Supporting information for:

An Efficient Multistrategy DNA Decontamination Procedure of PCR reagents for Hypersensitive PCR Applications

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Supporting Information and Methods

1. Ancient DNA facility

PCRs for the detection of reagent's contamination with animal, human and bacterial DNA were prepared in PCR flow hoods equipped with UV light and Biozone[TM] AirCare[TM] devices in a high containment facility with positive air pressure (http://www.ijm.fr/ijm/plates-formes/pole-paleogenomique-et-taphonomie-moleculaire/) using highly stringent experimental procedures as described [1].

2. qPCR Amplification system

PCR amplifications were performed using the UNG-coupled quantitative real-time PCR system UQPCR [2]. QPCR reaction buffer (10 x) was prepared as described by Lutfalla and Uze [3], except for a total replacement of dTTP by dUTP, and addition of *Taq DNA polymerase (*FastStart Taq DNA polymerase, Roche Applied Science, Mannheim, Germany) prior to PCR rather than in the 10 x qPCR buffer. dNTPs were from Roche Applied Science, (Mannheim, Germany) since the latter are certified to originate from chemical synthesis. In contrast, other manufacturers confirmed to produce parts of their dNTPs from animal products (e.g., InVitrogen, pers. comm.), which therefore bear a high potential of being contaminated with DNA from domestic animals. The final composition of the 1 x qPCR reaction buffer is 1.6 % glycerol, 40 mM 2-amino pH 8.3, 5 mM KCl, 50 µg/ml horse serum albumin HSA (Sigma A 3434; Sigma-Aldrich Inc., St. Louis, MO, USA), 0.024 % Lubrol (2-dodecoxyethanol;Sigma-Aldrich). The qPCR conditions used with primers BB3/4 were 1 U FastStart Taq (Roche Applied Science), 1 x qPCR buffer, 2 mM $MgCl_2$, 1 x GC-rich solution (Roche Applied Science), 1 µM of each primer, 30µM dA/G/CTPs and 60 µM dUTP. The qPCR experiments were carried out in a Lightcycler 2.0 apparatus (Roche Applied Science Mannheim, Germany) with the following program: a 10 minute-activation step at 95°C was followed by 60 cycles of denaturation (95°C, 15s), annealing (56°C, 15s) and elongation (67°C, 20s), followed by one melting step (95°C for 15s, then 60°C for 10s and temperature increase of 0.1°C per 10s with continuous fluorescence measurement) when the melting temperature Tm of the amplification products were measured. These conditions varied according to the primers (e.g., GC-rich solution was used only for primers BB3/4 and BB1/2). All PCRs were amplified for 60 cycles.

Decontamination tests were carried out on various DNA species and primer sequences are given in Table S1. To test the degradation of DNA by irradiation, we used phage λ DNA as a PCR template, with PCR primers amplifying regions whose lengths (including the primer annealing sites) range from 73 bp (L9/L5), 104 bp (L1/L3), 150 bp (L1/L4), 153 bp (L9/L10), 188 bp (L1/L5), 244 bp (L1/L6) to 307 bp (L1/L7).

To amplify contaminating bovine DNA from commercial reagents, we used PLATINUM® Quantitative PCR SUPERMIX-UDG (Invitrogen, St. Louis, MO, USA) and FastStart Taq DNA polymerase (Roche Applied Science, Mannheim, Germany), and primers that had been designed to amplify the D-loop region of bovine mitochondrial DNA: BB1 and BB2 amplify a 201 bp fragment (including the primer annealing sites) and cover nucleotide positions 16,160-16,361 of the taurine mitochondrial consensus sequence according to Anderson [4]; BB3 and BB4 amplify a 153 bp fragment (including the primer annealing sites) and cover nucleotide positions 16,022-16,175. The contamination of LightCycler® FastStart DNA Master^{PLUS} SYBR Green I mix (Roche Applied Science, Mannheim, Germany) was assayed likewise using a primer pair targeting a 1107 bp fragment of the mitochondrial D-loop region between nucleotide positions 15,623 and 390 with primers BOUT2 and BOUT3, a 702 bp fragment with a combination of primers BB3 and BOUT3, and a 555 bp fragment with primers BOUT2 and BB4 and the aforementioned primers BB3 and BB4 (Table S1).

To test for the efficiency of the DNase I treatment, we amplified a 103 bp fragment of the *tet* sequence of plasmid pBR322 using PLATINUM® Quantitative PCR SUPERMIX-UDG and primers BR1 and BR2 (Table S1).

To test for the efficiency of various treatments designed to minimize spread of contamination via contact of the experimenter, a 107 bp fragment of the ribosomal protein 49 (*rp49* gene) of *D. melanogaster* was amplified in the presence of dUTP from genomic DNA of *D. melanogaster* using the FastStart DNA Master^{PLUS} SYBR Green mix (Roche Applied Science, Mannheim, Germany) and primers RPF and RPR.

Various *Taq* polymerases were tested for contamination with bovine DNA using primers BB3/4 and the decontaminated home-made PCR buffer as described above, according to the manufacturer's recommendations: AmpliTaq Gold® (Applied Biosystems, CA, USA), FastStart Taq DNA polymerase (Roche Applied Science, Mannheim, Germany), HotStarTaq® DNA polymerase (Qiagen, Hilden, Germany), GoTaq® Hot Start Polymerase (Promega Corporation, Madison, WI, USA).

