SUPPLEMENTARY INFORMATION for "Two-step multiplex PCR improves the speed and accuracy of genotyping using DNA from noninvasive and museum samples" by

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Proportion of successful PCRs (black circles) and PCRs with allelic dropout (white circles) according to initial template DNA amount in the PCR reaction, for four primate taxa.

a) Mountain gorillas b) Western lowland gorillas c) Western chimpanzees and d) Black and white colobus. Data from 0-2500 pg/rxn DNA shown only.

Supplementary Table 1: Primers used in study

Locus	T_a (^oC) singleplex PCR	Primer Sequences[a] (5' – 3')	Reference[b]
Mountain, Western & Cross River Gorillas (*G.b. berengei, G.g. gorilla & G.g. diehli**)			
D1s550*	60	F: CCTGTTGCCACCTACAAAAG R: TAAGTTAGTTCAAATTCATCAGTGC R: $_{nest:}$ TCAAATTCATCAGTGCTATGTG	Bradley et al., 2000
D1s2130	57	F: GAACTCAGGCAGCTTTCTCA R: TAATTGTGTGAGCCAGTTTCC	Morin et al. 1998
D2s1326	57	F: GAACTCAGGCAGCTTTCTCA R: TAATTGTGTGAGCCAGTTTCC	Bradley et al., 2000
D3s2459	55	F: CTGGTTTGGGTCTGTTATGG R: AGGGACTTAGAAAGATAGCAGG R: $_{nest:}$ TTCAAACAATGTAATGGGAAGC	Zhang et al. 2001
D4s1627	55	F: AGCATTAGCATTTGTCCTGG R: GACTAACCTGACTCCCCCTC R: $_{nest:}$ CCC TCC TGA AAA ACA CAA AC	Bradley et al., 2000
D5s1457*	55	F: TAG GTT CTG GGC ATG TCT GT R: TGC TTG GCA CAC TTC AGG	CHLC
D5s1470*	58	F: CATGCACAGTGTGTTTACTGG R: TAGGATTTTACTATATTCCCCAGG $R_{nest:}$ CTGGAATATGTATGTGTAGGTAGG	Bradley et al., 2000
D6s474	55	F: TGTACAAAAGCCTATTTAGTCAGG R: TCATGTGAGCCAATTCCTCT	Bradley et al. 2000
D6s1056	58	F: ACAAGAACAGCATGGGGTAA R: CCTGGATCATGAATTGCTAT $R_{nest:}$ GTACATGTGTATGTCTCTGTGTC	Morin et al. 1998
D7s817*	60	F: TAAATCTCTTTATGGCTGACTG R: GGG TTC TGC AGA GAA ACA GA	Bradley et al., 2000
D7s2204	59	F: TCATGACAAAACAGAAATTAAGTG R: AGTAAATGGAATTGCTTGTTACC $R_{nest:}$ GTTTCAGTGTAGAGTTCCTTTATTC	Bradley et al., 2000
D8s1106*	59	F: TTG TTT ACC CCT GCA TCA CT R: TTC TCA GAA TTG CTC ATA GTG C	Bradley et al., 2000
D10s1432*	55	F: CAGTGGACACTAAACACAATCC R: TAGATTATCTAAATGGTGGATTTCC $R_{nest:}$ CTT TTG AGT ATG GAG AAC CCC	Bradley et al., 2000
D14s306	55	F: AAAGCTACATCCAAATTAGGTAGG R: TGACAAAGAAACTAAAATGTCCC	Morin et al. 1998
D16s2624*	58	F: TGAGGCAATTTGTTACAGAGC R: TAATGTACCTGGTACCAAAAACA	Bradley et al., 2000
vWf*	57	F: CCCTAGTGGATGATAAGAATAATC R: GGACAGATGATAAATACATAGGATGGATGG $R_{nest:}$ GGACAGATGATAAATACATAGGATG	Bradley et al., 2000
Western Chimpanzees *(P.t. verus)*			
D1s1622	59	F: CCCTCTGTCTCCAGCTGTAA R: TCACCCTCACATGATGCC $R_{nest:}$ CCTCACATGATGCCCTTTT	Morin et al. 1998
D1s1656	58	F: GTGTTGCTCAAGGGTCAACT R: GAGAAATAGAATCACTAGGGAACC $R_{nest:}$ AAATAGAATCACTAGGGAACCAAA	Morin et al. 1998
D2s1326	58	F: AGACAGTCAAGAATAACTGCCC R2: AGGGAATTCTCTGAGCTAATAC $R_{nest:}$ GGAATTCTTCTGAGCTAATACACAG	Bradley et al., 2000
D2s1329	60	F2: ACCGTTCTCAAATACCAGGAATC R4: CCTGGGTTCTTAATTTAACCATAATTC $R_{nest:}$ GGGTTCTTAATTTAGCCATAATTCTTAC	Bradley et al., 2000
D3s2459	59	F: CTGGTTTGGGTCTGTTATGG R: AGGGACTTAGAAAGATAGCAGG $R_{nest:}$ GGGACTTAGAAAGATAGCAGGATT	Zhang et al. 2001
D3s3038	58	F: CATCTTTCTTTTCCTGTTCCC R: GATACCATATTCAACATGAAGAGG $R_{nest:}$ CCATATTCAACATGAAGAGGAAA	CHLC
D4s1627	59	F: AGCATTAGCATTTGTCCTGG	Bradley et al., 2000

