Main specifications for the DS3000

■ Main unit specifications

Item	Details
Number of capillaries	4
Capillary length	36 cm
Sample format	8-tube strip × 4
Device control	Touch panel PC
Number of Dyes	6
Application	Sequencing analysis / Fragment analysis
Size	400 (W) × 600 (D) × 600 (H) mm
Weight	45 kg
Performance guarantee temperature	15 – 30˚C
Performance guarantee humidity	20 – 80% RH (no condensation)
Power input	100 – 240 ±10% VAC, 50/60 Hz
Rated power	260 VA
Supported secondary analysis software	• Mutation Surveyor (SoftGenetics, LLC, sold separately) • GeneMarker (SoftGenetics, LLC, sold separately) • GeneMarker HID (SoftGenetics, LLC, sold separately)

■ Run module specifications

Run Module	Application	Polymer type	Contiguous Read Length*1 (bp, QV20 CRL)	Average run time (minutes)
Fast_Sequence36_Polymer7	Sequencing analysis	Polymer7	≥600	≤32
Standard_Sequence36_Polymer7	Sequencing analysis	Polymer7	≥700	≤60
BDx_Fast_Sequence36_Polymer7	BDx sequencing analysis	Polymer7	≥600	≤32
BDx_Standard_Sequence36_Polymer7	BDx sequencing analysis	Polymer7	≥700	≤60

Run Module	Application	Polymer type	Average run time (minutes)	Sizing precision*2 (bp, 50 – 400 bp)
Fragment_Analysis36_Polymer7	Fragment analysis	Polymer7	≤35	NA
Fragment_Analysis36_Polymer4	Fragment analysis	Polymer4	≤44	<0.16

*1 Contiguous Read Length (bp, QV20 CRL) is measured with BigDye® Terminator v3.1. Sequencing Standard Kit (Thermo Fisher Scientific, sold separately)

*2 Sizing precision (bp, 50 - 400 bp) is measured with PowerPlex™ ESI17 Fast Allelic Ladder and WEN ILS 500 ESS. (Promega®, sold separately)

· BigDye is a registered trademark of Thermo Fisher Scientific Inc.

· Promega is a registered trademark of Promega Corporation.

■ Consumables specifications

Product name	Part number	Details	Remarks
Capillary Cartridge 36 cm	613-0330	1 pcs	Storage temperature: 15 – 30˚C
Buffer	613-0252	Anode Buffer × 2 cartridges Cathode Buffer × 2 cartridges	Storage temperature: 2 – 10˚C
Polymer7	613-0251	4 cartridges	Storage temperature: 2 – 10˚C
Polymer4	613-0250	4 cartridges	Storage temperature: 2 – 10˚C
Septa for Cathode Buffer Cartridge	613-7231	10 pcs	
Retainer for Cathode Buffer Cartridge	613-7233	4 pcs	
Septa for 8 well tubes	613-7230	24 pcs	
Base and Retainer for 8 well tubes	613-7232	4 pcs	
Anode Electrode Assembly	613-7263	1 pcs	

Specifications in this catalog are subject to change with or without notice,
as Hitachi High-Tech Corporation continues to develop the latest technologies and product for its customers.

CAUTION:For correct operation, follow the instruction manual when using the instrument.

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Technical Report 4

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Detection of Shellfish Allergens using DS3000 Compact CE Sequencer

Abstract

Food allergies occur in approximately 1-3% of adults and 4-6% of children [1] and occasionally can cause life-threatening symptoms. Thus, detection of food allergens and labeling of them on food products are critical issue for food safety. Although protein allergen can be detected directly by a conventional method such as ELISA, nucleic acid-based assay, which enables detect DNA sequences coding allergens, is sometimes employed because of its higher sensitivity, less cost and better ease-of-use.

We here describe a high-sensitive multiplex PCR assay combined with capillary electrophoresis (CE) that targets the tropomyosin genes, which are widely present in mollusk species, including shellfish, as a fragment analysis application of the DS3000 Compact CE Sequencer [2]

DS3000
Compact CE Sequencer

Results and Discussion

- Tropomyosin genes and the eukaryotic 18S rRNA gene (positive control) from products of four mollusk species (clam, abalone, mussel, oyster) were detected simultaneously by performing multiplex PCR and capillary electrophoresis on DS3000 Compact CE Sequencer.
- To evaluate the detection range of the multiplex PCR, the amount of the input DNA was varied from 0.01 to 10 ng. Following the electrophoresis, amplicon peaks of each gene were detected as expected irrespective of the amount of the input (Figure 1).
- On the other hand, number of PCR cycle and the condition of the sample injection need to be tweaked to prevent the saturation of the peaks.

