Technical Note
for the identification, prevention, and elimination of DNA and RNA contaminations
(Decontamination Guideline)

May 2006

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1. Introduction

The detection of pathogens using polymerase chain reaction (PCR) and the subsequent detection of the PCR product by means of fluorescence labeled single-stranded oligo nucleotides (probes) has become an important pillar in human diagnostics. The special advantages of this technology, called real-time PCR, are above all its rapidness, its large linear quantification range as well as its very high specificity and sensitivity. Due to the high detection sensitivity, less than one copy of a DNA or RNA sequence per microliter of sample material may already be detected (Mackay, 2004). This high sensitivity at the same time bears the risk that through inappropriate handling of samples even minute DNA or RNA quantities of the pathogen from another sample may result in a positive signal in real-time PCR (cross contamination). These signals lead to false positive results in molecular diagnostics with potentially severe consequences for the patient. It is, therefore, of utmost importance to identify possible contaminations, to protect oneself from contaminations (prevention), and to eliminate already existing DNA or RNA contaminations (decontamination) (Mifflin, 2003).

1.1. General detection of nucleic acids

The detection of an existing contamination is of significant relevance in human diagnostics in order to avoid false positive results. Detection of contaminating DNA is achieved by using intercalating and fluorescing stains (e.g. ethidium bromide, SYBR Green I) and UV light. For this purpose, the working area in the darkened room is exposed to UV light. Double-stranded DNA, which has been labeled with one of the intercalating stains mentioned above, may thus be visualized as a fluorescing spot. Radioactively labeled nucleic acid may be detected with a Geiger or scintillation counter (wipe test). For health safety and economic reasons, however, this way of labeling or detection of nucleic acids is not suitable for the identification of contaminations in the laboratory or in and on laboratory materials and reagents. However, the detection of unlabeled nucleic acid is difficult and requires a suitable, highly sensitive detection procedure. Real-time PCR represents such a sufficiently high-sensitive system for the detection of very low quantities of DNA or RNA.

1.2. Presentation of results using real-time PCR

The basis for the determination of the quantity of DNA or RNA initially present in a sample with real-time PCR is the Ct value (threshold cycle = Ct) or the crossing point (Cp). The DNA or RNA is amplified and the intensity of the fluorescence in the sample increases in direct correlation to the increase of the amplification product.

The Ct value represents the PCR cycle at which the fluorescence signal of a sample exceeds a threshold value (threshold cycle = Ct) due to the amplification of the DNA or RNA in the sample. From this cycle onward, the increase in the fluorescent signal takes the shape of a curve as presented on the computer screen. Further information on this subject is available in the instruction manuals of the real-time PCR instruments and in our *Technical Notes for the Quantification of Pathogen Copy Numbers* (www.qiagen-diagnostics.com).

Unlike conventional methods such as ELISA (enzyme-linked immunosorbent assay), real-time PCR does not feature a so-called 'cut-off value' below which a signal may be interpreted as negative. If a Ct value is assigned to a sample in real-time PCR analysis, this PCR must be assessed as being positive. The Ct value of a sample correlates with the initial DNA or RNA concentration. The lower the concentration, the higher the Ct value of the sample and vice versa. A relatively high Ct value is thus assigned to low positive material (e.g. Ct > 38).

The cause of a low signal, besides a low pathogen load in the tested sample, may also be a contamination. The representation of such low positive signals is the same, thus a differentiation between a contamination and a low positive sample on the basis of the amplification curve can not be made.

1.3. Summary: Introduction

- Real-time PCR is a highly sensitive molecular detection procedure for RNA and DNA.
- In comparison to conventional methods such as ELISA, real-time PCR does not feature a 'cut-off value', on account of which even low signals must be assessed as being positive.
- The reasons for low signals, which are caused by either a small pathogen load in the sample or by a contamination with a very low concentration, may not be differentiated by the presentation of the results.

2. Contaminations

2.1. Identification of contaminations

If low positive signals appear in larger numbers within one PCR run, a contamination may not be excluded. In this case, the result of the real-time PCR should always be compared with the clinical data or possibly the symptoms of the patient in order to avoid assessing real positive samples as false positive ones.

2.1.1. Positive negative controls

If the low positive signals appear in one or several negative controls (NTC = non template control, water controls), a contamination is definitely present (see Figure 1).

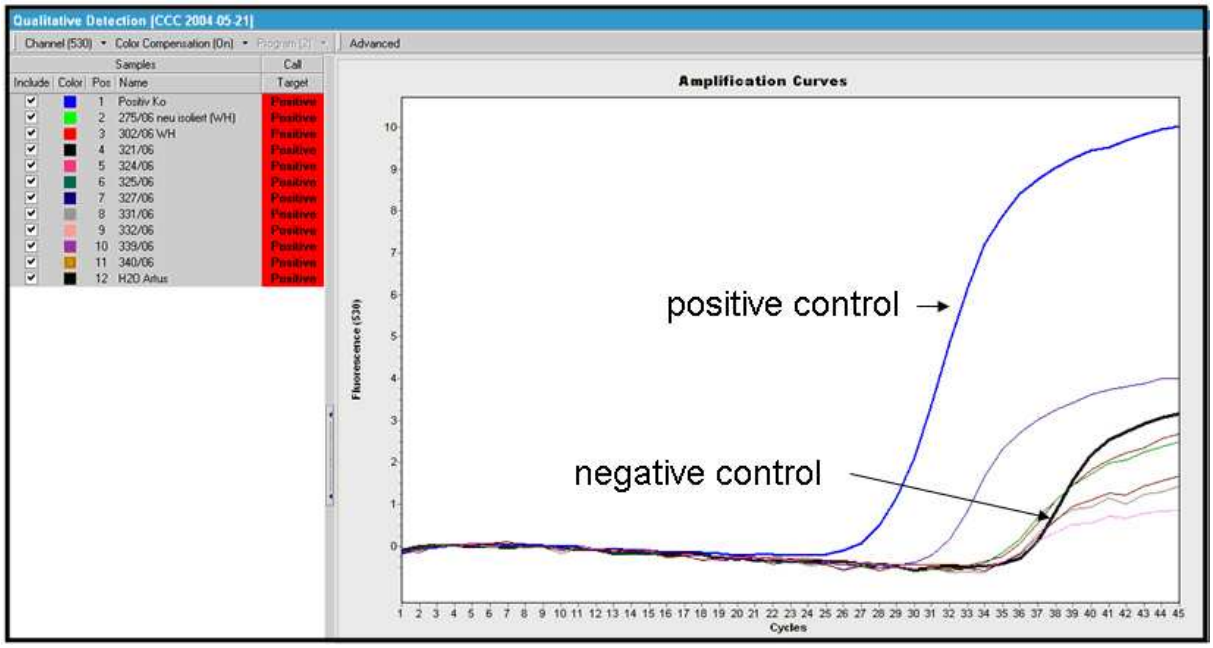

Figure 1: Analysis of a malaria PCR with a quantification standard as the positive control of the *artus*® Malaria LC PCR Kit using the LightCycler® 2.0 Instrument. Due to a contamination, the negative control (NTC, water control) and the samples to be examined revealed positive signals.

