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Microchip Electrophoresis System MultiNA™ II MCE-301

Application News

# Campylobacter Species Identification Using a Microchip Electrophoresis System

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## User Benefits

- Streamlines the electrophoresis workflow from gel preparation through data analysis, reducing hands-on work.
- Fingerprinting Analysis minimizes manual interpretation and supports standardized result calls.
- Automated dilution makes it easy to bring high-concentration samples into the optimal range while helping reduce stress on the system.

## ■ Introduction

*Campylobacter* is one of the leading causes of foodborne illness associated with poultry and related foods and is therefore an important target in food safety and public health. In particular, accurate differentiation of *Campylobacter jejuni* and *C. coli*, which account for most cases, is important, not only for species identification itself but also for contamination source tracking and epidemiological investigations. PCR-based genotyping is widely used as a rapid and reliable approach, and multiplex PCR, which amplifies multiple target genes in a single reaction, is especially efficient.

However, when processing large numbers of samples, manual gel preparation and visual interpretation can become cumbersome. In this application, a microchip electrophoresis system, MultiNA II MCE-301, was used to automatically analyze multiplex PCR amplicons from *Campylobacter*. We also leveraged the system's Fingerprinting Analysis function. By combining automated analysis with objective fragment comparison, this approach streamlines the workflow and enables simple, highly accurate species identification.

## ■ Sample Preparation

The workflow from sample preparation to species identification is shown in Fig. 1.

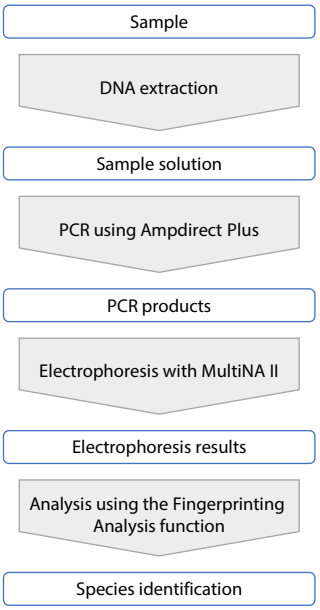



Fig. 1 Analytical workflow

Samples consisted of DNA extracted and purified from bacterial isolates. For species identification, we used the primer set reported by Wang et al.[1)], targeting three *Campylobacter* species: *C. jejuni*, *C. coli*, and *C. fetus* subsp. *fetus*. The target genes, primer names, and expected amplicon sizes are listed in Table 1.

Table 1 Primer names and expected amplicon sizes

| Species | Target Gene | Primers | Amplicon Size (bp) |
|---|---|---|---|
| *C. coli* | *C. coli glyA* | CCF, CCR | 126 |
| *C. jejuni* | *C. jejuni hipO* | CJF, CJR | 323 |
| *C. fetus* | *C. fetus sapB2* | CFF, CFR | 435 |
| Common | *C. jejuni* 23S rRNA | 23SF, 23SR | 650 |

PCR was performed using the Ampdirect™ Plus / BIOTAQ HS DNA Polymerase Set (P/N: 241-08890-92), which has strong tolerance to PCR inhibitors present in samples. Multiplex PCR was carried out using the primer set described above. The reaction mixture and PCR cycling conditions are shown in Fig. 2. Based on the reference, some cycling parameters were adjusted for BIOTAQ HS DNA Polymerase.

| | | | |
|---|---|---|---|
| 2x Ampdirect Plus | 12.5 µL | | |
| BIOTAQ HS DNA Polymerase | 0.5 U | | |
| CJF primer | 0.5 µM | | |
| CJR primer | 0.5 µM | 95˚C, 10 min | |
| CCF primer | 1.0 µM | 94˚C, 30 sec | } 35 cycles |
| CCR primer | 1.0 µM | 59˚C, 60 sec | |
| CFF primer | 1.0 µM | 72˚C, 90 sec | |
| CFR primer | 1.0 µM | 72˚C, 7min | |
| 23SF primer | 0.2 µM | | |
| 23SR primer | 0.2 µM | | |
| Template DNA | 2.0 µL | | |

Fig. 2 PCR reaction mixture and cycling conditions

## ■ Automated Dilution Function

Running high-concentration samples such as PCR products without dilution can overload the system, potentially affecting separation quality and subsequent sample analysis. To maintain good separation performance, samples should be run within an appropriate concentration range.

MultiNA II (Fig. 3) includes an automated dilution function, allowing the user to select a 5-fold, 10-fold, or 20-fold dilution. Dilution can be performed simply by preparing an empty tube to receive the diluted sample; there is no need to aliquot dilution solution in advance.

In this study, the amplicon concentration was known in advance to be high, so samples were diluted 20-fold using this function prior to electrophoresis. This helps promote good separation while also reducing stress on the microchip.

Fig. 3 Microchip electrophoresis system MultiNA™ II MCE-301

## ■ Electrophoresis Conditions

PCR products were automatically diluted using MultiNA II and loaded directly for electrophoresis to confirm the amplicon sizes. The analytical conditions and reagents used are listed in Table 2. The DNA-1000 Kit was used together with GelStar as the fluorescent dye. A 100 bp DNA Ladder (Takara Bio, P/N: 3407A) was used as the size standard. This ladder is pre-registered in the software, and after the run, the calibration curve used for size calling is generated automatically.

