Stability Testing of Casework Direct Lysate Following Long-Term Storage and Freeze-Thaw Cycles

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Casework Direct System (Cat.# DC4560, DC4561)

DNA present in both mock SAK and touch CWD lysates is stable for 30 months when stored at 4°C. Touch DNA samples started to show signs of degradation at 28°C. It is likely that the nature of the sample itself can affect the stability of DNA in the resulting lysate.

Introduction

The Casework Direct Kit, Custom (now available as the Casework Direct System) is used for rapid initial processing of swabs from casework samples, cuttings of sexual assault swabs or cuttings of stained fabric. Subsequent quantification of human DNA is performed using either the PowerQuant® System or Plexor® HY System and amplification of normalized template is performed with the PowerPlex® Systems for human short tandem repeat (STR) genotyping. For casework samples, no subsequent purification of the Casework Direct (CWD) lysate is required prior to STR amplification, unless data from the quantification indicate the presence of possible PCR inhibitors.

Since there is no purification process involved and the sample is used as a crude lysate, we performed experiments to determine if the DNA in the CWD lysate would be stable upon storage. This white paper presents our results from long-term storage at 4°C and 28°C, as well as freeze-thaw studies. We included representatives of the most challenging sample types, such as Y-screening samples and touch DNA samples. Depending on sample types typically encountered, we recommend labs conduct their own internal validation studies to determine the optimal stability and storage of CWD lysates.

Materials and Methods

Long-Term Storage at 4°C and 28°C

We used a representative touch sample (swab from a car steering wheel) and a mock sexual assault kit (SAK) sample (semen spotted on vaginal swab) for this study. CWD lysate was prepared from the two samples following the recommended protocol using a 400µl lysis volume. An aliquot of the lysate was tested immediately, and the remainder of the lysate was stored at 4°C and 28°C for testing at later time points (6, 18 and 30 months).

For human DNA quantification, we used 2µl of the lysate in quadruplicate with the PowerQuant® System. The normalized volume was amplified in quadruplicate using the PowerPlex® Fusion System on the GeneAmp® PCR System 9700 Thermal Cycler. We used cycling profiles recommended in the *PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* #TMD039, and calculated average peak heights and peak height ratios.

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Freeze-Thaw Cycles

For mock SAK samples, we collected buccal cells on 15 cotton swabs from female individuals. Semen samples were diluted tenfold in Nuclease-Free Water; 10µl of each dilution was spotted on the 15 cotton swabs and allowed to dry overnight. For touch samples, 15 swabs were moistened with Nuclease-Free Water and used to swab a computer, monitor and docking station, then allowed to dry overnight.

We pooled CWD lysates from the mock SAK samples, vortexed and re-aliquoted them into 12 × 200µl samples in 1.5ml microcentrifuge tubes. Three tubes were placed at 4°C as controls. Three tubes were frozen at –20°C one time, then thawed immediately prior to quantitation and kept at 4°C. Three tubes went through five freeze/thaw (FT) cycles where the tubes were kept frozen or at room temperature for at least 1 hour for each cycle. The final three tubes went through 10 FT cycles. We performed the same procedure for the CWD lysates from the touch samples.

Following the FT cycling, we quantitated DNA using the PowerQuant® System. A 2µl aliquot of each sample was analyzed in duplicate using the Applied Biosystems® 7500 Real-Time PCR System. PowerPlex® Fusion and Y23 Systems require 0.5ng of input DNA, and a maximum of 15µl of CWD lysate can be added to the amplification reactions. We calculated average peak heights and peak height ratios.

Data Analysis

All data were analyzed initially on the 7500 Software ver. 2.3. The results were imported into the PowerQuant® Analysis Tool ver. 1.0.0.0 for further calculations and evaluation. The following quality flags were monitored throughout the studies: 1) the ratio of autosomal DNA target concentration to that of the target for assessing DNA degradation ([Auto]/[Deg]); 2) the ratio of autosomal DNA target concentration to that of the Y-chromosomal target ([Auto]/[Y]); and 3) the Internal PCR Control (IPC) Cq. These quality flags provide information about the sample and probable STR profile quality. A threshold of 2 was used for the [Auto]/[Deg] and [Auto]/[Y] ratios to flag a sample as possibly degraded or as a potential mixture, respectively. A shift of 0.3 for the IPC Cq was used to flag a sample for possible inhibition.

