Supplementary Materials

Supplementary Materials and Methods

Protocol for DNA extraction from historical/ancient skin samples (Lab protocol: method LL)

The following method for the isolation and purification of DNA from historical/ancient skin samples was based on protocols used to extract and sequence ancient DNA from animal and human remains (Dabney et al., 2013; Rohland et al., 2018). The modified method has been successfully employed for ancient bone samples at the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Chinese Academy of Sciences.

Reagents

EDTA, 0.5M, pH 8.0 (Fermentas, cat.no. R102)

Ethanol, 100% (Sigma, cat.no. E7023)

Guanidine hydrochloride (Sigma, cat.no. G3272)

Isopropanol (Sigma, cat.no. 59304)

PE buffer (Qiagen, cat.no. 19065)

Proteinase K (Carl Roth, cat.no. 7528.1)

Sodium acetate, 3M, pH 5.2 (Sigma, cat.no. S7899)

Tris-HCl, 1M, pH 8.0 (AppliChem, cat.no. A4577,1000)

Tween 20 (Sigma, cat.no. T2700-100ML)

Nuclease free water (Invitrogen, cat.no.10977-015)

Consumables

2.0 ml LoBind tubes (Eppendorf, cat.no. 0030108078)

1.5 ml LoBind tubes (Eppendorf, cat.no. 0030108051)

50 ml Falcon tubes (Corning, cat.no. 430897)

High Pure Viral Nucleic Acid Large Volume Kit (Roche, cat.no. 05114403001)

MinElute PCR purification kit-spin columns (Qiagen, cat.no. 28006)

High pure collection tube: 2.0 ml Tube Extenders (Qiagen, cat.no. 1029526)

Note: Before use, all consumables were UV irradiated for 40 min.

Buffers

Supplementary Table S1.1. Lysis buffer [10 ml] ***(UV irradiation for 20 min)***

Reagent	Volume	Final concentration
Nuclease free water	745 µl	
0.5 M EDTA, pH 8.0	9 ml	0.45 M
Tween 20	5 µl	0.05%
10 mg/ml Proteinase K	250 µl	0.25 mg/ml

Note: Proteinase K was added after UV irradiation.

Supplementary Table S1.2. Binding buffer [50 ml] ***(UV irradiation for 20 min)***

Reagent	Volume/amount	Final concentration
Guanidine hydrochloride	23.88 g	5 M
Nuclease free water	to 30 ml	
Isopropanol	to 50 ml	40%
Tween 20	25 µl	0.05%

Note: Guanidine hydrochloride was added to a 50 ml tube. Nuclease free water was added to 30 ml (using graduation of the tube). Salt was dissolved by mixing (heated in a microwave for ca. 1 min). Isopropanol was added to 50 ml, with Tween 20 then added, followed by 20 min of UV irradiation.

Supplementary Table S1.3. TET buffer [50 ml] ***(UV irradiation for 20 min)***

Reagent	Volume	Final concentration
Nuclease free water	~49.4 ml	
0.5 M EDTA, pH 8.0	100 µl	1 mM
1 M Tris-HCl, pH 8.0	500 µl	10 mM
Tween 20	25 µl	0.05%

DNA extraction procedure

Day 1:

1. Add 1 ml of lysis buffer to each prepared sample. Prepare an extraction blank (add 1.0 ml of lysis buffer to a new 2.0 ml LoBind tube).
2. Suspend samples by vortexing and rotation (12 rpm) overnight (14–18 h) at 37 °C.

Day 2:

