Supplementary Material

In situ labeling of DNA reveals inter-individual variation in nuclear DNA breakdown in hair and may be useful to predict success of forensic genotyping of hair

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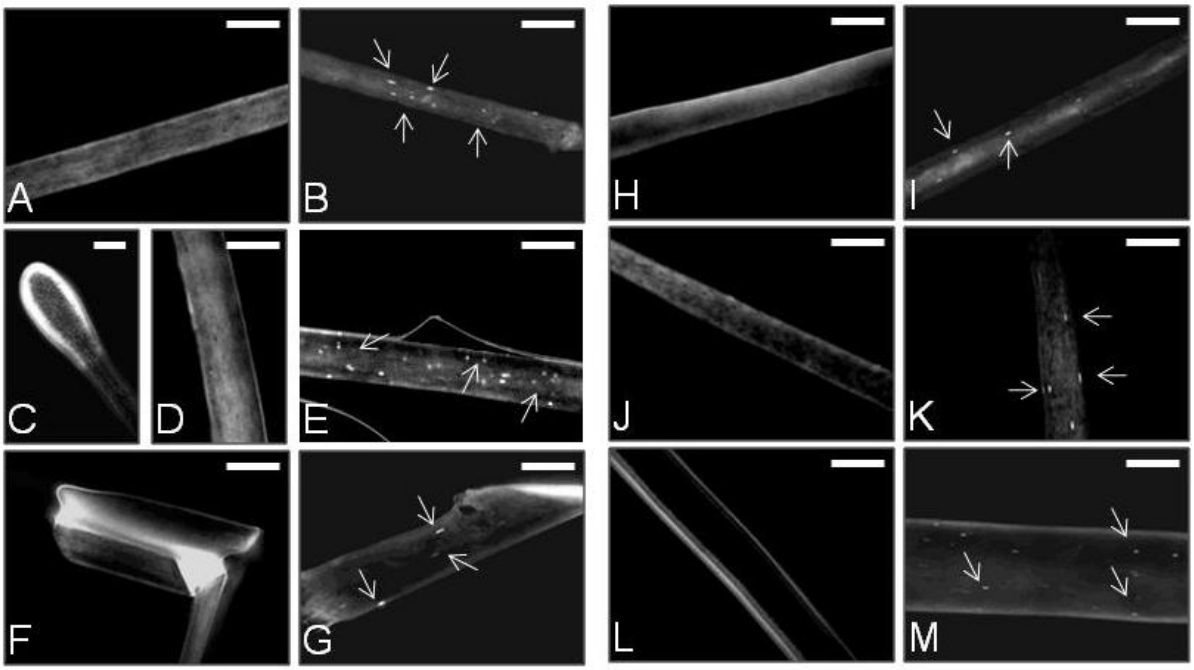
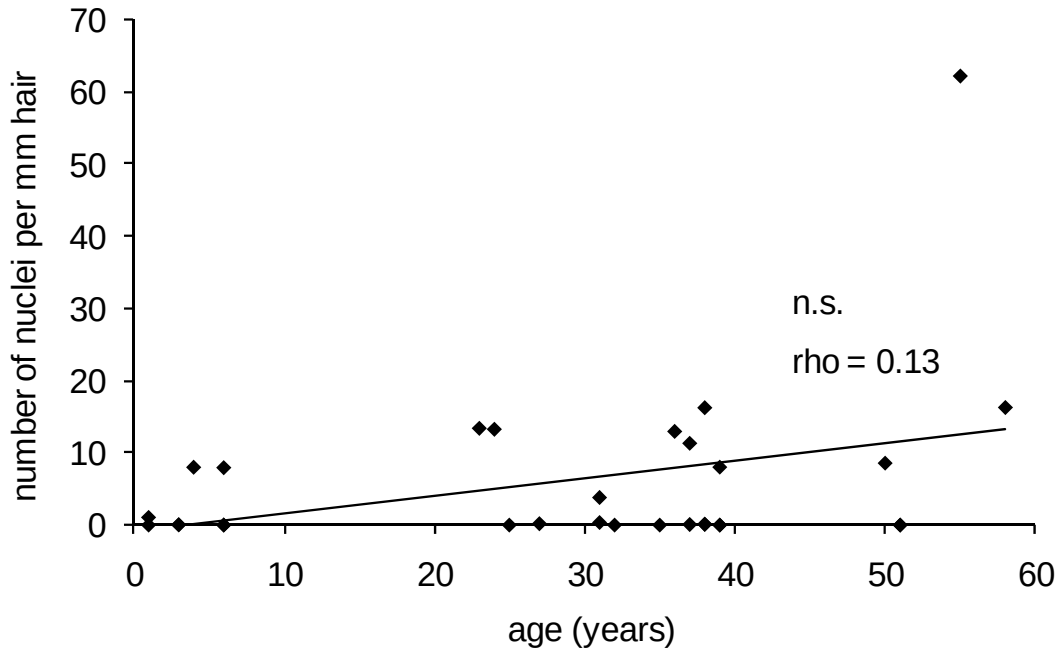
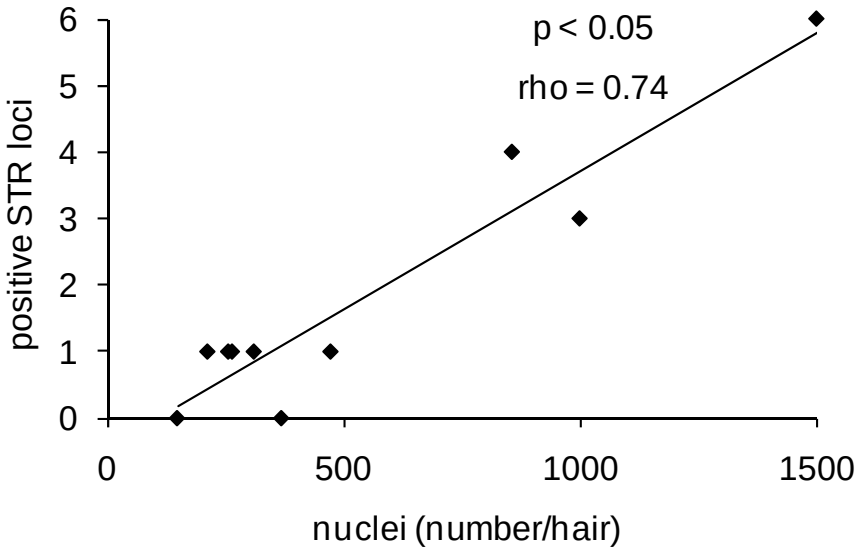


