## Supplementary legends

### Supplementary Table S1 to S6

Supplementary Table S1. the results of bioinformatics analysis for mining species-specific sequences in genomes of *Cervus nippon*, *Cervus elaphus*, and *Rangifer tarandus*.

Supplementary Table S2. primers and PCR amplification program of each species-specific target sequence.

Supplementary Table S3. sample information of fresh animal samples.

Supplementary Table S4. off targets prediction of Cn_target1 in the genomes of *Cervus nippon*, *Cervus elaphus*, and *Rangifer tarandus*.

Supplementary Table S5. off targets prediction of Cn_target2 in the genomes of *Cervus nippon*, *Cervus elaphus*, and *Rangifer tarandus*.

Supplementary Table S6. sample information of commercially available antler products.

### Supplementary Figure S1 to S2

Supplementary Figure S1. AGE accurately and specifically identified *Cervus elaphus*, *Cervus nippon*, and *Rangifer tarandus* in fresh animal samples with Sanger sequencing platform.

Supplementary Figure S2. through the CRISPR-Cas12a system, AGE identified *Cervus elaphus*, *Cervus nippon*, and *Rangifer tarandus* in fresh animal samples based on Ce_target1 and -2, Cn_target1 and -2 and Rt_target1 and -2.

### Supplementary File S1

Supplementary File S1. Codes used in the bioinformatics analysis steps.

# Supplementary Information

**Table S1. The results of bioinformatics analysis for mining species-specific sequences in genomes of *Cervus nippon*, *Cervus elaphus*, and *Rangifer tarandus.***

| Species | Genome | Fragment sequence | Candidate fragment sequences | Specific sequences |
|---|---|---|---|---|
| *Cervus nippon* | nuclear | 3,447,186,722 | 158,287,562 | 7670053 |
| | mitochondrion | 16,410 | 645 | 87 |
| *Cervus elaphus* | nuclear | 2,090,198,320 | 94,378,541 | 570878 |
| | mitochondrion | 16,332 | 657 | 103 |
| *Rangifer tarandus* | nuclear | 2,108,086,750 | 95,321,027 | 8446736 |
| | mitochondrion | 16,338 | 700 | 377 |

**Table S2. Primers and PCR amplification program of each species-specific target sequence.**

| Specific target sequence | Primer | Primer sequence (5'→3') | PCR amplification program |
|---|---|---|---|
| Ce_target1 | Ce1F | GCATTAGAGGACTGCTGCC | 95℃ 5min<br>94℃ 30s, 60℃ 30s, 72℃ 30s, 35 cycles<br>72℃ 7min |
| | Ce1R | TGGAGTTCTAAGCTGGTCAC | |
| Ce_target2 | Ce2F | CCTCAGTGTACTGTTCAGAGAT | 95℃ 5min<br>94℃ 30s, 56℃ 30s, 72℃ 30s, 35 cycles<br>72℃ 7min |
| | Ce2R | CATACAGAGTCCTCATGAATGAG | |
| Ce_target3 | Ce3F | CTACTCCAACCTATTGCAGA | 95℃ 5min<br>94℃ 30s, 58℃ 30s, 72℃ 1 min, 35 cycles<br>72℃ 7min |
| | Ce3R | TTTGTGGAGGGATGCTTGAT | |
| Cn_target1 | Cn1F | AGCTGTAACCTGATGCTGATG | 95℃ 5min<br>94℃ 30s, 56℃ 30s, 72℃ 30s, 35 cycles<br>72℃ 7min |
| | Cn1R | GCAACTTGTTTTCCTACCATT | |