Identification and characterization of the PCR products were performed by analysis of the fusion temperature Tm of the products using the Lightcycler® and when necessary, by electrophoresis of 1 μL of the qPCR mix in a 10 % polyacrylamide gel.

The efficiency of the PCR was evaluated by determining the slope of the regression line obtained when plotting the Cts (crossing points at threshold) as a function of the logarithm of the concentration of the relative initial template quantity. The amplification efficiency was deduced from the slope of the linear regression analysis using the formula $1+E=10^{-1/slope}$ [5].

The efficiency of DNA degradation (in %) was calculated using the following equation [6]:

$$Edeg = \frac{100}{(1+E)^{\Delta Ct}}$$

where E is the efficiency of the PCR amplification varying between 0 and 1 as deduced from the slope of a standard curve, and ΔCt is the difference between the Ct of treated and control samples.

The test of the efficiency of the UNG treatment was performed according to Pruvost et al. [2] using amplicons of the tetracycline resistance gene of pBR322 in which thymines had

been replaced by uracil. The activity of the UNG from a marine bacterium (Roche Applied Science, Mannheim, Germany) and the UNG extracted from cod (*G. morhua*; Biotec Marine Biochemicals, Norway) was compared by quantifying the DNA before and after treatment using qPCR.

3. Decontamination treatment of surfaces and equipment

To test for the efficiency of decontamination procedures of surfaces, six decontamination agents were tested: (1) UV light, (2) DNA away®, (3) "CoPA solution", (4) bleach, (5) detergent and (6) air showering.

As a source for UV light we either used a UV Stratalinker (Stratalinker® UV Crosslinker 2400 device Stratagene) or a calibrated manual UV lamp.

According to US patent n° 5858650, a 10 mM copper-bis-(phenanthroline)-sulfate solution was prepared by dissolving 10 mM anhydrous copper sulfate and 20 mM 1,10-phenanthroline hydrate by sonication in water. The 10 mM copper-bis-(phenanthroline)-sulfate solution was mixed with two volumes of 6.8 % H_2O_2 to yield the "CoPA solution" used to wipe working surfaces and equipment.

We tested for the efficiency of various decontamination treatments currently in use in the forensic and ancient DNA field to prevent dispersal of exogenous DNA attached to gloves and the skin of the experimenters. The 107 bp *rp49* gene of *D. melanogaster* was amplified from genomic DNA. The amplification product was quantified in a nanodrop device (NanoDrop™ 2000, Thermo Fisher Scientific Inc., Wilmington, DE, USA) and serially diluted. Squares of 1 cm^2 were cut from powderless latex gloves. 10 µl of the serial dilutions of the *rp49* amplicon (between 170 pg and 17 fg) were applied to each square and air-dried. For the negative controls, 10 µl of water was applied. The squares containing different quantities of DNA were then treated in different conditions as followed: (i) Some were exposed for 1 hour at a distance of 10 cm to a manual UV lamp that delivered at this distance an energy of 4 J/m^2, thus corresponding to a total delivery of 1.45 J/cm^2. Other squares were rubbed with a cotton swab soaked in either (ii) water (negative controls), (iii) "CoPA solution", (iv) Bleach ("Eau de Javel", La Croix, containing 2.6 % active chlorine), (v) DNA away® (Molecular Bioproducts, San Diego, CA, USA) and (vi) detergent (a 10% dilution of dish-washing detergent containing 5-15% anionic non-ionic surfactants). To test recommended air showering procedure (http://www.chem.ox.ac.uk/OxfordTour/abc/node9.html), air in a syringe was pressed through a filter on a glove square containing dried DNA. To recover the DNA the glove squares were then incubated over-night in a microtube at 37°C in 200 µl TE buffer (10 mM Tris/HCl pH 8; 1 mM EDTA). Using a heated needle, a hole was introduced in the bottom of the microtube while it was placed over a fresh microtube. After centrifugation in an Eppendorf centrifuge at maximum speed for 1 minute, DNA was ethanol-precipitated using 20 µl 0.25% linear polyacrylamide as a carrier [7], washed, dried, dissolved in water and amplified by qPCR using primers PRF/R.

4. Reagent's contamination

Negative PCR controls (NTCs) with primer pairs BB3/4 or BB1/2 that yielded a product were sequenced. The sequences obtained were analysed via the median joining network approach [8] and are shown in Figure S1.

5. Irradiation

5.1. UV-irradiation

UV-irradiation tests were carried out in a Stratalinker® UV Crosslinker 2400 device (Stratagene, Cedar Creek, USA) and a Spectrolinker XL 1500 UV crosslinker device (Spectronics Corp. Westbury, NY, USA), both operated by five 254 nm UV bulbs (15 W). With brand new UV bulbs, a 10 minute irradiation corresponds to an energy of 1.20 J/cm^2 as measured by a cell at 11 cm from the bulbs when using an old generation Stratalinker® UV Crosslinker 2400 devices that have no reflecting walls. In the new generation Spectrolinker XL 1500 UV crosslinker devices with their reflecting aluminium walls, more UV light is received by the measuring cell and 10 minutes correspond to 4.8 J/cm^2. Since reflecting walls do little to improve the light received by microtubes at 1 cm of the UV bulbs, the value of the energy received by the measuring cell is not accurately reflecting that received by the sample. To accommodate different generations of UV crosslinkers, we preferred to refer to the irradiation time instead, and to use the measurement of the energy received by the cell to monitor the decrease in performance due to aging of the UV bulbs. This value is then used to increase the irradiation time accordingly.

