ABSTRACT

FORENSIC DNA TYPING USING A TREHALOSE STABILIZED PCR REACTION MIXTURE

Perhaps one of the greatest challenges faced by the forensic DNA laboratory today is that of backlog reduction. Automation is the key to reducing backlog, but even with current automated methods much time is spent on liquid handling. The goal of this study was to demonstrate a proof-of-concept that would reduce liquid handling by creating a stable, dry, and ready-to-use PCR reagent mixture by incorporating the sugar trehalose into the mixture. This study was conducted to determine whether: 1) the addition of trehalose affected amplification of forensically relevant STR loci, 2) freeze-drying and adverse storage conditions affected the reagent mixture, and 3) long term storage of the PCR reagent mixture affected the quality of forensic DNA profiles. The addition of trehalose to the ABI Identifiler™ kit PCR master mix did not adversely affect the PCR reaction. In the absence of trehalose, PCR master mix stored at -20˚C and 22-25˚C failed to yield complete DNA profiles (with the exception of a single frozen sample). In stark contrast, complete DNA profiles were obtained for trehalose preserved lyophilized PCR master mix stored at -20˚C and at 22-25˚C. A minimum of 8 of the 16 loci were profiled at 50˚C, with fewer peaks found at 60˚C. DNA profiles were not obtained from lyophilized reaction mixtures stored at 90˚C. ABI Identifiler™ master mix preserved with trehalose was viable for up to 6 weeks at 18-23˚C and may be viable for longer periods of time. After further optimization, trehalose preserved master mix can potentially lead to preloaded 96 or 384-well plates, which would lend them for use in automating the PCR reaction process.

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