QIAxcel vs. qPCR: Evaluating Versatility for Forensic DNA Screening

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INTRODUCTION

DNA analysis is vital in forensic investigations, yet traditional methods like STR analysis often encounter challenges with degraded samples, resulting in incomplete genetic profiles. Alternative genotyping methods, such as SNPs, mini-STRs, and mtDNA typing, target shorter DNA regions, making them more effective for degraded samples. However, a robust screening tool is essential to determine whether a sample is a good candidate for alternative methods.

Environmental factors, including UV exposure, heat, and enzymatic activity, contribute to DNA degradation, leading to allele dropout (1). While real-time qPCR remains the gold standard for measuring DNA quality through metrics like the degradation index (DI), its limitations—including variability across kits—make it insufficient as a standalone tool for predicting a sample's suitability for alternate genotyping methods (2).

This study evaluates the QIAxcel Connect, paired with the QIAxcel DNA High Sensitivity kit, as a complementary screening tool for assessing degraded DNA. The evaluation includes artificially degraded control DNA, burned skeletal remains, and chemically treated cadavers. The QIAxcel system provides assessments of DNA concentrations and degradation levels. By integrating this system into forensic workflows, we aim to enhance the evaluation of degraded samples, enabling more informed decisions about genotyping approaches and improving efficiency in challenging sample processing.

MATERIALS & METHODS

Sensitivity Study

CEPH Individual 1347-02 control DNA was used to create an 8-point dilution series ranging from 5 ng/µL to 0.5 pg/µL. Five replicates were prepared for each concentration.

Artificially Degraded Samples

Artificially degraded DNA samples were prepared using CEPH Individual 1347-02 control DNA at a concentration of 5 ng/µL in 60 µL per replicate. Twenty replicates were divided into four time points (n=5 per time point): 0 minutes, 20 minutes, 40 minutes, and 60 minutes of incubation at 95°C.

Thermally Degraded Skeletal Remains

In a previous study, femurs from two donors at the Southeast Texas Applied Forensic Science Facility (STAFS) were selected. For unburned controls, one window cut was collected from each femur. The femurs were then sectioned along the diaphysis and burned to the desired color **(Fig. 1)**. After burning, the cross-sections were washed, chipped, and powdered. Powdered samples (250 mg per replicate, n=5) were lysed and purified using the EZ2 Connect Fx Extra Large-Volume Protocol.

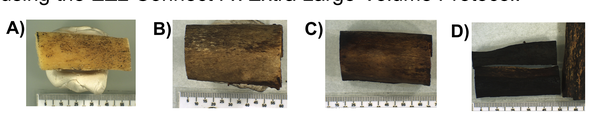


Figure 1. Representative Photos of Femur Cross-sections Thermally Degraded to Different Stages Based on Bone Color A) Unburned control; **B)** Light Brown; **C)** Brown; and **D)** Black color stages.

RESULTS & DISCUSSION

Sensitivity Study

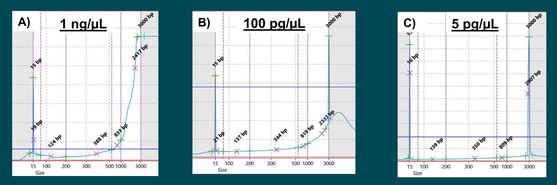


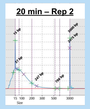
Figure 3. Representative electropherograms from QIAxcel at various levels of DNA input: A) 1 ng/µL; **B) 100** pg/µL; **C) 5** pg/µL.

- Oversaturation of the QIAxcel started to occur at 1 ng/µL and would require dilution and re-injection **(Fig. 3A).**
- QIAxcel was sensitive down to 5 pg/µL as marketed **(Fig. 3C).**

Artificially Degraded Samples

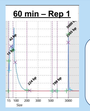
Table 1. Comparison of Average STR Recovery Across Artificially Degraded Samples Using Quantiplex Pro DI and QIAxcel DI Metrics. Green represents DIs below 10 and STR recovery above 70%, while red denotes DIs above 10 and STR recovery below 70%.

