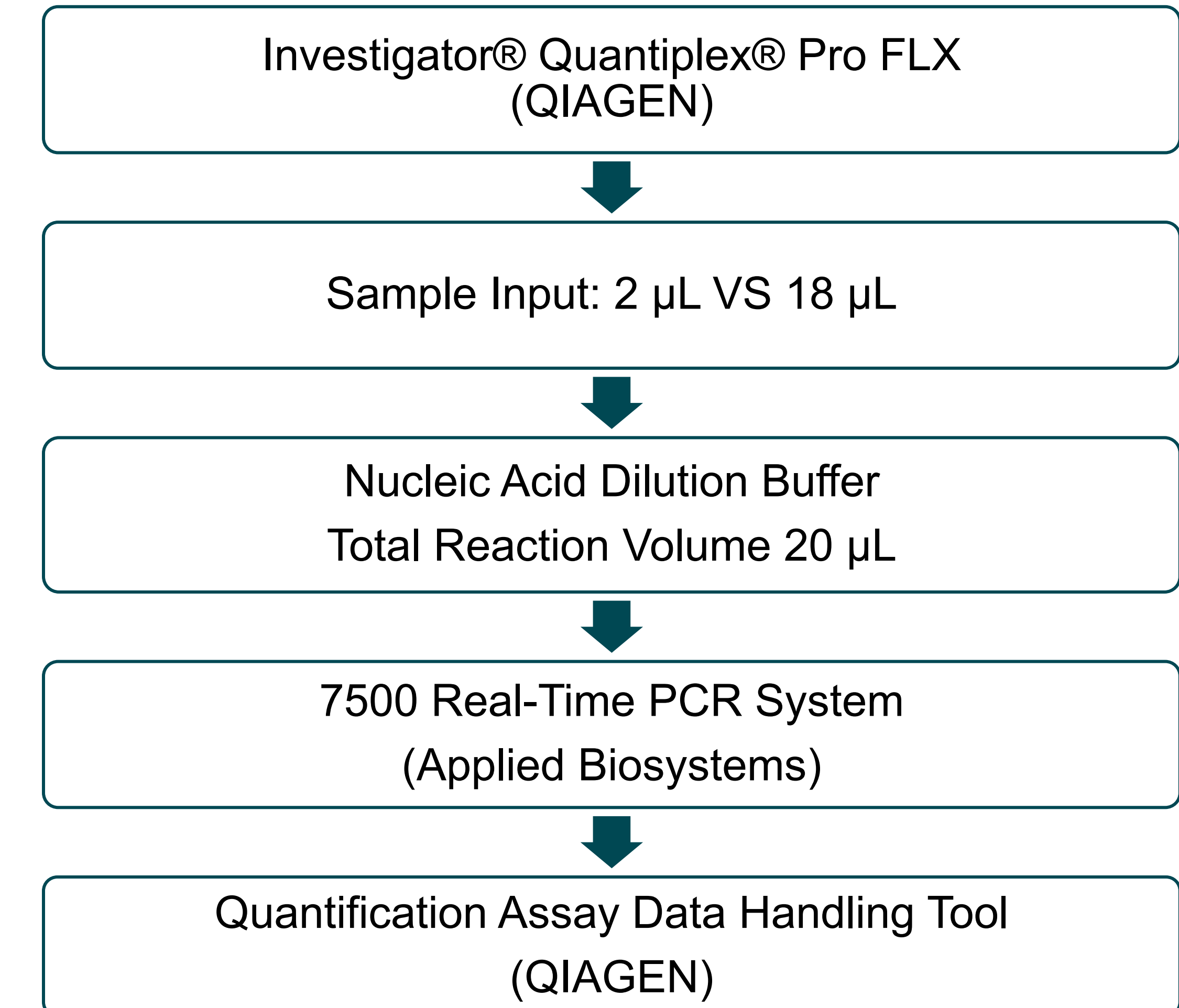
**Table 2. Sensitivity of Male DNA Detection in DNA Mixtures.** Successful detection of male DNA is highlighted in green; failed detection is shown in orange. Each sample input volume was tested in replicate: 1:10,000 (N = 2), 1:400,000 (N = 4), 1:1,000,000 (N = 6).

### Male Target Detection in Vaginal Swabs with Semen Spike

Expected Male DNA (ng)	Sample Input	
	2 µL	18 µL
500	Green	Green
100	Green	Green
20	Green	Green
4	Green	Green
0.8	Green	Green
0.16	Green	Green
0.032	Green	Green
0.0064	Orange (X)	Green

**Table 3. Sensitivity of Male DNA Detection in Y-Screening.** Successful detection of male DNA is highlighted in green; failed detection is shown in orange. Each sample input volume was tested in triplicate (N = 3).

## MATERIALS & METHODS



## CONCLUSIONS

- Reduced assay preparation time compared to non-lyophilized DNA quantification kits that require master mix preparation and aliquoting.
- Increased (18 µL) sample input increased accuracy of detection at extreme low concentrations (Table 1).
- Increased (18 µL) sample input improved precision of male DNA detection in both mock DNA mixtures and vaginal swabs spiked with semen (Tables 2 & 3).

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Lee, S.B., McCord, B. and Buel, E. (2014), Advances in forensic DNA quantification: A review. ELECTROPHORESIS, 35: 3044-3052. <https://doi.org/10.1002/elps.201400187>
2. Developmental validation of the Investigator® Quantiplex® Pro FLX. (2024). QIAGEN.