UV-irradiation was performed in thin-wall, UV-clear 0.2 ml PCR tubes (Abgene, Epsom, UK) for small volumes (10 µL – 200 µL) or in thin-wall, UV-clear 0.6 mL Qubit™ tubes (Invitrogen, Carlsbad, USA) for larger volumes, as other tubes gave poor results due to inefficient UV-light transmission. Tubes were irradiated lying on the side on an aluminium sheet at around 1 cm from the bulbs.

The efficiency of UV-rays to inactivate DNA for PCR was assayed by irradiating different volumes of bacteriophage λ DNA and performing qPCR as described above on a titration series (50 pg, 10 pg, 2 pg and 400 fg) of irradiated DNA to assess the extent of inactivation of the template, compared to a non-irradiated standard range. The primers used were L9-L5 targeting a 73 bp region (Table S1). Such experiments were carried out on phage λ DNA buffered either with water, 10 x qPCR buffer (without SYBR Green I dye), 25 mM MgCl$_2$, or 5 x GC-rich solution (Roche Applied Science), in order to assess any UV-protective effect that could interfere with the decontamination process.

The effect of UV-rays on PCR performance of the qPCR buffer reagents and of the FastStart Taq DNA polymerase was assessed by carrying out PCR experiments using these irradiated reagents separately or together, and comparing the PCR efficiency to experiments performed with non-irradiated reagents. Phage λ DNA was used as a template and amplified using the L9-L5 primer couple as described above. Whenever 10 x qPCR buffer was irradiated, the SYBR Green I dye was added only after irradiation but before the −80°C freezing step [3], to ensure efficient DNA detection.

5.2. γ-irradiation

Irradiation experiments were carried out in an IBL 637 device with a ^{137}Cs source that had been calibrated by Fricke dosimetry.

QPCR reaction buffer (10 x), FastStart Taq DNA polymerase (Roche Applied Science), bacteriophage λ DNA and λ-specific PCR primers were γ-irradiated with 1, 2 or 4 kGy. When relevant for the tests, different reagents (HSA, dNTPs, detergent Lubrol, KCl, 2-aminopropanediol, glycerol, water) were irradiated separately, and used to prepare buffers

containing subsets or all of the irradiated reagents. QPCR reaction buffer (10 x), FastStart Taq DNA polymerase, bacteriophage λ DNA and λ-specific PCR primers were γ-irradiated with 1, 2 or 4 kGy.

The efficiency of γ-irradiation to destroy double-strand DNA was assayed by performing qPCR on a serial dilution (260 pg, 26 pg, 2.6 pg and 260 fg) of irradiated phage λ DNA as a PCR template, with PCR primers L9-L5, L1-L3, L1L4, L1-L5 and L1-L7 described above, compared to non-irradiated controls.

Degradation of DNA through γ- and UV-irradiation in various buffers was tested by irradiating 25 ng/µL of λ DNA with 4 kGy γ-irradiation and for 10 minutes UV irradiation in a UV crosslinker in water complemented with 2.5 M 2-aminopropanediol, 50 % glycerol, 10 mg/mL horse serum albumin (HSA) in water, 10 % Lubrol in water, or 20 mM dNTPs (dATP, dCTP, dGTP) and 40 mM dUTP, followed by amplification of a 73-bp DNA fragment.

6. Endonuclease treatment

6.1. DNase I digestion of primers

DNase I digestion was performed on primers BR1/2 amplifying a 107 bp fragment of plasmid pBR322. Radiolabeling of the primers was achieved using T4 polynucleotide kinase (New England Biolabs, Ipswich, MA, USA) and γ-^{32}P-dATP. DNase I solution was prepared from DNase I powder (Roche Applied Science, Mannheim, Germany) at a concentration of 15 U/µl. Radiolabeled primer DNA was incubated at room temperature for 10 minutes, respectively, with 3 U of DNase I solution in the presence of 3 mM MgCl$_2$ (Figure S2A). In the example shown in Figure S2A, primer BR1 is highly sensitive to DNase I, which could be due to the formation of a hairpin and a very stable primer-dimer (ΔG = - 6,9 kcal/mol) that can be predicted by the software "Oligo6" (Molecular Biology Insights, Inc., Cascade, CO, USA) and that is shown below the gel picture in Figure S2A.

A 10 minute incubation of the PCR mix with DNase I at room temperature was sufficient to completely degrade any DNA present in the reaction mixture, as evaluated by gel electrophoresis analysis of a radiolabelled 103 bp pBR322 fragment that had been incubated with DNase I at different incubation times between 5 and 45 minutes (data not shown). The following heat-inactivation at 95°C for 10 minutes irreversibly denatured the DNase I prior to PCR as evaluated by gel electrophoresis of the same radiolabelled pBR322 fragment incubated with 3 U DNase I that had been heat-denatured between 5 and 20 minutes (data not shown). This denaturation treatment, however, prematurely activated the Hot-Start *Taq* polymerase. Consequently, the *Taq* DNA polymerase must be added after inactivation of the DNase I, which is not practical with large numbers of reactions and increases the risk of contamination. The compulsory heat denaturing of the DNase I also prematurely denatured the stabilizing proteins such as BSA leading to a largely delayed amplification (14 cycles on average) with a significantly decreased efficiency. When adding the albumin after DNase I treatment, the efficiency of the PCR was recovered but the saturation plateau was reached much earlier and only half of the quantity of DNA molecules were newly synthesized (Figure S2B).