| | | | |
|---|---|---|---|
| | | TGC | |
| Cn_target2 | Cn2F | AGACTTGAGGAAAGAATGTTCTTTC | 95℃ 5min<br>94℃ 30s, 54℃ 30s, 72℃ 30s, 35 cycles |
| | Cn2R | CCAGTGTGAACAAGCATTTACTTAC | 72℃ 7min |
| Cn_target3 | Cn3F | TACAGCTTTCTACTCAACAC | 95℃ 5min<br>94℃ 30s, 56℃ 30s, 72℃ 90s, 35 cycles |
| | Cn3R | CCACAGTTATGTGTGAGCAT | 72℃ 7min |
| Rt_target1 | Rt1F | AGGCACGTGACTGGTTTATTAGG | 95℃ 5min<br>94℃ 30s, 58℃ 30s, 72℃ 30s, 35 cycles |
| | Rt1R | CAGTCCTGCAGCTAAACCTCTG | 72℃ 7min |
| Rt_target2 | Rt2F | GTGCTTGCTTCTTGGCATCC | 95℃ 5min |
| | Rt2R | CAGGCAGATTCTTTAGCACTGC | 94℃ 30s, 58℃ 30s, 72℃ 30s, 35 cycles<br>72℃ 7min |
| Rt_target3 | Rt3F | GGTCAACAAATCATAAAGATATTGG | 94℃ 1min<br>94℃ 1min, 45℃ 90s, 72℃ 90s, 5 cycles |
| | Rt3R | TAAACTTCAGGGTGACCAAAAAATCA | 94℃ 1min, 50℃ 90s, 72℃ 1min, 35 cycles<br>72℃ 5min |
| Pa_target1 | Pa1F | AATATAAAACCCCCTGCCATTTCACA | 95℃ 5min<br>94℃ 30s, 58℃ 30s, 72℃ 30s, 35 cycles |
| | Pa1R | GTAATTCCGGCTGCTAGTACAGG | 72℃ 7min |

**Table S3. Sample information of fresh animal samples.**

| No. | Sample type | Market label | Locality | Barcode ID |
|---|---|---|---|---|
| 1 | Fresh sample | Red deer antler | Changchun, Jilin | *Cervus elaphus* |
| 2 | Fresh sample | Red deer antler | Changchun, Jilin | *Cervus elaphus* |
| 3 | Fresh sample | Red deer meat | Changchun, Jilin | *Cervus elaphus* |

| | | | | |
|---|---|---|---|---|
| 4 | Fresh sample | Red deer meat | Changchun, Jilin | *Cervus elaphus* |
| 5 | Fresh sample | Sika deer antler | Fushun, Liaoning | *Cervus nippon* |
| 6 | Fresh sample | Sika deer antler | Changchun, Jilin | *Cervus nippon* |
| 7 | Fresh sample | Sika deer meat | Changchun, Jilin | *Cervus nippon* |
| 8 | Fresh sample | Reindeer antler | Changchun, Jilin | *Rangifer tarandus* |
| 9 | Fresh sample | Reindeer antler | Changchun, Jilin | *Rangifer tarandus* |
| 10 | Fresh sample | Reindeer antler | Changchun, Jilin | *Rangifer tarandus* |
| 11 | Fresh sample | Reindeer antler | Changchun, Jilin | *Rangifer tarandus* |

**Table S4. Off targets prediction of Cn_target1 in the genomes of *Cervus nippon*, *Cervus elaphus*, and *Rangifer tarandus.***

| Species | Number of mismatches | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| *Cervus nippon* | 0 | 1 | 0 | 0 | 3 | 48 |
| *Cervus elaphus* | 0 | 0 | 0 | 0 | 4 | 63 |
| *Rangifer tarandus* | 0 | 0 | 0 | 0 | 7 | 65 |

**Table S5. Off targets prediction of Cn_target2 in the genomes of *Cervus nippon*, *Cervus elaphus*, and *Rangifer tarandus.***

| Species | Number of mismatches | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| *Cervus nippon* | 1 | 0 | 0 | 0 | 3 | 35 |
| *Cervus elaphus* | 0 | 0 | 0 | 0 | 4 | 34 |
| *Rangifer tarandus* | 0 | 0 | 0 | 0 | 3 | 38 |