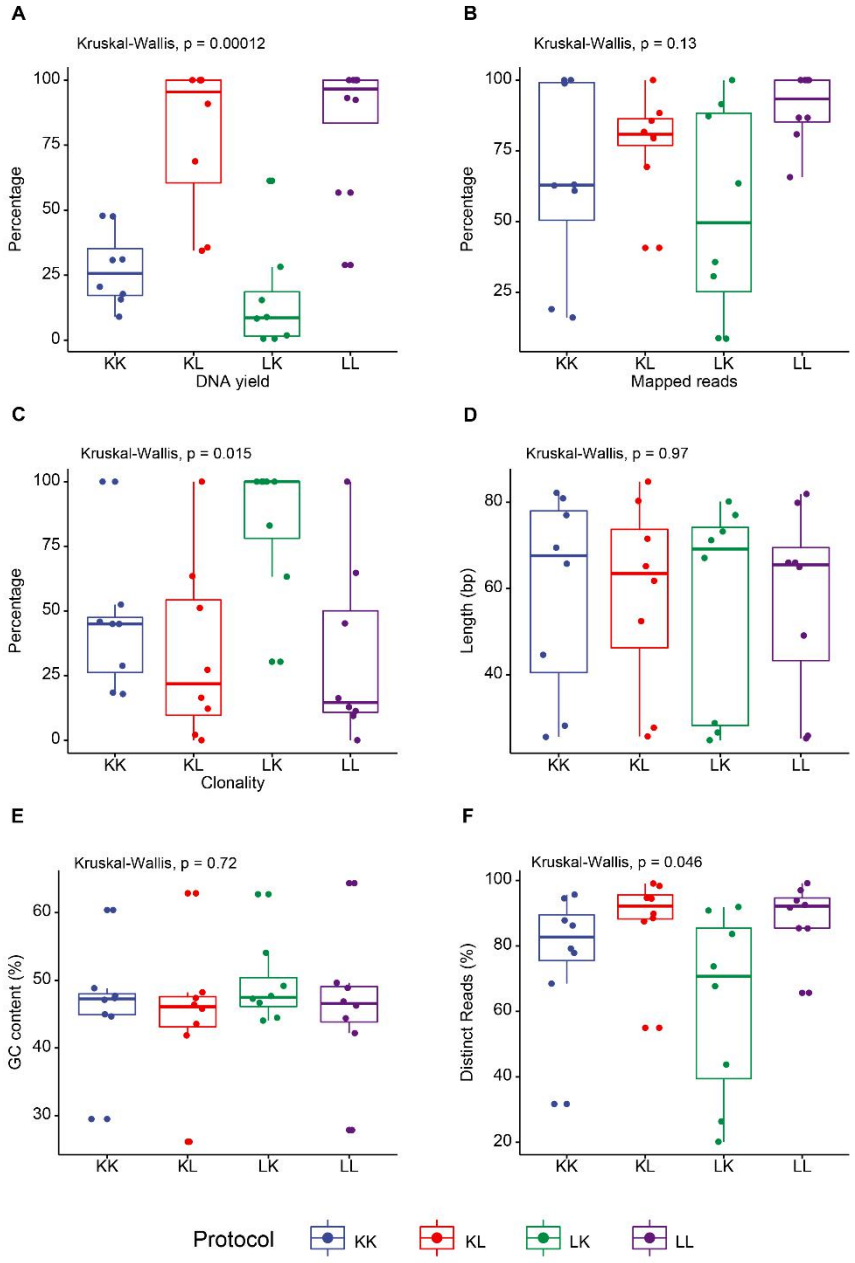
3. For each sample (and extraction blank), transfer 10 ml of binding buffer to a 50 ml tube and add 400 µl of 3 M sodium acetate, pH 5.2.
4. Spin the tubes containing sample and lysis buffer for 5 min at 13 200 rpm to pellet residual sample material.
5. Transfer the supernatant to a 50 ml tube containing 10 ml of binding buffer/sodium acetate mix. Mix gently by shaking.
6. Pour the mixture from step 5 into the extension reservoir of the pre-assembled spin column (High Pure Viral Nucleic Acid Large Volume Kit) and close the 50 ml tube with a screw cap. Centrifuge for 4 min at 1 500 rpm. Change the position of the tubes by 180° and centrifuge for 2 min at 1 500 rpm.
7. Remove the screw cap from the 50 ml tube. Carefully remove the extension reservoir from the pre-assembled spin column and close the spin column cap. Place the spin column into a collection tube.
8. Spin samples for 1 min at 6 000 rpm. Discard the flow-through.
9. Add 750 µl of PE buffer, spin for 30 s at 6 000 rpm. Discard the flow-through. Place the spin column back in the collection tube.
10. Repeat step 9.
11. Turn the spin column by 180° and spin for 1 min at 13 200 rpm. Transfer the spin column to a new collection tube.
12. Add 25 µl of TET on top of the silica membrane of the spin column and let the tube stand for 2–5 min. Spin for 30 s at 13 200 rpm.
13. Repeat step 12.
14. Transfer the final 50 µl of eluted DNA to a 1.5 ml LoBind tube and freeze at -20 °C.

REFERENCES

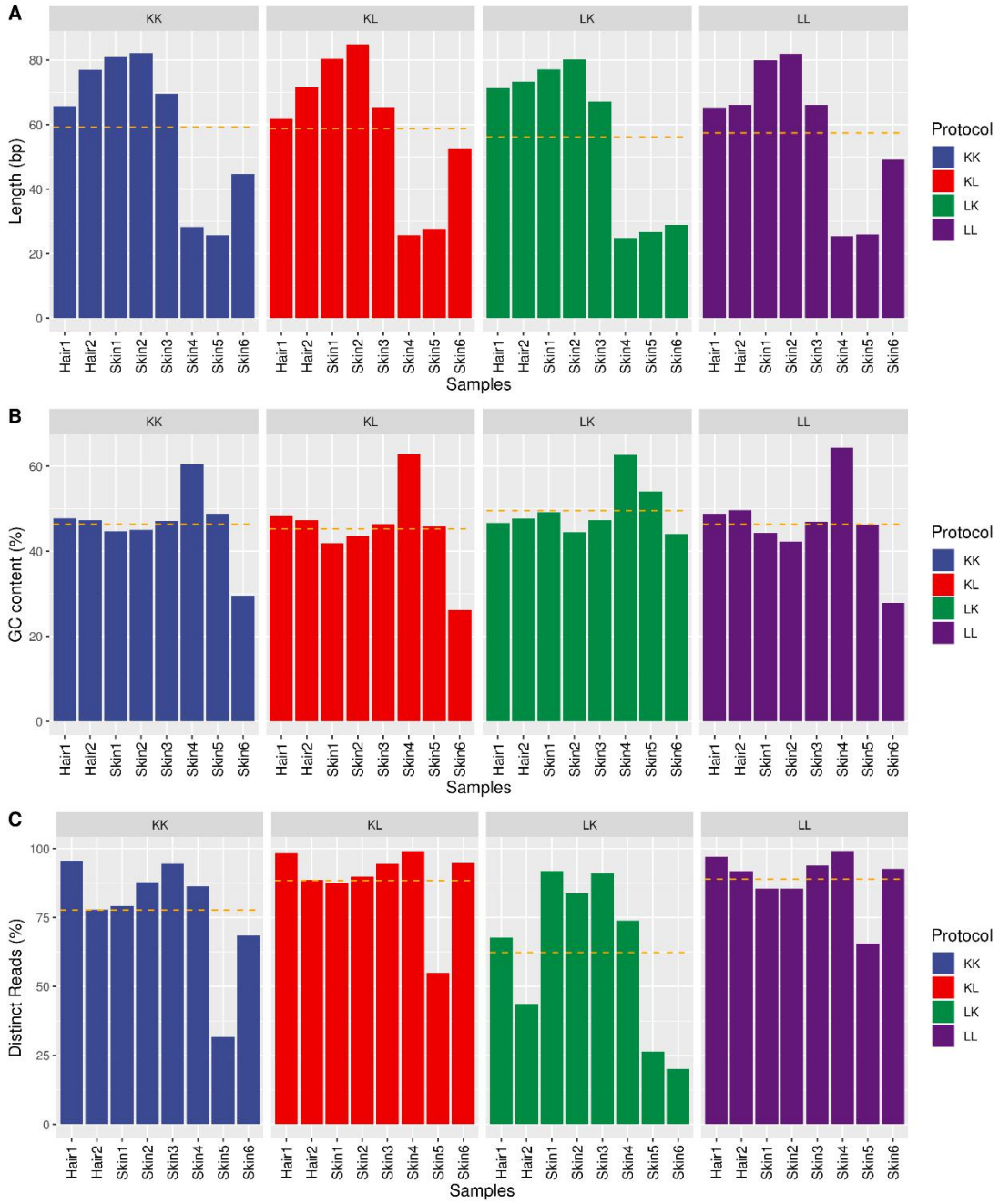
Dabney J, Meyer M, Pääbo S. 2013. Ancient DNA damage. *Cold Spring Harbor Perspectives in Biology*, **5**(7): a012567.

