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Genotyping with a 16-locus STR multiplex using 12-cm plates on an ABI PRISM 377 DNA Sequencer

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1. Introduction

Genotyping of 15 STR loci plus Amelogenin by use of the commercial kit Powerplex® 16 system Promega [1], on an ABI PRISM™ 377 DNA Sequencer (Perkin Elmer), is generally carried out on 36-cm plates.

We demonstrate that genotyping of samples amplified with Powerplex 16 can be accomplished efficiently on 12-cm plates [2]. To guarantee sufficient accuracy, different conditions were tested in over 300 runs in which 12-cm plates were used. There are two advantages of performing electrophoresis, genotyping 16 loci per lane, with 12-cm plates rather than 36-cm plates with the ABI PRISM 377 DNA Sequencer: firstly, gel casting is more practical; secondly and more importantly, the same gel can be used for up to three consecutive runs because the lower voltage of the run exposes the gel to lower stress, maintaining sensitivity, separation, and correct allele determination.

2. Materials and methods

In a polyacrylamide gel, DNA mobility is influenced by several factors such as acrylamide gel concentration, voltage, ionic strength, temperature, acrylamide–bisacrylamide ratio, etc. [4]. After attempting several different changes to the system to obtain efficient separation of STR fragments with 12-cm instead of 36-cm plates, we chose to modify two parameters: voltage and acrylamide concentration. We reduced the voltage to 650 V, to have an effective 610 V on the plates, for two reasons: first, to obtain sharper bands; second, in order to reduce gel extrusion caused by electro-endosmotic flow between the polyacrylamide and the two plates. Reducing gel extrusion enables the gel to be re-utilized for a total of three consecutive runs. On the other hand, we increased the

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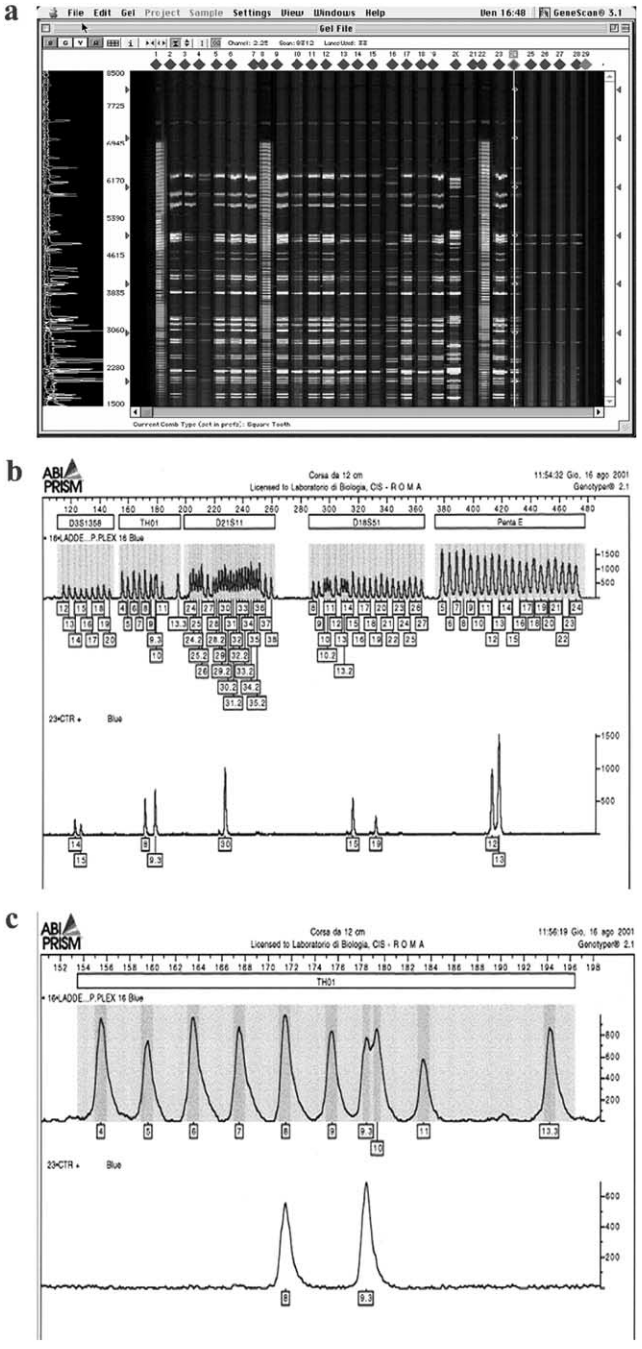
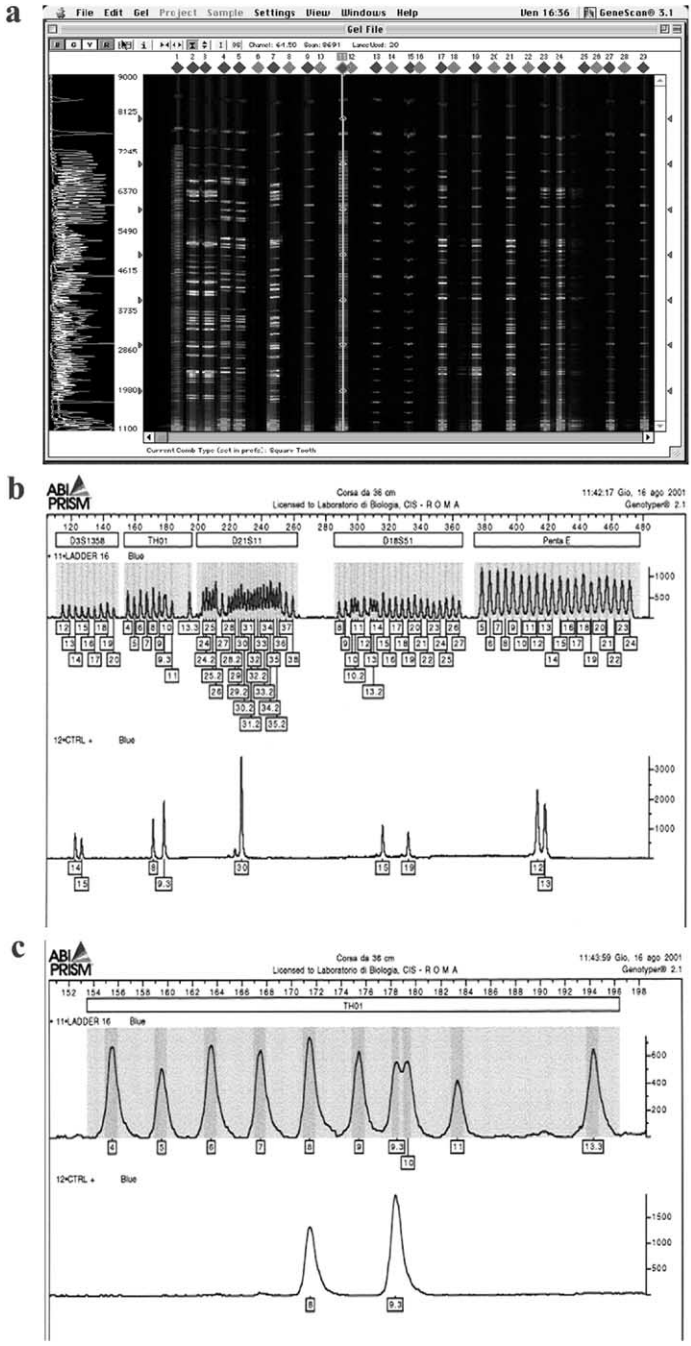


