

STRboost™: A Novel STR Amplification Enhancer

Introduction

Successful forensic analysis depends on the ability to identify and individualize biological evidence. Unfortunately, many forensic sample types (e.g. degraded samples, touch evidence, or those derived from hairs, bone and teeth), yield low quality and/or low quantity DNA. Degradation resulting from sub-optimal storage conditions can further exacerbate difficulties associated with successful forensic DNA typing using multiplex STR analysis. STRboost is a novel reagent specifically formulated to enhance multiplex PCR amplification reactions used for human identification applications to facilitate successful recovery of forensic DNA profiles from compromised sample types. STRboost was evaluated for its ability to recover genetic profiles from low quantity sample types and also those containing inhibitors. Results indicate that using STRboost can overcome limitations associated with multiplex STR analysis using compromised sample types to resolve stochastic effects such as peak balance issues, allele dropout and sub-optimal peak height definition.

Materials and Methods

Amplification Reactions: Various amounts of human genomic DNA (62.5, 31.25, 15.62, 7.81, and 2.00 pg) were used as templates in amplification reactions using primers specific for the β -actin gene product (490 bp) in the presence or absence of STRboost. Each reaction contained 2.5 U Platinum Taq DNA polymerase (Invitrogen), 10x reaction buffer, 10 mM dNTPs mix (Invitrogen), 50 mM $MgCl_2$, 10 μ M final concentration of human β -actin forward (5' ctacctcatgaagatcctcacc) and β -actin reverse (5' gtacttgcgctcaggaggagc); and either water or STRboost in a final volume of 30 μ l. Cycling parameters were: 94°C for 2 min followed by 40 cycles of 94°C for 15 sec, 55°C for 30 sec, ending with 72°C for 7 min. A 10 μ l aliquot of each reaction was run on a 0.8% agarose/ethidium bromide gel.

Multiplex STR Analysis: Human genomic DNA (100 pg) was amplified using the AmpF λ STR® Identifier kit (ABI) with or without STRboost included in the reaction. Amplified products (1.5 μ l) were then analyzed on an ABI PRISM 310 Genetic Analyzer to generate genetic profiles. For reactions containing hematin, the inhibitor was added to a final concentration of 5 μ M in each reaction containing STRboost or water. Cycling parameters: 95°C for 11 min followed by 28 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min and a final extension of 60 min at 60°C.

Results

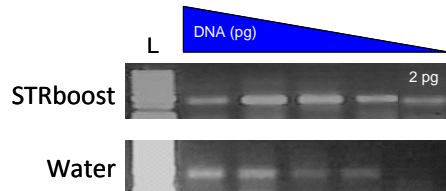


Figure 1. STRboost enhances amplification of low quantity samples (picogram). Decreasing amounts of DNA were used as templates in reactions containing STRboost (top) or water (bottom) for amplification of the β -actin gene product (490 bp).

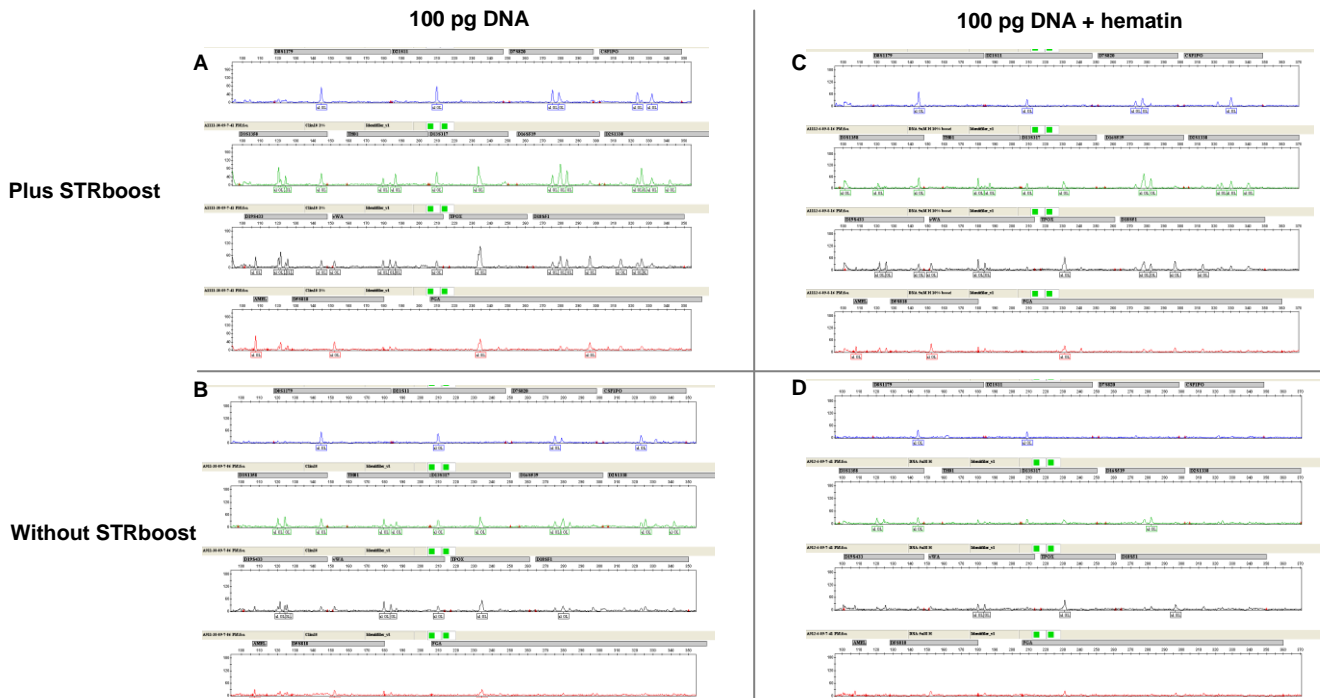


Figure 2. STRboost enhances amplification and recovery of genetic profiles from challenging sample types, including low quantity DNA sample containing inhibitors. (A) 100 pg DNA + STRboost; (B) 100 pg DNA without STRboost; (C) 100 pg DNA + 5 μ M hematin + STRboost; (D) 100 pg DNA + 5 μ M hematin without STRboost.

Discussion

We have evaluated the use of STRboost for forensic DNA analysis to enhance amplification and recovery of STR profiles from challenging sample types. Results presented in Figure 1 indicate that the yield of amplified products is significantly improved by including STRboost in end-point PCR reactions containing extremely low quantities (picogram) of DNA as might be recovered from trace evidence of low copy number samples in forensic evidence. STRboost was then included in amplification reactions using the Identifiler kit to assess the ability of the enhancing reagent to successfully recover DNA profiles from challenging sample types, such as those containing inhibitors or low quantity of input DNA. The STR profiles obtained from these reactions are shown in Figure 2. Results indicate enhancement of amplification from as little as 100 pg DNA (panel A, compare to panel B), and also samples containing inhibitors (e.g. hematin; compare panels C and D). Profiles generated from reactions containing STRboost resulted in increased peak height at loci and improved recovery of alleles, thus enabling the identification of individuals in instances where traditional methods fail to provide a useable profile. STRboost can be easily incorporated into existing workflows to enhance the accuracy, sensitivity and precision of multiplex STR analysis used for genetic identification. Including STRboost in amplification reactions helps resolve stochastic effects typically associated with sample degradation or inhibition (e.g. peak balance issues, allele dropout, and suboptimal peak height definition), thus facilitating recovery of complete DNA profiles used for forensic identification purposes.