In order to clarify whether the contamination originated during the purification or later, for example during the preparation of the PCR, non-purified negative controls (NTC, water controls) should be analyzed in parallel to newly purified negative controls (NTC, water controls) in a subsequent PCR. Multiple determinations (at least triplicates) should be carried out for statistical reasons. If only single or double determinations are carried out, the results are not conclusive: Very low contaminations in the sample material mean that the distribution of the DNA or RNA molecules in the sample material and thus into the PCR occurs

randomly. As a result, the same sample may show a positive PCR result in one case and also a negative one in the other case (see chapter **2.1.3 Statistical contaminations**).

2.1.2. Simultaneous increase of amplification curves

An additional indication for a contamination are an increased number of positive signals with approximately the same Ct values (see Figure 2), independently of whether these signals are observed in the sample material or in negative controls.

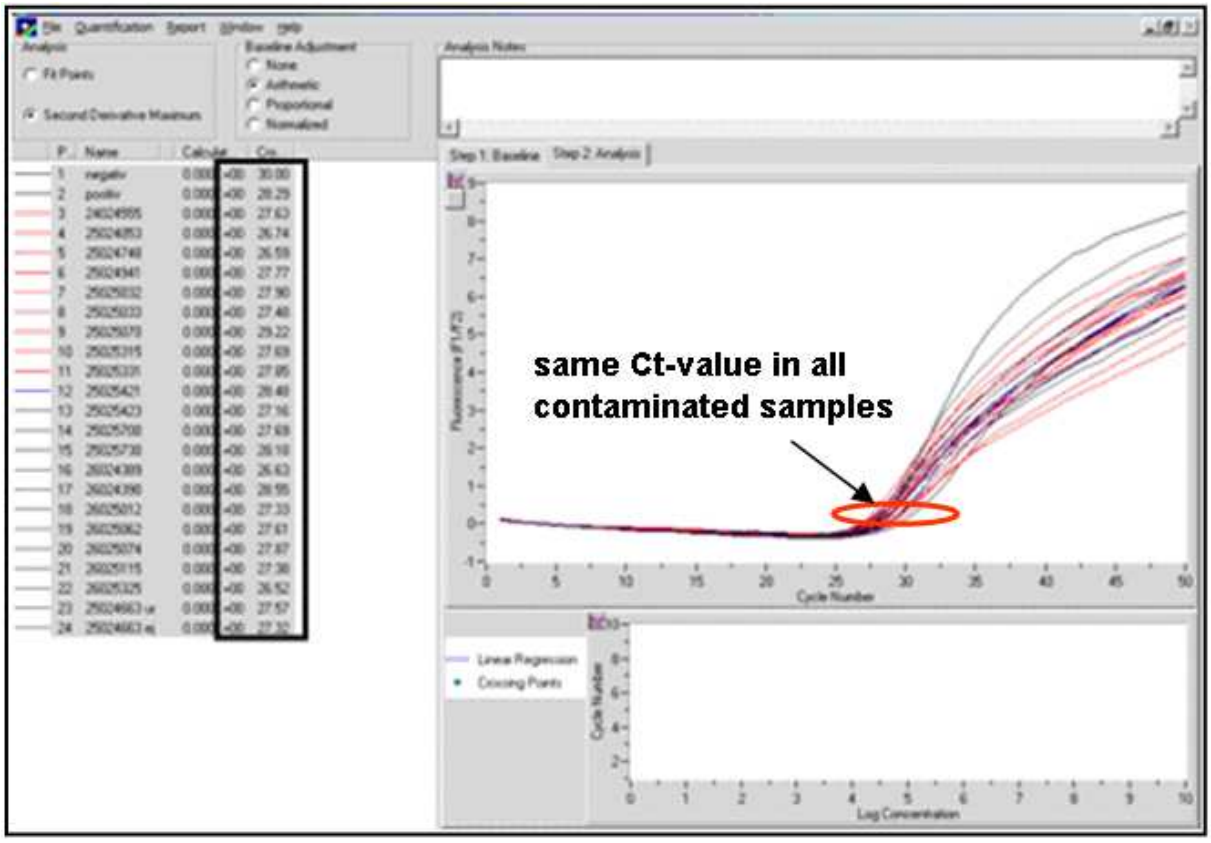

Figure 2: Analysis of an HBV PCR with the *artus*® HBV LC PCR Kit using the LightCycler® 1.2 Instrument. The contamination had the same intensity in all samples which is obvious from the nearly simultaneous increase of the curves (approximately the same Ct values in all samples).

2.1.3. Statistical contaminations

For the determination of the analytical detection limit of the *artus*® PCR kits, the pathogen load is given in copies or international units (IU) per microliter of the sample material, which may be detected with a probability of 95%. Pathogen loads below the specified analytical detection limit may be detected with a corresponding lower probability. If a very small quantity of positive material is present in a sample (e.g. due to a contamination), the

statistical distribution of the material in the sample may lead to an assignment of a Ct value in one PCR and to the failure of the reaction in another one (see Figure 3).

