Use of 12cm Plates on an ABI PRISM® 377 DNA Sequencer For Eight-Loci STR Multiplex Genotyping

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Our objective was to modify the standard protocols slightly in order to enable efficient genotyping of samples amplified with PowerPlex® 1.2 using 12cm rather than 36cm plates.

INTRODUCTION

Genotyping of 8 STR loci plus Amelogenin using the PowerPlex® 1.2 System[a,b] (1) on an ABI PRISM® 377 DNA sequencer is generally carried out on 36cm plates. When using kits that amplify three loci simultaneously, 12cm plates can be used (2). In the latter case, gel casting is simpler and less time consuming. Our objective was to modify the standard protocols slightly in order to enable efficient genotyping of samples amplified with PowerPlex® 1.2 using 12cm rather than 36cm plates.

MATERIALS AND METHODS

DNA mobility in a polyacrylamide gel is influenced by several factors such as acrylamide concentration, voltage, ionic strength, temperature, acrylamide:bisacrylamide ratio, etc. (3). In order to obtain efficient separation of STR fragments using 12cm instead of 36cm plates (having tried several different changes to the system) we chose to modify two parameters: voltage and acrylamide concentration. We reduced voltage down to 650 volts, which gives an effective 610 volts on the plates, for two reasons: first to obtain sharper bands; and second to reduce gel extrusion caused by electro-endosmotic flow between polyacrylamide and the two plates. Reducing gel extrusion allows reuse of the gel for a total of three consecutive runs. We also increased the polyacrylamide concentration to 6.75% to enhance separation of the amplified DNA fragments. All the other parameters follow the standard protocols provided in the ABI PRISM® 377 DNA sequencer manual.

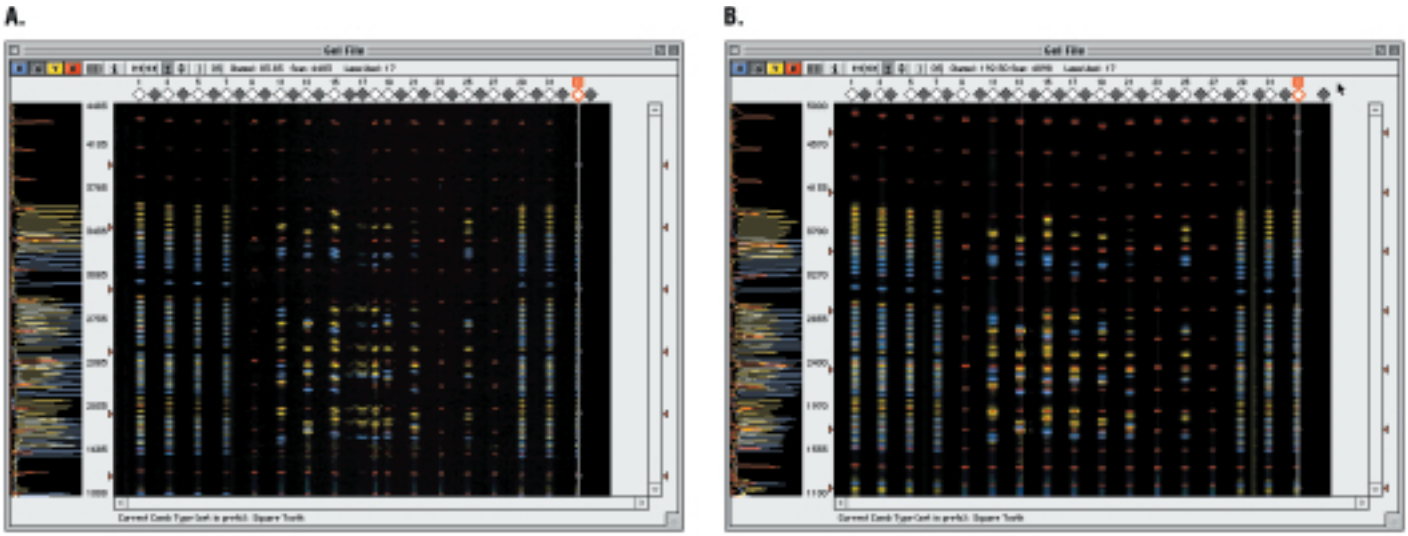

Figure 1. ABI PRISM® GeneScan® 3.1 collection gel of 36cm plate (Panel A) and 12cm plate (Panel B) showing samples and ladders amplified with the PowerPlex® 1.2 System.