Table 2 Electrophoresis Conditions

| Item | Condition |
|---|---|
| System | MultiNA II MCE-301 |
| Reagent kit | DNA-1000 Kit (P/N: 292-27911-91) |
| Fluorescent dye | GelStar (Lonza, P/N: 50535) |
| Size standard (size marker) | 100 bp DNA Ladder (Takara Bio, P/N: 3407A) |
| Analysis mode | On-chip |

## ■ Electrophoresis Results

Fig. 4 shows the electrophoresis results obtained for a negative control (NC), in which water was added instead of template DNA, and for PCR products generated using DNA extracted from *C. jejuni*, *C. coli*, and *C. fetus* as templates.

In the NC, no amplicon-derived fragments were detected, indicating a clean result with no evidence of contamination. In the samples prepared from extracted DNA, a common fragment at approximately 650 bp, corresponding to 23S rRNA, was clearly detected, along with a distinct species-specific fragment for each target.

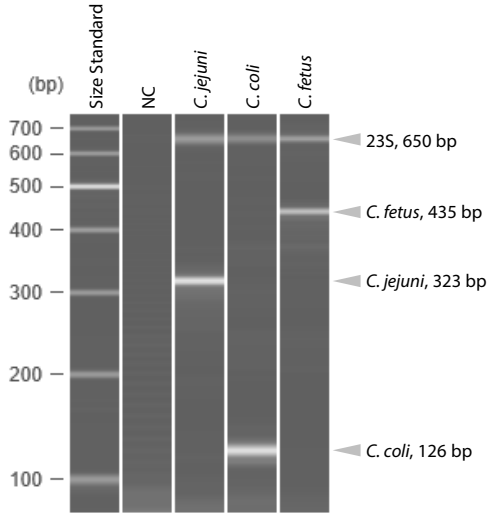
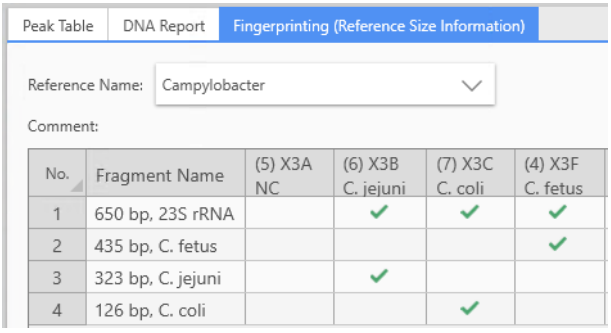



Fig. 4 Gel image of the electrophoresis results

## ■ Fingerprinting Analysis

Next, the results were analyzed using the Fingerprinting Analysis function of MultiNA II (Fig. 5). This function automatically compares fragments detected in each sample against registered reference fragment-size data and displays the results in a list format.




| No. | Fragment Name | (5) X3A NC | (6) X3B C. jejuni | (7) X3C C. coli | (4) X3F C. fetus |
|---|---|---|---|---|---|
| 1 | 650 bp, 23S rRNA | | ✓ | ✓ | ✓ |
| 2 | 435 bp, C. fetus | | | | ✓ |
| 3 | 323 bp, C. jejuni | | ✓ | | |
| 4 | 126 bp, C. coli | | | ✓ | |

Fig. 5 Fingerprinting Analysis results

As shown in Fig. 5, the species-specific fragment corresponding to each organism was detected in *C. jejuni*, *C. coli*, and *C. fetus*. In addition, the common 23S rRNA-derived fragment was detected in all positive samples. By contrast, none of these fragments were detected in the NC. This enables objective confirmation of the presence or absence of species-characteristic fragments without relying solely on visual review.

In addition, by switching the display to Full Screen mode, the electrophoresis results can be shown in the upper panel and the Fingerprinting Analysis results in the lower panel, allowing band positions in the gel image to be directly cross-checked against the automated calls on a single screen. Because the presence or absence of fragments of specific sizes can be assessed using both visual review and automated interpretation, this feature supports more efficient and standardized result evaluation.

## ■ Conclusion

In this application, we demonstrated that multiplex PCR products of *Campylobacter* can be analyzed via electrophoresis using MultiNA II to differentiate the three major species: *C. jejuni*, *C. coli*, and *C. fetus*. MultiNA II automates the workflow from gel preparation and sample loading through data analysis, and its automated dilution function enables even high-concentration PCR products to be analyzed at appropriate concentrations.

Furthermore, the Fingerprinting Analysis function automatically compares target fragments against reference fragment-size data, allowing results to be confirmed objectively without relying solely on visual interpretation. Because the results can also be displayed in a list view, this approach is useful for improving throughput, standardizing interpretation, and reducing human error in multi-sample workflows, thereby contributing to more efficient routine testing.

## References

1) Gehua Wang, et al., *J. Clin. Microbiol.*, 40(12), 4744-4747 (2002).



01-01265-EN First Edition: Jul. 2026

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