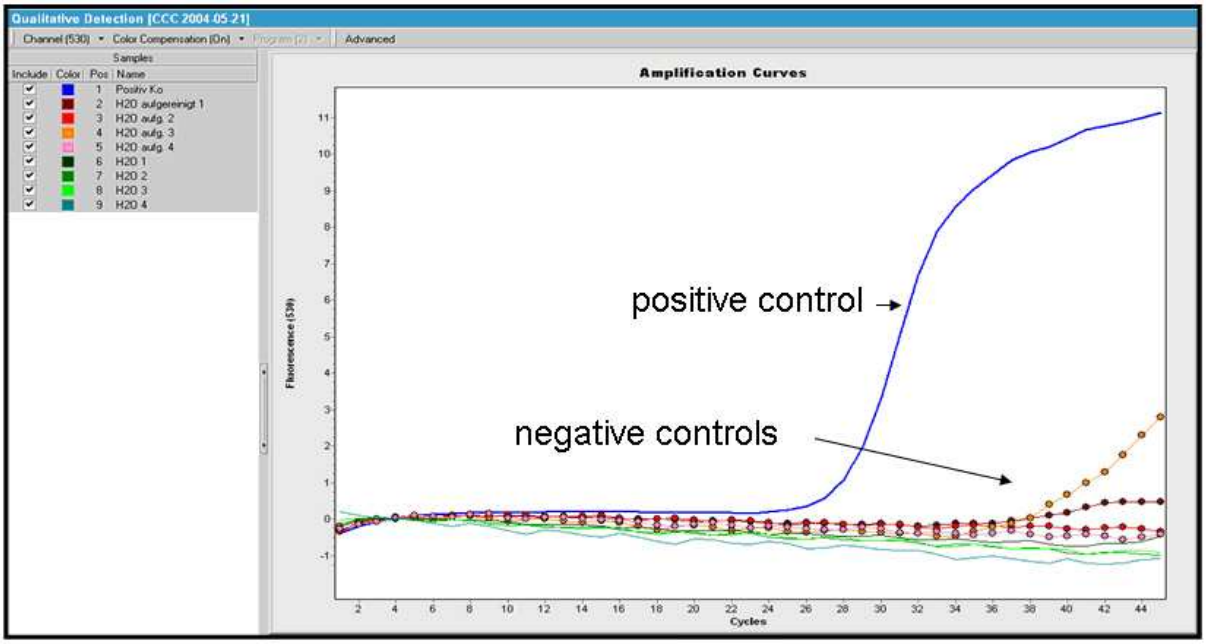

Figure 3: Analysis of a malaria PCR with a quantification standard as the positive control of the *artus*® Malaria LC PCR Kit using the LightCycler® 2.0 instrument. A low contamination results in both positive and negative signals in the negative controls.

2.1.4. Identification of the contamination source

The probability of being able to detect a very low quantity of positive material may be increased by the use of multiple determinations. It is recommended to use at least threefold or better fivefold replicates of one sample, the results of which ought to be clearly negative. If the newly purified negative controls (NTC, water controls) but not the non-purified negative controls show a positive signal after the evaluation of the PCR results, it may be assumed that the contamination occurred during the purification process (see Figure 4).

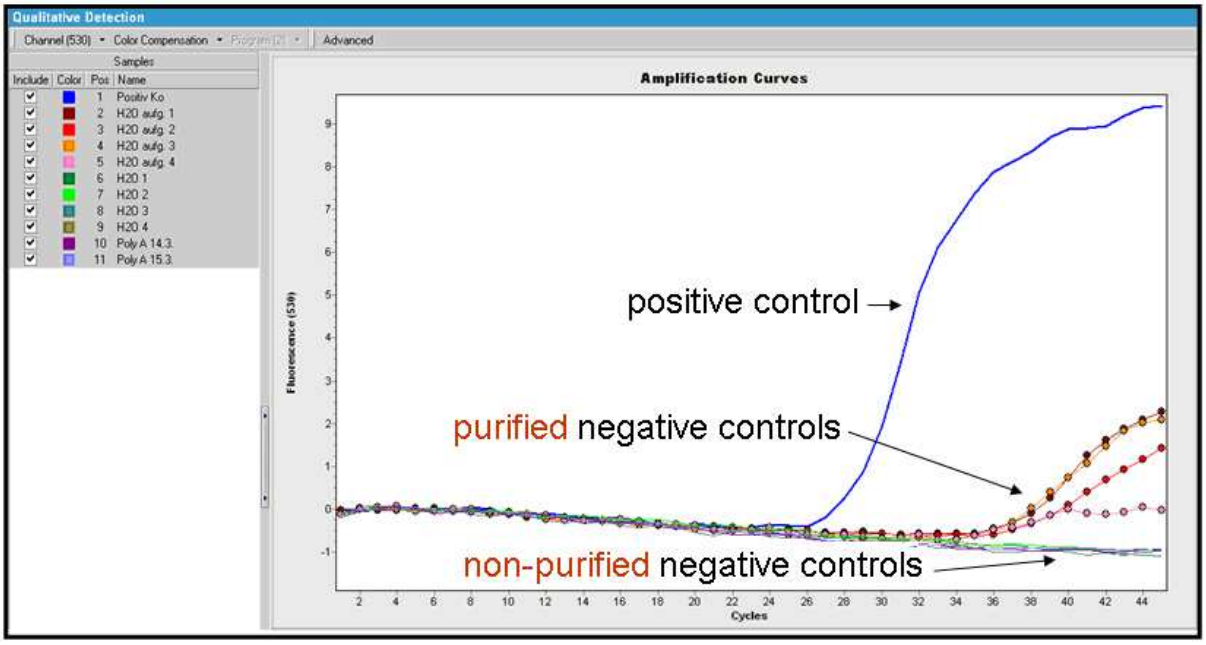

Figure 4: Analysis of a malaria PCR with a quantification standard as the positive control of the *artus*® Malaria LC PCR Kit using the LightCycler® Instrument. The PCR results of the newly purified negative controls but not the PCR results with the non-purified negative controls show a positive signal. A contamination during the purification process may be assumed.

Such a contamination is dealt with by cleaning the working areas, the PCR equipment (pipettes etc.), and the instrumentation used (see following chapters) as well as by replacing already opened or used reagents by new ones. If the contamination source is to be determined more precisely, samples of the working areas for example may be taken with swabs before the cleaning process and subjected to a suitable DNA/RNA purification (e.g. QIAamp® DNA Mini Kit or QIAamp Viral RNA Mini Kit) and a PCR analysis. In such a case, pipette pistons may be incubated in a vessel with molecular biology grade pure water which is subsequently used as sample material.

If both the newly purified and the PCR negative controls are positive, a contamination during the PCR preparation may rather be assumed. This contamination type may simply be eliminated by working with new PCR reagents or master mixes. In this case also the complete PCR equipment should preventively be cleaned before use.

In each case it should be examined whether the contamination has completely been eliminated by carrying out a PCR run with negative controls (NTC, water controls) in multiple determinations.