We also tested whether a milder DNase I inactivation step could be performed without causing premature activation of the hot start DNA polymerase. Indeed, it was shown that DNAse I could be fully inactivated in the presence of 30 mM DTT by a heat treatment of 30 minutes at 80°C [9]. We tested the effect of a 30-minute incubation at 80°C of the PCR

reaction prior to perform the standard PCR protocol using primer pair EA51/61 amplifying 83 bp of horse mitochondrial DNA amplified using FastStart Taq DNA polymerase with a prior UNG treatment. Comparative analysis with a reaction mix containing the same concentration of DTT but that was not incubated for 30 minutes at 80°C reveals clearly that the Hot start DNA polymerase was significantly activated and primer dimers were appearing much earlier, thereby reducing the sensitivity of detection of low amounts of DNA (Fig. S2C). The reaction efficiency at the beginning of the PCR appears higher upon preincubation at 80°C, since the control DNA is detected earlier at the highest concentration, presumably because more active DNA polymerase is available in the initial phase of the PCR. However, dimers appear very early, in the experiment shown at least 15 cycles earlier than in the control reaction. Consequently, the low amounts of DNA cannot be measured because dimers are amplified earlier. Thus, this inactivation procedure is detrimental to the sensitivity of the PCR.

6.2. Heat-labile dsDNase activity test

To compare the activity of the hl-dsDNase® and the wild type dsDNase® (Biotec Marine Biochemicals, Tromsø, Norway), DNA degradation was measured by treating 100 pg of bacteriophage λ DNA with the dsDNases for 30 min at 25°C. DNase activity was tested in the following conditions. (i) In 25 mM Tris/HCl, pH 8, 10 mM or 20 mM $MgCl_2$ and 1 mM $CaCl_2$ in a final volume of 10 µl, with serial dilutions (with a $\sqrt{10}$ serial dilution factor) of either dsDNase with concentrations ranging from 2×10^{-3} to 2×10^{-5} U/µl. (ii) In 20 µl FastStart Taq DNA polymerase storage buffer (20 mM Tris-HCl pH 9.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.2 % Tween 20, 50 % glycerol) or in FastStart Taq DNA polymerase solution supplemented with 10 mM $MgCl_2$, 1 mM $CaCl_2$ and 1 mM DTT, were treated with 0.09, 0.01, 0.005, 0.0015 U/µl of dsDNase. The nuclease was inactivated by incubation for 30 minutes at 65°C.

QPCR was performed as described above on 1 µl of each of these assays in order to quantify the level of degradation of the λ DNA by the dsDNase. The extent of DNA degradation was assayed by performing qPCR on the treated DNA samples using the L9-L5 primer pair as previously described. Control qPCRs were performed on DNA samples where the DNase had been replaced by water.

6.3. DNase inactivation test

To test the efficiency of inactivation of both wild-type and hl-dsDNase (Biotec Marine Biochemicals, Tromsø, Norway), parallel experiments were performed with the two enzymes. Inactivation tests were carried out in different buffer conditions. (i) In 10 µl of 25 mM Tris/HCl, pH 8, 20 mM $MgCl_2$ and 1 mM $CaCl_2$, 1 U of nuclease was incubated for 10 or 30 minutes at 60°C or for 10 or 30 minutes at 55°C with or without 1 mM DTT. Residual activity was tested by an incubation step of the last mix with 100 pg of the bacteriophage λ in 250 mM Tris/HCl, pH8, 10 mM $MgCl_2$ and 1 mM $CaCl_2$ for 30 minutes at 25°C followed by a 30 minute-inactivation step at 65°C. (ii) In the storage buffer of the FastStart Taq DNA polymerase, within a final volume of 20 µl, 2 U of nuclease were incubated for 10 or 30 minutes at 55°C or for 10, 15, or 30 minutes at 50°C with or without 1 mM DTT in 10 mM $MgCl_2$ and 1 mM $CaCl_2$. Residual activity was tested by an incubation step of this mix with 100 pg of the bacteriophage λ in 250 mM Tris/HCl, pH 8, 10 mM $MgCl_2$ and 1 mM $CaCl_2$ for

30 minutes at 25°C followed by a 30 minute-inactivation step at 65°C. QPCR was performed as described above using the primers L9-5 and L9-10 with 1 µl of each of these assays to quantify to which degree the DNases degraded the λ DNA.

6.4. Activity test of dsDNases on endogenous contaminations

The efficiency of DNA degradation by the dsDNases on endogenous bovine contaminants co-purified with reagents was tested with the incubation of 1µl of the FastStart DNA Master^{PLUS} SYBR Green mix with or without 1 U of hl-dsDNase in a final volume of 10 µl of 25 mM Tris/HCl, pH 8 with 10 mM MgCl$_2$, 1 mM CaCl$_2$ and 1 mM DTT, for 30 minutes at 25°C and 30 minutes at 55°C. The treated and untreated Master^{PLUS} SYBR Green mixes were used for qPCR targeting the bovine mitochondrial control sequences with the primer pair BB3/4.