		R: GACTAACCTGACTCCCCCTC $R_{nest:}$ CTAACCTGACTCCCTCTCCTG	
D5s1457	59	F: TAGGTTCTGGGCATGTCTGT R: TGCTTGGCACACTTCAGG $R_{nest:}$ TTGGCACACTTCAGGGG	CHLC
D5s1470	64	F: CATGCACAGTGTGTTTACTGG R: TAGGATTTTACTATATTCCCCAGG $R_{nest:}$ **GTTTCTT**TATATTCCCCAGGCTGGAG[§]	Bradley et al., 2000
D6s1056	58	F: ACAAGAACAGCATGGGGTAA R: CCTGGATCATGAATTGCTAT $R_{nest:}$ CCTGGATCATGAATTGCTATATG	CHLC
D7s2204	59	F: TCATGACAAAACAGAAATTAAGTG R4: GTTCACTGTAGAGTTCCTTTATGC $R_{nest:}$ TTCACTGTAGAGTTCCTTTATGCTATTT	Bradley et al., 2000
D7s817	54	F2: TAAATCTCTTTATGGCTGACTG R: GGGTTCTGCAGAGAAACAGA $R_{nest:}$ GCAGAGAAACAGAACCAGTAG	Bradley et al., 2000
D9s910	58	F: AAGTCAGTTAGCTGAAGGTTGC R: TATATGAAGTGCTTAGAAAAAGTGC $R_{nest:}$ TGAAGTGCTTAGAAAAGTGCTG	Bradley et al., 2000
D10s676	58	F: GAGAACAGACCCCCAAATCT R: ATTTCAGTTTTACTATGTGCATGC $R_{nest:}$ TGCATGCACAGATCTATCTATCTATC	CHLC
D11s2002	57	F: CATGGCCCTTCTTTTCATAG R2: AGTGTGACCACCACACCAGC $R_{nest:}$ CACCACACCAGCCAGTTAT	Bradley et al., 2000
D12s66	57	F: TCATTTAAGCATTTGAGGGAA R: AGACTTCAAAACAGACACTT $R_{nest:}$ ACTTCAAAACAGACACTTTGGTC	Bradley et al., 2000
D14s306	57	F: AAAGCTACATCCAAATTAGGTAGG R: TGACAAAGAAACTAAAATGTCCC $R_{nest:}$ CAAAGAAACTAAAATGTCCCAAAT	CHLC
D16s2624	58	F: TGAGGCAATTTGTTACAGAGC R: TAATGTACCTGGTACCAAAAACA $R_{nest:}$ CCTGGTACCAAAAACAAAAGG	Bradley et al., 2000
D18s536	58	F: ATTATCACTGGTGTTAGTCCTCTG R: CACAGTTGTGTGAGCCAGTC $R_{nest:}$ TTGTGTGAGCCAGTCCTTC	CHLC
Black and White Colobus (*C. guereza)*			
D1s548	55	F: GAACTCATTGGCAAAAGGAA R:GCCTCTTTGTTGCAGTGATT	CHLC
D1s1665	57	F: TAAGTAAGTTCAAATTCATCAGTGC R:TTCCAAGCTTCACAGTGTCA R_{nest}:TCCACAAAGAAGGGAAAGGA	CHLC
D2s442	55	F: AAGGGAAGGAGCATAGCAAC R:CACCAATAGGATTAGATAGATTAGACA R_{nest}:GATTTGGTAGATAGACAGATGTGA	CHLC
D2s1326	56	F: AGACAGTCAAGAATAACTGCCC R:ATCTGCTGTGACCCAAAAGC R_{nest}:AGGGAATTCTCTGAGCTAATAC	CHLC
D4s2408	57	F: AATAAACTTCAACTTCAATTCATCC R:AGGTAAAGGCTCTTCTTGGC R_{nest}:ACCACATGTTCAGGTGCATT	CHLC
D6s474	60	F: TGTACAAAAGCCTATTTAGTCAGG R:TCATGTGAGCCAATTCCTCT	CHLC
D6s503	55	F: CGGTTCAGTCCATAGCAACT R:TCCAAACTTTAAAATGTCCTAACAA R_{nest}:AGCTCTGAGTTTCAGGGTAGC	CHLC
D6s1056	55	F: ACAAGAACAGCATGGGGTAA R:GCATGGTGGACTATTTGGAT R_{nest}:CCTGGATCATGAATTGCTAT	CHLC
D10s611	55	F: CATACAGGAAACTGTGTAGTGC R:TTACACAAAATATTTACATTCACTTATG R_{nest}:CTGTATTTATGTGTGTGGATGG	CHLC
D10s676	60	F: GAGAACAGACCCCCAAATCT R:TGCAATAAAATAGAAAATGTCAGA R_{nest}:ATTTCAGTTTTACTATGTGCATGC	CHLC