2.1.5. Summary: Identification of contaminations

- Low positive signals in the negative control or repeatedly appearing low signals at a similar Ct value, which potentially indicate a pathogen load below the detection limit, indicate a contamination.
- In order to determine whether the contamination took place during the purification or at a later stage, for example during the PCR preparation, non-purified negative controls and newly purified negative controls have to be analyzed in multiple determinations in a PCR.
  - If the newly purified negative controls but not the PCR with the non-purified negative controls show a positive signal, a contamination during the purification process may be assumed.
  - If newly purified samples and the negative controls of the PCR are positive, a contamination during the PCR preparation is indicated.

2.2. Problems of data interpretation due to software adjustments

2.2.1. Import of standard curves

In some cases a positive signal may be observed despite the lack of a Ct value. This may also occur rarely in the case of very low positive sample material or contaminations dependent on the real-time PCR instrument and its software used. Such a result may also occur after the import of external standard curves (note: the import of external standard curves is not possible in the case of the ABI PRISM® SDS instrument). The software positions the imported standard curve according to a calibrator – defined as a standard – which is carried along in the PCR and automatically a fixed position of the threshold ensues. This position may be relatively high above the background and low positive amplification curves. Consequently, it is possible that no Ct value may be assigned to e.g. a low positive sample (see Figure 5).

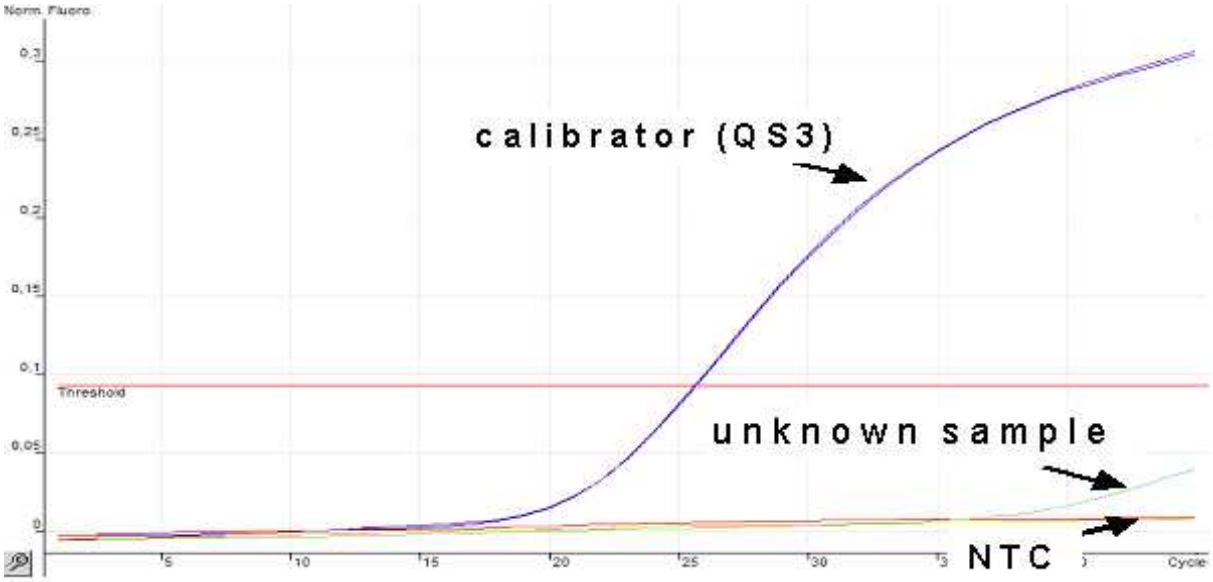

Figure 5: Analysis of a sample with the *artus* HBV RG PCR Kit using an *artus* 3000™ Instrument. The amplification curves of a quantification standard (QS3) are depicted in a double determination which was used as a calibrator for the import of a standard curve, as well as a sample to be determined (with a low rise in fluorescence) and two negative controls (NTC). No Ct value could be assigned to the amplification signal of the unknown sample so that, it might mistakenly be assessed as negative. The shape of the curve, however, indicates a positive PCR.

In order to get a more revealing picture, an analysis of the relevant data by means of a single curve analysis (SACA: single amplification curve analysis) is useful (see Figure 6). The shapes of the curves of the samples in question are directly compared to those of the distinctly negative ones. If the shape of the curve permanently runs nearly parallel to those of the negative controls during the whole PCR, it may be interpreted as negative. If, on the contrary, a rise in fluorescence takes place, even at a late timepoint, this must be assessed as a positive result, whereby a contamination still has to be excluded.

Figure 6: Single amplification curve analysis (SACA) of a sample with the *artus* HBV RG PCR Kit using an *artus* 3000 Instrument. The amplification curves of an unknown sample and those of the negative controls (NTC) are depicted. In order to increase the resolution of the presentation, the raw data (Raw Channel) were analyzed and the scaling of the depiction was automatically carried out (Auto-Scale). The comparison between the shape of the amplification curve of the sample and those of the negative controls allows an assessment (positive or negative) of the unknown sample. After this analysis the PCR of this unknown sample may be assessed as low positive.

A Ct value may be assigned to such uncertain samples by lowering the threshold manually. In the case of the LightCycler software for example, this is achieved by choosing the analysis mode *Fit Points*. However, it is not available when using external standard curves. Neither do the software of *artus* 3000™ and Rotor-Gene™ allow a quantitative determination of the pathogen load in the case of a manual adaptation of the threshold if an external standard curve is used. Further information on this subject is available in the *Technical Notes for the Quantification of the Pathogen Copy Numbers* (www.qiagen-diagnostics.com).

2.2.2. Summary: Problems of data interpretation due to software adjustment

- After the examination of the analysis adjustments such as the PCR profile and the analysis options of the fluorescence measurements (analysis channels, respectively filters), it is helpful to carry out single curve analyses in which uncertain samples may directly be compared to a negative control (e.g. water). The comparison between the shape of the amplification curve of the sample and those of the negative controls allows an assessment of the PCR (positive or negative) of the unknown sample.

3. Prevention

The identification of DNA or RNA contaminations, the search for their origins and their elimination can be a very time-consuming and cost-intensive procedure. Therefore, preventive measures to protect against a contamination are of significant importance. These measures concern the personnel, the facilities and laboratory equipment as well as the materials used.