Table 1. Average DNA quantification values of mock SAK and touch samples stored for 0, 6, 18 and 30 months at 4°C and 28°C. Orange values indicate threshold flags.

Sample Name	[Auto]	[Deg]	[Y]	IPC Cq	IPC Shift	IPC Threshold	[Auto]/[Y]	[Auto]/[Y] Threshold	[Auto]/[Deg]	[Auto]/[Deg] Threshold
Mock SAK 0	24.5536	14.4627	0.6175	20.59	–0.05	Below	39.76	At or Above	1.7	Below
Touch DNA 0	0.1315	0.0317	0.065	20.47	0.17	Below	2.02	At or Above	4.15	At or Above
Mock SAK 6 Mnths 28C	23.3159	12.3094	0.641	20.31	–0.16	Below	36.38	At or Above	1.89	Below
Mock SAK 6 Mnths 4C	22.9624	14.2677	0.6626	20.36	–0.11	Below	34.65	At or Above	1.61	Below
Touch DNA 6 Mnths 28C	0.1187	0.0309	0.0631	20.32	–0.21	Below	1.88	Below	3.84	At or Above
Touch DNA 6 Mnths 4C	0.1224	0.0394	0.0736	20.43	–0.09	Below	1.66	Below	3.11	At or Above
Mock SAK 18 Mnths 28C	28.6722	14.2199	0.8884	20.18	–0.34	Below	32.27	At or Above	2.02	At or Above
Mock SAK 18 Mnths 4C	24.9329	14.3858	0.8318	20.26	–0.14	Below	29.98	At or Above	1.73	Below
Touch DNA 18 Mnths 28C	0.1115	0.0232	0.0678	20.23	–0.13	Below	1.64	Below	4.8	At or Above
Touch DNA 18 Mnths 4C	0.1365	0.0473	0.0966	20.26	–0.11	Below	1.41	Below	2.89	At or Above
Mock SAK 30 Mnths 28C	22.8146	11.2893	0.6552	20.39	–0.26	Below	34.82	At or Above	2.02	At or Above
Mock SAK 30 Mnths 4C	18.6877	11.2972	0.6044	20.38	–0.27	Below	30.92	At or Above	1.65	Below
Touch DNA 30 Mnths 28C	0.0753	0.0127	0.0389	20.45	–0.21	Below	1.94	Below	5.94	At or Above
Touch DNA 30 Mnths 4C	0.1093	0.037	0.0672	20.53	–0.12	Below	1.63	Below	2.95	At or Above

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Results

Quantification Using PowerQuant® System

Table 1 shows the results of the DNA analyzed in the long-term storage study using the PowerQuant® System. We observed that extracts prepared from SAK and touch samples were stable at 4°C for 30 months. Also, the extracts prepared from SAK samples were stable up to 30 months when stored at 28°C. While the quantification values for all the three targets did not change significantly for the 6- and 18-month time points, we observed a progressive decrease in [Auto] and [Deg] values when touch samples were stored at 28°C. Interestingly, we observed no significant change in [Auto] and [Deg] values when SAK samples were stored at 28°C. The [Auto]/[Deg] ratio trended higher for touch samples stored at 28°C (as compared to time zero; Figure 1). We are testing further time points to see if this trend continues. It is important to note that the stability of DNA in CWD extracts is affected by the sample type, as shown by the difference in stability at 28°C of SAK vs. touch samples. This difference should be taken into consideration when considering long-term storage at ambient temperature.

Figure 2 shows the DNA quantitation results for samples subjected to FT, analyzed using the PowerQuant® System.

