

# Application Data Sheet

**No. AD-0059**

**MCE-202/ MultiNA**

## A High Throughput Platform for Identification of Crop Varieties using Microchip Electrophoresis & AutoFinder Program

Crop breeding programs aim to produce new species with the desirable traits that are difficult to breed for. They include enhanced crop productivity yields, better crop nutritive value and the ability to withstand damage from biotic factors (e.g: insect resistance). The conventional breeding approach is highly subjected to fluctuating environmental conditions and a relatively long period of breeding time. The recent development of DNA molecular markers has greatly improved the selection of potentially useful gene candidates within a much shorter period of time. Here, we present a high throughput platform for quick and easy identification crop varieties sample using Microchip Electrophoresis System, MultiNA in combination with AutoFinder program. The platform has the advantages of providing not only fast and easy but also unambiguous identification results directly.

### □ Experimental Procedure

First, sample analysis of PCR products was performed on MultiNA by on-chip mixing method and point-to-point calibration curve using 25 bp step ladder. The obtained data (peak intensity and bp size) including sample information (names and well numbers) were exported as CSV format (MultiNA Viewer). Then, the CSV data was imported to AutoFinder software for identification of each sample based on pre-set targeted sizing, tolerance and intensity threshold. Figure 1 shows the workflow of this high throughput automatic identification platform.

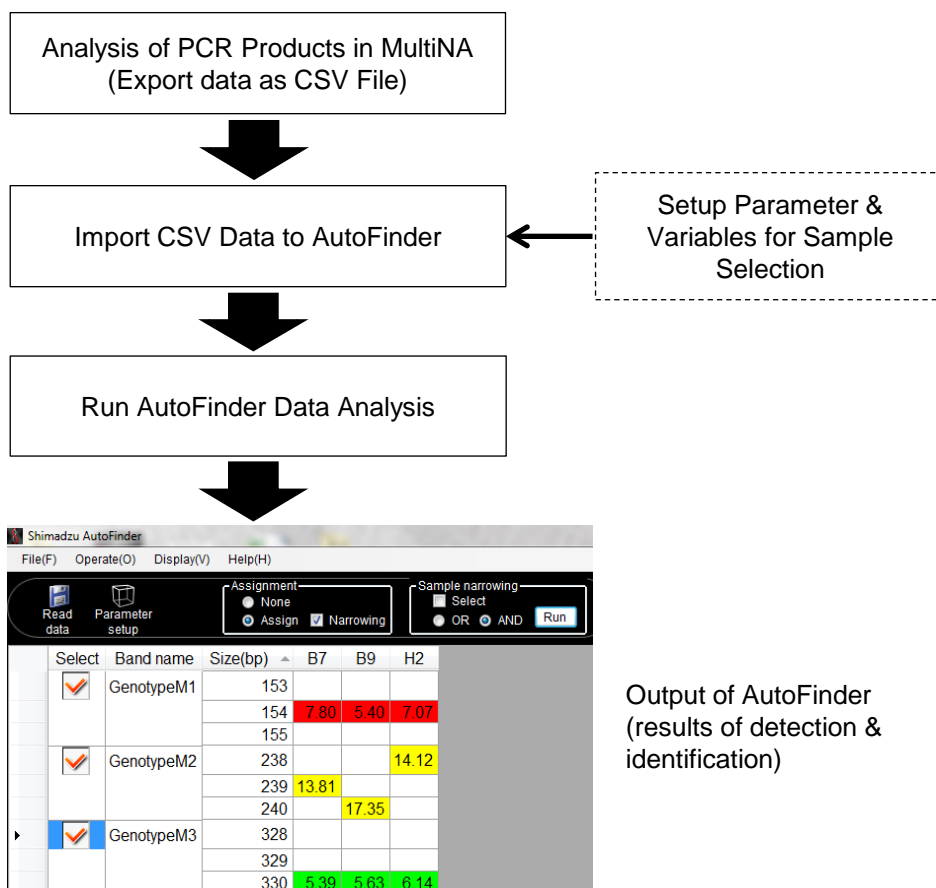


Fig 1: Workflow of MultiNA with AutoFinder for high throughput Identification of Crop Varieties

## ☐ Analytical Instruments and Conditions

Instrument : MCE-202 MultiNA  
Analysis mode : DNA 500 on-chip mixing  
Ladder : 25 bp step ladder  
Reagents : DNA-500 Reagent Kit  
Fluorescent dye, SYBR® Gold gel stain  
TE Buffer

## ☐ Parameter settings of AutoFinder

The CSV data output were analyzed using pre-set parameters as shown on table 1. In this sample analysis, three genotypes M1, M2 and M3 were the targets. The parameters to be set were target size, genotype name, accuracy tolerance (bp) and detection threshold (mV). Color codes were also assigned for every targets displaying the results in different colors.

Table 1: Parameters for automatic detection and identification of target genotypes in crop variables study

Size of target band (bp)	Name of target band	Tolerance (-)	Tolerance (+)	Threshold (mV)	Color
154	Genotype M1	1	1	5	
239	Genotype M2	1	1	5	
329	Genotype M3	1	1	5	

## ☐ Identification of crop varieties by AutoFinder

The output from AutoFinder is shown in Figure 1. A total of 96 crop samples (PCR products) was analyzed of which 85 samples were detected and identified. The analysis results are compiled into Table 2. As can be seen from the table, 3 samples exhibited Genotype M1, 52 samples Genotype M2 and 3 samples Genotype M3. Other samples exhibited multiple genotypes. It is worth to note that optimization of the parameter settings such as threshold (mV) and tolerance (bp) are critical not only to avoid false positive results but also detect the low intensity target bands due to any reason.

Table 2: Analysis results of 85 PCR products from crop samples by MultiNA with AutoFinder program

Target genotype	Number of samples
Genotype M1 (154 bp)	3
Genotype M2 (239 bp)	52
Genotype M3 (329 bp)	3
Genotype M1 and M2	17
Genotype M1 and M3	1
Genotype M2 and M3	6
Genotype M1, M2 and M3	3

## ☐ Summary

MultiNA (MCE 202) in combination with AutoFinder software is a high throughput platform that enables rapid and easy analysis of data for determining genetic variations in crop breeding programs.

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