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Comparison of DNA Quality Between Gray and White Matter in Postmortem Brain Tissue for Forensic Analysis

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Abstract

Postmortem brain tissue remains a valuable source of forensic DNA despite progressive decomposition. Because gray matter and white matter differ substantially in cellular composition and postmortem degradation patterns, these regional differences may influence DNA quantification and downstream genetic analyses.

This study compared DNA quality and quantity between gray and white matter from 50 autopsy cases, including 13 decomposed bodies, using gel electrophoresis, spectrophotometry, fluorometry, and quantitative PCR (qPCR). The effect of RNase treatment was evaluated in selected samples, followed by short tandem repeat (STR) analysis.

In spectrophotometric analysis of non-RNase treated samples, gray matter exhibited higher nucleic acid concentrations than white matter; however, this pattern was reversed in RNase-treated samples, indicating substantial RNA contamination in gray matter. Fluorometric analysis showed no regional difference before RNase treatment, whereas white matter exhibited higher dsDNA concentrations with RNA removal. In contrast, qPCR consistently demonstrated higher concentrations of amplifiable DNA in white matter regardless of RNase treatment. STR profiling remained highly successful, with higher peak heights in decomposed white matter and improved peak heights in RNase-treated gray matter.

These findings indicate that white matter preserves amplifiable DNA more effectively than gray matter and may represent a preferable target for forensic DNA analysis of decomposed brains. In addition, RNA removal and dsDNA-specific quantification can improve the accuracy of DNA assessment and STR profiling.

INTRODUCTION

The brain is anatomically protected by the skull, which acts as a barrier against environmental contaminants, microbial invasion, and physical damage, and contributes to better DNA preservation compared to more exposed tissues [1]. Notably, even in pulpified brains resulting from advanced decomposition, satisfactory histological results can often be obtained [2]. Several studies have confirmed its utility in forensic DNA typing even after prolonged postmortem intervals [3–6]. Previous studies have confirmed that decomposed soft tissues can yield DNA results comparable to those of fresh samples or hard tissues [7]. In particular, brain tissue has demonstrated DNA recovery rates equivalent to dental samples but with the advantage of significantly shorter processing times [8], suggesting its potential as a highly valuable resource in forensic analysis. Despite this potential, existing literature remains remarkably scare; to date, only eight studies have focused on decomposed soft tissues [9–16], most of which are constrained by small sample sizes or a restricted focus on nuclear DNA (nDNA). Furthermore, research involving larger cohorts remains even more limited, with only one study analyzing more than 30 individuals [11].

The brain represents a unique tissue with distinct structural regions—gray matter and white matter—each with different cellular constituents, which may lead to variation in DNA integrity. Gray matter, which is more cellular, contains neuron cell bodies, dendrites of efferent neurons, interneurons, astrocytes, and microglia. In contrast, white matter primarily consists of myelinated axons and contains large numbers of oligodendrocytes and other supportive glial cells [17–19]. These histological differences also translate into distinct postmortem degradation patterns. In gray matter, particularly in the cerebral cortex, structure is lost with neuronal features becoming indistinct and eosinophilic. White matter, on the other hand, tends to be relatively better preserved and more resistant to decomposition [2], which may have implications for DNA preservation in forensic contexts. This regional variation in preservation underscores the need to evaluate DNA quality separately in gray and white matter when designing forensic DNA workflows, particularly when working with degraded samples.

While numerous studies have investigated the cellular and structural differences between gray and white matter [11, 20, 21], relatively few have examined the quality of nucleic acids, particularly in terms of quantification differences between these distinct brain regions. A previous study has demonstrated that spectrophotometry measured higher DNA concentrations than fluorometry, and the results did not align with those obtained via qPCR. This discrepancy was also observed in degraded samples [22]. This finding highlights the need for a comparative evaluation of DNA quantification methods across different brain regions.

Given that the brain is the most metabolically active organ and exhibits relatively high preservation potential, it represents a valuable target for postmortem DNA analysis. Accordingly, there is a clear need to better understand DNA quality variation across brain regions. Therefore, this study aims to compare DNA quality and quantity between gray and white matter and to discuss the implications of these findings for the forensic utility of brain-derived DNA.

MATERIALS AND METHODS

1. Sample collection

Brain tissues were collected from a total of 50 autopsy cases conducted by the authors from May 2023 to July 2024. The cases included 38 males and 12 females, with a mean age of 59.6 years (range: 24–85 years). The sex distribution was unbalanced due to a limited number of autopsy requests involving female. According to the total body score (TBS) proposed by Moffatt et al [23], 37 cases with TBS 2 or less were classified as “fresh” and 13 cases with TBS 3 or more as “decomposition.” Decomposed bodies were included only when the gray and white matter could be morphologically distinguished, and their maximum TBS was 17. In seven cases in which brain integrity could have been affected due to head trauma or intracranial hemorrhage, brain tissues were carefully collected from unaffected regions. Gray and white matter were separated and finely minced, and then a total of 100 samples were stored in a deep freezer at -80 ℃ until DNA extraction. This study was approved by the Institutional Review Board of Sungkyunkwan University (SKKU 2021-06-001).

2. DNA extraction and quantification

1) DNA extraction

DNA was extracted using 50 mg of tissues from each sample with a silica membrane-based kit, GeneAll® Exgene™ Clinic SV mini kit (GeneAll Biotechnology, Seoul, Republic of Korea). The manufacturer's protocol was modified to improve both quality and quantity of DNA from brain tissue. Specifically, this involved optimizing buffer volumes, omitting the 70 ℃ incubation, meticulously separating supernatants, and extending centrifugation durations. To evaluate the effect of RNA contamination, 20 ㎕ of RNase was added to 13 randomly selected fresh cases and all 13 decomposed cases and incubated for 2 minutes at room temperature.

2) DNA quantification

Gel electrophoresis was performed using a 1% agarose gel prepared in 0.5X Tris-acetate-EDTA buffer. High-concentration samples were normalized to 20 ng/㎕ for fresh samples or 50 ng/㎕ for decomposed samples prior to loading to minimize the influence of DNA quantity on band intensity. They were then mixed with 6X LoadingSTAR dye (DyneBio, Seongnam, Republic of Korea) and loaded onto the gel, and electrophoresis was conducted at 200 V for 28–33 minutes. DNA integrity was assessed by visualizing band patterns using a Gel Doc™ XR+ System with Image Lab™ Software v3.0 (Bio-Rad Laboratories, Hercules, CA, USA) and comparing them with a Genesta™ 1 kb DNA Ladder (GeneAll Biotechnology).