In this context it should be emphasized that the QIAGEN Hamburg GmbH company regularly offers training for sample preparation and real-time PCR in which also measures for the prevention of contaminations are treated. If you are interested in participating in such a training please contact your QIAGEN sales representative.

3.1. Use of UDG (uracil DNA glycosylase) in PCR

A known strategy to eliminate contaminations of PCR products (amplicons) is the partial replacement of dTTP nucleotides (thymidine) by dUTP nucleotides (uracil) in PCR (Pierce & Wangh, 2004). If contaminations should occur with amplicons containing uracil, they may be eliminated by uracil DNA glycosylase (UDG) in a digestion step inserted before the subsequent PCRs. Corresponding to its specificity, UDG accepts only sequences containing dUTPs as a substrate. A possible destruction of the DNA to be detected by the following PCR is thus excluded. The efficiency of this method, however, is highly dependent on the specific UDG used, but also on its reaction and inactivation conditions (digestion temperature). High temperatures have frequently to be employed for several minutes for uracil DNA glycosylase inactivation, which may be disadvantageous for the subsequent PCR. In this case, sensitivity and specificity of the actual PCR may be diminished, on account of which this method is not applied in *artus* PCR Kits. In addition, the PCR products in the real-time PCR are mostly very short, which means that possibly only a few dUTP nucleotides are inserted, especially if the amplicons are GC-rich sequences. The digestion of the amplicons by uracil DNA glycosylase may partially be ineffective in such cases.

This form of contamination prevention has a limited application range since it can only stop the repeated amplification of PCR products with incorporated dUTPs. The general appearance of a contamination and the by far larger portion of contaminations, which arise for example during the purification process, are not stopped by this kind of prevention.

3.1.1. Summary: Use of UDG (uracil DNA glycosylase) in PCR

- PCR products are very short in the real-time PCR, on account of which the insertion of uracil into the amplificons during PCR and the treatment of subsequent PCRs with uracil DNA glycosylase may possibly be insufficient. Moreover, the efficiency of this method is very much dependent on the UDG used, its reaction and inactivation conditions (digestion temperature), which may have a disadvantageous effect on the sensitivity and specificity of the actual PCR.
- The application range of this prevention of contaminations is limited since it only stops the repeated amplification of PCR products with incorporated dUTPs. It does not prevent the general appearance of contaminations.

3.2. Personnel and clothing

Both the directive and the executing personnel hold an outstanding position in the prevention of contaminations. Above all, these may be avoided through expertise, caution and awareness for the particular requirements for the handling of PCR technology.

Working with PCR methods and especially with real-time PCR requires an extensive training and experience of the personnel. This includes a regular theoretical and practical continuing education as well as a sensitization for the particularities of the work with DNA or RNA. Furthermore, this comprises among others the handling of sample material as well as its preparation, the extraction of nucleic acid, the set up and run of the PCR and the evaluation of the experiment.

In order to guarantee a proper operating procedure, the activities in the laboratory should to be standardized and controlled (e. g. SOP, standard operation procedure). This allows a faultless and traceable procedure in the laboratory, but also the recognition and preventive avoidance of errors in the operation procedure. Avoiding causes of error (e.g. concerning the operation procedure) and the handling of possibly arising errors may be specified and structured by means of SOPs.

The wear and regular change of laboratory clothing (laboratory coats, disposable sleeve protectors, overshoes, masks, hoods) and gloves should be a standard. Powder-free gloves lower the risk of a contamination since possibly contaminated material may especially attach to the coated gloves. Above that, the coating may contain potential PCR inhibitors (e.g. starch) possibly leading to a reaction failure. Door handles and other objects, which are not directly linked to the working activities, should not be touched with gloves. In the case of

uncertainty during the handling of sample material or equipment (e.g. touching the rim of a tube with the glove), the gloves should strictly be changed.

Restrictions for the access to the laboratories may be imposed as another measure for the avoidance of contaminations.

Separate laboratory wear should be available for every laboratory (extraction, PCR set-up, and equipment room, see chapter **3.3 Facilities, three-room strategy**) and for every staff member. For this purpose, a color differentiation of the laboratory wear for the individual working areas is advantageous. This wear has always to be changed when entering or leaving a room. The laboratory clothing should regularly be cleaned (once per week) or one-way clothing for a subsequent disposal may be used (e.g. overalls, disposable sleeve protectors). For the storage of laboratory wear care should be taken that the laboratory coats do not get out of the room and that coat hooks are available at a sufficient distance from each other. The coats should not hang one above the other in order to avoid a transfer of possibly contaminated material.

According to general laboratory practices, hands should be washed with soap solution (e.g. Lifosan® Soft) before and after every activity. In addition, it is to be attended to the fact that a thorough disinfection (e.g. with Sterillium®) precedes the washing with soap solution in the case of handling infectious material.

The particularities of the work in a molecular biological laboratory should also be pointed out to the cleaning personnel. This includes the change of clothing (cleaning coat) when entering or leaving a laboratory as well as following an order according to which the relevant rooms should be cleaned (see chapter **4.2. Cleaning of contaminated laboratory rooms and materials**). Separate cleaning equipment should be available for every laboratory (bucket, floorcloth etc.).

3.3. Facilities

Local facilities permitting, one particular laboratory should be established for every working step in the execution of a PCR analysis (**three-room strategy**, see Figure 7). The focus ought to be on the spatial separation of sample preparation (nucleic acid isolation, extraction), PCR set-up, and PCR run (analysis, detection, instrument room). Materials and objects should only be transported unidirectionally, i.e. from the sample preparation to the PCR set-up laboratory and finally into the instrument room. If this is not possible in exceptional cases, the relevant materials and objects should be thoroughly decontaminated (see chapter **4.2. Cleaning of contaminated laboratories and materials**).

Three-room strategy

Nucleic acid isolation **PCR Set-up** **PCR run**

Figure 7: The nucleic acid purification as well as the PCR set-up should be carried out spatially separated from the detection.

Nucleic acids also attach to particles (dust) and may be distributed by draught. Therefore the laboratory doors should be kept closed in order to avoid intense air exchange between the rooms. A connection between the air-conditioning systems of the laboratories should not exist either. The use of aerators is generally not recommended. If possible, a sluice chamber system may precede the laboratories (possibly low pressure in the laboratories).

The use of work benches (see Figure 8: PCR hoods) with integrated UV lamps is another possibility to prevent contaminations. This is, above all, useful if a consequent spatial separation of the working areas is not possible.