■ Figure 1

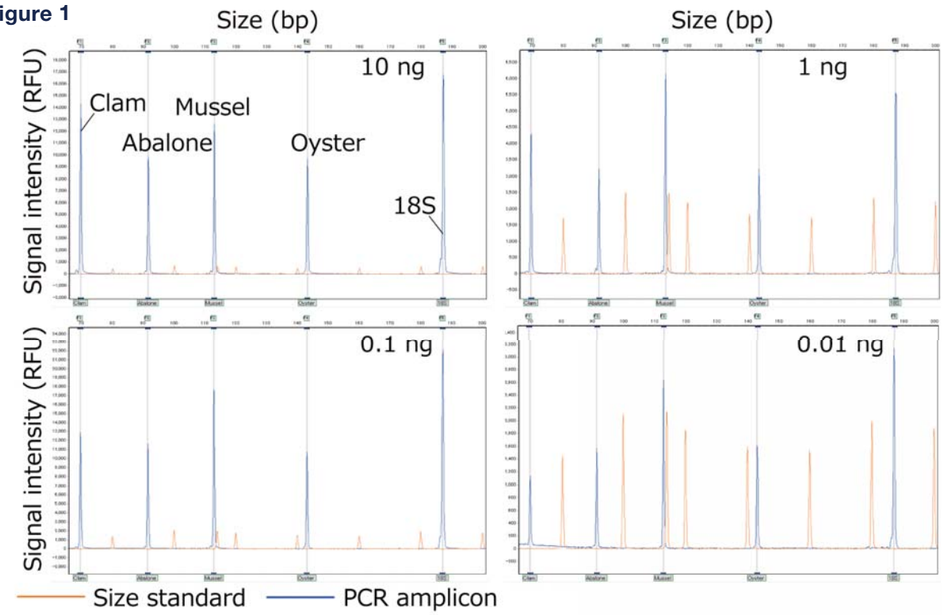

Figure 1: Detection of tropomyosin genes along with the 18S rRNA gene as a positive control
Electropherograms obtained from 0.01, 0.1, 1 or 10 ng of mixed genomic DNA of 4 mollusk species (clam, abalone, mussel and oyster) are shown. Amplicon peak was not detected in a non-template control (data not shown).

Materials and Methods

Sections of 10-20 mg were obtained from commercially available shellfishes (clam, abalone, mussel and oyster). Genomic DNA was extracted from each section using DNeasy® Blood & Tissue Kit (Qiagen, sold separately). DNAs were quantified and equally mixed in equal amounts. Multiplex PCR was performed with mixed DNA following the protocol of Reference 2 with modifications described below. 1) Forward primers were labeled with FAM. (2) DNA polymerase was replaced with Phusion™ Hot Start II DNA Polymerase (Thermo Fisher Scientific) (3) Primer concentration and the number of PCR cycle were optimized to unify the signal intensities. PCR conditions are detailed in Table 1, 2 and 3. The PCR products mixed with the size standard, GeneScan™ 600 LIZ® dye Size Standard v2.0 (Themo Fisher Scientific), were analyzed on DS3000 Compact CE Sequencer on Polymer4 with assay described in Table 4. Peak detection and its sizing were performed on GeneMarker v.3.0.1 (SoftGenetics). Note that, to display peaks of interest clearly, only a part of an entire electropherogram was shown.

Table 1：PCR reaction

Solution	Liquid volume
x5 HF buffer	8
2.5 mM dNTPs	2
primer Fw	1
primer Rv	1
Phusion HS II	0.4
Amp. grade water	26.6

Temp.(℃)	Time(s)	Cycle
98	30	1
98	10	26 or 31
62	30	
72	30	
72	300	1
4	hold	

total reaction time：50-60 min

Table 2：Primer concentration

Target	Final conc. (nM)
18S rDNA	60
Clam	500
Abalone	125
Mussel	250
Oyster	500

Table 3：PCR cycle and injection

Input DNA(ng)	Cycle	Injection time(s)
10	26	3
1	26	9
0.1	31	9
0.01	31	9

Technical Report 4

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Table 4：Performance of Polymer4

Polymer	Run Module	Run time	Run voltage
Polymer 4	AB_5Dye_LIZ600(60-600)_36_P4	～45min	13 kV

Reference

[1] Jablonski, J. E., Fu, T. J., Jackson, L. S., & Gendel, S.M.(2010).
Determination of protein levels in Soy and Peanut oils by colorimetric assay and ELISA. Journal of AOAC INTERATIONAL, 93(1), 213–220.

[2] Suh S-M., Kim M-J., Kim H-I., Kim H-J., Kim H-Y., (2020). A multiplex PCR assay combined with capillary electrophoresis for the simultaneous detection of tropomyosin allergens from oyster, mussel, abalone, and clam mollusk species. Food Chemistry, 317, 126451

Caution

- DS3000 Compact CE Sequencer is for Research Use Only and not intended for diagnostic procedures.
- This document shows an example of verification under limited samples and environments.
- It does not guarantee that data equivalent to those in this document can be obtained with every sample under every environment.