Spectrophotometry was performed using the Nabi UV/Vis NANO Spectrophotometer (MicroDigital, Seongnam, Republic of Korea). Absorbance at 260 nm, along with the 260/280 and 260/230 ratios, was recorded to assess nucleic acid quantity and potential contamination. Samples were considered to be of acceptable quality when the A260/280 ratio ranged from 1.8 to 2.0 and the A260/230 ratio ranged from 2.0 to 2.2.

Fluorometry targeting double-stranded DNA (dsDNA) was performed using the Qubit™ Flex Fluorometer with the Qubit™ 1X dsDNA High Sensitivity Assay Kit (Invitrogen™, Waltham, MA, USA) according to the manufacturer's protocols.

Quantitative PCR (qPCR)-based quantification was performed using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. Reactions were run on the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (Thermo Fisher Scientific), including all samples, standards, and negative controls. Data were processed using QuantStudio™ Design & Analysis Software v1.3 (Thermo Fisher Scientific), and the degradation index (DI) was calculated as the ratio of the small autosomal target (SA) to large autosomal target (LA) concentrations.

For spectrophotometry, fluorometry, and qPCR, the means of duplicate measurements were used for analysis. A summary of each method is represented in Table 1.

Table 1
Comparison of DNA quality assessment methods.

Methods	Gel electrophoresis	Spectrophotometry	Fluorometry	qPCR
Principle	Size-based separation of DNA fragments	UV absorbance at 260 nm	Fluorescent dye specific to target	Real-time amplification of target
Target	All DNA	Total nucleic acids (dsDNA, ssDNA, RNA)	dsDNA	Sequence-specific dsDNA
Output data	Concentration Visual integrity	Concentration A260/280 ratio A260/230 ratio	Concentration	Concentration (LA, SA) Degradation (DI)

UV, ultraviolet; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; qPCR, quantitative PCR; LA, large autosomal target; SA, small autosomal target; DI, degradation index

3. STR analysis

STR genotyping was performed using the PowerPlex® Fusion System (Promega, Madison, WI, USA), which includes 22 autosomal loci (CSF1PO, FGA, TH01, TPOX, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, Penta E, Penta D) along with the sex-determining locus Amelogenin and the Y-chromosomal marker DYS391.

DNA inputs were adjusted to 0.5 ng/□ based on the LA concentration. PCR amplification was conducted using a VeritiPro™ Thermal Cycler (Thermo Fisher Scientific), and capillary electrophoresis was performed on the 3500 Genetic Analyzer (Thermo Fisher Scientific), following the manufacturer's guidelines. The electrophoresis data were analyzed using GeneMarker HID v3.2.0 (SoftGenetics LLC, State College, PA, USA). Allele detection was confirmed by peak heights exceeding 50 relative fluorescence units (RFU) and base number deviations within ± 0.5 bp of the corresponding allele in the ladder.

To evaluate the quality of STR results, peak height, peak height ratio (PHR), and stutter were analyzed for each locus. Loci were categorized into three groups by size: short (< 200 bp; AMEL, D3S1358, D1S1656, D16S539, D18S51, TH01, vWA, D8S1179, D12S391), medium (200–400 bp; D2S441, D10S1248, D13S317, D2S1338, CSF1PO, D21S11, D7S820, D5S818, D19S433, FGA), and long (> 400 bp; Penta E, Penta D, TPOX, DYS391, D22S1045). PHR was calculated in heterozygous loci as the ratio of the minor peak height to the major peak height. Peaks were considered stutter if they appeared at the expected stutter position (typically N-1, with additional positions N-0.5× for D1S1656 and N+× for D22S1045), exhibited an intensity greater than 50 RFU, and did not exceed the stutter threshold defined for each locus. Stutter peaks were quantified by calculating the ratio of stutter peak height to the height of the corresponding main allele.

Because two RNase-treated white matter samples showed abnormal peaks in the internal lane standard, these samples and their corresponding gray matter and untreated counterparts were excluded from analyses. The STR profiling success rate was calculated as the percentage of analyzable samples yielding a complete STR profiles at all targeted loci.

4. Data analysis

The normality of continuous variables was assessed using the Shapiro-Wilk test. The study focused on group-level differences according to tissue type, postmortem status, and RNase treatment rather than paired within-case comparisons. Therefore, all analyses were conducted using independent group comparisons. Continuous variables were compared using independent t-tests for normally distributed variables or Wilcoxon rank-sum tests for non-normally distributed variables. Spearman correlation analysis was conducted to evaluate associations among the quantification methods. All statistical analyses were performed using StataNow/SE v18.5 (StataCorp LLC, College Station, TX, USA) and Microsoft 365 Excel version 2406 Build 16.0.17726.20078 (Microsoft Corp., Redmond, WA, USA). Statistical significance was set at $p < 0.05$. Figures were generated using GraphPad Prism v10.4.1 (GraphPad Software, San Diego, CA, USA).

RESULTS

Spectrophotometric analysis revealed significant differences between gray matter and white matter, as well as marked changes in nucleic acid quantification and purity profiles following RNase treatment (Fig. 1). In non-treated samples, gray matter exhibited significantly higher nucleic acid concentration (Fig. 1A; $p < 0.001$), A260/280 ratios (Fig. 1C; $p < 0.001$), and A260/230 ratios (Fig. 1E; $p = 0.004$) than white matter. These differences remained significant across all variables in fresh samples ($p < 0.001$), whereas among decomposed samples, only the A260/280 ratio remained significantly higher in gray matter ($p = 0.005$). Within gray matter, fresh samples showed significantly higher nucleic acid concentrations and A260/280 ratios than decomposed samples ($p = 0.002$ and $p = 0.045$, respectively), while no significant differences between fresh and decomposed samples were observed in white matter for any variables.

In RNase-treated samples, these trends were reversed. Nucleic acid concentrations became significantly higher in white matter than in gray matter in both fresh and decomposed groups (Fig. 1B; $p = 0.002$ for total samples, $p = 0.003$ for fresh samples, and $p = 0.020$ for decomposed samples). In addition, the initial differences in A260/280 ratios (Fig. 1D) and A260/230 ratios (Fig. 1F) between gray and white matter largely disappeared and these ratios moved toward the acceptable range, primarily due to a relatively low A260 absorbance values in RNase-treated gray matter samples.

In gel electrophoresis, distinct patterns were observed according to tissue types and RNase treatment (Fig. 2). After concentration normalization based on spectrophotometric measurements, fresh gray matter samples exhibited markedly weaker bands than white matter samples in non-treated samples (Fig. 2A). In RNase-treated samples, however, this difference disappeared, and most normalized fresh

samples showed similarly distinct bands without observable smear patterns, indicating the presence of relatively intact DNA fragments (Fig. 2B).