D10s1432	56	F: CAGTGGACACTAAACACAATCC R:AGCCTGGGTGACAGAGTGAG R_{nest}:TAGATTATCTAAATGGTGGATTTCC	CHLC
D11s2002	64	F: CATGGCCCTTCTTTTCATAG R:CCTCCCCCTAATGCTGGTAT R_{nest}:AGTGTGAGCCACCACACCAGC	CHLC
D12s372	55	F: TGGACCACAGGGTATCATCT R:AGGGCTTGGGTGAATTGAG R_{nest}:TCCAATGGAAAGAAATGGAG	CHLC
D13s321	57	F:TACCAACATGTTCATTGTAGATAGA R:CATACACCTGTGGACCCATC R_{nest}:GAGGCAGGAAGATTGTTGGA	CHLC
Fesps	57	F: GGAAGATGGAGTGGCTGTTA R:CTCCAGCCTGGCGAAAGAAT R_{nest}:CCTGGCGAAAGAATGAGACT	Polymeropoulos et al.,1991

[a] - F = forward primer (the forward primer used in the multiplex and singleplex PCRs are identical except that the forward primer used in the singleplex PCR is fluorescently labeled with either FAM, HEX or NED), R = reverse primer, R_{nest} = reverse nested primer (if used)
[b] - all nested primers were designed for this study, reference refers to forward and reverse primers only
* - loci used to amplify *G.g. diehli* historical DNA
§ - pig-tailed primer, pig-tail sequence in bold
CHLC = Cooperative Human Linkage Center

Supplementary Table 2. Number of PCR replicates necessary for various categories of initial DNA template amount, to ensure with high confidence (>99% certainty) that homozygote genotypes are authentic and not the result of allelic dropout, for each primate taxa

Template DNA amount (pg/rxn)	Mountain gorilla	Western lowland gorilla	Western chimpanzee	Black and white colobus
≤ 25	**5** (665)	**4** (90)	**4** (78)	**4** (108)
26-50	**4** (416)	**4** (438)	**4** (226)	**3** (139)
51-100	**3** (749)	**3** (760)	**3** (382)	**3** (216)
101-200	**2** (1693)	**2** (1131)	**2** (615)	**2** (596)
≥ 201	**2** (6233)	**2** (4989)	**2** (5286)	**2** (2784)