Rohland N, Glocke I, Aximu-Petri A, Meyer M. 2018. Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nature Protocols*, **13**(11): 2447–2461.

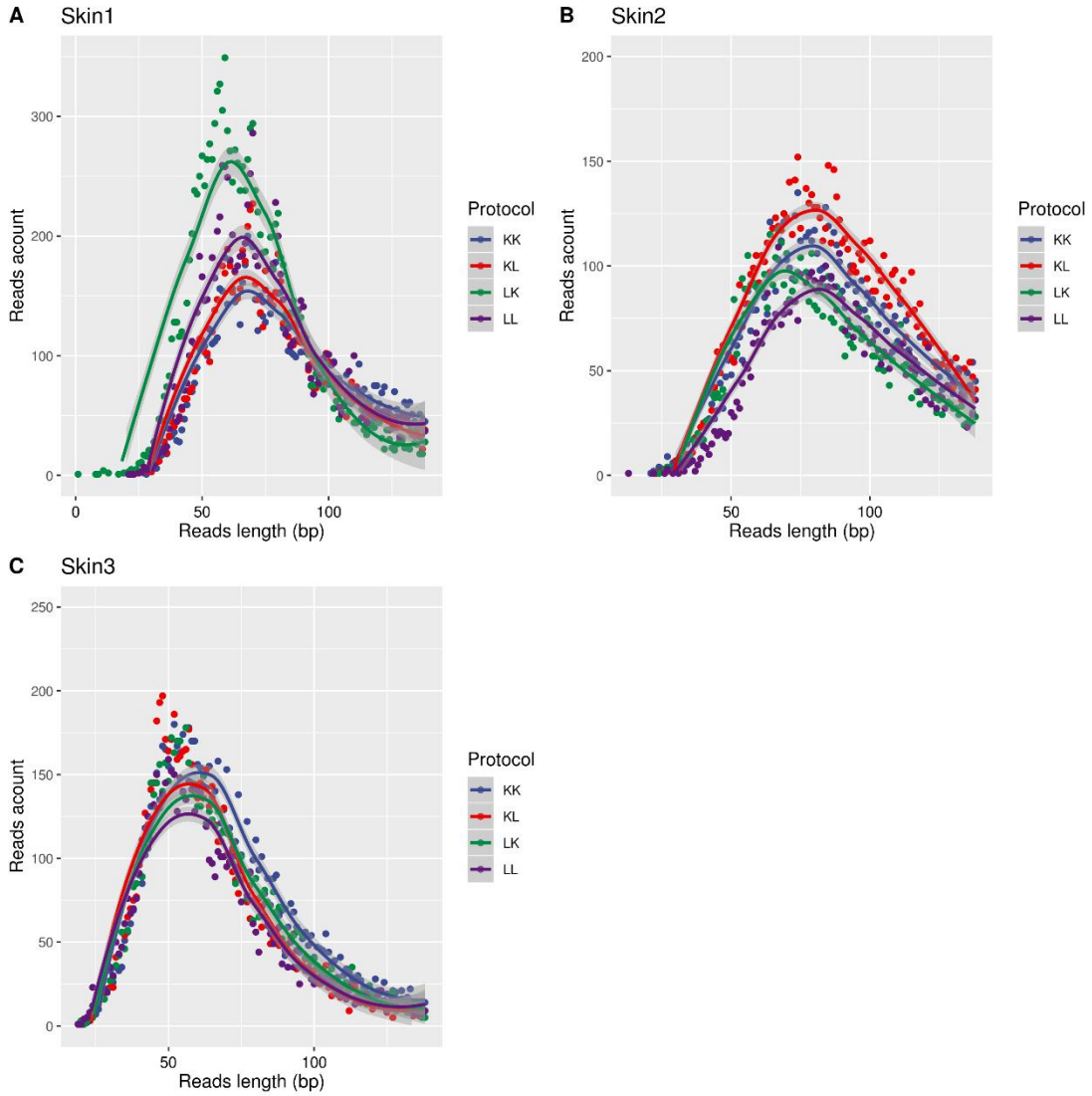
Supplementary Figures



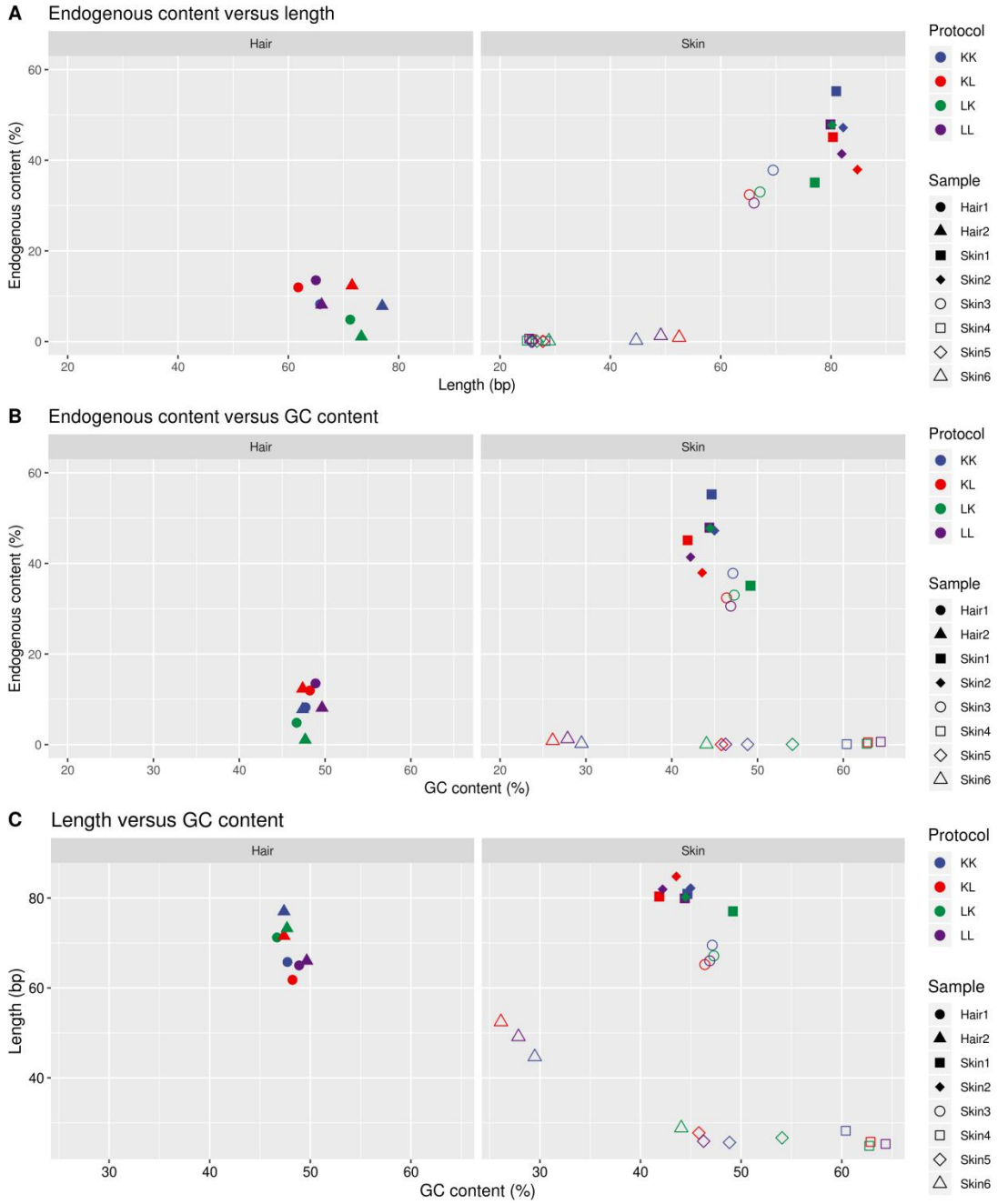
Supplementary Figure S1. Box plots of (A) DNA yield, (B) Number of uniquely mapped reads, (C) clonality, (D) read length (bp), (E) GC content (%), and (F) distinct read rate (%) for each DNA extraction method and eight samples. These plots used the same data as Figure 3 and Supplementary Figure 2. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made). Dotted horizontal bar represents average values of each method.



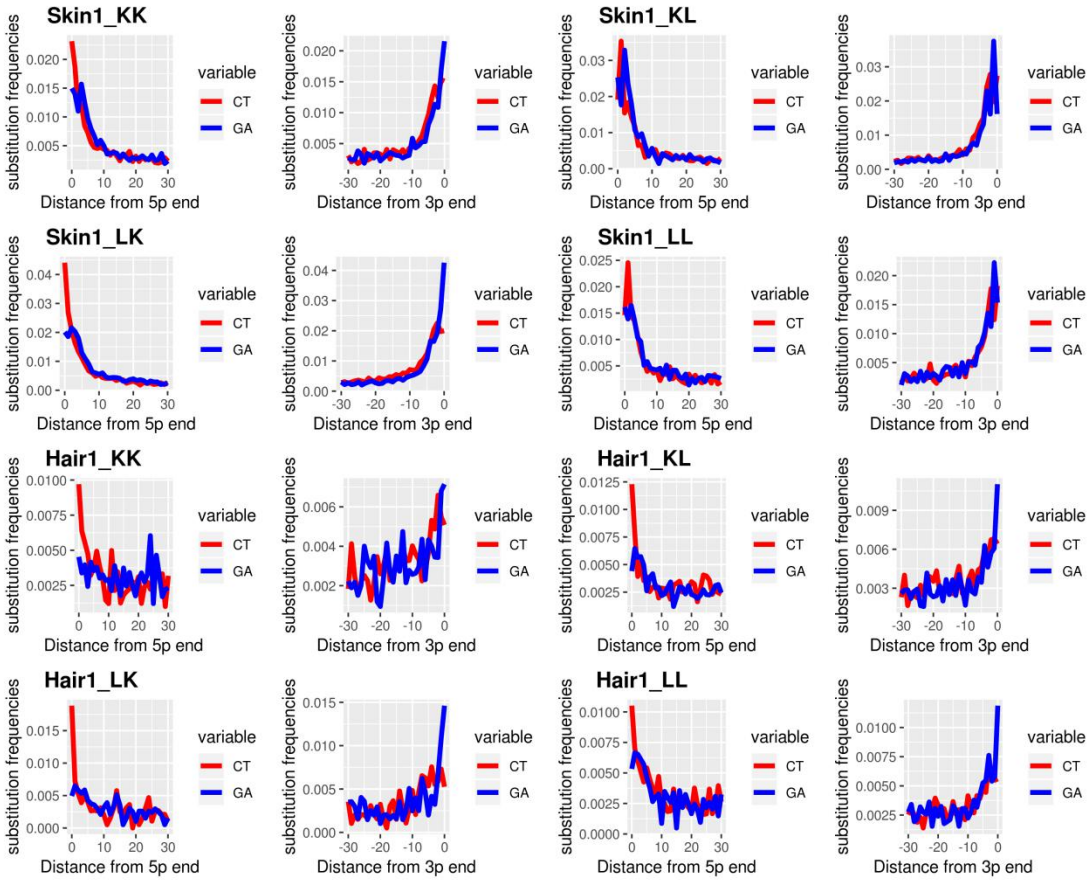
Supplementary Figure S2. Bar plots of (A) read length (bp), (B) GC content (%), and (C) distinct read rate (%) for each DNA extraction method and eight samples. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made). Dotted horizontal bar represents average values of each method.