6.5. Decontamination of Taq DNA polymerase, primers and dNTPs using hl-dsDNase

The FastStart Taq DNA polymerase supplemented with 10 mM MgCl$_2$, 1 mM CaCl$_2$ and 1 mM DTT was incubated with hl-dsDNase (0,1 U/µl) for 30 minutes at 25°C followed by a 20 minute-inactivation at 50°C.

Primers were incubated with hl-dsDNase (0,001 U/µl) of nuclease for 800 pmol of primers) in 25 mM Tris/HCl, pH 8 buffer, 10 mM MgCl$_2$, and 1 mM CaCl$_2$, for 30 minutes at 25°C. The nuclease was inactivated for 30 minutes at 55°C.

dNTPs were incubated with hl-dsDNase (0,001 U/µl) for 2 mM of dATP, dCTP, dGTP and 4 mM of dUTP in 25 mM Tris/HCl pH 8 buffer, 1 mM CaCl$_2$ and either 10 or 20 mM MgCl$_2$, for 30 minutes at 25°C. The efficiency of decontamination was slightly better with 20 mM MgCl$_2$, 98 % of the DNA was degraded whereas only 97.70 % with 10 mM MgCl$_2$. The nuclease was inactivated for 30 minutes at 55°C.

The efficiency of the hl-dsDNase for decontamination of the *Taq* DNA polymerase, the primers and the dNTPs were assayed on a series of blank qPCR controls carried out with the primer pair BB3/4. The reagents, treated or untreated by DNAse, were tested in qPCR buffer where all other components but SYBR Green I were UV irradiated and where the volume of MgCl$_2$ was adjusted so that the MgCl$_2$ concentration provided by the nuclease-treated reagents was taken into account. A series of blank qPCR controls was carried out.

We also evaluated whether the *Taq* DNA polymerase and the primers could be treated together with hl-dsDNase. We found that under the conditions described before and at a MgCl$_2$ concentration between 10 and 20 mM, 98 % of λ DNA was degraded when the treatment with hl-dsDNase was performed in the presence of 90 µM primers prior to PCR. Thus, the presence of primers does not interfere with the hl-dsDNase treatment. Subsequent PCR amplifications did not show a lower efficiency (difference in the efficiency ∆E=± 1%). Despite these results, separate treatment of the *Taq* DNA polymerase and of the PCR primers with hl-dsDNase is preferable if different primers are used. Furthermore, separate treatment minimizes the risk of primer-dimer formation during the inactivation step of the hl-dsDNase.

7. Efficiency of the UVD decontamination procedure

To assess the efficiency of the UVD decontamination procedure, we tested a large number of blank controls. In particular, no amplification products were obtained out of 409 NTCs

using bovine-specific primers BB3/4 amplifying a 153 bp fragment, out of 164 NTCs using primers amplifying a 79 bp product, 70 NTCs using primers amplifying a 94 bp product from the bovine D-loop mitochondrial DNA, and 45 NTCs amplified with 7 different primer pairs of the bovine mitochondrial DNA amplifying products from 57 to 92 bp.

8. Sequencing

Sequencing was performed by capillary electrophoresis at Eurofins MWG Operon (Ebersberg, Germany) and by manual sequencing using the Sanger sequencing method and $5'-^{32}P$-phosphorylated sequencing primers [10].

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Table S1: Primers used for amplification of various target molecules.

target Sequence	primer name	primer sequence
phage λ	L1	5'-AAGGAAACGACAGGTGCTGA-3
phage λ	L3	5'-CCAGCTGCTTTTTGTTGACTT-3
phage λ	L4	5'-TCCTGCCAG TTCTGA ATGGT-3'
phage λ	L5	5'-TACCCTTGCCACCGCCT-3'
phage λ	L6	5'-AGCATCCCTTTCGGCATA-3'
phage λ	L7	5'-ATCTGCCTCGCTGGCCT-3'
phage λ	L9	5'-GGTGCGAGTATCCGTACCATT-3'
phage λ	L10	5'-GCGCAGCTTTTCGTTCTC-3'
mitochondrial D-loop B. taurus	BB1	5'-TTAATTACCATGCCGCGTGA-3'
mitochondrial D-loop *B. taurus*	BB2	5'-CATGGGCTGATTAGCCATTAGT-3'
mitochondrial D-loop *B. taurus*	BB3	5'- CCATGCATATAAGCAAGTACATGA-3'
mitochondrial D-loop *B. taurus*	BB4	5'-GCGGCATGGTAATTAAGCTC-3'
mitochondrial D-loop *B. taurus*	BOUT2	5'-GGCACAATCGAAAACAAATTACT-3'
mitochondrial D-loop *B. taurus*	BOUT3	5'-CTTGCTTTGGGTTAAGCTACATC-3'
mitochondrial D-loop *B. taurus*	BB15m	5'-TGA CTG TAC ATA GTA CAT TAT GTC AA-3'
mitochondrial D-loop *B. taurus*	BB16m	5'-GCT CGT GAT CTA ATG GTA AGG-3'
mitochondrial D-loop *B. taurus*	BB17	5'-CCC CAT GCA TAT AAG CAA G-3'
mitochondrial D-loop *B. taurus*	BB18	5'-AAG AAT GAA TTT GAC ATA ATG TAC TA-3'
mitochondrial D-loop *E. caballus*	EA5.1	5'-CATCCAAGTCAAATCATTTCCA-3'
mitochondrial D-loop *E. caballus*	EA6.1	5'-CTGATTTCCCGCGGCTT-3'
pBR322	BR1	5'-ATGCGTTGATGCAATTCT-3'
pBR322	BR2	5'-GTCGATAGTGGCTCCAAGTA-3'
rp49 gene *D. melanogaster*	RPF	5'-CCGCTTCAAGGGACAGTATCTG-3'
rp49 gene *D. melanogaster*	RPR	5'-CACGTTGTGCACCAGGAACTT-3'