Sample	Average Human Quant (ng/µL)	Quantiplex Pro Average DI	QIAxcel Average DI (S-M)	Average % STR Allele Recovery
0 Min Average	3.32	1.27	0.42	100
20 Min Average	1.41	44.28	5.22	87.73
40 Min Average	0.60	2637.59	11.13	65.00
60 Min Average	0.19	Undetermined	131.29	46.36



Human Quant: 1.29 ng/µL
Quantiplex Pro DI: 71.80
QIAxcel DI (S-M): 5.41
% STR Allele Recovery: 77.27%

Figure 4. QIAxcel electropherogram of a 20-minute degraded sample, showing STR allele recovery and DI metrics from Quantiplex Pro DI and QIAxcel DI.



Human Quant: 0.21 ng/µL
Quantiplex Pro DI: Und.
QIAxcel DI (S-M): 79.66
% STR Allele Recovery: 50%

Figure 5. QIAxcel electropherogram of a 60-minute degraded sample, showing STR allele recovery and DI metrics from Quantiplex Pro DI and QIAxcel DI.

Burned and Chemically Damaged Remains

Table 2. Comparison of Average STR Recovery Across Burned Skeletal Samples Using Quantiplex Pro DI and QIAxcel DI Metrics. Green represents DIs below 10 and STR recovery above 70%, while red denotes DIs above 10 and STR recovery below 70%.

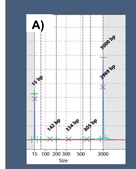
Sample	Average Human Quant (ng/µL)	Quantiplex Pro Average DI	QIAxcel Average DI (XS-S)	Average % STR Allele Recovery
Cadaver 1, Unburned	0.041	2.60	2.75	100
Cadaver 1, Light Brown	0.0071	8.56	1.61	99.56
Cadaver 1, Dark Brown	0.0011	15.05	2.08	72.44
Cadaver 1, Black	0.0002	27.73	2.05	4.89
Cadaver 2, Unburned	0.030	4.43	2.27	100
Cadaver 2, Light Brown	0.0041	6.81	4.04	95.11
Cadaver 2, Dark Brown	0.0021	11.95	0.87	67.11
Cadaver 2, Black	0.00046	16.96	39.84	0

- Human DNA Quant from Quantiplex Pro correlated most strongly with STR Allele Recovery **(Table 2)**.
- QIAxcel DI (for extra small to small DNA fragments) did not predict STR success, whereas Quantiplex Pro DI did **(Table 2)**.

Table 3. Comparison of STR Recovery Across Chemically Treated Remains Using Quantiplex Pro DI and QIAxcel DI Metrics. Green represents DIs below 10 and STR recovery above 70%, while red denotes DIs above 10 and STR recovery below 70%.

Sample	Human Quant (ng/µL)	Quantiplex Pro DI	QIAxcel DI (S-L)	% STR Allele Recovery
Rid-X (Day 1) - Tooth	8.48	611.54	34.07	52.27
Rid-X (Day 3) - Tooth	0.66	24.67	21.39	77.27
Rid-X (Day 5) - Tooth	7.95	85.52	25.11	61.36
Rid-X (Day 7) - Tooth	0.23	34.15	14.07	90.91
Rid-X (Day 28) - Tooth	0.18	5.84	7.47	100
Rid-X (Day 1) - Ulna	326.41	9.26	13.85	75
Rid-X (Day 3) - Ulna	22.35	22.24	43.03	68.18
Rid-X (Day 5) - Ulna	11.09	13.16	35.21	77.27
Rid-X (Day 7) - Ulna	4.54	15.64	52.39	90.91
Rid-X (Day 28) - Ulna	1.85	6.29	7.39	95.45
Hydrochloric Acid (Day 1) - Radius	95.68	17.39	18.52	84.09
Hydrochloric Acid (Day 3) - Forearm	70.65	8.91	14.62	93.18
Lye (Day 1) - Tissue	76.58	1.67	1.93	95.45
Lye (Day 3) - Tissue	11.74	5.63	3.44	72.73
Lye (Day 5) - Tissue	3.33	8.65	2.73	65.91
Sulfuric Acid (Day 1) - Tooth	3.62	3.30	6.52	100
Sulfuric Acid (Day 3) - Tooth	1.15	6.04	11.56	97.7
Sulfuric Acid (Day 5) - Tooth	0.23	25.52	16.12	95.5

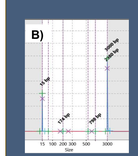
Utility of QIAxcel Screening for mtDNA Typing