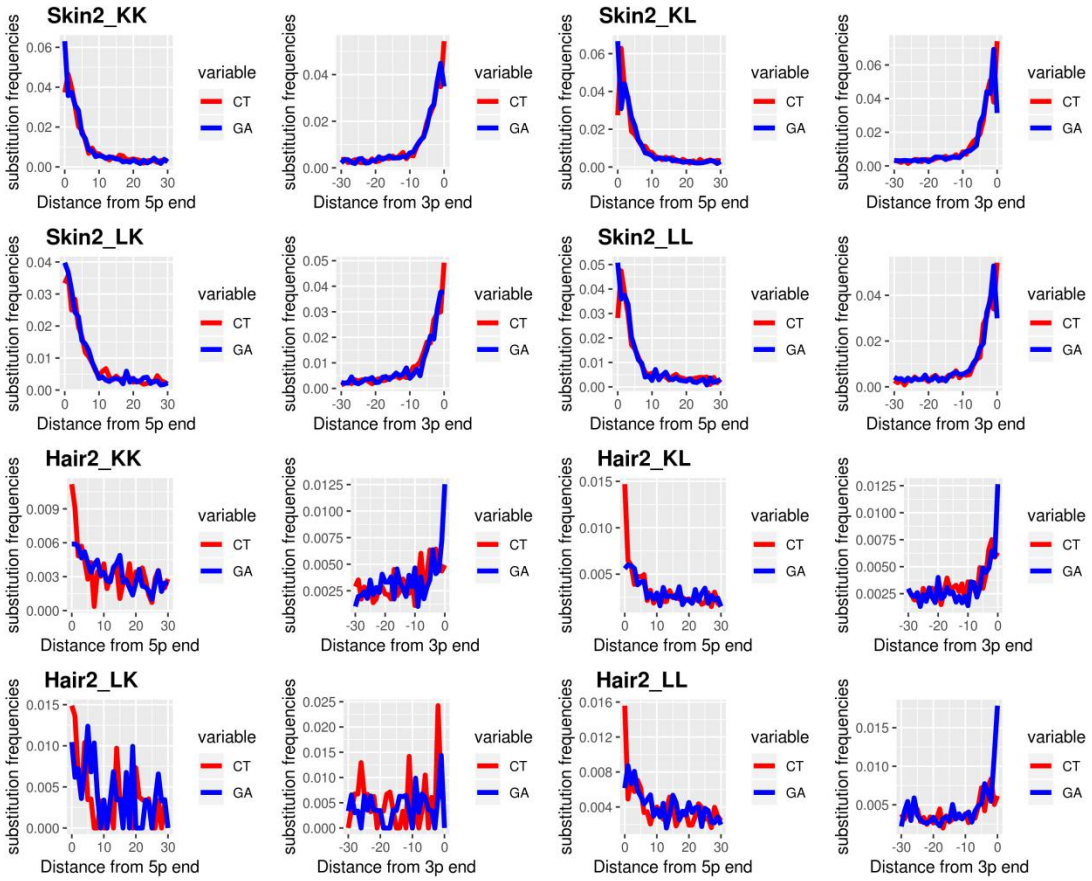
Supplementary Figure S3. Scatter plots showing read length distribution for three historic monkey skin samples and different DNA extraction methods that produced the most reads among eight tested samples. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



