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Research article

Principles of STR multiplex amplification

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Abstract

We performed a systematic evaluation of template amount, multiplex primer concentration, amplification cycle number, and amplification volume on commercial multiplex performance. We determined that, except for stochastic variation with limited template amounts, the same quality, intensity, and accuracy of DNA profiles can be obtained while varying each of these parameters over a broad range. Each parameter can be used to compensate for changes in the others, allowing us to provide specific recommendations with use of limited sample material.

Keywords: STR; Multiplex amplification; Forensic

1. Introduction

Several publications indicate that amplification volumes lower than the manufacturers' recommendations yield excellent DNA profiles. Gaines et al. [1] reported some success with as little as 30 pg of DNA in a 5 μl AmpFlSTR® Profiler Plus® amplification reaction. Frégeau et al. [2] showed effective 25 μl Profiler Plus amplification reactions and Leclair et al. [3] suggested enhanced detection and sensitivity with 5 μl Profiler Plus amplification volume reduction. Recently, Schmidt et al. [4] demonstrated effective 1 μl amplifications on specially designed glass chips. In this work, we systematically show that: (1) reduced reaction volume is inversely proportional to signal strength of amplification products, (2) this principle applies generally across several multiplexes, and (3) the increased signal strength of lower volume reactions can be counter-balanced with use of a modified reaction mix and decreased amplification cycle number to produce a profile equivalent in quality and strength to that created under manufacturer-recommended conditions.

2. Materials and methods

Strain 9947 DNA was used in all experiments. DNA template amounts as indicated were amplified in volumes indicated either in manufacturer-recommend reaction mix or a modified reaction mix. Amplifications of 50, 25, 15, and 12.5 μl were performed in 96-well trays. Amplifications of 2 μl were performed in 384-well trays. Amplifications of 6 μl were performed in both tray formats (as labeled). All materials were amplified by holding temperature at 95 °C for 11 min, then cycling at 94 °C for 1 min, 59 °C for 1 min, 72 °C for 1 min for the number of cycles indicated, followed by 60 °C for 60 min, and indefinite hold at 10 °C. Amplification products of 2 μl reactions were diluted to 6 μl with a proprietary CE set-up buffer prior to separation. All products were separated on either AB 3100 or 3130xl Genetic Analyzers.

3. Results and discussion

3.1. Amplification volume

We used AmpFlSTR® Identifiler® PCR Amplification Kit components (Applied Biosystems, Foster City, CA USA) to amplify a range of DNA templates from 2 pg to 2 ng in triplicate amplifications using 2, 6, 12.5, 15, and 25 μl amplification reaction volumes, respectively, in 28 cycles of amplification. All reaction components were used at the concentration recommended by the manufacturer. Fig. 1 displays the results showing that the product yield and corresponding fluorescent intensity, measured in rfu per allele, increases with each reduction in amplification reaction volume. The slope of the signal increase is steeper and the instrument saturation plateau is reached at lower template amount when lower amplification volumes are used (Fig. 1). This effect is

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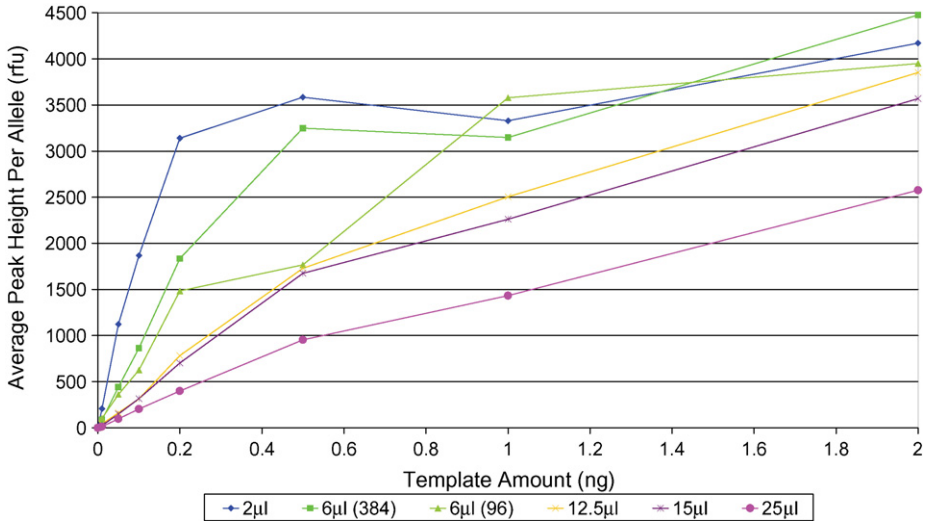

Fig. 1. Identifiler amplifications of 28 cycles were performed in triplicate in the reaction volumes shown using manufacturer reagents and protocol as described in Section 2 using 2 ng, 1 ng, 500 pg, 200 pg, 100 pg, 50 pg, 10 pg, 2 pg, and no DNA template, respectively.

observed with all multiplex sets tested including the AmpFlSTR® Identifiler®, Profiler Plus®, and COfiler® PCR Amplification Kit components, respectively (data not shown).