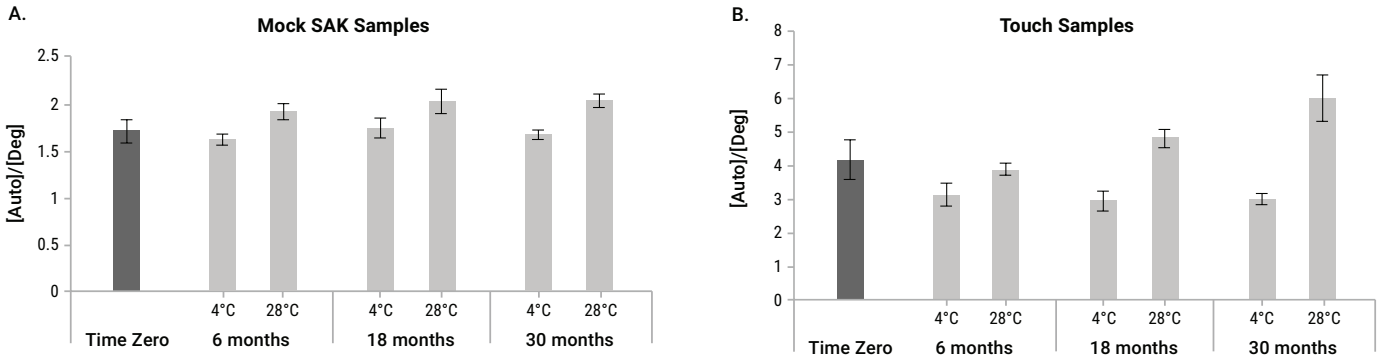

Figure 1. Effects of long-term storage on DNA quantification with the PowerQuant® System. Average [Auto]/[Deg] values are shown for CWD lysates from mock SAK (Panel A) and touch samples (Panel B) stored for 6, 18 and 30 months at 4°C and 28°C.

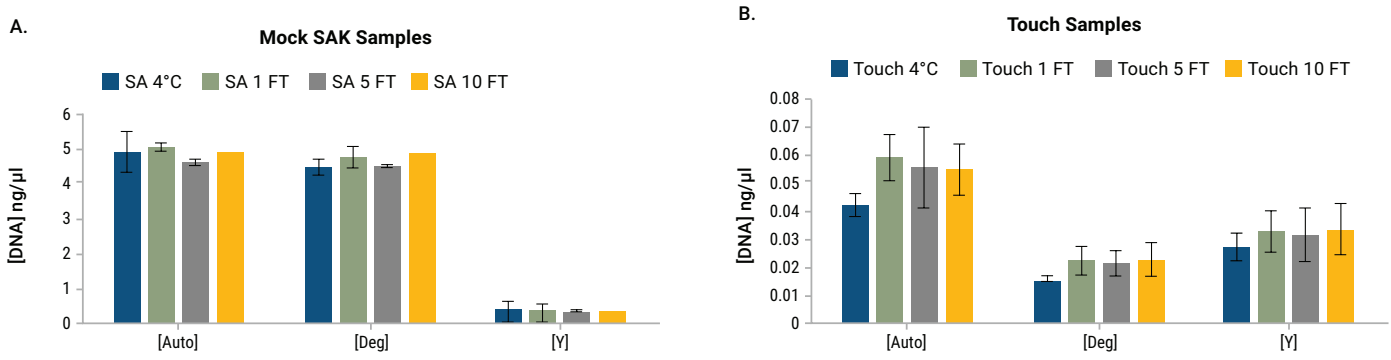

Figure 2. Effects of freeze-thaw cycles on DNA quantification with the PowerQuant® System. DNA concentrations (average ± 1SD) are shown for CWD lysates from mock SAK (Panel A) and touch samples (Panel B) following 0, 1, 5 and 10 freeze-thaw (FT) cycles.