In decomposed samples, some RNase-treated specimens exhibited clearer electrophoretic bands than non-treated samples despite a reduction in measured nucleic acid concentration. In these samples, the post-treatment concentration fell below the normalization threshold, eliminating the need for dilution prior to gel loading and resulting in improved band visibility.

Unlike spectrophotometric measurements, fluorometric analysis revealed no significant difference in dsDNA concentration between non-treated samples from gray matter and white matter (Fig. 3A). Nevertheless, RNase treatment resulted in higher dsDNA concentrations in white matter than in gray matter (Fig. 3B; $p = 0.003$ for total samples, $p = 0.005$ for fresh samples, and $p = 0.031$ for decomposed samples), mirroring the pattern observed with spectrophotometric analysis.

While dsDNA concentrations did not differ significantly between fresh and decomposed samples within either tissue type, decomposed samples showed substantially greater dispersion, suggesting increased heterogeneity in DNA preservation.

qPCR analysis demonstrated higher dsDNA concentrations in white matter than in gray matter even in non-treated samples, mirroring the patterns of RNase-treated samples observed in both spectrophotometric and fluorometric analyses (Fig. 4A–D). Specifically, in non-treated samples, LA concentrations were significantly higher in white matter than in gray matter in the total sample set ($p = 0.002$) and fresh samples ($p < 0.001$), whereas no significant difference was observed in decomposed samples (Fig. 4A). This pattern was maintained in RNase-treated samples (Fig. 4B; $p = 0.007$ for both the total sample set and fresh samples). Similarly, SA concentrations were significantly higher in white matter than in gray matter in the total sample set and fresh samples (both $p < 0.001$), but not in non-treated decomposed samples (Fig. 4C). In RNase-treated samples, white matter continued to show significantly higher SA concentrations across all groups, including decomposed samples (Fig. 4D; $p = 0.006$ for total samples; $p = 0.006$ for fresh samples; $p = 0.032$ for decomposed samples).

No significant differences in the DI were observed between gray matter and white matter irrespective of postmortem status or RNase treatment (Fig. 4E–F). Nevertheless, decomposed samples exhibited significantly higher DI values than fresh samples. In non-treated samples, this increase was significant only in white matter treatment (Fig. 4E; $p < 0.001$), whereas in RNase-treated samples, significantly elevated DI values were observed in both gray and white matter (Fig. 4F; $p = 0.016$ and $p = 0.005$, respectively).

Spearman correlation analysis of nucleic acid and dsDNA concentrations measured by all methods provided a concise summary of the preceding findings (Fig. 5). In non-treated samples, spectrophotometric measurements in gray matter correlated poorly with concentrations obtained by fluorometry and qPCR, particularly with qPCR-derived targets (Fig. 5A). In RNase-treated samples, strong correlations emerged across all analytical methods (Fig. 5B). By contrast, white matter exhibited

consistently high correlations among spectrophotometric, fluorometric, and qPCR measurements irrespective of RNase treatment, suggesting that spectrophotometric measurements in white matter were less affected by non-dsDNA nucleic acids **(**Fig. 5C **and D)**.

The STR profiling success rate remained exceptionally high regardless of RNase treatment, achieving 100% in non-treated samples and 99.9% in RNase-treated samples. When STR peak heights were compared according to RNase treatment status, the RNase-treated gray matter samples showed significantly higher results than non-treated samples (Fig. 6A). In contrast, no significant differences were observed between non-treated and RNase-treated samples in white matter, indicating that RNase treatment had a limited impact on STR amplification in white matter (Fig. 6B).

Specifically, analysis of STR peak heights revealed that, in decomposed samples, white matter generally yielded higher peak heights than gray matter, although the level of statistical significance varied according to RNase treatment status ($p < 0.001$ in non-treated samples and $p = 0.003$ in RNase-treated samples). When STR loci were categorized by amplicon length into short, medium, and long groups, this trend was observed across all groups of non-treated samples (Fig. 7A, C, **and E**; $p < 0.001$ for short loci; $p = 0.006$ for medium loci; $p = 0.050$ for long loci). In RNase-treated samples, however, the difference between gray and white matter remained significant only for medium-length loci (Fig. 7D; $p = 0.011$).

When non-treated samples were compared between fresh and decomposed groups, medium and long loci exhibited significantly lower peak heights in decomposed samples than in fresh samples (Fig. 7C **and E**; gray matter: $p < 0.001$ for both medium and long loci; white matter: $p = 0.014$ for medium loci and $p = 0.015$ for long loci), reflecting the effects of DNA degradation. In RNase-treated samples, these differences were no longer observed in white matter. In contrast, short loci demonstrated an unexpected pattern, with decomposed samples showing higher peak heights than fresh samples (Fig. 7A **and B**). This difference was significant in white matter regardless of RNase treatment ($p < 0.001$ for both) and in RNase-treated gray matter ($p = 0.034$).

Analysis of STR PHRs revealed only minor differences between gray and white matter which were largely attenuated by RNase treatment. No consistent pattern according to locus length was observed. Stutter analysis also revealed no significant differences according to the type of tissue, postmortem status, RNase treatment, or amplicon size.

DISCUSSION

Since forensic samples are often limited and irreplaceable, precise DNA quantification prior to destructive testing is essential for maximizing sample preservation and ensure successful downstream analysis. Various quantification methods are used in forensic laboratories; however, their results often differ when applied to degraded or heterogeneous samples because each method targets different nucleic acid components. Such discrepancies are particularly relevant in brain tissue, where gray and white matter differ substantially in cellular composition and postmortem degradation patterns. Despite

the forensic importance of the brain, few studies have evaluated DNA quantification separately in these two regions.

In the present study, spectrophotometric analysis demonstrated substantially higher nucleic acid concentrations in gray matter than in white matter when RNase treatment was not applied, particularly in fresh samples. However, this pattern was reversed with RNase treatment, with white matter exhibiting higher concentrations than gray matter. The marked reduction in spectrophotometric measurements observed in gray matter following RNase treatment indicates that a considerable proportion of the measured nucleic acids consisted of RNA. These findings are consistent with previous reports showing that RNA co-extraction can substantially inflate spectrophotometric measurements of DNA [24]. Gel electrophoresis findings further supported this interpretation, as the differences in band intensity between gray and white matter largely disappeared with RNase treatment. Together, these results suggest that spectrophotometric quantification alone may overestimate DNA quantity in gray matter because of RNA contamination.

