

## Introduction

DNA template quality is a critical factor in the successful amplification of target sequences via PCR. DNA is often damaged when stored or exposed to conditions such as acid, alkylating agents, heat, light, phenol/chloroform extraction, reactive oxygen species or simply time. Standard storage and extraction procedures result in base damage and/or loss that can impede primer annealing, polymerase fidelity and polymerase processivity, resulting in inefficient or failed amplification.

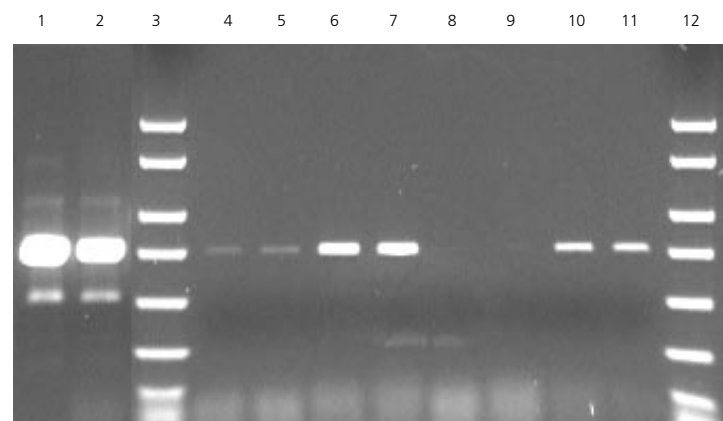
This poster highlights both the increased yield and specificity of PCR amplification from both damaged and undamaged DNA template using Restorase™, Sigma's new DNA repair and amplification polymerase blend. Restorase™ combines a DNA repair enzyme with Sigma's high-quality long PCR DNA polymerase blend AccuTaq™ to repair damaged DNA sites. The result is an increased amplicon specificity and yield. Restorase™ is designed to amplify long DNA fragments from damaged template DNA that is unable to be amplified using standard PCR enzymes. Restorase™ improves both the yield and specificity of PCR amplification from both damaged and undamaged template, and has been proven effective on amplicons ranging from 200 to 20,000 bp.

## Features and Benefits

- Repairs damaged DNA
- Amplifies sequence where other thermostable polymerases fail
- Amplifies sequences in multiplex reactions
- Increases amplicon specificity and yield
- Applicable over a wide range of amplicon size (200 bp to 20 kb)

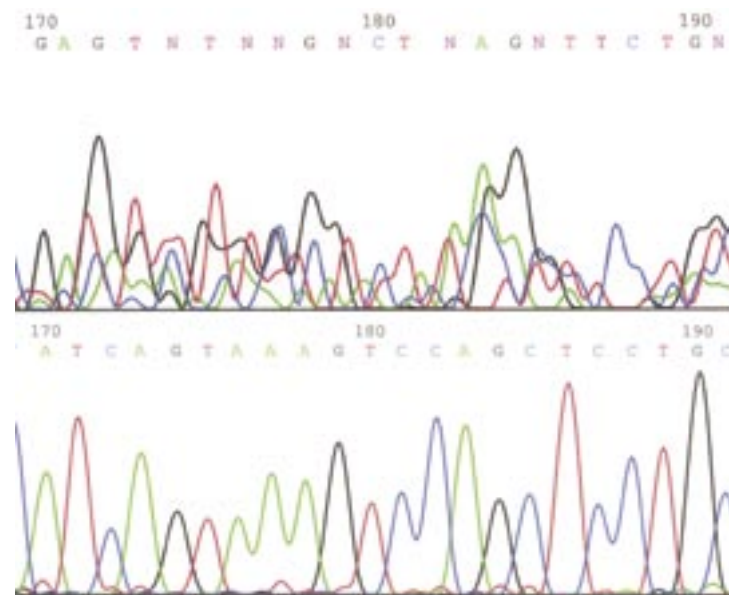
## Repair and Amplification of Highly Degraded DNA Template