Figure 8: PCR hoods of the Herolab GmbH. High-energy UV lamps combined with special reflectors for an irradiation of the whole working area from 5 to 30 minutes serve for the destruction of nucleic acids. The doors are equipped with security switches in order to protect the user from UV irradiation.

Air circulation out of the work bench into the room should absolutely be avoided. For this reason, work benches with an air suction device and an integrated carbon/HEPA filter may only partially be recommended. After suction and passing through the filter the air is again released into the room. This may lead to disturbing air circulations. Furthermore, dependent on the pore density of the filter used, smaller potentially contaminated particles may pass the filter and lead to DNA/RNA contaminations of the air.

If possible, the working area should preventively be decontaminated before and after an activity (e.g. UV light, see chapter **4.1.1.2 Decontamination using UV light**).

A color labeling may be useful for the particular sensitization of the personnel and for the demarcation of the working areas with the corresponding equipment such as pipettes. It is recommended to prepare the master mix in a special work bench, separated from the potentially positive material.

Further, it is recommended to read the equipment and product manuals of the manufacturers carefully and to follow the indications given there.

3.4. Materials

As already mentioned in chapter **3.3 Facilities**, all materials and devices should only be transported unidirectionally between the laboratories (from the extraction to the PCR set-up and finally into the instrument room). Otherwise, the relevant materials and devices (e.g. plastic holders for reaction tubes, cooling blocks or carousel of the LightCycler Instrument) are carefully to be decontaminated preventively. Each working place should, thus, have its own equipment (pipettes, pipette tips, centrifuges, vortexers a. o.).

Pipette tips with aerosol filters should exclusively be used (see Figure 9).

Figure 9: Example for pipette tips with aerosol filters: EPPENDORF “epT.I.P.s.“, STANDARDTIPS

A manual placement of the tips onto the pipette is to be avoided. The pipette piston may be protected against contaminations by the use of direct displacement pipettes and corresponding tips. All materials ought to be stored in closed containers. Especially reaction tubes should not be taken manually out of the stock packing (not even with gloves).

All surfaces of the working areas should be cleaned before and after the activity, for which purpose the measures listed in chapter **4.2. Cleaning of contaminated laboratories and materials** may serve. The execution of the extraction of nucleic acids may also be transferred into a security work bench. The internal air of the work bench should not escape outwards in order to avoid a distribution of the potentially positive material within the room.

The same applies also for the preparation of the PCR set-up. The use of PCR work benches offers the possibility to separate the working area and to decontaminate it with an integrated UV lamp. Pipettes, consumables as well as waste containers should always be in the work bench and be decontaminated with the methods mentioned above.

Waste (e.g. LightCycler capillaries) ought to be disposed of in lockable containers and additionally in a plastic bag. When using the LightCycler Instrument, the breaking of a used capillary is to be avoided by all means, since a large quantity of the PCR product (amplicon) may be in the capillary after the PCR. In the case of such a contamination, all objects (e.g. LightCycler Instrument, carousel) should thoroughly be cleaned (see chapter **4.3. Decontamination of a real-time instrument**).

For a better organization and control of the cleanliness, a cleaning plan should be established. All equipment should be cleaned regularly. It is recommended to carry out a thorough cleaning of the laboratories and a decontamination of all materials and devices once per week. During this process, pipettes should also be disassembled, as far as possible, and thoroughly cleaned.

Furthermore, the installing of UV lamps (see Figure 10) irradiating the whole room is recommended.

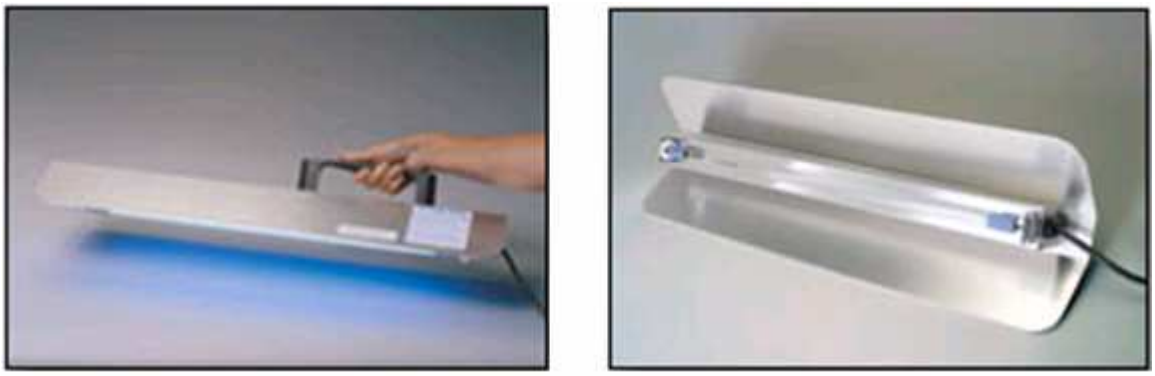

Figure 10: SterilWand™ UVC Emitter™ of the steril-Aire Inc. company. On the left: A flexible UV lamp for the irradiation of inaccessible areas. On the right: UV lamp with reflector for the irradiation of larger areas.

For the decontamination of the working areas, the UV lamps should be switched on overnight or at least for 30 minutes. Materials and equipment which could react sensitively to UV light (e.g. centrifuge plastic lids), should be protected against UV light and be de-contaminated separately. In addition, „dead spaces“ within the laboratory, which are not reached by the UV light, ought to be cleaned from possible contaminations. An example for a cleaning schedule can be found in chapter **6 Appendix**.

The rotor of used centrifuges is to be closed by a lid during use to limit a distribution of the material within the room in the case of an unintentional opening of a reaction tube during the run.

Stocks of liquids (e.g. lysis buffer, ethanol) have to be aliquoted and regularly be replaced. The *artus* Master Mix and the *artus* IC (internal control) should as well be aliquoted if required in order to avoid a contamination of the whole preparation and to minimize the disadvantages (costs, effort) resulting from it.

Wooden racks for storage of reaction tubes should not be used. Potentially contaminated material may attach on wood, due to its surface characteristics. For the same reason, wood is more difficult to decontaminate. Therefore, material made of plastic should always be used.

4. Elimination of contaminations (decontamination)

DNA and RNA contaminations are not evidently recognizable. After the work with DNA and RNA, all objects and materials should thus be regarded as potentially contaminated and thoroughly be cleaned.