Table S2: Examples of significantly different distributions between sample and control PCRs

Positive NTCs/Total NTCs	Positive Sample PCRs/Total Sample PCRs					
	1 Sample		10 Samples Bonferroni		50 Samples Bonferroni	
0/2	5/5		19/19		44/44	
0/5	2/2	3/4	6/6		8/8	
0/10	2/3	3/6	3/3	4/5	4/4	
0/20	2/6		2/2	3/5	3/3	
0/50	2/15		2/4	3/11	2/2	
0/100	2/29		2/8		2/3	3/12
0/279	2/81		2/21		2/9	
0/409	2/118		2/31		2/14	
0/1500	2/435		2/114		2/49	
15/372	2/8		2/2	3/8	3/5	4/10
151/1170	2/3	3/6	3/3		4/4	
50/1000	2/7		2/2		4/4	
15/1000	2/22		2/6		2/3	3/12
5/1000	2/57		2/16		2/7	

When sample and contaminating sequences are indistinguishable, to ensure authenticity of the sample amplification with a 95% confidence level it is necessary to demonstrate that the rate of success of sample amplification is significantly higher than the background of contamination detected in the PCR blank controls (NTCs). The table presents various examples of threshold values that are different with a P-level of significance (α) of 0.05 or lower as determined using the Fisher's exact test. The left column with the blue background contains examples of various total numbers of NTCs performed and numbers of contaminated PCRs obtained (Positive NTCs/Total NTCs) that were chosen to either show cases (i) where only a small number of NTCs is carried out, even without obtaining positive amplification (from 0/2 to 0/100), or (ii) that were observed in the present study (from 0/279 to 151/1170), or (iii) that were chosen to present hypothetical situations where a very high number of NTCs was performed allowing detection of a few contaminated amplifications (from 50/1000 to 5/1000). For each of these NTCs values, we determined the threshold values of the minimal number of successful sample replications that must be obtained following a maximal number of PCR attempts (Positive Sample PCRs/Total Sample PCRs) to ensure that the chance that all sample amplifications are due to contamination is no more than 5%. The P-value will be lower than 0.05 when more replications are successful or when successful replications are obtained with fewer PCR attempts. When the rate of PCR success required is very high, this minimal number could prevent the validation of sequences obtained from samples that do not show very good DNA preservation and do not yield a product with every PCR attempt. Thus, the table also indicates for these cases the alternative replication number just above this minimal number, which would allow validation of sequences obtained from such less well-preserved samples. Finally, when several samples are compared to a single series of NTCs, there is an increased probability that an apparently significant difference can be obtained by chance and it is necessary to correct for multiple comparisons. This correction was performed using the conservative

Bonferroni correction where the P-value α is adjusted by the number n of samples analyzed using α/n [11]. The table presents the numbers required to validate any sample using three examples of multiple comparisons: either when analyzing a single sample (no correction), or 10 or 50 samples.