Apurinic/aprymidinic (AP) sites represent some of the most common forms of DNA damage. To recreate this damage, Lambda DNA was treated with formic acid for 7.5 minutes and 10 minutes. At low pH (<4.0), DNA undergoes depurination. By controlling the duration of exposure to acid conditions, we can control the extent of depurination. Reactions were quenched by raising the pH to physiological levels (7.0) with the addition of sodium hydroxide. The ability of Restorase™ to amplify a 742-bp fragment of DNA from these samples was compared to that of Taq DNA polymerase (Figure 1A). Restorase™ increased amplicon yield over Taq when amplifying from the 7.5-minute formic acid treated DNA, and produced ample product from the 10-minute formic acid treated DNA that Taq was unable to amplify. Sequence analysis of products from lanes 5 and 6 demonstrate the ability of Restorase™ to repair damaged DNA (Figure 1B).



**Figure 1A. Restorase™ allows for PCR amplification of DNA that could not be amplified using Taq DNA polymerase.** Lambda DNA was damaged with formic acid for 7.5 minutes and 10 minutes.

A 742-bp fragment was amplified from DNA damaged for 0 (control; lanes 1–2), 7.5 (lanes 4–7), and 10 minutes (lanes 8–11). Taq-amplified products were run in duplicate in lanes 1–2, 4–5, and 8–9, while Restorase™-amplified products were run in duplicate in lanes 6–7 and 10–11. Lanes 3 and 12 show PCR marker (Sigma product P9577).

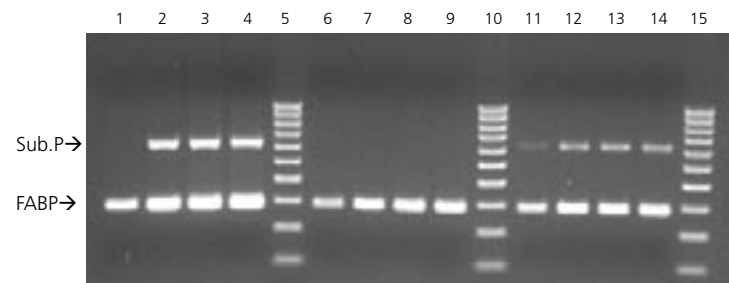


**Figure 1B. Restorase™ allows for PCR amplification of DNA that could not be amplified using Taq DNA polymerase.** Lambda DNA was damaged with formic acid for 7.5 minutes and 10 minutes.

Sequencing of Taq-amplified product from 7.5-minute damaged template (lane 5) resulted in a high percentage of mis-calls and unreadable sequence (top picture), while sequencing of Restorase™-amplified product from 7.5-minute damaged template (lane 6) resulted in high-quality sequence (bottom picture).