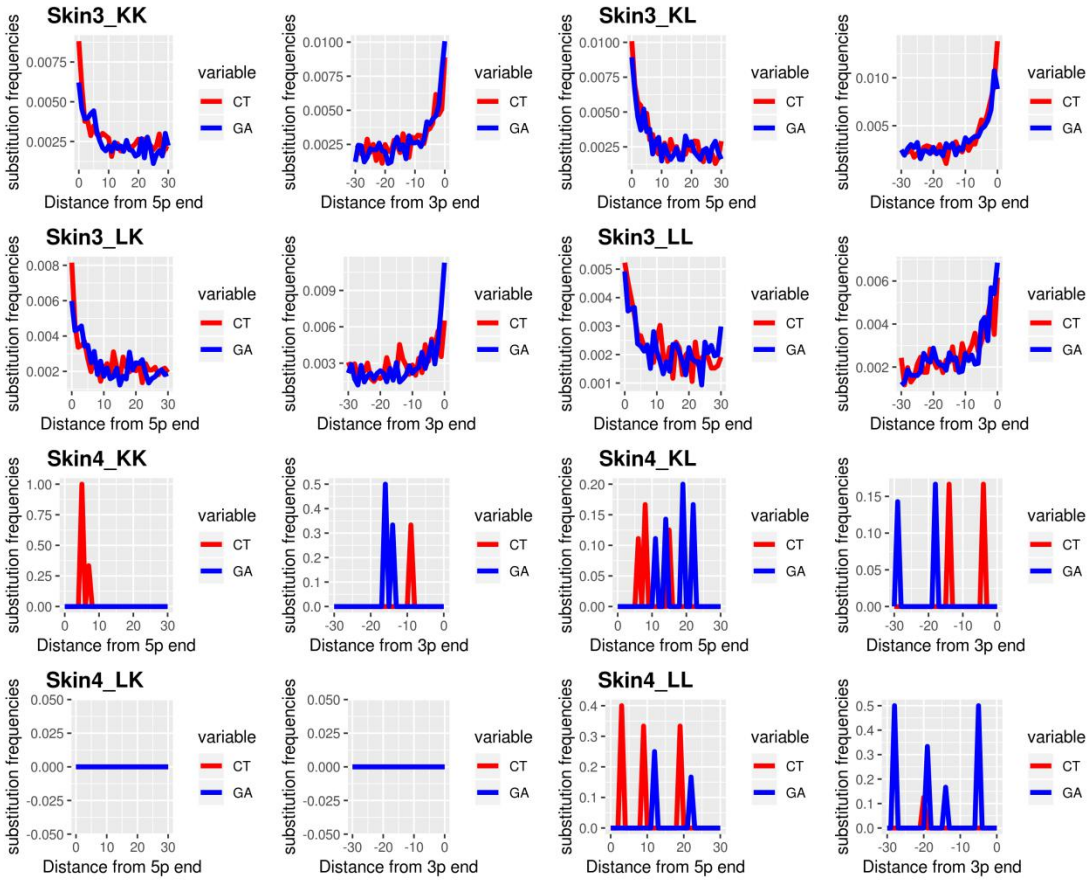
Supplementary Figure S4. Scatter plots for (A) uniquely mapped endogenous content versus read length, (B) uniquely mapped endogenous content versus GC content, and (C) read length versus GC content for each sample and different DNA extraction method. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



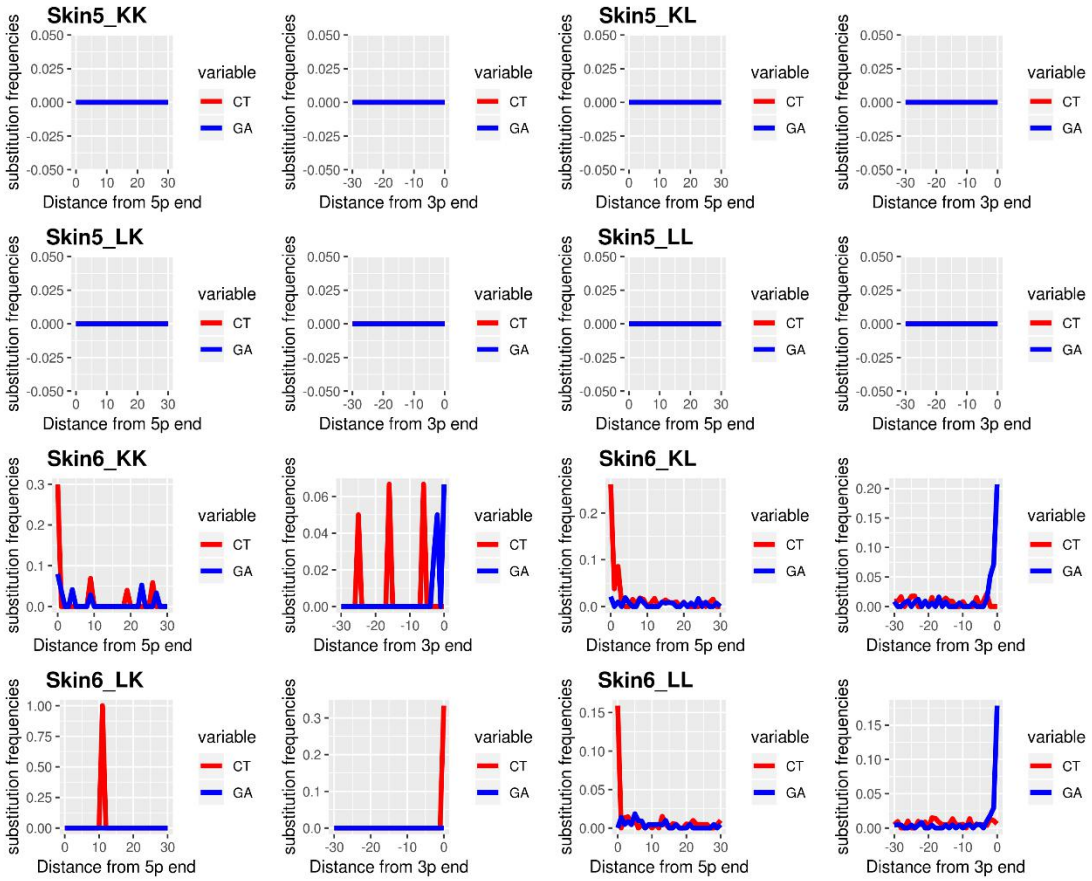
Supplementary Figure S5. C-T and G-A substitution frequencies for samples Skin-1 and Hair-1 and different DNA extraction methods. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



