D1K ScreenTape allows Mycoplasma PCR analysis for cell line screening

Application Note

Nucleic Acid Analysis

Author

Donna McDade Walker

Agilent Technologies, Inc.

Loanhead, United Kingdom

Abstract

The Agilent 2200 TapeStation and the D1K ScreenTape streamline PCR screening of tissue culture S/N for *Mycoplasma spp*.. Lengthy gel electrophoresis analysis is replaced with a 10 minute, automated, hands-free analysis step without any reagent or gel preparation. Cell line results are immediately archived thanks to the 2200 TapeStation software, which was specifically developed for ScreenTape.

Introduction

Mycoplasma spp. are microorganisms that commonly infect cells in tissue culture, potentially leading to spurious experimental results, the loss of crucial data, and unplanned costs. Laboratories routinely use mycoplasma specific PCR to screen cultures for these contaminants and the resulting DNA fragments are analyzed by gel electrophoresis. The D1K ScreenTape system is a novel, automated method for mycoplasma PCR analysis.

Experimental

Material

Venor GEM-Mycoplasma Detection kit was purchased for PCR from Minerva Biolabs GmbH (Berlin, Germany); CHO cells were kindly provided by the Division of Pathway Medicine, Edinburgh University (Edinburgh, UK); 2200 TapeStation System, D1K ScreenTape and D1K Reagents were obtained from Agilent Technologies (Waldbronn, Germany).

PCR assay

For each cell line, 100 µL of tissue culture S/N from transfected CHO cells was boiled for 10 minutes. Using the instructions from the Mycoplasma Detection kit, reactions were set up in thin walled 0.2 mL tubes.

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D1K ScreenTape analysis procedure

Samples from the PCR block were mixed 1:3 with D1K sample buffer and placed in the Agilent 2200 TapeStation with D1K ScreenTape and tips. The analysis was started from the software. The full analysis of the samples was achieved and archived, with no user intervention, within 10 minutes.

Results and Discussion

Analyzed results from only 1 µL sample were visualized immediately (Figure 1). The time to result for seven samples is less than 10 minutes. The band sizes are automatically highlighted for each of the PCR reactions. Samples in lanes 1 (positive control), and 5 show fragments of 182 and 269 bp, that are diagnostic of Mycoplasma infection, whereas samples in lanes 2 (negative control), 3, 4 and 6, that only show a band at 182 bp, are free of infection.

Conclusion

The Agilent 2200 TapeStation system allows significant time saving for mycoplasma PCR analysis, by providing a fully automated, walk-away solution. No reagent preparation is needed, and the use of ethidium bromide is eliminated. Data on the presence or absence of mycoplasma for each cell line is automatically archived and easily retrieved. Reports can be generated and easily saved and printed.

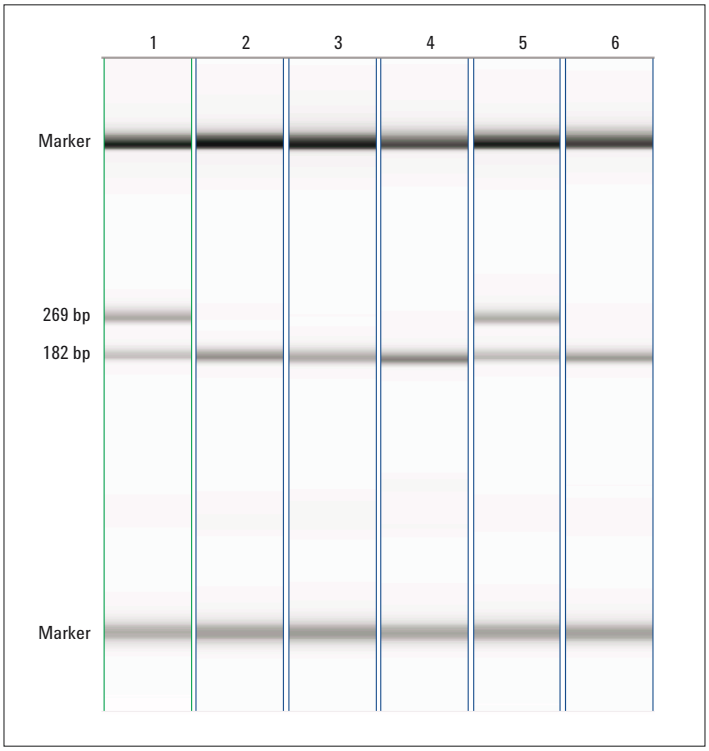

Figure 1
Analysis of mycoplasma PCR reactions with D1K ScreenTape including positive control (lane 1) and negative control (lane 2). The band sizes are indicated in bp.

www. agilent.com/genomics/tapestation

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