Fluorometric quantification showed a different pattern. In non-RNase-treated samples, no significant difference in dsDNA concentration was observed between gray and white matter. In RNase-treated samples, however, white matter exhibited significantly higher dsDNA concentrations than gray matter. Furthermore, RNase treatment significantly reduced dsDNA concentrations in gray matter, which was less definite in white matter. A previous study reported that fluorometric quantification can also be affected by RNA contamination when the DNA:RNA ratio ≥ 1:4 [22]. The marked decrease observed only in gray matter after RNase treatment is consistent with this observation and further supports the presence of substantial RNA contamination in this region. Notably, spectrophotometric measurements yielded higher nucleic acid concentrations than fluorometric measurements, in agreement with previous findings [25]. This discrepancy reflects fundamental methodological differences: spectrophotometry measures total nucleic acids and is susceptible to interference from RNA, single-strand DNA (ssDNA), proteins, and other contaminants, whereas fluorometry more selectively detects dsDNA [26]. Such interferences may become more pronounced during decomposition, leading to overestimation of nucleic acid concentration, reduced A260/230 ratios, and elevated A260/280 ratios when RNA or ssDNA is abundant [27]. Consistent with these methodological characteristics, stronger correlations among all quantification methods were observed in RNase-treated samples, particularly in gray matter, highlighting the importance of RNA removal for accurate DNA quantification from brain tissue.

Unlike spectrophotometric and fluorometric analyses, qPCR consistently demonstrated higher LA and SA concentrations in white matter than in gray matter regardless of RNase treatment. Because qPCR selectively quantifies amplifiable human DNA targets, these findings suggest that white matter preserves intact DNA more effectively than gray matter. Previous studies support the delayed nuclear degradation of white matter compared to gray matter. One study reported that neuronal nuclei in gray matter frequently undergo pyknosis and karyorrhexis during postmortem autolysis, whereas white matter initially exhibits myelin sheath disintegration with relatively delayed nuclear breakdown [28]. Another study similarly found that general cellular architecture deteriorated earlier than myelin and axonal

structures, indicating greater structural persistence of white matter components during decomposition [29]. These histological observations provide a plausible explanation for the consistently higher LA and SA concentrations observed in white matter and suggest that DNA-containing cellular structures may remain intact for longer periods in white matter than in gray matter. Therefore, the differences observed among quantification methods likely reflect both RNA contamination and intrinsic differences in DNA preservation between the two brain regions.

The STR results were largely consistent with the qPCR findings. STR profile parameters such as peak height, PHR, and stutter are important indicators of the quality and reliability of STR profiles and are closely associated with both the quantity and integrity of template DNA. Therefore, accurate quantification of human DNA before amplification is critical for achieving reliable STR typing results [30]. Although overall profiling success remained high, decomposed white matter generally yielded higher peak heights than gray matter. This pattern was most evident in non-treated samples and was observed across multiple locus size groups. The superior peak height performance observed in white matter is consistent with previous studies showing that DNA degradation adversely affects STR amplification efficiency and signal intensity [31]. Medium- and long-sized loci showed reduced peak heights in decomposed samples, reflecting the effects of DNA degradation, whereas short loci were relatively preserved. This finding is consistent with the well-known “ski-slope” pattern of degraded DNA, in which shorter STR amplicons are preferentially amplified because longer template fragments are progressively lost during degradation [32]. The persistence of relatively higher peak heights in white matter may be attributable to delayed nuclear breakdown following myelin disintegration [28], allowing amplifiable DNA fragments to remain detectable for longer periods during decomposition. RNase treatment significantly increased peak heights in gray matter but had little effect in white matter. These findings suggest that RNA contamination may reduce amplification efficiency in gray matter and that RNA removal can improve STR performance without substantially altering allele balance or stutter characteristics. Collectively, the STR results further support the superior preservation of amplifiable DNA in white matter during decomposition

CONCLUSION

This study demonstrates that DNA quantification results differ substantially between gray and white matter because of regional differences in nucleic acid composition and postmortem preservation. Gray matter contained higher levels of RNA, resulting in overestimation of nucleic acid concentration without RNase treatment, whereas white matter consistently showed higher amounts of amplifiable DNA confirmed by qPCR and RNase treatment. STR analysis further indicated better preservation of DNA in white matter, particularly in decomposed samples. These findings suggest that white matter is a more suitable target for forensic DNA analysis in decomposed brains and that RNA removal and dsDNA-specific quantification can improve the accuracy of DNA assessment and STR profiling.

Declarations

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CRediT authorship contribution statement:

Dong Yeon Lee: Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Ga Eun Kim:** Investigation, Methodology. **Eun-Byoul Jung:** Investigation, Methodology. **Eugene Choi:** Validation, Resources. **Sojung Lim:** Investigation, Validation. **Soong Deok Lee:** Investigation, Validation. **Moon-Young Kim:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing.