## Rescue of Correct Mouse Genotyping

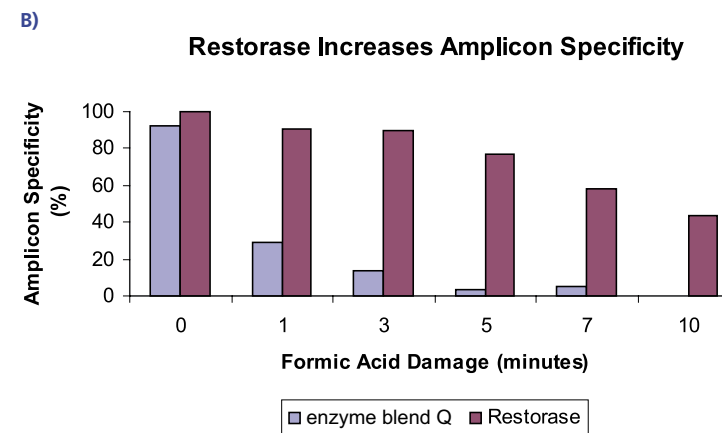
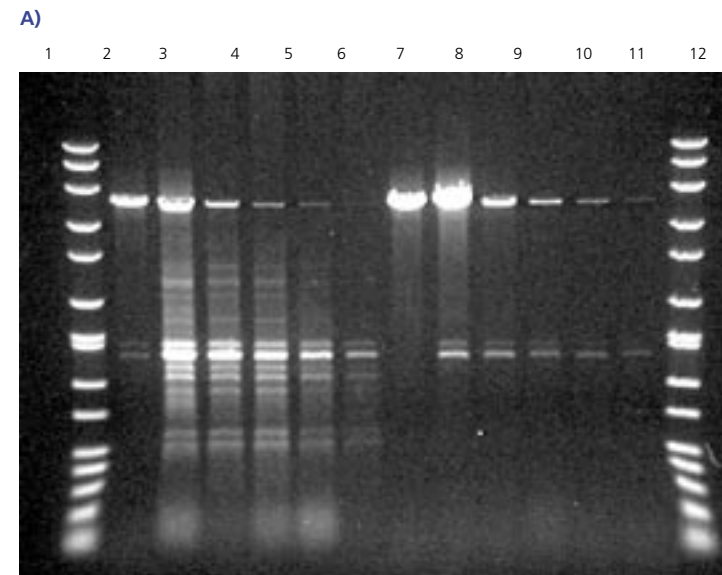
Meaningful results often rely on the accurate genotyping of experimental organisms during the early stages of an experiment. Incorrect genotyping can result in the loss of both time and rare experimental individuals. We tested the ability of both Taq DNA polymerase and Restorase™ to correctly genotype both Substance P (Sub. P) positive and negative (knockout) mice from phenol chloroform extracted mouse genomic DNA samples (Figure 2). A 289-bp fragment of fatty acid binding protein (FABP) was amplified (positive control) in multiplex reactions with a 627-bp fragment of Sub.P. Pup 1 (lanes 1–4) and pup 24 (lanes 11–14) are wild type (contain sequences for both FABP and Sub.P), while pup 2 (lanes 6–9) is a Sub. P knockout (contains only FABP sequence). While Restorase™ correctly identified both wt mice, Taq DNA polymerase failed to amplify detectable Sub.P bands, potentially leading to the incorrect genotyping of both wt mice.



**Figure 2. Restorase™ rescues correct genotyping where Taq DNA polymerase fails.** Lanes 5, 10, and 15 show 100-bp PCR low ladder (Sigma product P1473). Lanes 1–4 show products amplified from wild type pup 1, lanes 6–9 show products amplified from Sub.P knockout pup 2, and lanes 11–14 show products amplified from wild type pup 24. Products in lanes 1, 6, and 11 were amplified using Taq, failing to produce Sub.P bands, resulting in the incorrect genotyping of pups 1 and 24. Products in lanes 2–4, 7–9, and 12–14 were amplified using Restorase™, producing proper bands in all cases resulting in the proper genotyping of each mouse. Restorase™ samples for each mouse were pre-incubated with Restorase™ PCR mix at 0 °C for increasing amounts of time (from left to right; 1, 3, and 5 minutes, respectively).

## Enhanced Yield and Specificity

Specificity and yield are two important considerations of PCR performance. The inefficient amplification of sequence can lead researchers to increase PCR cycle number, thus increasing the probability of misincorporation of nucleotides. This can be problematic when ultimately sequencing a PCR product. Likewise, poor specificity can result in numerous unwanted bands, both interfering with and confusing the interpretation of experimental results. We compared yield and specificity of Restorase™ to that of a high-fidelity enzyme blend (Figures 3A and B). Each enzyme was used to amplify a 5-kb fragment of human genomic DNA from 0, 1, 3, 5, 7, and 10-minute formic acid treated samples. Restorase™ produced a ≥2-fold increase in amplicon yield over the high fidelity enzyme blend (Figure 3A, quantification data not shown), and greatly increased primer specificity in all samples tested (Figure 3B).