Suppl. Fig. S1 *In situ* labeling of nuclear DNA in hair from various body regions. Five volunteers donated hairs from the arm (A, B) and axilla (C-E) as well as beard hairs (F,G), eyebrows (H, I), lashes (J, K) and pubic hairs (L, M). The hairs were *in situ*-labeled for DNA with Hoechst 33258, and nuclei were counted under the microscope. Examples of hairs lacking DNA-positive nuclei (A, D, F, H, J, L) and of hairs containing Hoechst-positive nuclei (B, E, G, I, K, M) are shown. Panel C shows the hair bulb in which nuclei of living cells are labeled intensely. Bars = 100 μm



Suppl. Fig. S2 Lack of correlation between the number of *in situ*-labeled nuclei and the age of the donor of hairs. Hair from individuals of different age was *in situ*-labeled for DNA with Hoechst 33258, and nuclei were counted under the microscope. The frequency of nuclear remnants was plotted over the age of the donors. rho, Spearman's rank correlation coefficient. n.s., not significant



Suppl. Fig. S3 Correlation between the number of *in situ*-labeled nuclei and the number of typeable STR loci in hairs. STR typing was performed on DNA extracted from 11 hairs that contained more than 8 nuclei per mm. Hairs in which no nuclei could be detected by *in situ* labeling of DNA (Fig. 4c, nuclei (number per hair) = 0) were excluded from the correlation analysis. rho, Spearman's rank correlation coefficient

Supplementary Tables. Ct (cycle threshold) values used for DNA quantification

Suppl. Table S1. Standard curve for nuclear DNA

nDNA concentration (pg/µl)	Ct
$6.6*10^{3}$	9.57
$6.6*10^{2}$	13.03
$6.6*10^{1}$	16.62
6.6	20.39
$6.6*10^{-1}$	23.36
$6.6*10^{-2}$	26.12
$6.6*10^{-3}$	29.02