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Figures

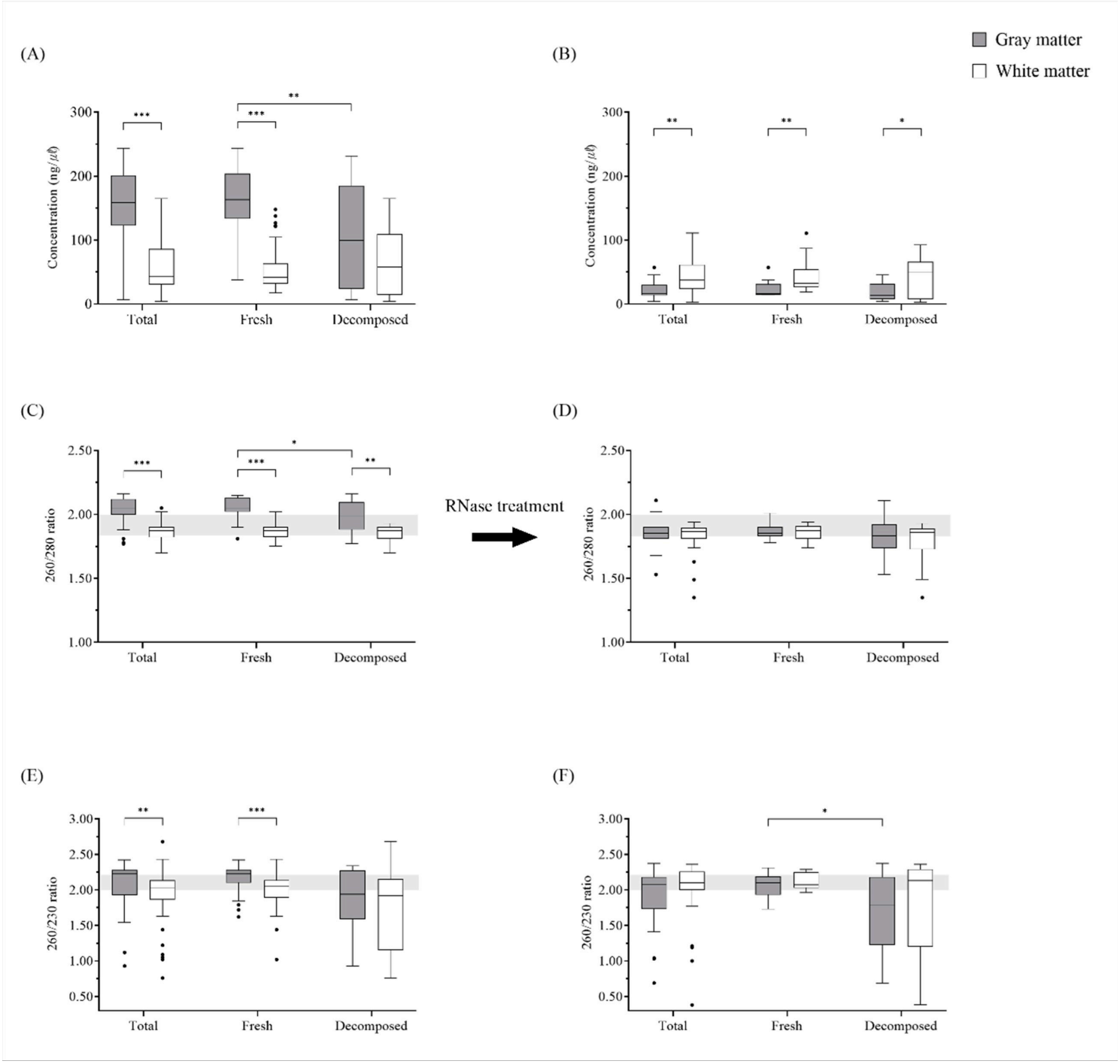


Figure 1

Spectrophotometry analysis of nucleic acid concentration and purity in gray and white matter. Box plots of nucleic acid concentrations (A and B), A260/280 ratios (C and D), and A260/230 ratios (E and F) are shown in non-treated (A, C, E) and RNase-treated samples (B, D, F). In non-treated samples, gray matter exhibited higher nucleic acid concentrations than white matter (A); however, this trend was reversed in RNase-treated samples (B). Differences in A260/280 and A260/230 ratios between gray and white matter were generally reduced and these ratios approached the acceptable range in RNase-treated samples (C–F). Each box plot displays the median, interquartile range, whiskers, and outliers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

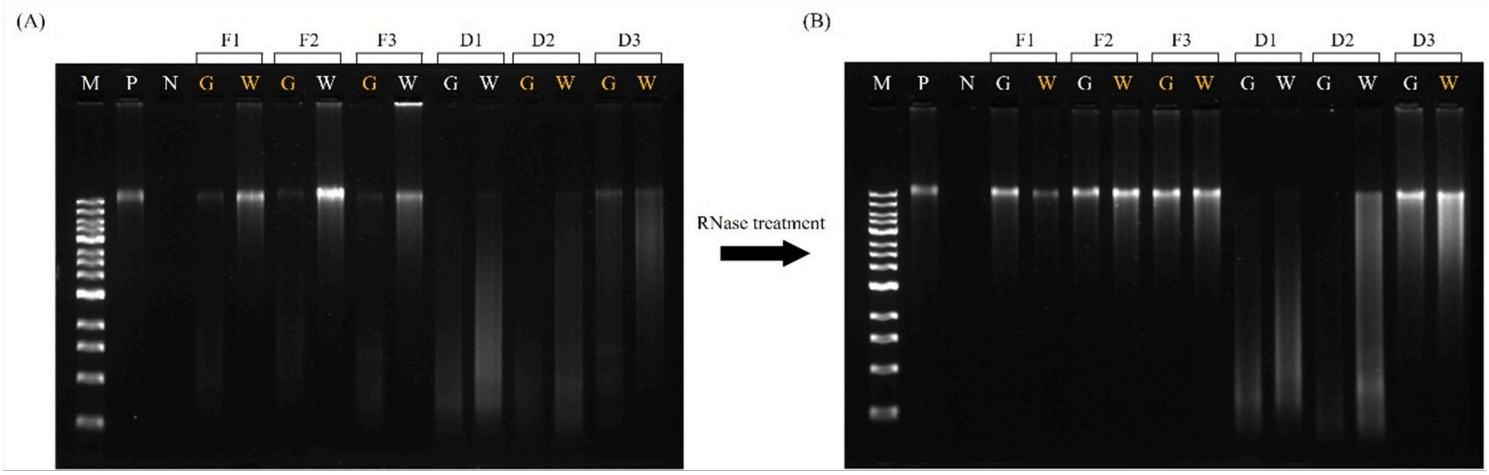


Figure 2

Gel electrophoresis results of nucleic acid extracted from gray and white matter. Yellow letters indicate samples exceeding the concentration thresholds and diluted to 20 ng/□ for fresh samples and 50 ng/□ for decomposed samples. In non-treated samples (A), fresh samples (F1–F3) showed weaker bands in gray matter than in white matter following concentration normalization based on spectrophotometric measurements. In RNase-treated samples (B), most fresh samples no longer required dilution because of reduced measured nucleic acid concentrations, and the differences in band intensity between gray and white matter were no longer observed. In decomposed samples (D1–D3), no consistent differences in band intensity were observed between gray and white matter. Notably, some RNase-treated samples exhibited clearer bands despite lower measured nucleic acid concentrations, likely because the reduced concentrations decreased the need for dilution prior to gel loading. F, fresh sample; D, decomposed sample; M, 1 kb DNA size ladder; P, positive control; N, negative control; G, gray matter; W, white matter.