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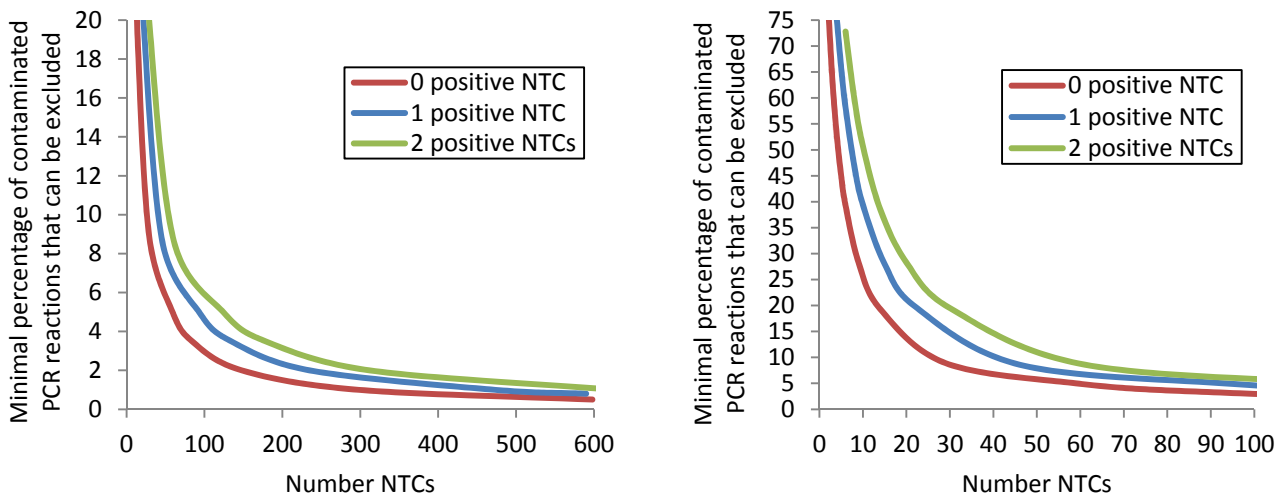
Figure S1: Relationships between the numbers of NTCs performed and the PCR contamination level that can be excluded with a 95% confidence level. The exact binomial test [11] was used to calculate the one tailed probability that a certain number of NTCs performed is significantly lower than a certain theoretical proportion of contaminated PCR reactions. The simulation was performed for various proportions when 0, 1 or 2 NTCs gave an amplification product (red, blue and green lines, respectively), and the minimal number of NTCs that gave a probability of 0.05 or lower was plotted. The curves can be used to determine the lowest percentage of contamination that can be excluded as a function of the number of NTCs performed. For best precision, two versions of the curves are shown with different scales in the abscissa and the ordinate. For example, if 100 NTCs have been performed and only one yielded a positive PCR, then one can assume with a 95% confidence level that the absolute contamination level of the PCRs is lower than 4.7%, whereas if only 30 NTCs are performed, even without yielding a single positive PCR, then one can only assume that the contamination level is below 9.5%.
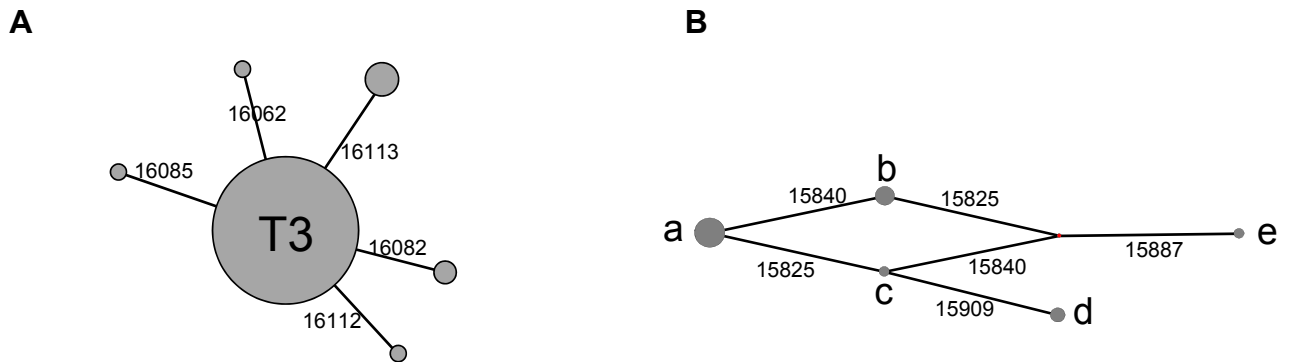
Figure S2: Genetic diversity of mitochondrial sequences contaminating PCR reagents. Median Joining networks [8] of products from negative PCR controls (NTCs) using primer pairs BB3/4 and BB1/2. A. *Bos taurus* sequences. The sequence distribution observed in the contaminant resembles that of the European bovine mitochondrial sequences: the bovine sequences show a predominance of the T3 haplogroup with a few closely related sequences [12]. B. *Sus scrofa.* The porcine sequences are more diverse and include, at a lower resolution, haplogroups described by [13]: **a** is contained in haplogroups EH1, 2, 3, 7, 9, 10, 11, 13, 14, 18, 19, 22, 23, 24, 25; **b** is contained in haplogroups EH16 and 20; **c** is contained in EAH3, 4 and AH18, 24 and 27; **d** is contained in haplogroup EAH1; **e** is contained in haplogroups EAH2 and AH19. Haplogroups starting with letter E correspond to European breeds, whereas haplogroups starting with letter A correspond to Chinese breeds.
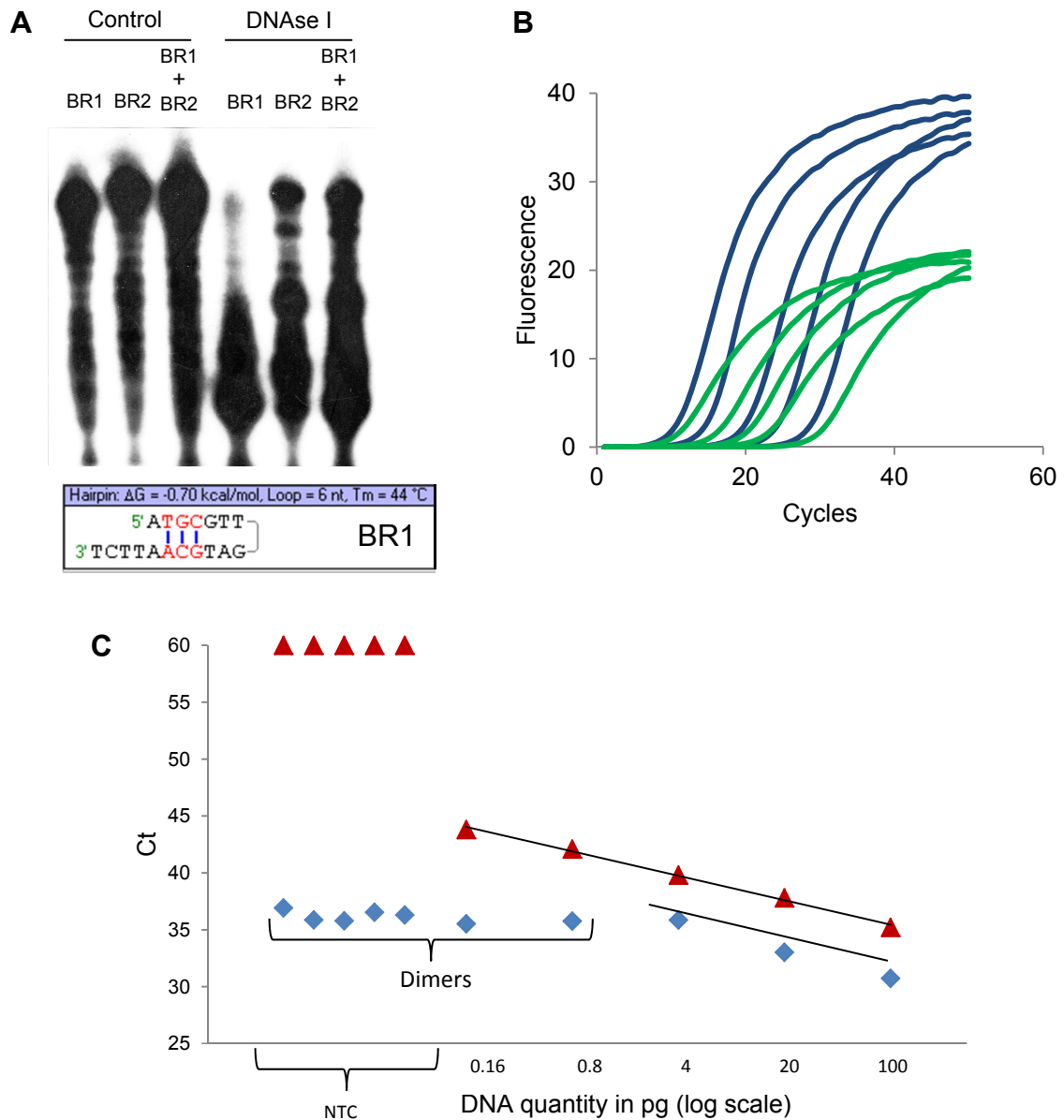