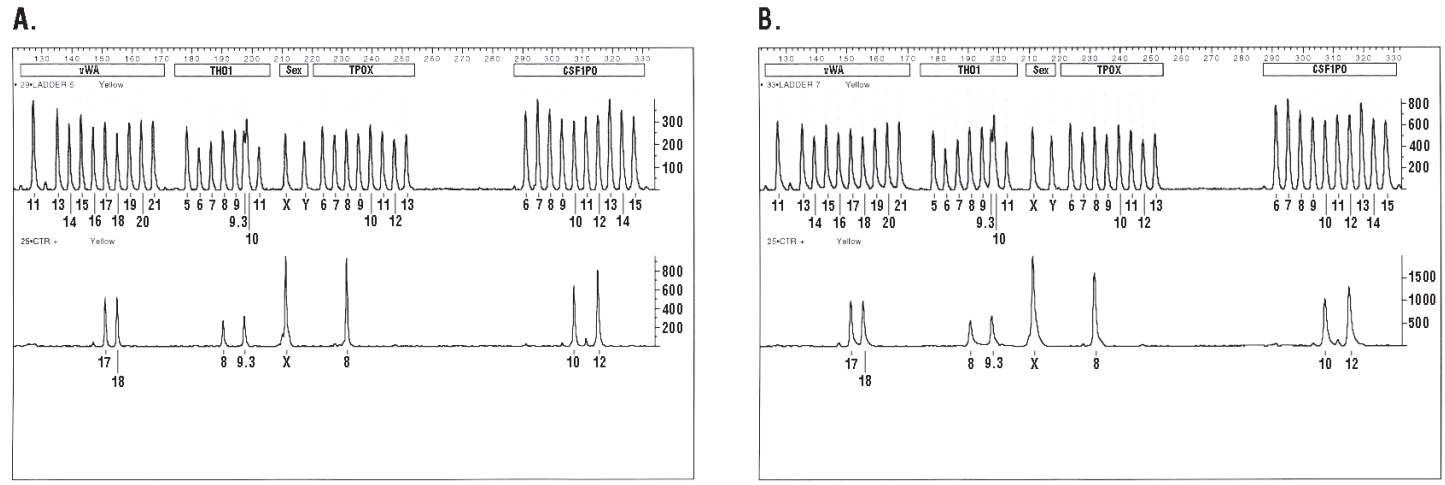

Figure 2. Electropherograms of ladder and sample amplified with the PowerPlex® 1.2 System on a 36cm plate (Panel A) and a 12cm plate (Panel B) genotyped with ABI PRISM® Genotyper® 2.1 software.

RESULTS AND DISCUSSION

To validate and guarantee accuracy in genotyping, these new conditions were tested in over 300 runs of known samples amplified with the PowerPlex® 1.2 System and run on 12cm plates. Figure 1 shows ABI PRISM® 377 data collection gel images of the 36cm and 12cm plate runs, respectively. Figure 2 shows electropherograms of ladders and samples amplified with PowerPlex® 1.2, run on 36cm and 12cm plates, respectively, and genotyped with the Genotyper® software (4). It is evident that allele separation is equally efficient using both methods and that genotyping remains accurate. A typical delicate genotyping situation, shown in Figure 3, is the separation between the 9.3 and 10 alleles of TH01 from the PowerPlex® 1.2 ladder, first run on 36cm, then on 12cm plates. It is evident that similar resolution is achieved.