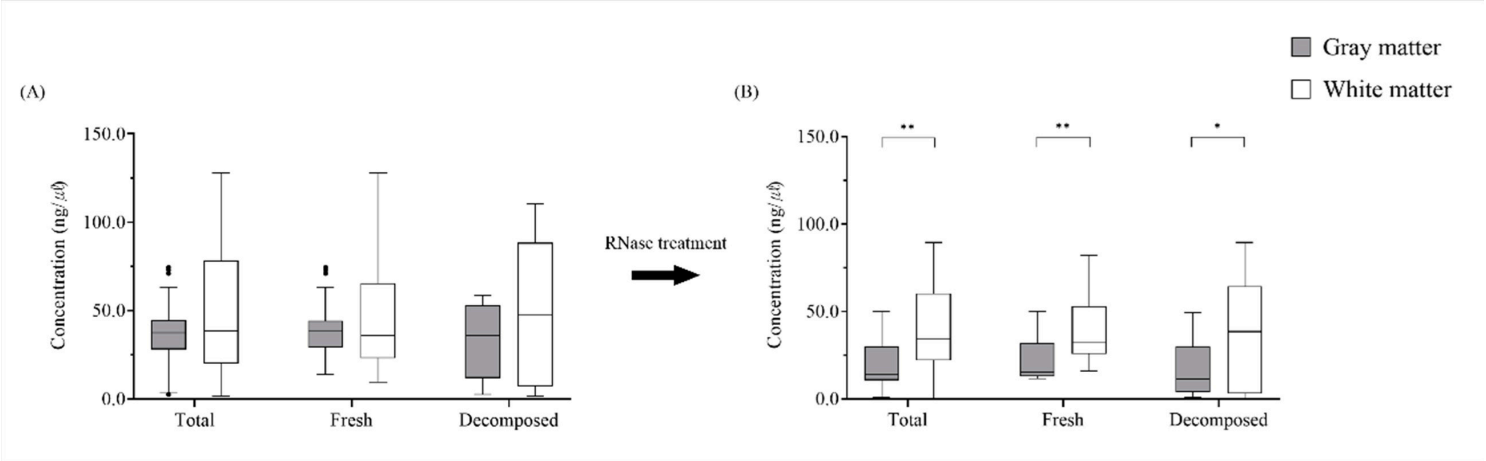


Figure 3

Fluorometry results of dsDNA concentration from gray and white matter. Box plots are showing dsDNA concentrations in non-treated (A) and RNase-treated (B) samples, grouped by total, fresh, and decomposed samples. In non-treated samples (A), no significant differences in dsDNA concentration

were observed between gray matter and white matter, in contrast to the spectrophotometric results. In RNase-treated samples (B), white matter exhibited significantly higher dsDNA concentrations than gray matter across all groups, consistent with the pattern observed in spectrophotometric analysis. Within each tissue type, no significant differences in dsDNA concentration were observed between fresh and decomposed samples; however, decomposed samples showed a wider distribution of values, indicating greater inter-sample variability. Each plot displays the median, interquartile range, whiskers, and outliers. * $p < 0.05$, ** $p < 0.01$.

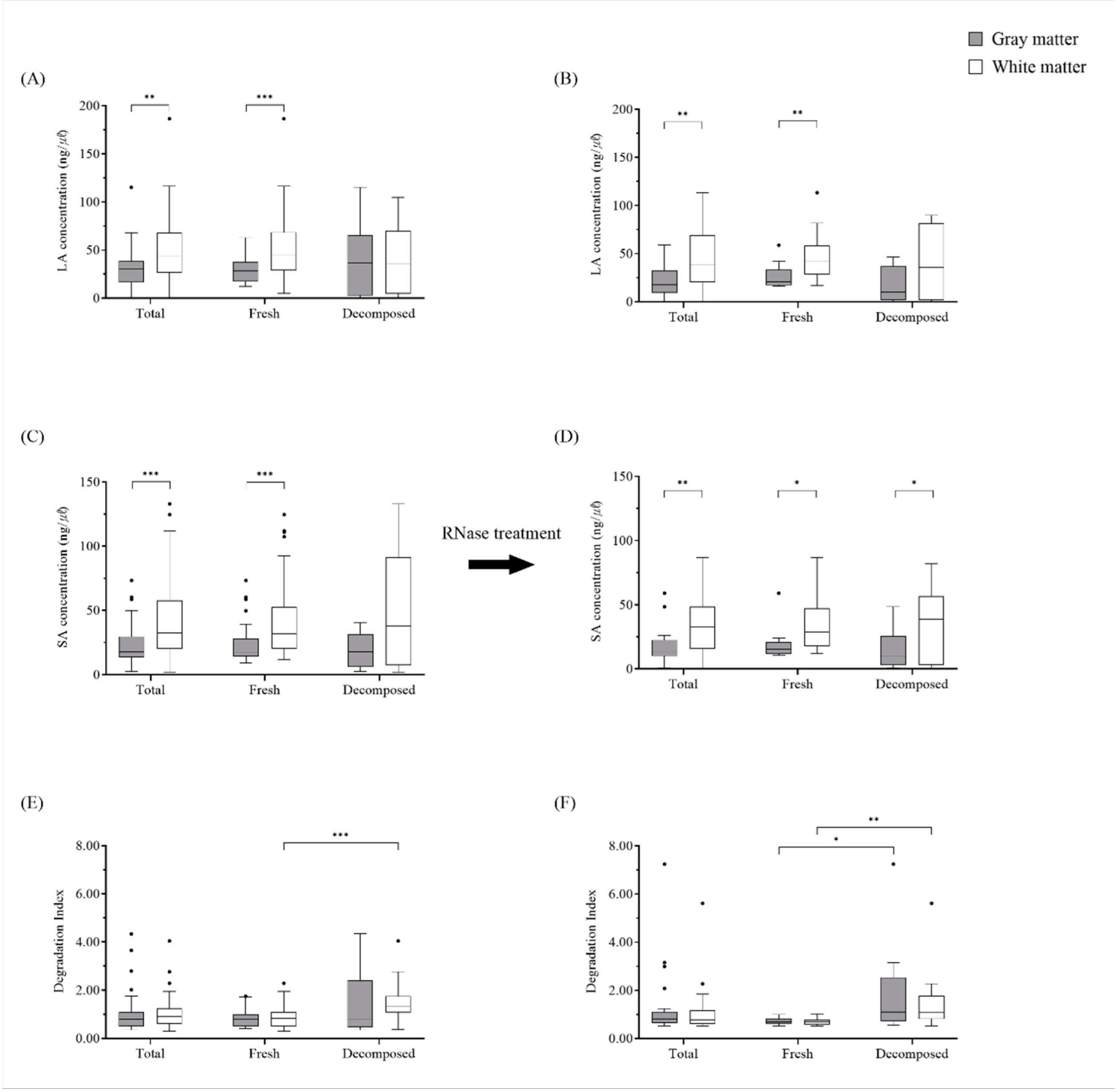


Figure 4

qPCR results of dsDNA concentration and degradation in gray and white matter. Box plots are showing long autosomal target (LA) concentrations (A and B), short autosomal target (SA) concentrations (C and D), and degradation index (DI) values (E and F) in non-treated (A, C, E) and RNase-treated (B, D, F) samples, grouped by total, fresh, and decomposed samples. White matter exhibited higher LA and SA concentrations than gray matter across most groups irrespective of RNase treatment, with the exception of LA concentrations in decomposed samples. No significant differences in DI were observed between gray matter and white matter regardless of postmortem status or RNase treatment. However, decomposed samples generally exhibited higher DI values than fresh samples. Each plot displays the median, interquartile range, whiskers, and outliers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