Only relatively small DNA/RNA molecules (100-500 bp) are generally necessary for real-time PCR. That is why the appropriate solutions for the decontamination of nucleic acid contaminations have to guarantee that the DNA or RNA will be reduced to sufficiently small fragments or nucleotides in order to prevent a subsequent amplification through the PCR. The cleaning of the surfaces with alcoholic solutions (e.g. Terralin®) is **not** suitable for the decontamination of DNA and RNA contaminations, since alcohols do not destroy the structure of nucleic acids. They merely serve for the disinfection. The autoclaving (heat sterilization, 121 °C at 1 bar excess pressure) of materials is also inappropriate for the effective destruction of nucleic acids, since it also only guarantees the killing of microorganisms. Additionally, contaminations may arise due to aerosol formation during the opening of the autoclave.

4.1. Principles of decontamination

4.1.1. General effects of decontamination agents

Reagents for the decontamination of DNA and/or RNA are generally based on three different principles of destruction or inactivation of the genetic material: modifications, in order to prevent a possible amplification, denaturation, whereby a destruction of the genetic information also does not occur, and degradation. Only the degradation leads to the destruction of the genetic information, for which reason the efficiency of any decontamination is dependent on how strong and fast the degradation of nucleic acids takes place.

4.1.1.1. Chemical decontamination

Acidic or alkaline solutions are especially suitable for a locally limited decontamination of surfaces. The acidic or alkaline hydrolysis of the phosphodiester bond between the individual nucleosides of a DNA/RNA leads to a fragmentation of the DNA or RNA strands into single nucleotides.

4.1.1.2. Decontamination using UV light (“UV Photolinking“)

UV light irradiation (wave length <280 nm) may and should be applied both in the pre-PCR (genomic DNA from humans or pathogens) and in the post-PCR stage (PCR products = amplicons) and serves for both the decontamination of laboratory surfaces (floor, working areas) and the ambient air. This method makes use of the fact that adjacent pyrimidines of a DNA strand are covalently linked to each other. Such pyrimidine dimers are generated by a UV light induced photoreaction of the double bonds mainly between the C5 and C6 atom of adjacent pyrimidine rings and are linked to a stretched cyclic compound of four (see Figure 11). On the one hand, the pyrimidine dimers cause a steric blockade of the DNA polymerase. On the other hand, the DNA may no longer be denaturated completely so that lastly the polymerization reaction (PCR) will be terminated.

This photoreaction preferentially occurs between thymidines and in a distinctly lower number of cases (1:10) also between cytidines. Therefore, AT-rich sequences are more effectively blocked than AT-poor DNA. Since the short amplicons, as they are usual in real-time PCR, do not necessarily have AT-rich regions, UV irradiation is less effective in single cases. Since in fact UV light treatment only leads to a deactivation, but not to an elimination of nucleic acids, an additional regular chemical cleaning may not be abstained from.

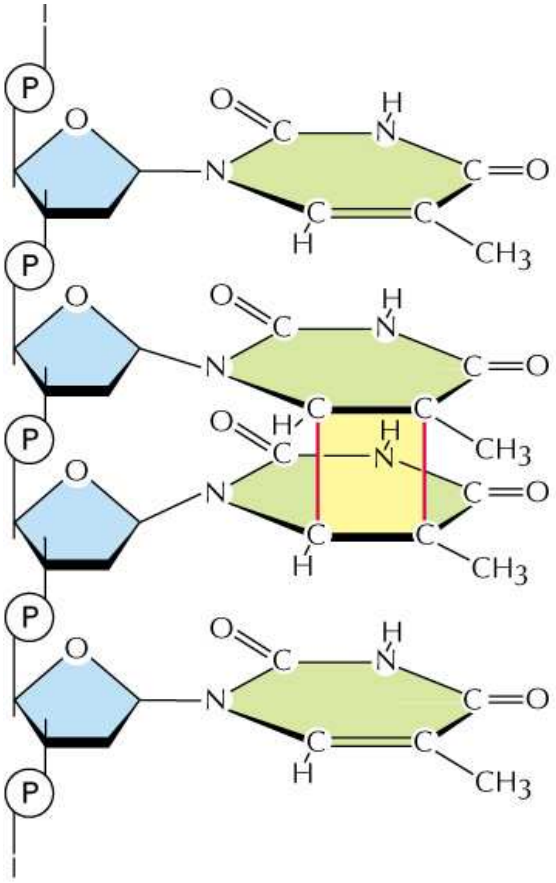

Figure 11: Schematic depiction of two adjacent pyrimidines which were covalently linked with each other to a stretched cyclic compound of four by UV light. Source: Alberts et al., Molecular Biology of the Cell, 4th ed., Fig. 5-48.

4.2. Cleaning of contaminated laboratory rooms and materials

The laboratories may be decontaminated by irradiation with appropriate UV light (< 280 nm). The use of a suitable reflector may increase the efficiency of the UV light by up to 90%. UV light with a low wave length (approximately 220 nm) leads to the formation of ozone gas (O_3). Ozone gas may also be used for the decontamination of laboratory rooms. Ozone, however, is injurious to health so that subsequently a sufficient exhaust of the gas has to be guaranteed.

A direct irradiation of the laboratory rooms and the equipment may be avoided by irradiating the room air flowing past a UV lamp (e.g. UV cleaner).

Since in this case only the circulating room air and the particles it contains are decontaminated, a separate cleaning of the equipment is necessary. This may be done with commercially available decontamination agents (e.g. DNA Zap®, DNA Away™, DNA-ExitusPlus™).

Compared to these agents, an alkaline (pH 12) 1% (v/v) solution from dishwasher powder (e.g. Calgonit) is an economical alternative. In order to avoid a corrosion of the purified surfaces by the Calgonit solution, a subsequent thorough washing with distilled water (aqua dest.) or 70% ethanol is necessary.

Furthermore, 0.5-2% hypochloric acid (sodium hypochlorite solution, NaOCl) destroys nucleic acids very effectively. Since hypochloric acid has also a corrosive effect, the decontaminated surfaces should subsequently carefully be washed with aqua dest.

The same applies for the use of a glycine HCl solution in order to decontaminate pipette pistons. For the decontamination of pipette pistons, a glycine HCl solution (pH 2) is used. The pipette pistons are incubated in the solution at 95 °C to 100 °C for 10 to 30 minutes and subsequently rinsed well with aqua dest. The drying of the pipette pistons occurs at a maximum of 60 °C (cabinet dryer). Afterwards the pistons should be slightly greased and be calibrated anew according to the manufacturer's indications.