Standard curve: concentration (nDNA) = $7.530*e^{-Ct/1.415}$ (pg/µl)

Suppl. Table S2. Standard curve for mitochondrial DNA

mtDNA copy number (copies / µl)*	mtDNA concentration (pg/µl)**	Ct
$2*10^{9}$	$3.6*10^{4}$	8.85
$2*10^{8}$	$3.6*10^{3}$	12.43
$2*10^{7}$	$3.6*10^{2}$	15.66
$2*10^{6}$	$3.6*10^{1}$	18.63
$2*10^{5}$	3.6	22.54
$2*10^{4}$	$3.6*10^{-1}$	25.80
$2*10^{2}$	$3.6*10^{-3}$	31.93

* As described in the Materials and Methods section, a fragment of the mitochondrial genome was cloned into the pCR2.1-Topo plasmid. A dilution series of this cloned mtDNA fragment was used to generate the standard curve.
** Concentration of mitochondrial DNA corresponding to the number of copies (1 copy of mtDNA corresponding to $1.8*10^{-5}$ pg).
Standard curve: concentration (mtDNA) = $1.877*e^{-Ct/1.438}$ (pg/µl)

Suppl. Table S3. Comparison of nuclear DNA contents of white and pigmented hair

	white hair			pigmented hair		
Donor	Ct	nDNA conc. (pg/µl)	nDNA content/hair (ng/mg)	Ct	nDNA conc. (pg/µl)	nDNA content/hair (ng/mg)
1	20.71	3.33	0.13	20.79	3.15	0.13
2	16.93	48.09	1.92	17.5	32.15	1.29
3	17.64	29.12	1.16	18.6	14.78	0.59
4	22.21	1.15	0.05	21.61	1.76	0.07
5	17.55	31.03	1.24	17.29	37.29	1.49
6	17.66	28.71	1.15	18.18	19.89	0.80
7	21.42	2.02	0.08	22.54	0.91	0.04
8	21.74	1.61	0.06	22.57	0.89	0.04

DNA was extracted from 5 mg white and pigmented hair of each donor, purified as described in the Materials and Methods section and finally eluted in 200 µl buffer. The concentration (conc.) of nDNA in this solution was determined by qPCR. The nDNA contents of hair correspond to the values shown in Figure 2b.

Suppl. Table S4. Nuclear DNA content of hair (batch of 5 mg)

Sample	Ct	nDNA conc. (pg/µl)	nDNA content/hair (ng/mg)
1	20.88	2.95	0.12
2	21.75	1.60	0.06
3	21.16	2.42	0.10
4	22.52	0.93	0.04
5	19.55	7.55	0.30
6	22.75	0.79	0.03
7	16.66	58.20	2.33
8	18.46	16.32	0.65
9	21.47	1.95	0.08
10	22.52	0.93	0.04
11	17.75	26.94	1.08
12	22.49	0.95	0.04
13	17.42	34.02	1.36
14	17.82	25.64	1.03
15	20.16	4.91	0.20
16	20.75	3.24	0.13
17	20.73	3.28	0.13
18	21.97	1.37	0.05
19	19.87	6.03	0.24
20	18.85	12.39	0.50
21	22.52	0.93	0.04
22	16.92	48.43	1.94
23	22.02	1.32	0.05
24	19.86	6.07	0.24
25	21.05	2.62	0.10
26	21.54	1.85	0.07
27	18.81	12.74	0.51
28	21.10	2.53	0.10
29	19.85	6.11	0.24
30	19.18	9.81	0.39

DNA was extracted from 5 mg hair of each donor, purified as described in the Materials and Methods section and finally eluted in 200 µl buffer. The nDNA contents of hair correspond to the values shown in Figures 3a and 3c.

