Deoxyuridine Triphosphate (dUTP)-Dependent Sizing Variance of Polymerase Chain Reaction (PCR) Products on the Agilent 2100 Bioanalyzer System

Technical Overview

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Abstract

To avoid false positive Polymerase Chain Reaction (PCR) detection, caused by cross-contamination with amplicons from earlier experiments, deoxyuridine triphosphate (dUTP) is commonly used as a substitute for deoxythymidine triphosphate (dTTP) in PCR mastermixes. This allows the performance of a decontamination step prior to PCR amplification. Analysis of PCR products on the Agilent 2100 Bioanalyzer system shows that there are sizing differences depending on the nucleotide used in the mastermix.

Introduction

The use of dUTP in PCR mastermixes is critical for many applications, such as pathogen detection and GMO analysis. It enables the use of uracil-N-glycosidase (UNG) to remove any uridine-containing DNA from PCR reactions prior to amplification, thus avoiding false positive detection due to contamination with amplicons from earlier runs.

When the PCR products were analyzed using a DNA 1000 assay on a 2100 Bioanalyzer system, it was observed that the 2100 Expert Software showed different sizing depending upon the nucleotide used in the mastermix.

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Materials and Methods

Amplification of the target sequences

The PCR was set up using two identical PCR mastermix formulations with the only difference being the use of dTTP in the first, and dUTP in the second mix.

Primers were at a final concentration of 200 nM. PCR was performed with the thermoprofile described in Table 1. The same starting material and amounts were used with both mastermixes on an Agilent 8800 SureCycler.

Analysis of PCR products on the Agilent 2100 Bioanalyzer system

The PCR products were analyzed with the DNA 1000 assay and 1 µL of the PCR reaction according to the DNA 1000 Quick Start Guide.

Results and Discussion

PCR reactions using either the standard dTTP-containing mix or the dUTP-containing mix were prepared using the same sample at the same starting amount with assays for an internal amplification control (IAC), soy lectin, and *A. tumefaciens* NOS terminator.

When the reactions were analyzed on the 2100 Bioanalyzer system, different fragment sizes were obtained for the amplicons derived from a dTTP reaction and a dUTP reaction, as shown in Figure 1, for the IAC and soy lectin reactions.

Table 1. Thermoprofile.

Temperature	Time	Action
95 °C	5 minutes	Enzyme activation
40 cycles of		
95 °C	30 seconds	Denaturation
60 °C	50 seconds	Annealing/Elongation

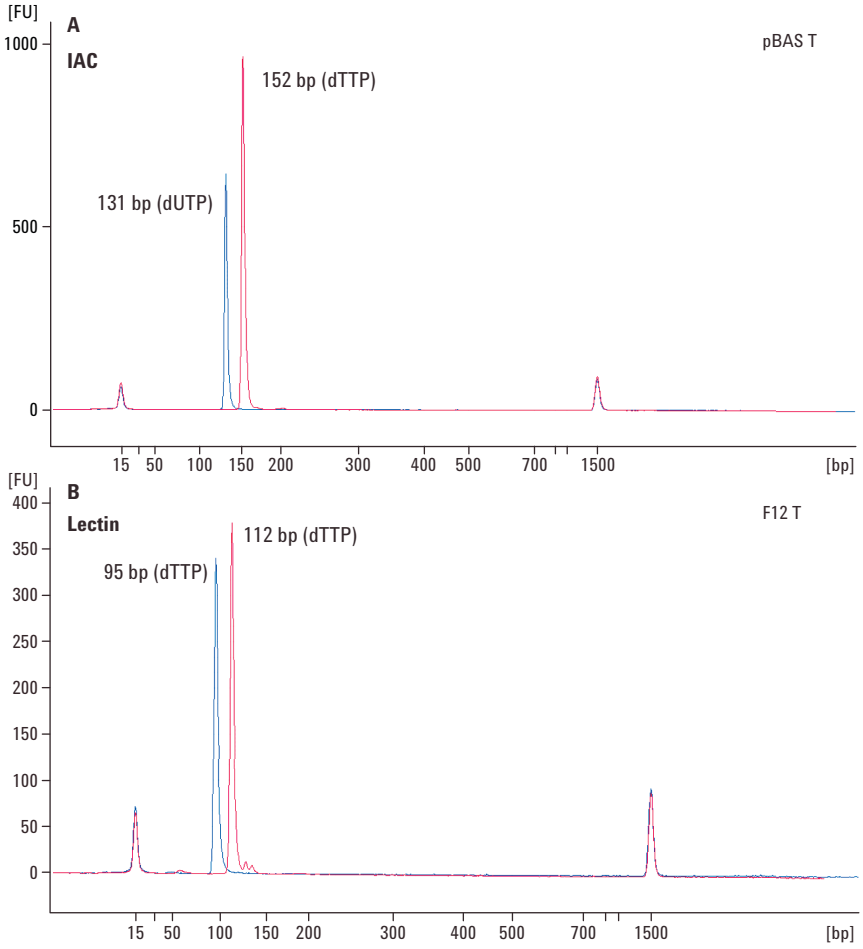

Figure 1. Exemplary electropherograms for IAC (A) and lectin (B). Red traces are from reactions using dTTP blue traces from those with dUTP.

Conclusion

Table 2 shows, for all targets, the size of the expected PCR products, AT percentage, and amplicon sizes determined using a DNA 1000 assay. The amplicon size from the dUTP reaction is consistently smaller than the one from a reaction containing dTTP.

There is no clear correlation between AT content and the extent of difference in sizing between a thymidine- or uridine-containing DNA.

In summary, when using amplicon-decontamination-enabled PCR mastermixes containing dUTP, the apparent size of the PCR products analyzed on the 2100 Bioanalyzer system appears, on average, approximately 15 % smaller than products from standard PCR mastermixes.

Table 2. Amplicon properties.

Amplicon	Size by sequence	% AT	Size dTTP	Size dUTP
IAC	146 bp	47.9	152 bp	131 bp
Lectin	104 bp	51.0	112 bp	95 bp
TNOS	105 bp	58.1	115 bp	101 bp

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