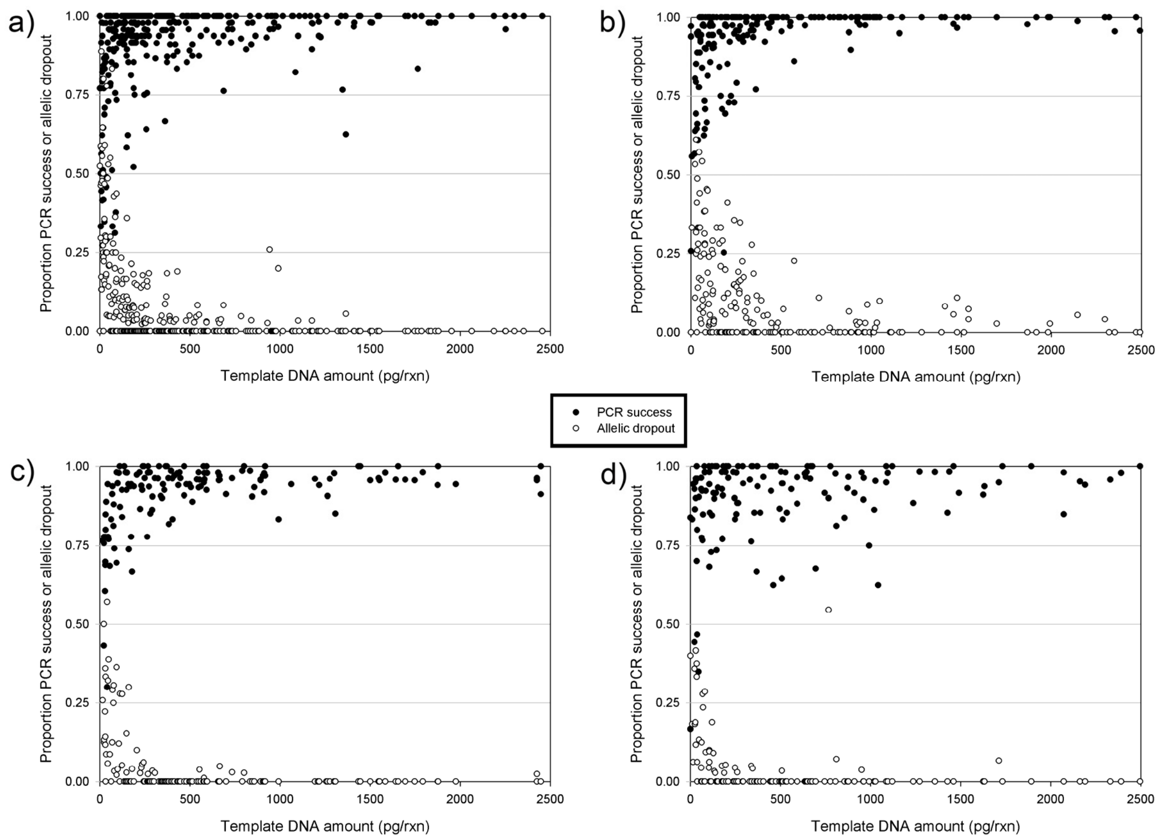
Number of replicates necessary given with total number heterozygote PCRs analyzed in parentheses.

Supplementary Table 3. Nested and unnested primers do not differ in their PCR success or allelic dropout rates.

Locus	Product size	Reverse primer	Proportion PCR success		Proportion allelic dropout	
			Mountain gorillas	Western lowland gorillas	Mountain gorillas	Western lowland gorillas
D1s2130	239-287	unnested	85% (800/936)	83% (639/771)	5% (35/732)	20% (79/405)
D2s1326	166-234	unnested	86% (875/1018)	93% (1173/1263)	8% (50/657)	11% (80/745)
D5s1457	104-140	unnested	98% (962/986)	99% (667/671)	4% (15/384)	2% (10/446)
D6s474	141-196	unnested	79% (619/780)	96% (707/734)	6% (50/808)	5% (33/620)
D7s817	118-150	unnested	94% (904/960)	nd	5% (28/591)	nd
D8s1106	137-157	unnested	93% (905/969)	90% (242/268)	11% (66/607)	4% (7/174)
D14s306	192-236	unnested	85% (828/972)	91% (718/786)	3% (27/775)	8% (46/582)
D16s2624	118-146	unnested	98% (948/963)	99% (748/753)	10% (47/468)	3% (17/550)
D1s550	140-188	nested	93% (966/1038)	98% (768/781)	3% (14/476)	9% (54/616)
D3s2459	139-207	nested	91% (874/959)	94% (890/943)	10% (71/725)	11% (68/606)
D4s1627	190-232	nested	90% (946/1050)	91% (947/1045)	5% (33/609)	12% (90/679)
D5s1470	152-184	nested	95% (1003/1051)	96% (754/784)	6% (29/495)	8% (44/559)
D6s1056	207-243	nested	88% (928/1055)	92% (643/702)	7% (42/642)	14% (75/539)
D7s2204	150-182	nested	89% (914/1027)	97% (168/174)	4% (27/728)	14% (12/84)
D10s1432	131-163	nested	97% (1089/1126)	94% (564/597)	3% (15/579)	10% (39/408)
vWf	121-169	nested	95% (1009/1060)	98% (720/735)	5% (22/480)	6% (24/395)
Mann Whitney U			**26 (p=0.574)**	**24 (p=0.694)**	**22 (p=0.328)**	**14 (p=0.121)**

PCR success and allelic dropout given as percentages, with proportion of PCRs given in parentheses. Mann-Whitney U values given with p-values in parentheses. nd = no data.

Supplementary Figure 1.



SUPPLEMENTARY REFERENCES

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