**Table S6. Sample information of commercially available antler products.**

| | | | | |
|---|---|---|---|---|
| 1 | commercially available products | Antler powder | Hebei | *Cervus nippon* |
| 2 | commercially available products | Sika deer antler powder | Kunming, Yunnan | *Rangifer tarandus* |
| 3 | commercially available products | Sika deer antler powder | Shuangyang, Jilin | *Rangifer tarandus* |
| 4 | commercially available products | Antler powder | Yinchuan, Ningxia | *Cervus elaphus* |
| 5 | commercially available products | Antler tablet | Nanjing, Jiangsu | *Cervus elaphus* |
| 6 | commercially available products | Antler powder | Nanjing, Jiangsu | *Cervus elaphus* |
| 7 | commercially available products | Red deer antler tablet | Luoyang,Henan | *Rangifer tarandus* |
| 8 | commercially available products | Antler tablet | Bozhou, Anhui | *Rangifer tarandus* |
| 9 | commercially available products | Antler tablet | Bozhou, Anhui | *Rangifer tarandus* |
| 10 | commercially available products | Antler powder | Hebei | *Cervus nippon* |
| 11 | commercially available products | Antler powder | Liaoning | *Rangifer tarandus* |
| 12 | commercially available products | Antler tablet | Bozhou, Anhui | *Rangifer tarandus* |
| 13 | commercially available products | Antler powder | Bozhou, Anhui | *Rangifer tarandus* |
| 14 | commercially available products | Red deer antler powder | Beijing | *Cervus elaphus* |

| | | | | |
|---|---|---|---|---|
| 15 | commercially available products | Sika deer antler tablet | Changchun, Jilin | *Cervus elaphus* |
| 16 | commercially available products | Red deer antler powder | Wushun, Liaoning | *Cervus elaphus* |
| 17 | commercially available products | Antler powder | Wuhan, Hubei | *Cervus elaphus* |
| 18 | commercially available products | Antler powder | Kunming, Yunnan | *Rangifer tarandus* |
| 19 | commercially available products | Antler powder | Changchun, Jilin | *Cervus nippon* |
| 20 | commercially available products | Antler tablet | Bozhou, Anhui | *Rangifer tarandus* |
| 21 | commercially available products | Antler tablet | Dunhua, Jilin | *Rangifer tarandus* |
| 22 | commercially available products | Antler powder | Luzhou, Sichuan | *Rangifer tarandus* |
| 23 | commercially available products | Antler tablet | Liwan, Guangdong | *Rangifer tarandus* |
| 24 | commercially available products | Sika deer antler powder | Tonghua | *Cervus elaphus* |
| 25 | commercially available products | Antler tablet | Kunming, Yunnan | *Rangifer tarandus* |
| 26 | commercially available products | Antler tablet | Jiangmen, Guangdong | *Rangifer tarandus* |
| 27 | commercially available products | Antler powder | Kunming, Yunnan | *Rangifer tarandus* |
| 28 | commercially available products | Antler tablet | Wenshan, Yunnan | *Cervus elaphus* |
| 29 | commercially | Antler tablet | Daxing, Beijing | *Cervus elaphus* |

| | | | | |
|---|---|---|---|---|
| | available products | | | |
| 30 | commercially available products | Antler tablet | Shanghai | *Cervus elaphus* |
| 31 | commercially available products | Antler powder | Changchun, Jilin | *Rangifer tarandus* |
| 32 | commercially available products | Antler powder | Kunming, Yunnan | *Rangifer tarandus* |
| 33 | commercially available products | Antler powder | Changchun, Jilin | *Rangifer tarandus* |
| 34 | commercially available products | Red deer antler | Wushun, Liaoning | *Cervus elaphus* |
| 35 | commercially available products | Red deer antler | Wushun, Liaoning | *Cervus elaphus* |