The modifications made for analysis on 12cm plates lengthen the run up to almost 3 hours, compared to the 2 hours and 30 minutes required applying the standard protocols. However, there are several advantages to genotyping 8 STR loci per lane using 12cm rather than 36cm plates with the ABI PRISM® 377 DNA sequencer: a) gel casting is more practical; b) a second and a third run can be performed immediately, one after another, without changing gel and run buffer, since the lower voltage of the run decreases gel stress, and the run buffer, for the same reason, has a longer life; c) the costs for buffer reagents, acrylamide, TEMED, APS and Amberlite are altogether reduced by over 80%; d) the production of hazardous waste is reduced (i.e., worn out acrylamide gel). The modifications to the running conditions maintain sensitivity, separation and correct allele determination.

REFERENCES

1. *PowerPlex® 1.2 System Technical Manual* #TMD009, Promega Corporation.
2. *ABI PRISM® 377 DNA Sequencer User's Manual*, The Perkin-Elmer Corporation.
3. Andrews, A.T. (1986) *Electrophoresis,* 2nd ed., Claredons Press.
4. *Genotyper® User's Manual*, The Perkin-Elmer Corporation.

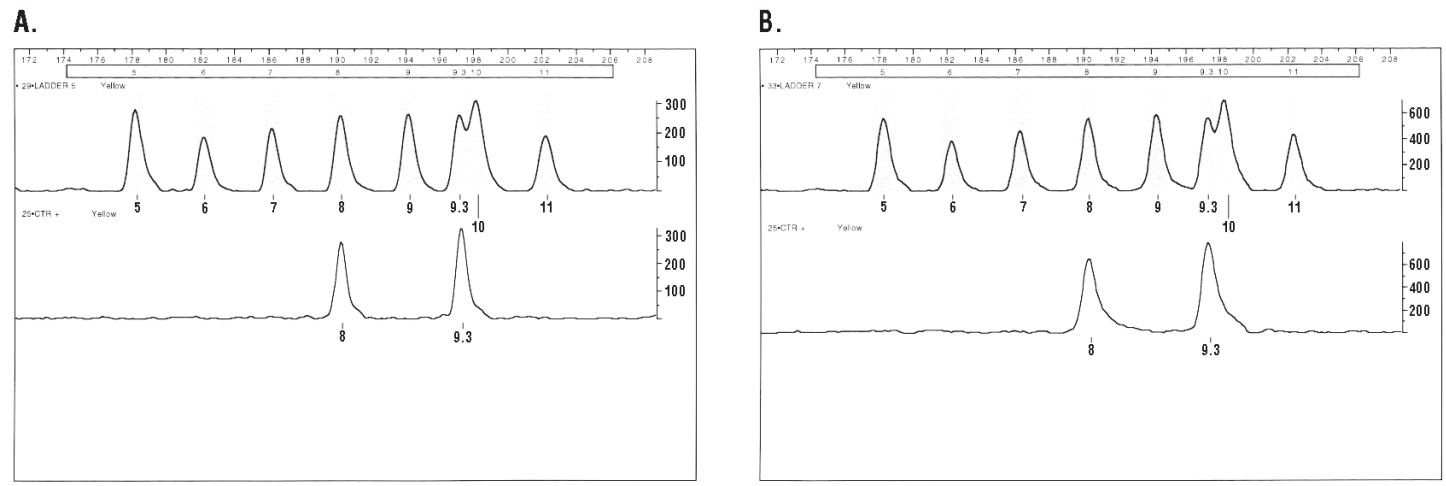

Figure 3. TH01 STR locus electropherograms of ladder and sample amplified with the PowerPlex® 1.2 System on a 36cm plate (Panel A) and a 12cm plate (Panel B) and genotyped using ABI PRISM® Genotyper® 2.1 software.