Suppl. Table S5. Mitochondrial DNA content of hair (batch of 5 mg)

Sample	Ct	mtDNA conc. (pg/µl)	mtDNA content/hair (pg/mg)
1	25.46	0.38	15
2	25.98	0.27	11
3	20.90	9.16	367
4	32.03	0.00	0
5	21.93	4.48	179
6	30.37	0.01	1
7	26.86	0.15	6
8	24.23	0.90	36
9	22.77	2.49	100
10	24.25	0.89	36
11	25.91	0.28	11
12	26.71	0.16	6
13	21.51	5.99	240
14	23.97	1.08	43
15	24.75	0.63	25
16	23.72	1.29	52
17	23.11	1.97	79
18	23.13	1.94	78
19	24.15	0.96	38
20	25.19	0.46	19
21	26.02	0.26	10
22	24.34	0.84	33
23	24.04	1.03	41
24	24.54	0.73	29
25	25.14	0.48	19
26	25.35	0.41	17
27	24.18	0.94	37
28	23.67	1.33	53
29	24.28	0.87	35
30	28.55	0.04	2

DNA was extracted from 5 mg hair of each donor, purified as described in the Materials and Methods section and finally eluted in 200 µl buffer. The mtDNA contents of hair correspond to the values shown in Figures 3b and 3c.

Suppl. Table S6. Nuclear DNA content of single hairs (length 2 cm)

Sample	Ct	nDNA conc. (pg/µl)	nDNA content/single hair (pg)
1	27.27	0.03	6.5
2	26.31	0.06	12.7
3	26.71	0.05	9.6
4	27.01	0.04	7.8
5	24.90	0.17	34.5
6	27.20	0.03	6.8
7	26.83	0.04	8.8
8	23.75	0.39	77.7
9	24.53	0.22	44.8
10	25.56	0.11	21.6
11	27.48	0.03	5.6
12	27.03	0.04	7.7
13	24.10	0.30	60.7
14	23.45	0.48	96.1
15	26.11	0.07	14.7
16	26.58	0.05	10.5
17	24.81	0.18	36.7
18	23.99	0.33	65.6
19	23.31	0.53	106.0
20	23.32	0.53	105.3
21	26.20	0.07	13.8
22	27.04	0.04	7.6
23	26.64	0.05	10.1
24	26.66	0.05	9.9
25	27.09	0.04	7.3
26	24.58	0.22	43.2
27	25.30	0.13	26.0
28	23.50	0.46	92.7
29	26.46	0.06	11.5
30	26.82	0.04	8.9
31	26.90	0.04	8.4
32	25.81	0.09	18.1
33	28.03	0.02	3.8
34	25.87	0.09	17.4
35	27.27	0.03	6.5
36	26.82	0.04	8.9
37	25.56	0.11	21.6
38	25.97	0.08	16.2
39	27.22	0.03	6.7
40	27.18	0.03	6.9

DNA was extracted from hair samples of 2 cm length, purified as described in the Materials and Methods section and finally eluted in 200 µl buffer. The nDNA contents of hairs correspond to the values shown in Figure 4a.

Suppl. Table S7. Mitochondrial DNA content of single hairs (length 2 cm)

Sample	Ct	mtDNA conc. (pg/µl)	mtDNA content/single hair (pg)
1	29.25	0.028	5.5
2	28.02	0.065	12.9
3	28.86	0.036	7.2
4	32.75	0.002	0.5
5	27.85	0.073	14.6
6	28.94	0.034	6.8
7	32.56	0.003	0.5
8	31.97	0.004	0.8
9	29.10	0.031	6.1
10	28.63	0.042	8.5
11	27.04	0.128	25.6
12	28.91	0.035	7.0
13	29.48	0.023	4.7
14	29.52	0.023	4.6
15	32.02	0.004	0.8
16	34.18	0.001	0.2
17	27.27	0.109	21.8
18	29.28	0.027	5.4
19	26.69	0.163	32.6
20	29.87	0.018	3.6
21	29.57	0.022	4.4
22	31.30	0.007	1.3
23	27.62	0.085	17.1
24	30.57	0.011	2.2
25	29.22	0.028	5.6
26	31.15	0.007	1.5
27	29.36	0.025	5.1
28	29.67	0.021	4.1
29	30.77	0.010	1.9
30	29.47	0.024	4.7
31	30.91	0.009	1.7
32	28.47	0.047	9.5
33	29.12	0.030	6.0
34	29.90	0.017	3.5
35	31.98	0.004	0.8
36	29.17	0.029	5.8
37	30.78	0.009	1.9
38	31.00	0.008	1.6
39	31.63	0.005	1.1
40	29.51	0.023	4.6

DNA was extracted from hair samples of 2 cm length, purified as described in the Materials and Methods section and finally eluted in 200 µl buffer. The mtDNA contents of hairs correspond to the values shown in Figure 4b.