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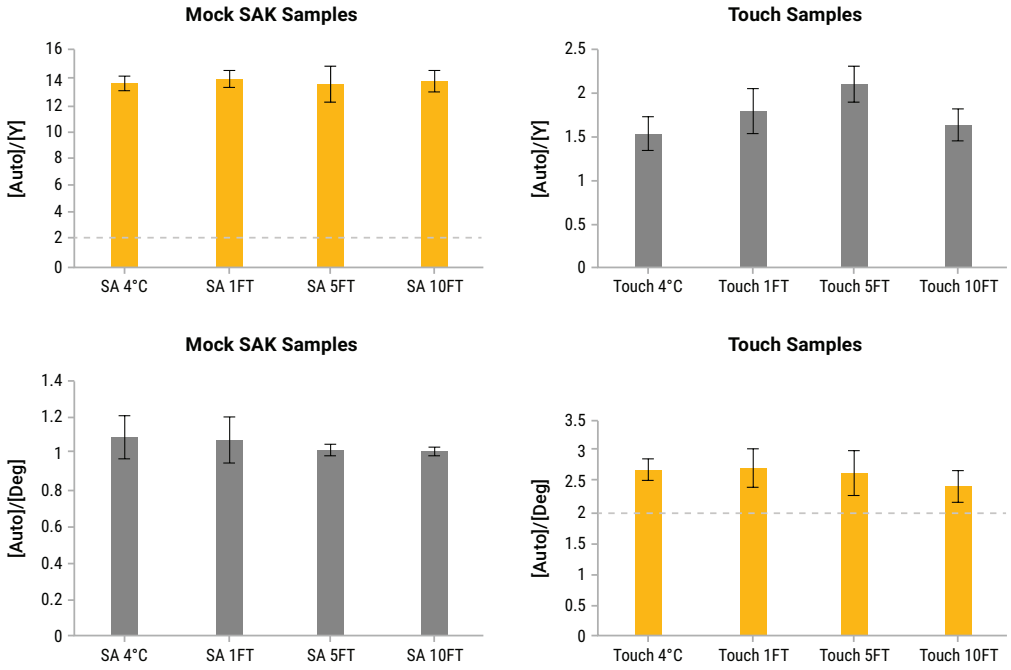

Figure 3. Effects of freeze/thaw cycles on PowerQuant® System [Auto]/[Y] and [Auto]/[Deg] ratios. Values (average ± 1SD) are shown for CWD lysates from mock SAK and touch samples with increasing freeze/thaw (FT) cycles. Yellow bars indicate samples with quality flags. Dotted lines indicate threshold values for quality flags.

No significant effect on quantification results was observed with any of the three PowerQuant® targets as a function of FT cycle with mock SAK or touch samples. Also, no significant effect of FT cycles was detected for [Auto]/[Y] or [Auto]/[Deg] ratios for both mock SAK and touch samples (Figure 3). Taken together, these data suggest that up to 10 FT cycles have little to no effect on the DNA quality of samples extracted with CWD.

STR Analysis of Stored CWD Samples

Long-term storage at 4°C and 28°C

For the touch samples, there is no obvious difference in peak height or locus-to-locus balance between the data generated from time zero and from lysate stored at 4°C for 30 months (Figure 4). There does not appear to be any loss of DNA in the Mock SAK extracts after storage at 28°C for 30 months whereas, for the touch DNA sample, loss of signal for the larger amplicons is noticeable under these conditions. This result correlates with the slightly higher [Auto]/[Deg] ratio observed with the touch sample lysates stored at 28°C as compared to 4°C (5.94 vs. 2.95).

When SAK and touch samples were amplified using the PowerPlex® Fusion System, minimal differences in peak height ratio for heterozygous loci were observed in the corresponding samples stored at 4°C and 28°C (Figure 5). Some variability in peak heights is to be expected among data analyzed many months apart, even if the samples were analyzed on the same instrument.

Freeze-thaw cycles

As seen in Figure 6, there was no observed difference in peak height between the data generated from the 4°C control sample or after 1, 5 and 10 FT cycles of the mock SAK or touch sample lysates. Upon STR amplification, samples subjected to 1, 5 or 10 FT cycles did not show any change in peak height ratio of heterozygous loci when compared to data from lysate stored at 4°C (Figure 7).