Figure S3: DNase I treatment. A. Effects of the DNase I treatment on the degradation of primers. Radio-labeled primers BR1 and BR2 had been incubated with DNase I for 10 minutes at 37°C at a concentration allowing complete degradation of a 103 bp fragment of plasmid pBR322, followed by 10 minutes of heat inactivation at 95°C prior to gel electrophoresis in a 12% polyacrylamide gel followed by autoradiography. Most of primer BR1 was degraded whereas primer BR2 was essentially unaffected. Primer BR1 was predicted to form a hairpin structure stable at 37°C, which is represented below. **B.** Efficiency of PCR after treatment of DNase I-sensitive primers**.** Primers (BR1 and BR2) were treated with DNase I in PCR reaction buffer in the absence of *Taq* followed by heat inactivation at 95°C for 10 minutes. Since the heat inactivation step lead to bovine serum albumin (BSA) precipitation, the reaction was centrifuged and fresh BSA and hot start *Taq* was added. QPCR was then performed on titration series of pBR322. Although DNase I treatment did not lead to a delay of the PCRs, the reaction plateau was reached much earlier and thus less DNA was synthesized. This effect is attributed to primer degradation. Green: control PCR; blue: DNase I-treated reaction mix. **C.** Hot start DNA polymerase is prematurely activated by a "mild" heat inactivation step that allows DNASE I inactivation. A horse DNA titration curve ranging from 100 to 0.16 pg, alongside 5 non-template controls (NTC), were amplified using primer pair EA51-61 (83 bp) in the Roche FastStart DNA Master^{PLUS} SYBR Green I mix supplemented with 30 mM DTT. PCR reactions were either incubated at 80°C for 30 minutes or not prior to PCR amplification with the following protocol: 15 min. at 37°C (UNG incubation step); 5 min. at 95°C (hot start activation step); followed by 60 cycles of 5 sec. at 95°C, 40 sec. at 65°C. Dimers were identified by a subsequent melting curve analysis. The figure represents the Ct of each sample as a function of the log of the concentration of DNA. The heat-treated samples are represented by blue diamonds, whereas the control samples are represented by red triangles.
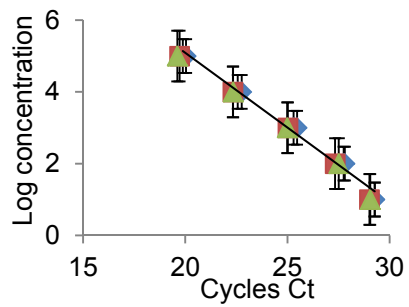
Figure S4: hl-dsDNase treatment of primers. PCR efficiency after treatment of DNase I-sensitive primers BR1/2 with hl-dsDNase. Blue diamonds = Control: PCR with untreated primers BR1/2; red squares= PCR with primers BR1/2 that were treated separately; green triangles = PCR with primers BR1/2 treated together.