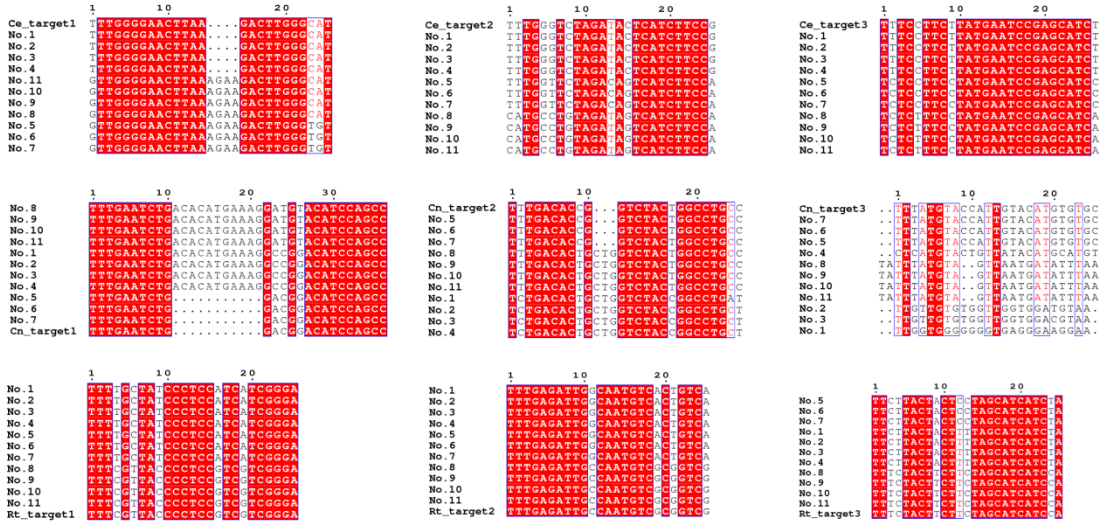


**Figure S1 AGE accurately and specifically identified *Cervus elaphus*, *Cervus nippon*, and *Rangifer tarandus* in fresh animal samples with Sanger sequencing platform. No.1, No.2, No.3, and No.4: fresh *Cervus elaphus* samples; No.5, No.6, and No.7: fresh *Cervus nippon* samples; No.8, No.9, No.10, and No.11: fresh *Rangifer tarandus* samples.**