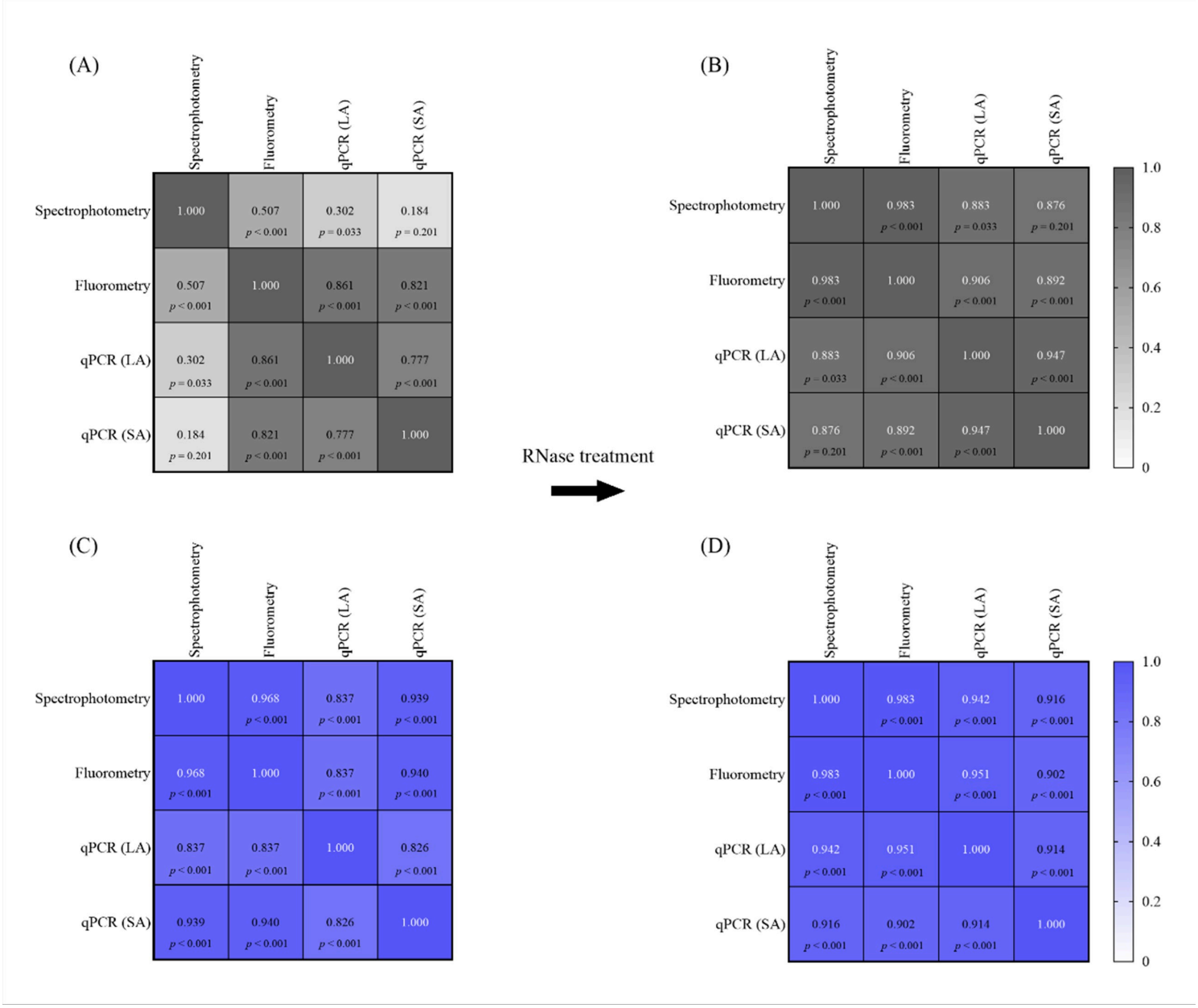


Figure 5

Correlation matrices among quantification methods in gray and white matter. Spearman correlation matrices comparing nucleic acid or dsDNA concentrations measured by spectrophotometry, fluorometry, and qPCR (large and small autosomal targets) in gray matter (A and B) and white matter (C and D), in non-treated (A and C) and RNase-treated samples (B and D). In non-treated samples, spectrophotometric nucleic acid measurements in gray matter showed relatively weak correlations with dsDNA concentrations measured by other methods, particularly qPCR-based measurements (A). In RNase-treated samples, strong correlations were observed among all quantification methods in gray matter (B). In contrast, white matter exhibited consistently strong correlations across all methods regardless of RNase treatment (C and D). Correlation coefficients (ρ) and corresponding *p*-values are shown in each cell. Color intensity reflects the strength of correlation, ranging from 0 (white) to 1. LA, large autosomal targets; SA, small autosomal targets.