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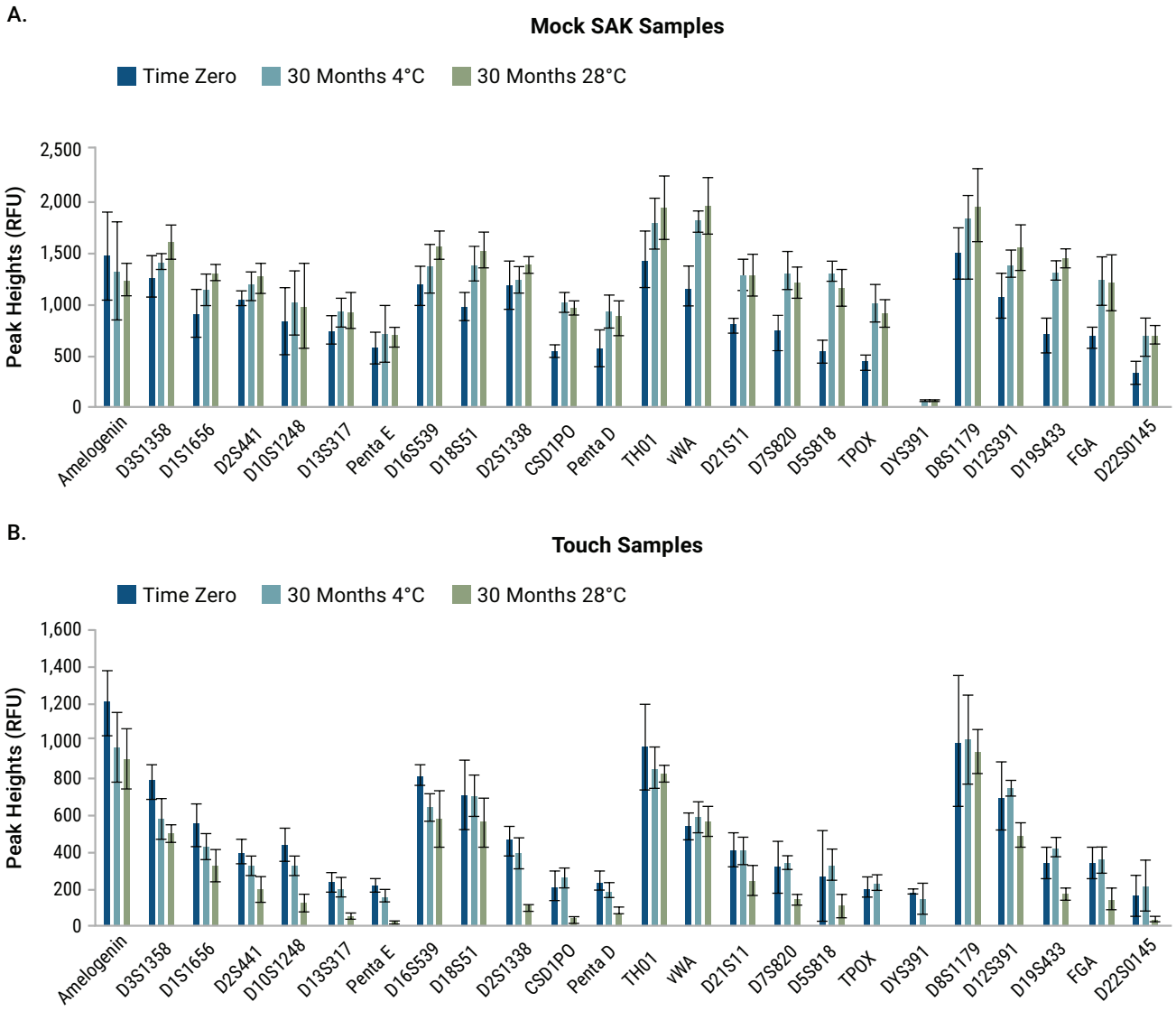

Figure 4. Effects of long-term storage on STR amplification with the PowerPlex® Fusion System (peak heights). Peak heights (average ± 1SD) are shown for PowerPlex® Fusion System amplification reactions generated from mock SAK and touch sample CWD lysates following long-term storage at 4°C and 28°C. RFU, relative fluorescence units.