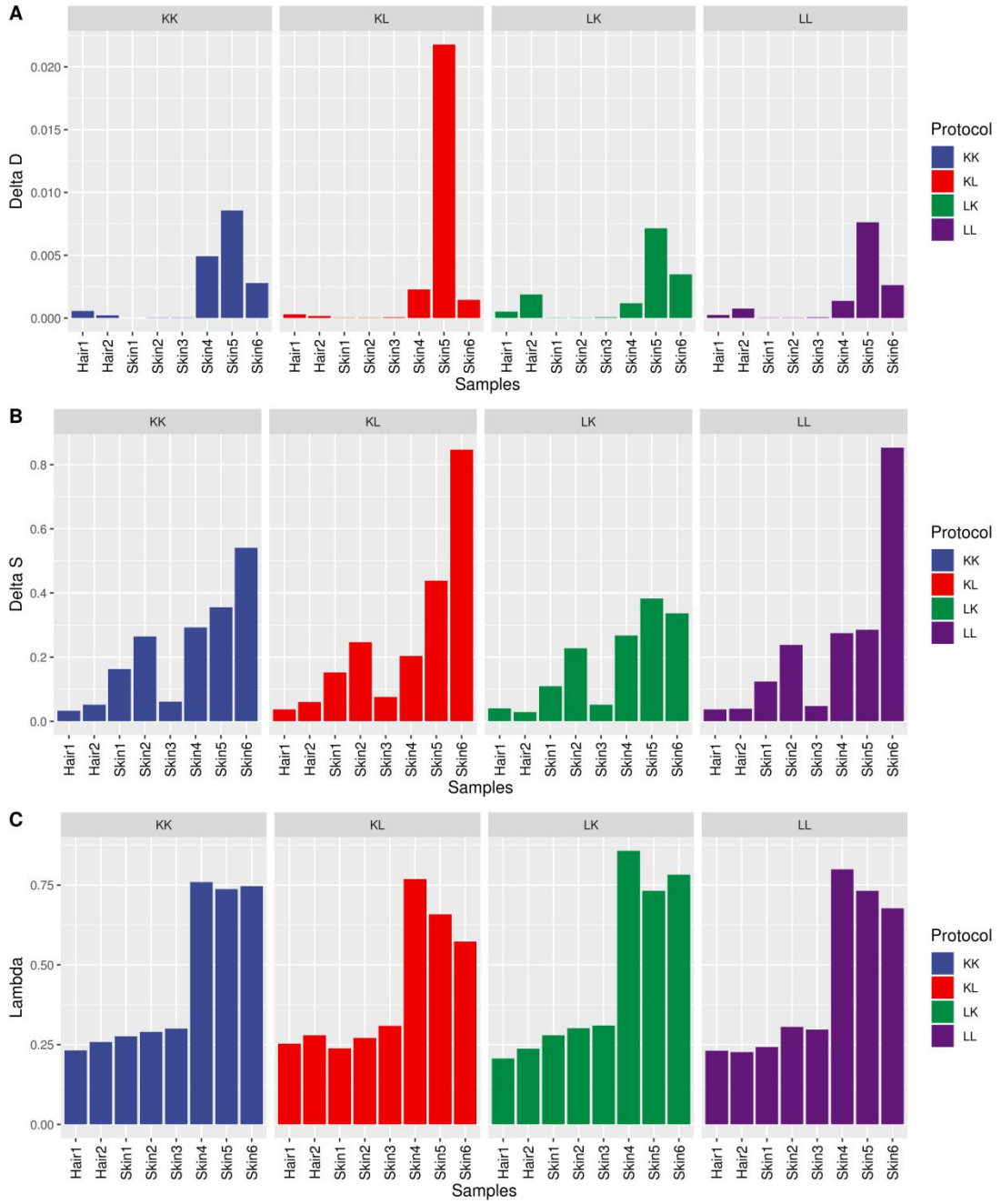
Supplementary Figure S5 (Continued). C-T and G-A substitution frequencies for samples Skin-2 and Hair-2 and different DNA extraction methods. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



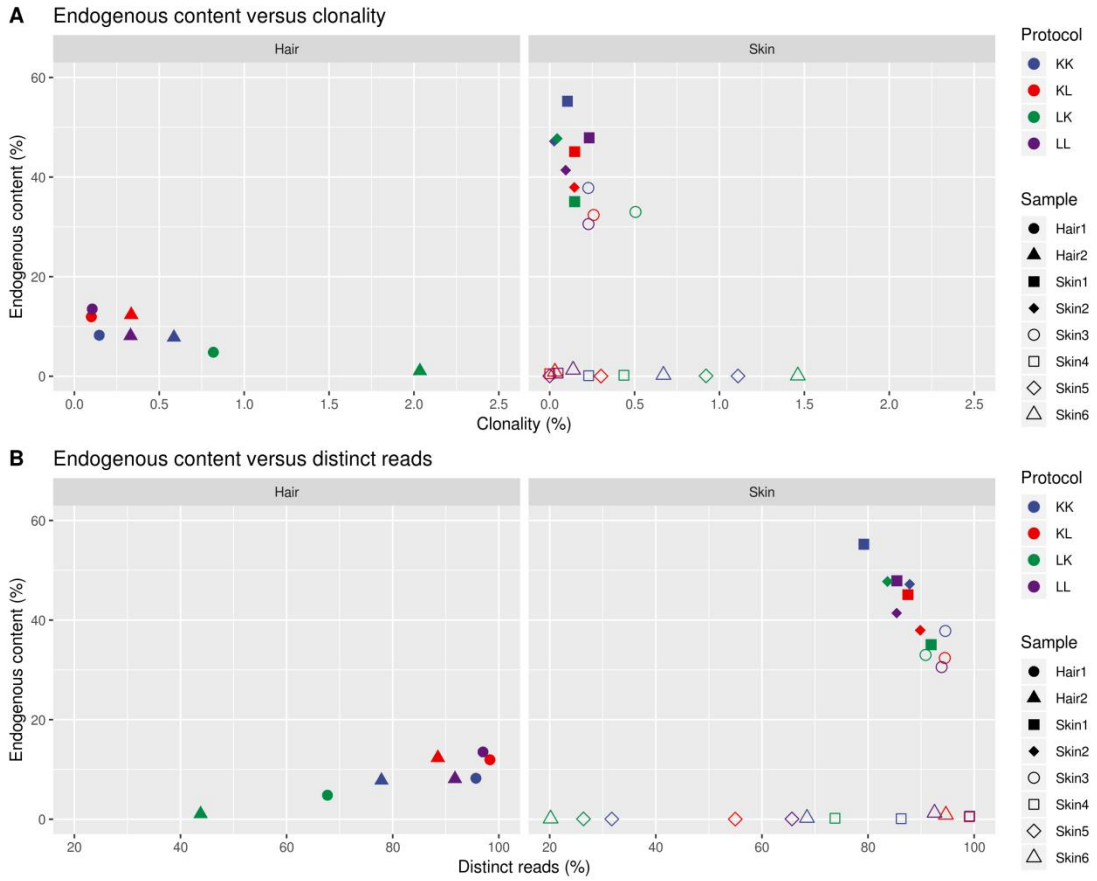
Supplementary Figure S5 (Continued). C-T and G-A substitution frequencies for samples Skin-3 and Skin-4 and different DNA extraction methods. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