Note that, generally, amplification of twice the template amount in twice the amplification volume generates similar signals. Thus, except for cases of low copy number samples, increasing amplification volume to analyze a proportionally increased sample volume does not improved allele detection. While this is a common practice, increased amplification volumes consume more evidentiary material to obtain the same result.

3.2. Counterbalance with other parameters

Increased signal strength while amplifying the same amount of template requires adjustment to interpretation guidelines because the same amount of template generates more fluorescent product and final signal intensity. Alternately, the increased signal intensity can be modulated with adjustments that decrease signal. We employed a combination of a modified reaction mix and 25 amplification cycles to accomplish this. Fig. 2 displays results with these conditions using the same ranges of template and amplification volumes. Note that all signals are much reduced in intensity and the lower volume reactions do not demonstrate signal saturation with templates up to 2 ng.

A close comparison of the 28 cycle-25 µl amplification reaction volume (Fig. 1) and 25 cycle-2 µl amplification reaction volume (Fig. 2) results reveals nearly identical fluorescent signal (rfu/allele) in response to use of various template amounts. The profile quality under the two conditions is also indistinguishable. Thus, except in circumstances of very limiting sample material, it saves both evidentiary sample and reagent expense to use the low amplification reaction volume approach. As has been described previously [5], stochastic

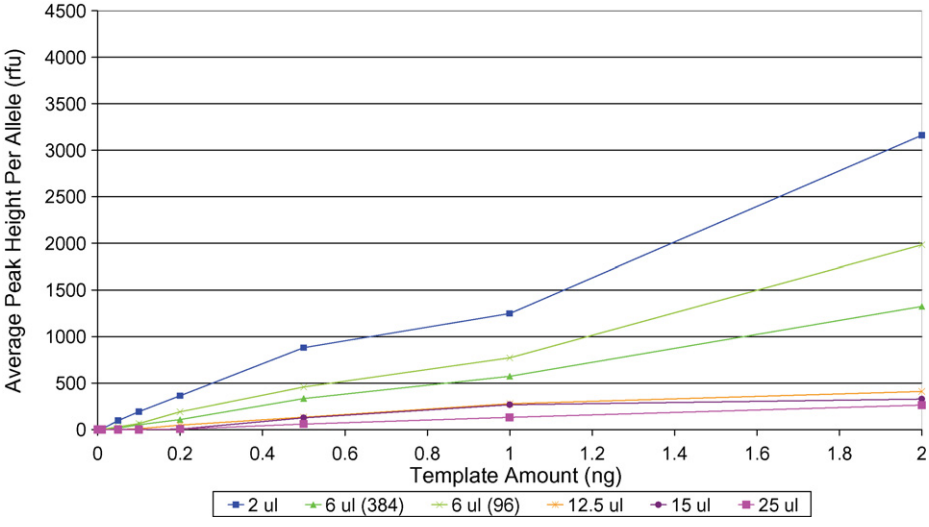

Fig. 2. Identifiler amplifications of 25 cycles were performed in triplicate in the reaction volumes shown using modified reagents and manufacturer protocol as described in Section 2 using 2 ng, 1 ng, 500 pg, 200 pg, 100 pg, 50 pg, 10 pg, 2 pg, and no DNA template, respectively.

issues prevail with small amounts of input template and in such circumstances, the larger reaction volume is the best choice.

4. Conclusions

Increased signal intensity per allele can be obtained for a constant template amount by lowering the amplification volume. Compensating decreases in intensity can be achieved by adjusting amplification mix and lowering the amplification cycle number. Making these compensating adjustments permits high quality forensic analyses while saving both sample material and reagent cost.

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Bode Technology (by employment). The sponsor was involved in the study design; collection, analysis and interpretation of data; the writing of the manuscript and the decision to submit the manuscript for publication.

Conflict of Interest

I'm an inventor on patents assigned to Promega Corporation, the manufacturer and supplier of a multiplex STR product that competes with the Applied Biosystems Identifiler product described in this work. This had no influence on my results or viewpoint.

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