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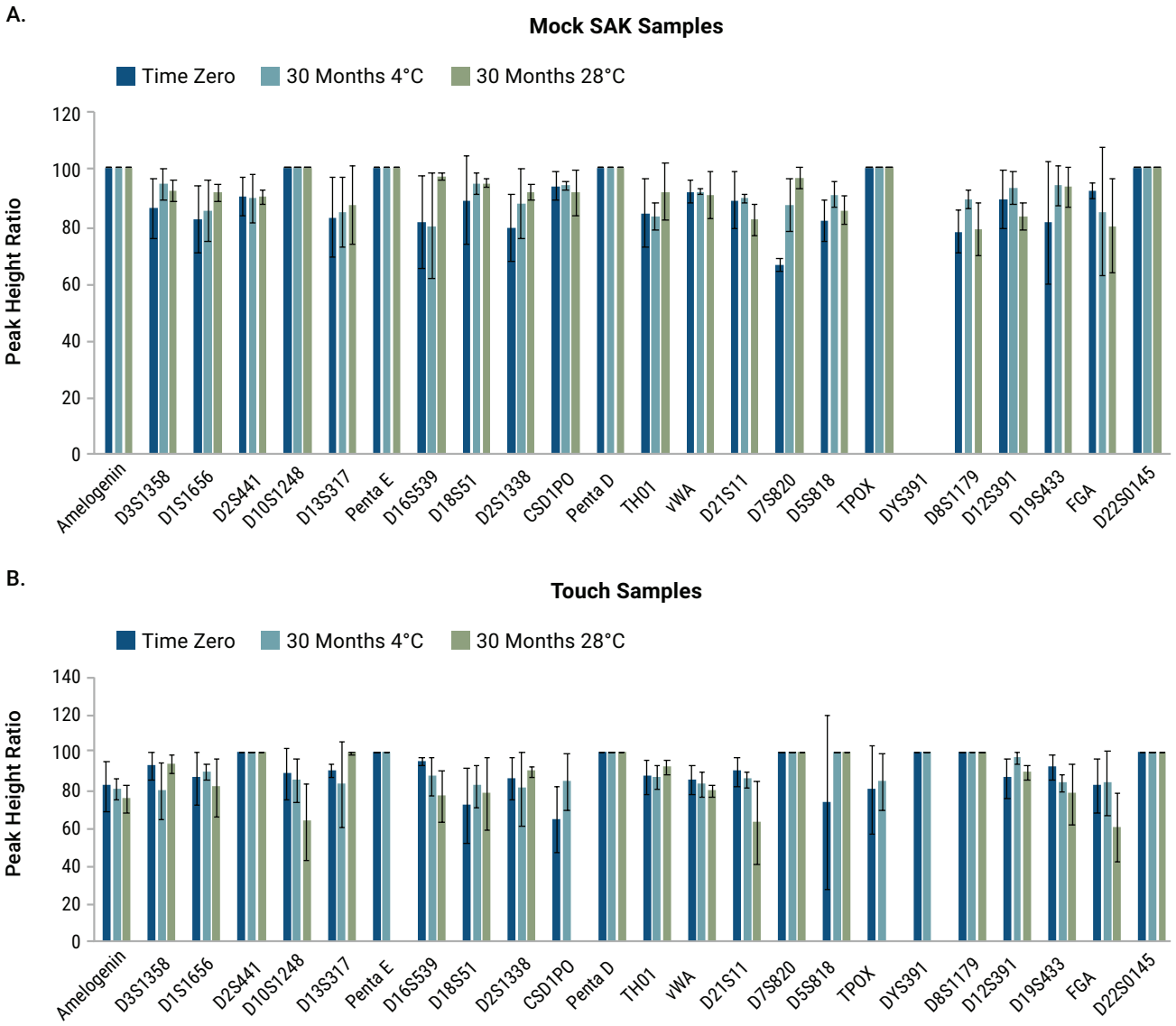

Figure 5. Effects of long-term storage on STR amplification with the PowerPlex® Fusion System (peak height ratios). Peak height ratios (average ± 1SD) are shown for PowerPlex® Fusion System amplification reactions from mock SAK and touch sample CWD lysates following 30-month storage at 4°C and 28°C.