Black Bone: Cadaver 1, replicate 1

Human Quant: 0.0001ng/µL
Quantiplex Pro DI: Und.
QIAxcel DI (XS-S): 1.96
% STR Allele Recovery: 8.89
mtDNA Results: Concordant T1a1 Haplogroup

mtDNA Coverage



Black Bone: Cadaver 2, replicate 2

Human Quant: 0.0003 ng/µL
Quantiplex Pro DI: 14.53
QIAxcel DI (XS-S): 46.85
% STR Allele Recovery: 0
mtDNA Results: Failed

mtDNA Coverage

Figure 6. Representative QIAxcel electropherograms and mtDNA results for (A) Cadaver 1, replicate 1 black bone, and (B) Cadaver 2, replicate 2 black bone, highlighting STR and mtDNA success in relation to DI metrics from Quantiplex Pro DI and QIAxcel DI.

- Chemically damaged samples showed variable and extreme DIs with both methods, but the QIAxcel DI better predicted STR success with Rid-X **(Table 3)**.
- The QIAxcel DI was a better predictor of success for alternative methods like mtDNA than DNA quantity (**Fig. 6**).
- The artificially degraded sample **(Table 1)** exhibited degradation patterns distinct from those of the burned and chemically damaged samples **(Tables 2 and 3)**.
- The QIAxcel allows custom distribution ranges to be set based on laboratory needs **(Table 4)**.

Table 4. Distribution ranges used for analysis with the QIAxcel High Sensitivity kit

Distribution Ranges	X-Small DNA	Small DNA	Medium DNA	Large DNA	X-Large DNA
	15 – 65 bp	65 – 200 bp	200 – 600 bp	600 – 1000 bp	1000 – 3000 bp

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MATERIALS & METHODS

Chemically Treated Remains

In a previous study, four cadavers at the STAFS facility were disarticulated (forearms and heads) and placed in HDPE buckets with 9–11 L of chemicals (3). Chemicals were purchased from local hardware stores and included Rid-X, Lye, Sulfuric Acid, and hydrochloric acid **(Fig. 2)**. Untreated samples (T=0) were collected before exposure, with sampling on days 1, 3, 5, 7, and 28 (day 21 for sulfuric acid). Bone and teeth were extracted using a modified Loreille et al. Total Demineralization method with Purification using MinElute® PCR Purification (QIAGEN). Tissue, fingernails, and hair were extracted using the EZ1&2® DNA Investigator® Kit (QIAGEN).

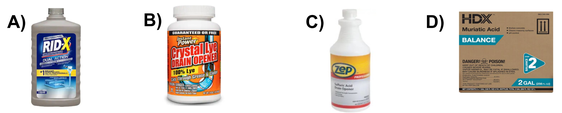

Figure 2. Commercial Products Used: A) Rid-X; **B)** Instant Power Crystal Lye Drain Opener; **C)** ZEP Sulfuric Acid Drain Opener; and **D)** HDX Muriatic Acid (Hydrochloric acid).

DNA Quantitation

Artificially degraded control DNA and degraded DNA extracts were quantified using Investigator Quantiplex® Pro (QIAGEN). Degradation of extracts was assessed using the QIAGEN Quantification Assay Data Handling and STR Setup Tool v.4.3.1

QIAxcel Extract Screening

Neat or diluted extracts (6 µL) were analyzed using the QIAxcel Connect with the QIAxcel DNA High Sensitivity Kit (QIAGEN). A custom distribution analysis method of fragment sizes was developed to calculate multiple Degradation Indices **(Table 4)**.

STR Typing

Each extract was amplified with the Investigator 24plex QS Kit (QIAGEN). Post-PCR products were separated and detected on an ABI 3500 (Thermo Fisher Scientific). Samples analyzed using Genemapper ID-X v1.6 (Thermo Fisher Scientific).

mtDNA Typing

A subset of burned bone samples were processed using the ForenSeq mtDNA Whole Genome Kit (QIAGEN) as per manufacturer's guidelines and sequenced on a MiSeq FGx® (QIAGEN) using a MiSeq FGx Reagent Kit (QIAGEN).

CONCLUSIONS

- QIAxcel DIs were generally less extreme than those from Quantiplex Pro.
- Future work will examine QIAxcel DI trends across different distribution ranges in relation to STR and mtDNA typing success.
- Future work will incorporate environmentally degraded samples.

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