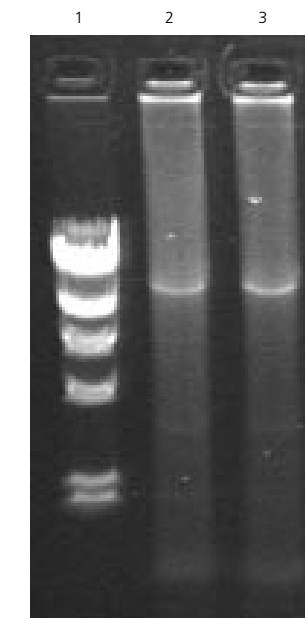
**Figure 3. Restorase™ increases both yield and specificity over high fidelity enzyme blend Q.**

**A)** Human genomic DNA was damaged with formic acid for 1, 3, 5, 7, and 10 minutes. Lanes 1 and 14 show a 1-kb DNA ladder (Sigma product D0428). Primers used were designed to target a 5-kb fragment of human genomic DNA. A standard high-fidelity enzyme blend was used to amplify product from template with 0, 1, 3, 5, 7, and 10 minutes of formic acid damage (lanes 2, 3, 4, 5, 6, and 7, respectively). Restorase™ was used to amplify product from template with 0, 1, 3, 5, 7, and 10 minutes of formic acid damage (lanes 8, 9, 10, 11, 12, and 13, respectively).

**B)** Restorase™ increases amplicon specificity compared to standard high-fidelity enzyme blend Q. Amplicon specificity was measured as a percentage of specific product [(5-kb amplicon yield/sum of all amplicon yields) x 100%], and was obtained from the reactions performed in Figure 3A.

## Enhanced Long Template Amplification

DNA damage from standard storage and extraction procedures can inhibit PCR. With increased amplicon length comes increased occurrences of polymerase stalling due to base damage and/or loss, rendering lengthy target amplification difficult. The ability of Restorase™ to repair damaged DNA makes it an effective tool in the amplification of lengthy target sequence. To demonstrate its utility in the amplification of extremely long stretches of DNA template, we used Restorase™ to amplify a 20-kb region from Human genomic DNA (Figure 4).



**Figure 4. Enhanced Long Template Amplification**

Each 50 µl PCR reaction contained 2.5 units of Restorase™, 7.5 ng of DNA template, 500 µM dntps, and 60 pmol of each forward and reverse BigBand™ primer in 1x Restorase™ PCR buffer. Two-step PCR was performed as follows: one cycle of 94 °C for 30 seconds; thirty cycles of 94 °C for 30 seconds followed by 68 °C for 20 minutes; 1 cycle of 68 °C for 20 minutes. Lane 1 contains Lambda DNA Hind III Digest (Sigma product D9780); Lanes 2–3 each contain 5 µl of Restorase™-amplified PCR product (20 kb). BigBand™ modified oligonucleotide primers increase the yield of Restorase™-amplified products and eliminate the need for a manual hotstart. BigBand™ primers are available only from Sigma-Genosys (www.sigma-genosys.com).

## Conclusion

In conclusion, Restorase™ allows for the amplification of highly degraded DNA samples unable to be amplified using other polymerases. As it increases both yield and specificity in all samples tested, it is also a powerful enzyme blend for day-to-day use. As demonstrated by experimental results, Sigma's Restorase™ is a powerful and superior enzyme blend in the amplification of both damaged and undamaged DNA.

## Related Sigma products

Product Name	Product #
Restorase™ (Patent Pending)	R1028
BigBand™ Primers	www.sigma-genosys.com
Precast 4% Agarose Gel	P5847
Precast 1% Agarose Gel	P5722
PCR Marker (50–2,000 bp)	P9577
PCR 100bp Low Ladder (100–1,000 bp)	P1473
1-kb Ladder (0.5–10 kb)	D0428
Lambda DNA Hind III Digest (125–23,130 bp)	D9780
SeqSaver™ Sequencing Premix Dilution Buffer	S3938
SigmaSpin™ Post-Reaction Clean-Up Columns	S5059
10x Capillary Electrophoresis Buffer	B4930
Formic Acid	F4636

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