Picture 1. Genotyping using 12-cm plates.



Picture 2. Genotyping using 36-cm plates.

polyacrylamide concentration up to 6.75% to enhance separation of the amplified DNA fragments. All the other parameters followed the standard protocols of the ABI PRISM 377 DNA Sequencer manual [3].

3. Results and discussion

To validate and guarantee accuracy in genotyping, the new different conditions were tested in over 300 runs of known samples amplified with the multi-locus kit Powerplex® 16 run on 12-cm plates. Shown in Pictures 1a and 2a are ABI PRISM 377 data collection gel images of the 12- and 36-cm plate runs, respectively, whereas Pictures 1b and 2b show electropherograms of ladders and samples amplified with Powerplex 16 run on the 12- and 36-cm plates, respectively, and typed with Genotyper [5] software. It is evident that allele separation is just as efficient and that genotyping remains accurate. Pictures 1c and 2c show a typical delicate genotyping situation between 9.3 and 10 alleles of TH01 from the ladder: first run on 12-cm, then on 36-cm plates. Moreover, Penta D and Penta E alleles are efficiently separated and genotyped. It is evident that with the modifications brought to the system, resolution is similar.

The modifications made in the system lengthen the run by up to almost 4 h compared to the 3 h required when standard protocols are applied. There are several advantages to performing electrophoresis, genotyping 16 STR loci per lane, with 12-cm plates rather than 36-cm plates with an ABI PRISM 377 DNA Sequencer: (a) gel casting is more practical; (b) a second and third run can be performed immediately one after another, without changing gel and run buffer, since the same gel can be used for up to three consecutive runs, the lower voltage of the run exposes the gel to lower stress, and the run buffer, for the same reason, has a longer life; (c) the costs for buffer reagents, acrylamide, TEMED, APS, Amberlite are, all together, reduced by over 80%; (d) the production of hazardous waste is reduced (i.e. worn-out acrylamide gel). The modifications to the system enable it to maintain sensitivity, separation, and correct allele determination.

References

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