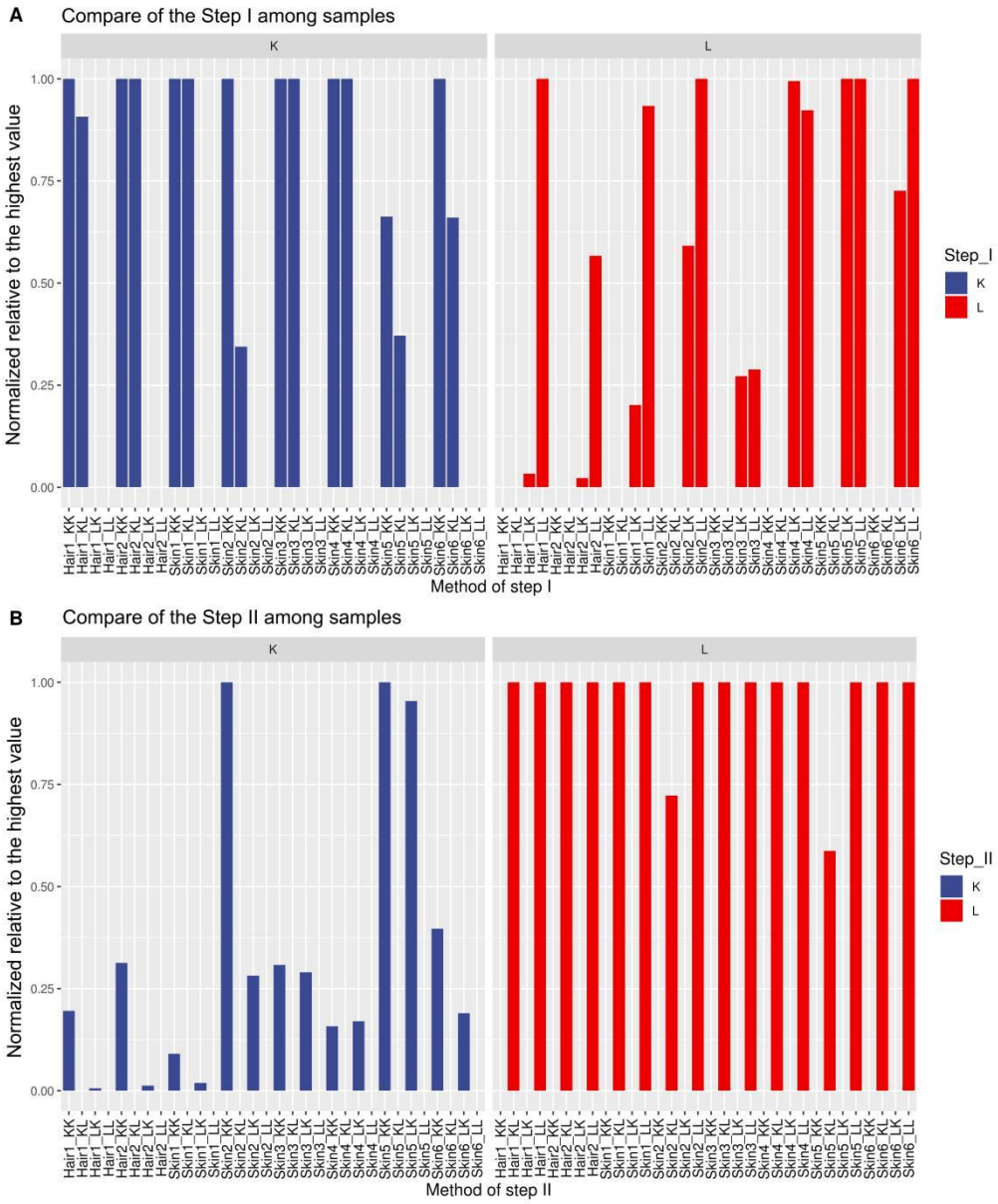
Supplementary Figure S5 (Continued). C-T and G-A substitution frequencies for samples Skin-5 and Skin-6 and different DNA extraction methods. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



Supplementary Figure S6. Bar plots showing DNA damage parameters (A: Delta D; B: Delta S; C: Lambda) for each DNA extraction method and eight samples. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



Supplementary Figure S7. Scatter plots of (A) uniquely mapped endogenous content versus clonality and (B) distinct reads (complexity) for each DNA extraction method and eight samples. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).



Supplementary Figure S8. Bar plots comparing DNA extraction of (A) step I and (B) step II as indicated by proportion of normalized reads relative to most reads for two DNA extraction methods (K and L) and eight samples. Abbreviation: KK (both buffers from commercial kit), KL (extraction buffer from kit, binding buffer laboratory-made), LK (extraction buffer laboratory-made, binding buffer from kit), and LL (both buffers laboratory-made).

Supplementary Table S1. Extraction and library quantification statistics.

Supplementary Table S2. Shotgun library sequencing and damage analysis statistics.

Supplementary Tables S1-S2 are listed as a separate file due to their large size.