Floors may be cleaned with commercial soap solution or sodium hypochlorite solution. Also in this case, a sensible order for the cleaning of the rooms should be fixed according to the **Three-room strategy** mentioned in chapter **3.3 Facilities**. Cleaning plans ought to be established for the rooms (see chapter **6 Appendix**).

4.3. Decontamination of a real-time device

In the case of a contamination of a real-time PCR device (e.g. breaking of a LightCycler capillary inside the apparatus), the indications of the corresponding manual should be observed or the manufacturer ought to be contacted. This applies as well for the use of an ABI PRISM® or an *artus* 3000/Rotor-Gene instrument. In general, it should be done without aggressive cleaning agents (e.g. Calgonit solution), acids or bases when decontaminating PCR devices. Instead, the use of commercial decontamination solutions (e.g. DNA Zap®) as well as of lint-free cloth (e.g. KIMTEC®) is recommended in order to avoid a damage of the optical system of the instrument. The same applies for the instrument's accessories (e.g. LightCycler carousel or rotor of an *artus* 3000/Rotor-Gene device). The holes in the LightCycler carousel for holding the capillaries may be reached and cleaned with moistened (decontamination solution) interdental or pipe bowl brushes (see Figure 12).

Figure 12: Commercially available interdental brushes for the cleaning of the carousels of the LightCycler Instrument.

4.4. Summary: Elimination of contaminations (decontamination)

- Working with PCR methods and especially with real-time PCR requires an extensive training and experience of the personnel. This includes a regular theoretical and practical continued education as well as a sensitization for the particularities of the work with DNA or RNA.
- SOPs (Standard Operation Procedures) allow a standardized and controlled working process.
- The **three-room strategy** should be striven for, but at least separated working areas should be installed if no separate rooms for the extraction, the PCR set-up, and the detection are available.
  - All materials and devices should only be transported unidirectionally (from the extraction to the PCR set-up and finally to the equipment room).
  - PCR hoods facilitate the separation of working areas within one laboratory. Every working place should have its own equipment (pipettes, pipette tips, centrifuges, vortexes a. o.).
  - The materials used should be appropriate for PCR diagnostics (e.g. aerosol filter tips) and be stored and used correspondingly.
- UV light should be applied in addition to the normal chemical cleaning procedure in order to decontaminate surfaces.

5. Product and manufacturer references

The products listed below serve for the prevention and decontamination of DNsA and RNA contaminations and were mentioned in the text:

Devices:

- DNA workstation Classic with carbon/HEPA filter for the exhaust air standard version, www.baack.de, cat.-no. BAA23001198W
- CleneCab PCR® Workstation, Herolab GmbH company, www.herolab.de, cat.-no. 30 00 10
- DNA-Workstation I, Kisker company, www.kisker-biotech.com, cat.-no. L020
- SterilWand™ UVC Emitter™ of the steril-Aire Inc. company, www.steril-aire.com

Materials and reagents:

- DNA Away™, BioExpress company, www.bioexpress.com, cat.-no. E-3071-1
- DNA-ExitusPlus™, AppliChem GmbH company and MultiBIND biotec GmbH company
- DNA Zap®, Ambion company, ww.ambion.com, cat.-no. 9890
- Hand disinfectant Sterillium®, Bode company, www.ratio-med.de
- KIMTEC® precision cloth, A. Hartensein company, www.laborversand.de, cat.-no. KW51
- Lifosan® Soft, B. Braun company, www.bbraun.de, cat.-no. 8505283
- Terralin®, Schülke&Mayr company, www.schuelke-mayr.com
- UV-Cleaner, Kisker company, www.kisker-biotech.com, cat.-no. L046-M
- Cloth, lint-free, 37 x 51 cm
- Calgonit, dishwasher powder

Cleaning agents:

- Soap solution: water-soap mixture (1% v/v), preparation: 5 strokes from Lifosan Soft dispenser (B. Braun company) in 1 l of water (1 stroke = ~2 ml)
- NaOCl solution: 0.5-2% sodium hypochlorite solution
- 10x Glycine HCl stock solution: 30.6 g NaCl, 39.2 g glycine (free acid), dilute in 523 ml aqua dest. and add 477 ml of 1 N HCl, pH 2. For the production of the 1x glycine HCl working solution, the 10x stock solution is diluted with an appropriate volume of aqua dest.

6. Appendix

Example for a cleaning schedule for the laboratory A:

Note:

- Laboratory doors and in-between doors are always kept closed.
- Every laboratory has its own cleaning materials and agents.
- Cleaning materials and agents remain in the respective laboratory.

In the sluice chamber, laboratory coats, protective gloves as well as overshoes are put on. Gloves must be worn in the laboratory!

Cleaning agents:

Mon, Wed, Thu:	Water-soap mixture (1% v/v), cloth (lint-free) preparation: 5 strokes from Lifosan Soft dispenser (Braun company) in 1 l of water. (1 stroke = ~ 2 ml)
Tue, Fri:	1. 1% sodium hypochlorite solution, cloth 2. soap, brand: Lifosan Soft, Braun company 3. water
Cleaning agents:	All necessary cleaning materials are below the sink in the respective laboratory sodium hypochlorite → white 5 l can water-soap mixture → pump bottle (1 l, PE)

On leaving the laboratory, switch on UV light for 20 min (ceiling, wall)

Cleaning:
All surfaces such as cupboards, refrigerators, working areas, equipment, window-sills, wheeled bins, Clene Cab from outside, big working benches from outside and cross struts, sink, sweep the floor and wipe with sodium hypochlorite solution or soap water, then finally clean with water!

Exchange trash bags, finally wipe sluice chamber. Dispose protective gloves and overshoes, send coats to the laundry.

7. References

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Mifflin, E.T., Setting Up a PCR Laboratory. PCR Primer, pp. 5-14, eds. Dieffenbach, C.W. & Dveksler, G.S., Cold Spring Harbor, 2003.

Pierce, K.E. & Wangh, L.J., Effectiveness and limitations of uracil-DNA glycosylases in sensitive real-time PCR assays. BioTechniques 2004, 36 (1): 44-48.

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In the case of further questions about real-time technology or other technical specifications of the *artus* products, please contact our technical service.

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