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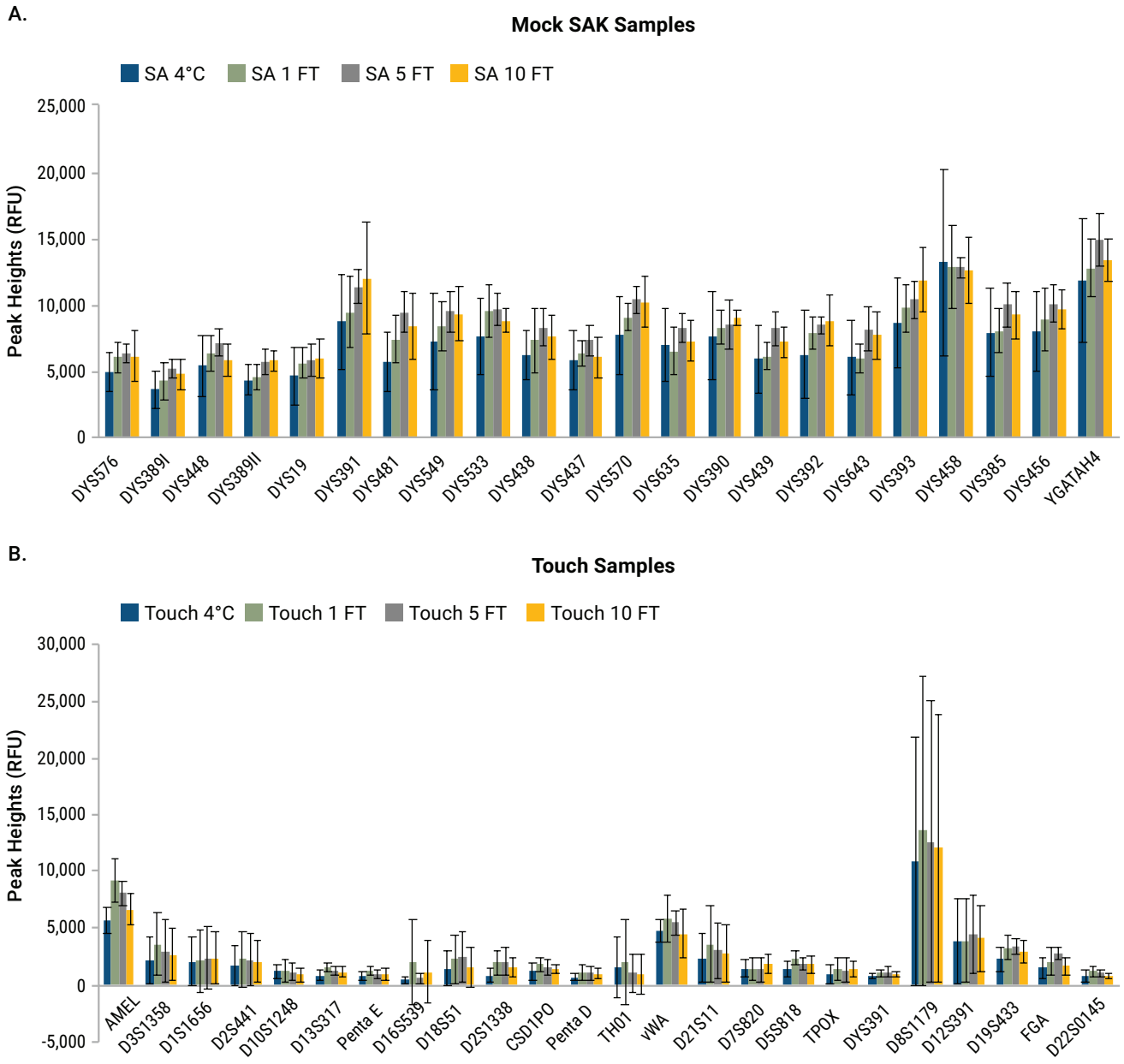

Figure 6. Effects of freeze-thaw cycles on on STR amplification with the PowerPlex® Y23 and PowerPlex® Fusion Systems (peak heights). Peak heights (average ± 1SD) are shown for PowerPlex® Y23 and PowerPlex® Fusion System amplification reactions generated from mock SAK and touch sample CWD lysates following multiple freeze-thaw (FT) cycles. RFU, relative fluorescence units.