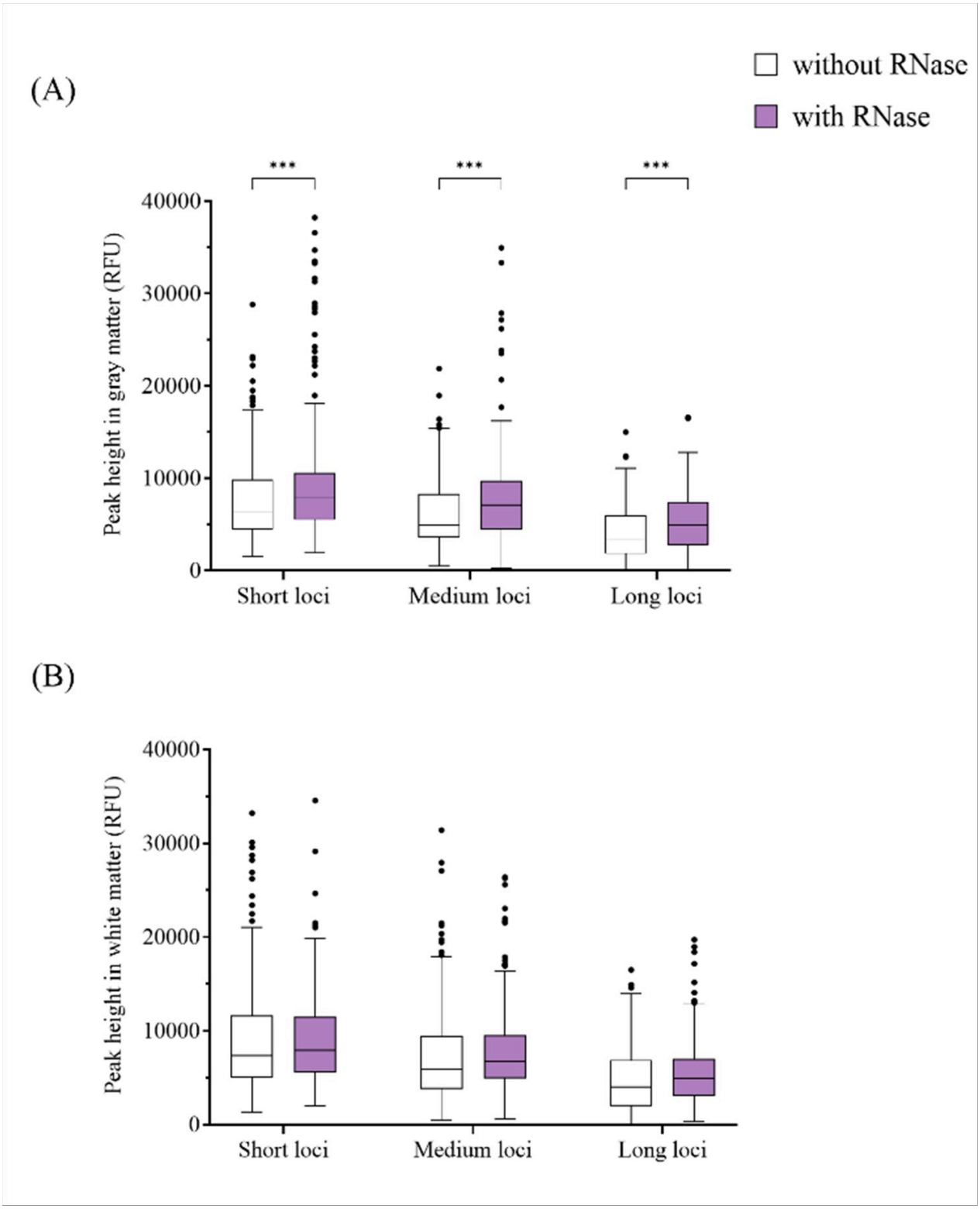


Figure 6

Comparison of STR peak heights between non-treated and RNase-treated brain samples. Box plots are showing STR peak heights grouped by locus size in gray matter (A) and white matter (B). RNase-treated samples showed significantly higher peak heights across all locus size groups in gray matter, whereas no significant differences were observed between non-treated and RNase-treated samples in white matter. Each plot displays the median, interquartile range, whiskers, and outliers. *** $p < 0.001$

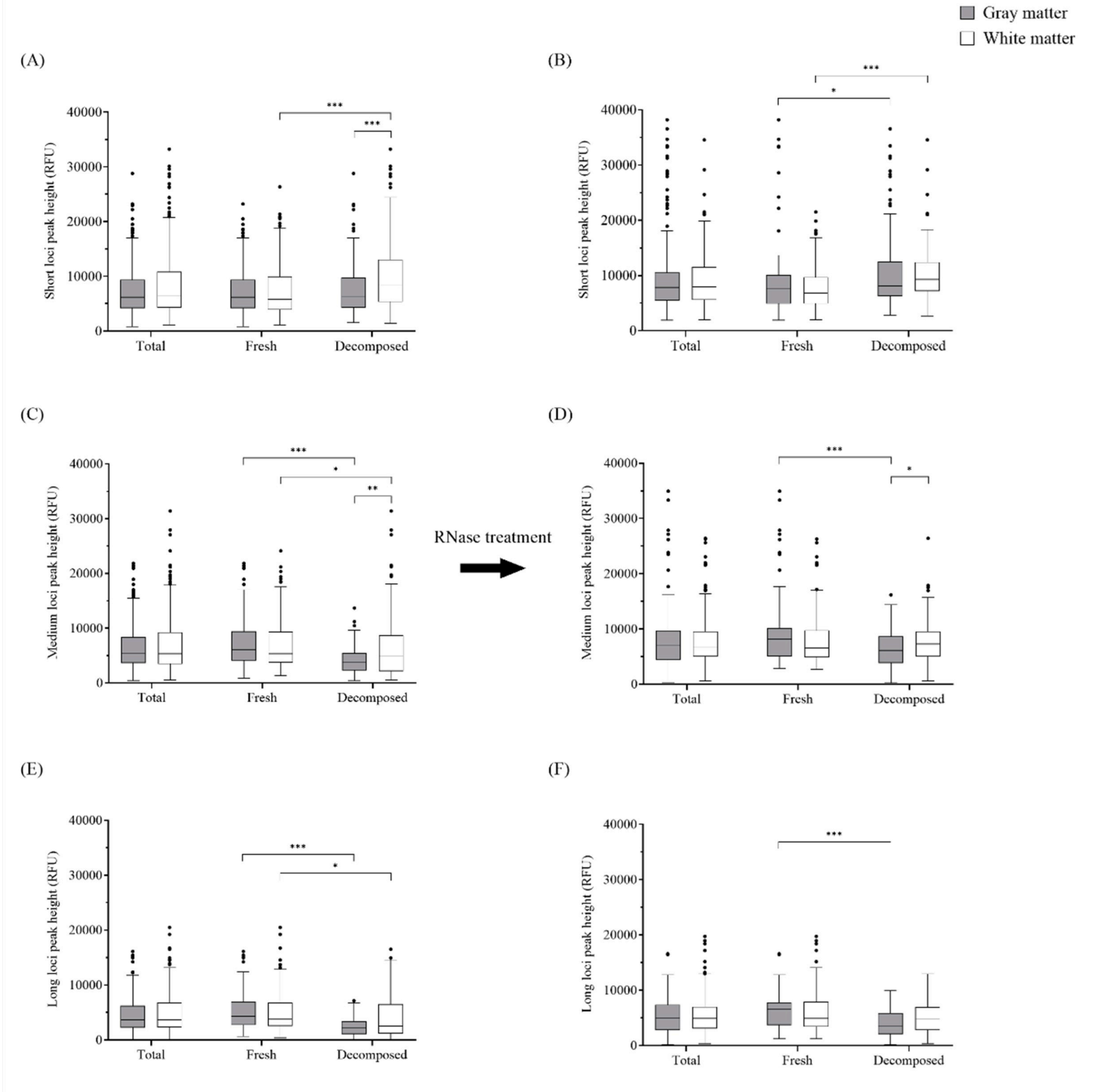


Figure 7

Peak height results grouped by locus size in gray and white matter. Box plots are showing peak heights of short (A and B), medium (C and D), and long (E and F) loci in gray and white matter in non-treated (A, C, E) and RNase-treated samples (B, D, F), grouped by total, fresh, and decomposed samples. White matter generally exhibited higher peak heights than gray matter in decomposed samples. Medium and long loci tended to show lower peak heights in decomposed samples than in fresh samples, whereas short loci tended to show higher peak heights in decomposed samples. In RNase-treated samples,

differences according to postmortem status were reduced in white matter. Each plot displays the median, interquartile range, whiskers, and outliers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$