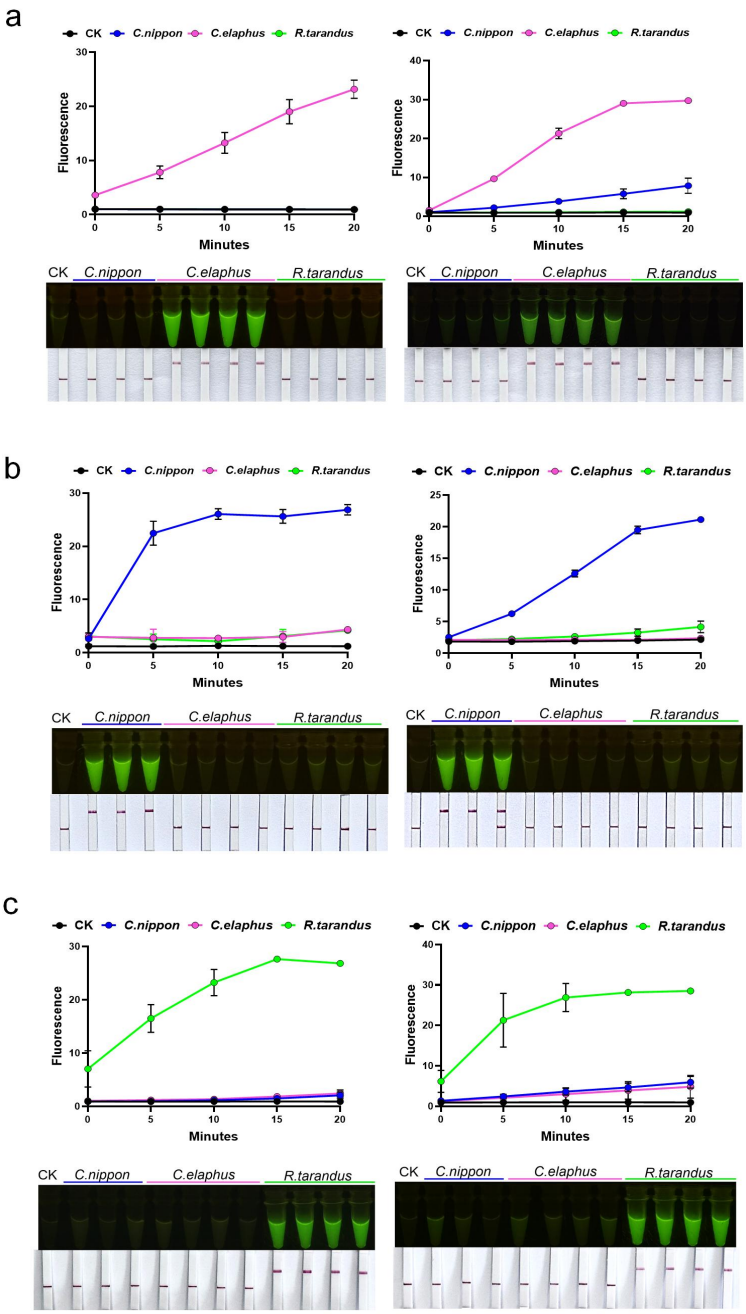



**Figure S2 Through the CRISPR-Cas12a system, AGE identified *Cervus elaphus*, *Cervus nippon*, and *Rangifer tarandus* in fresh animal samples based on Ce_target1, 2, Cn_target1, 2 and Rt_target1, 2. (a) The identification of *Cervus elaphus* in fresh animal samples using Ce_target1, 2. CK: nuclease-free water. (b) The identification of *Cervus nippon* in fresh animal samples using Cn_target1, 2. CK: nuclease-free water. (a) The identification of *Rangifer tarandus* in fresh animal samples using Rt_target1, 2. CK: nuclease-free water.**

**File S1. Codes used in the bioinformatics analysis steps.**

**#The genomes were cut into K bp fragments using Jellyfish to generate kmers.**

**/path/jellyfish count -C -m k -s 3G -t 6 -o x_kmer x_genome.fa # k, length of mer; x, one species**

**/path/jellyfish dump -c -t x_kmer > x_kmer.stat**

**#The kmers with PAM (TTTV starting or VAAA ending) sequences were extracted and compared to their genomes.**

**awk '$1~/^TTTA/||$1~/^TTTG/||$1~/^TTTC/||$1~/GAAA$/||$1~/CAAA$/||$1~/TAAA$/{print ">"NR"_"$2"\n"$1}' x_kmer.stat > x_kmer_PAM.fa**

**/path/bowtie-1.1.0/bowtie-build x_genome.fa x_genome.fa**

**/path/bowtie-1.1.0/bowtie -f -v 0 -p 8 x_genome.fa x_kmer_PAM.fa x_kmer_PAM_ genome.bwt**

**#The candidate fragment sequences of one species were mapped to these of two other species and only candidate fragment sequences with sequences differences of three or more mismatches and all indels were retained.**

**sed '$!N;s/\n/\t/' x_kmer_PAM.fa|awk '$2~/^TTTA/||$2~/^TTTG/||$2~/^TTTC/{print $1"\n"substr($2,5,21)}' > x_kmer_PAM_21_TTT.fa**

**sed '$!N;s/\n/\t/' x_kmer_PAM.fa|awk '$2~/GAAA$/||$2~/CAAA$/||$2~/TAAA$/{print $1"\n"substr($2,1,21)}' > x_kmer_PAM_21_AAA.fa**

**/path/bowtie-1.1.0/bowtie-build x_kmer_PAM_21_AAA.fa x_kmer_PAM_21_AAA.fa**

**/path/bowtie-1.1.0/bowtie-build x_kmer_PAM_21_TTT.fa x_kmer_PAM_21_TTT.fa**

**/path/bowtie-1.1.0/bowtie -f -v 2 -p 8 --norc b_kmer_PAM_21_TTT.fa a_kmer_PAM_21_TTT.fa a_kmer_PAM_21_TTT_b.bwt #a, target species; b, other species**

**/path/bowtie-1.1.0/bowtie -f -v 2 -p 8 --norc b_kmer_PAM_21_AAA.fa a_kmer_PAM_21_AAA.fa a_kmer_PAM_21_AAA_b.bwt**

**cat a_kmer_PAM_21_TTT_b.bwt a_kmer_PAM_21_AAA_b.bwt |awk '{print ">" $1}' |sort -u |awk 'FNR==NR{a[$1]=$0;next}{print $0"\t"a[$1]}' - a_kmer_PAM.fa |sed '$!N;s/\n/\t/'| awk '$2!~/>/ {print$1"\n"$2}' > a_kmer_PAM_uniq.fa**