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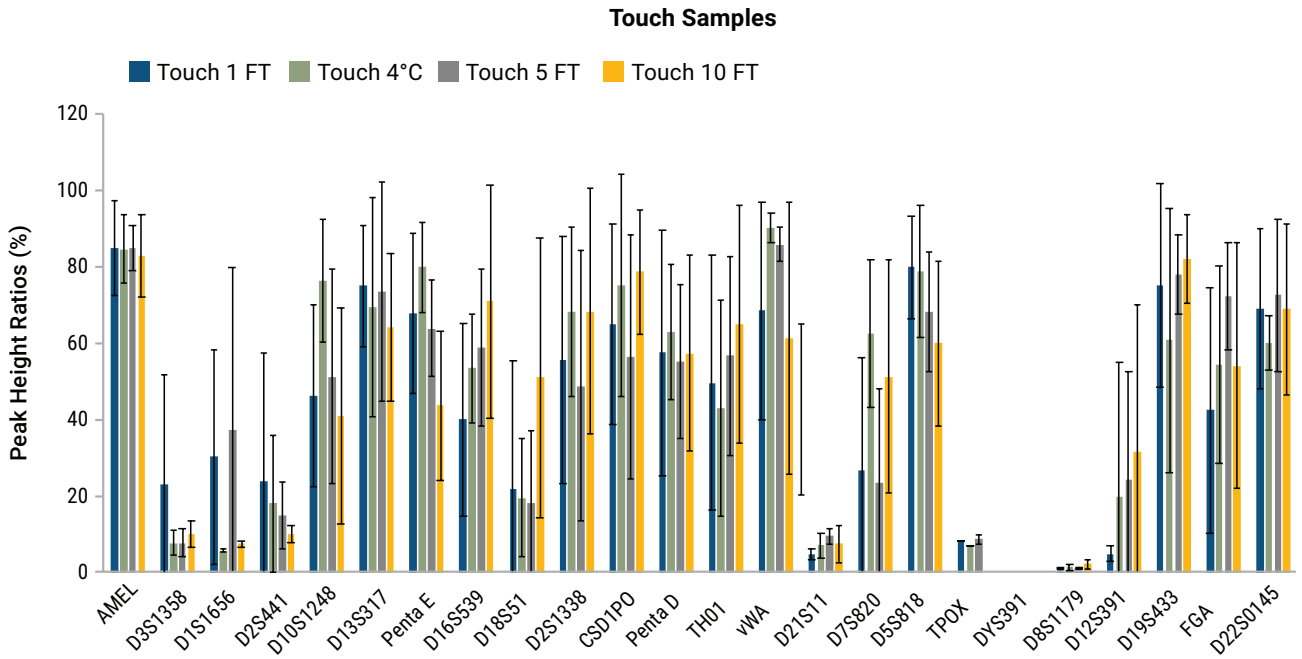

Figure 7. Effects of freeze-thaw cycles on on STR amplification with the PowerPlex® Fusion System (peak height ratios). Peak height ratios (average ± 1SD) are shown for PowerPlex® Fusion System amplification reactions generated from generated from touch sample CWD lysates following multiple freeze-thaw (FT) cycles.

Conclusions

DNA present in both mock SAK and touch CWD lysates is stable for 30 months when stored at 4°C. However, we detected an apparent degradation of touch DNA samples stored at 28°C.

DNA present in both mock SAK and touch CWD lysates can withstand up to 10 FT cycles with no change in quantification values.

No increase in [Auto]/[Deg] values was observed for mock SAK and touch samples with increasing FT cycles, suggesting that up to 10 FT cycles have little to no effect on the DNA quality of samples extracted with CWD.

There does not appear to be any difference in peak heights resulting from storage of the DNA from mock SAK or touch samples in CWD for up to 10 FT cycles or after storage at 4°C for 30 months. For samples stored at 28°C, we observed an increase in the [Auto]/[Deg] ratio of touch samples (but not mock SAK samples) with increased storage time. This increase in [Auto]/[Deg] ratio correlated with the decline in signal obtained from larger loci in STR amplification reactions from touch samples.

While mock SAK samples were stable at 28°C, touch DNA samples started to show signs of degradation at 28°C. These results indicate that, while DNA can be stable in CWD lysates at 28°C, it is likely that the nature of the sample itself can affect DNA stability in the resulting lysate at 28°C. At 4°C, the effects of the sample on DNA stability in the CWD extracts appears to be minimized.

This study shows that CWD lysates are stable at 4°C and are able to withstand up to 10 FT cycles. However, the stability of the lysate is dependent on the sample type. Internal stability studies are needed to determine the best storage conditions based on sample type.

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