

Practical examples of Sequencing / Fragment analysis under the wide range of ambient temperatures

Introduction

In the capillary electrophoresis, ambient temperature influences on the migration speed and the resolution, which fluctuates the data quality. The performance of HITACHI DS3000 Compact CE Sequencer was verified under the various ambient temperature. HITACHI DS3000 Compact CE Sequencer exhibited robust performance irrespective of the temperature.

Sequencing

[Electropherogram]

Sequencing analysis was performed within a range of guaranteed environmental temperature (15 °C, 21 °C, 30 °C). Figure 1 shows the results obtained at 21 °C.

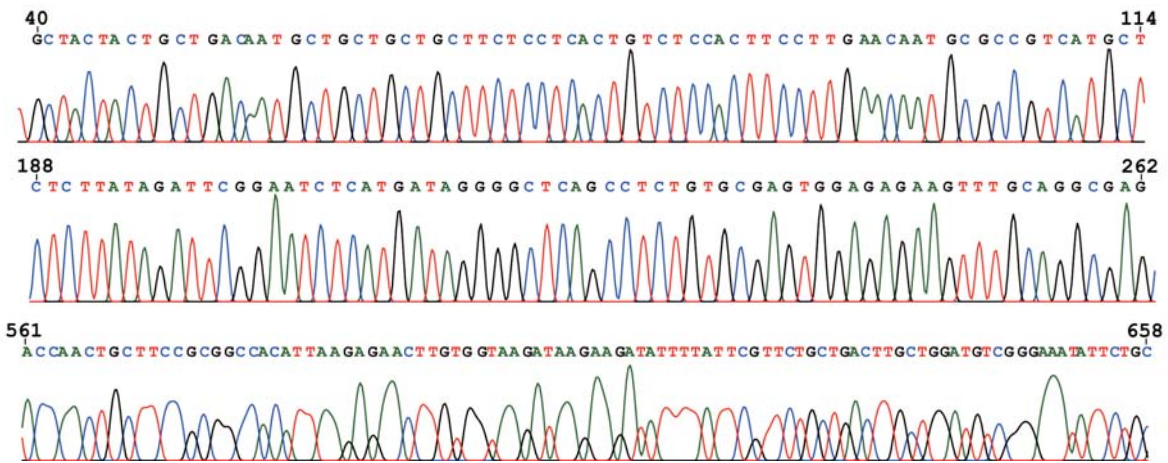
Peak qualities are satisfactory in a broad range of the electropherogram.

Equivalent performances were observed in the analysis at both 15 °C and 30 °C (data not shown).



DS3000
Compact CE Sequencer

■ Fast sequencing (approximately 30 minutes)



■ Standard sequencing (approximately 60 minutes)

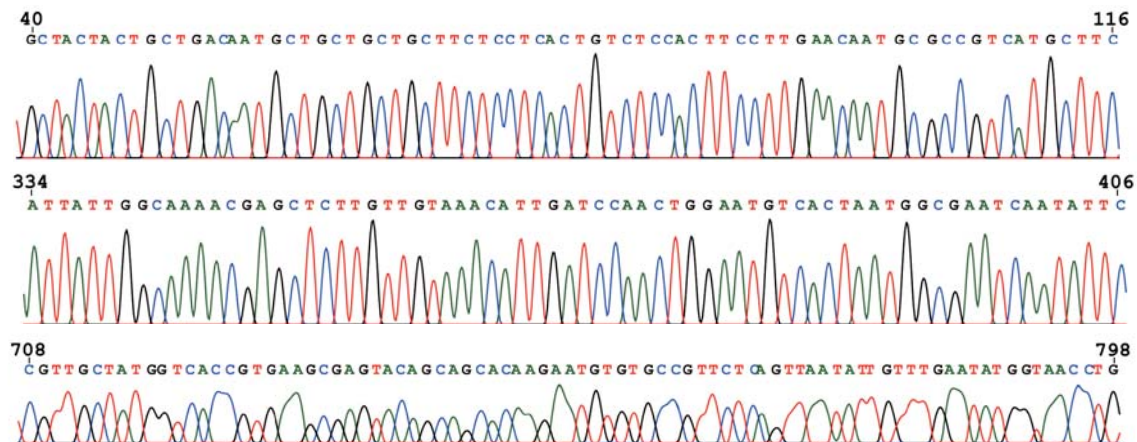


Figure 1: Example of sequencing data measured at 21 °C.

Sequencing was performed using the BigDye® Terminator v3.1, 3500/3500xL Sequencing Standard Kit.

[Contiguous Read Length]

The performance shown in Figure 1 was stably reproduced.

Minimum CRL was greater than 600 bp (Fast sequencing) or 700 bp (Standard sequencing) at all the temperature examined.

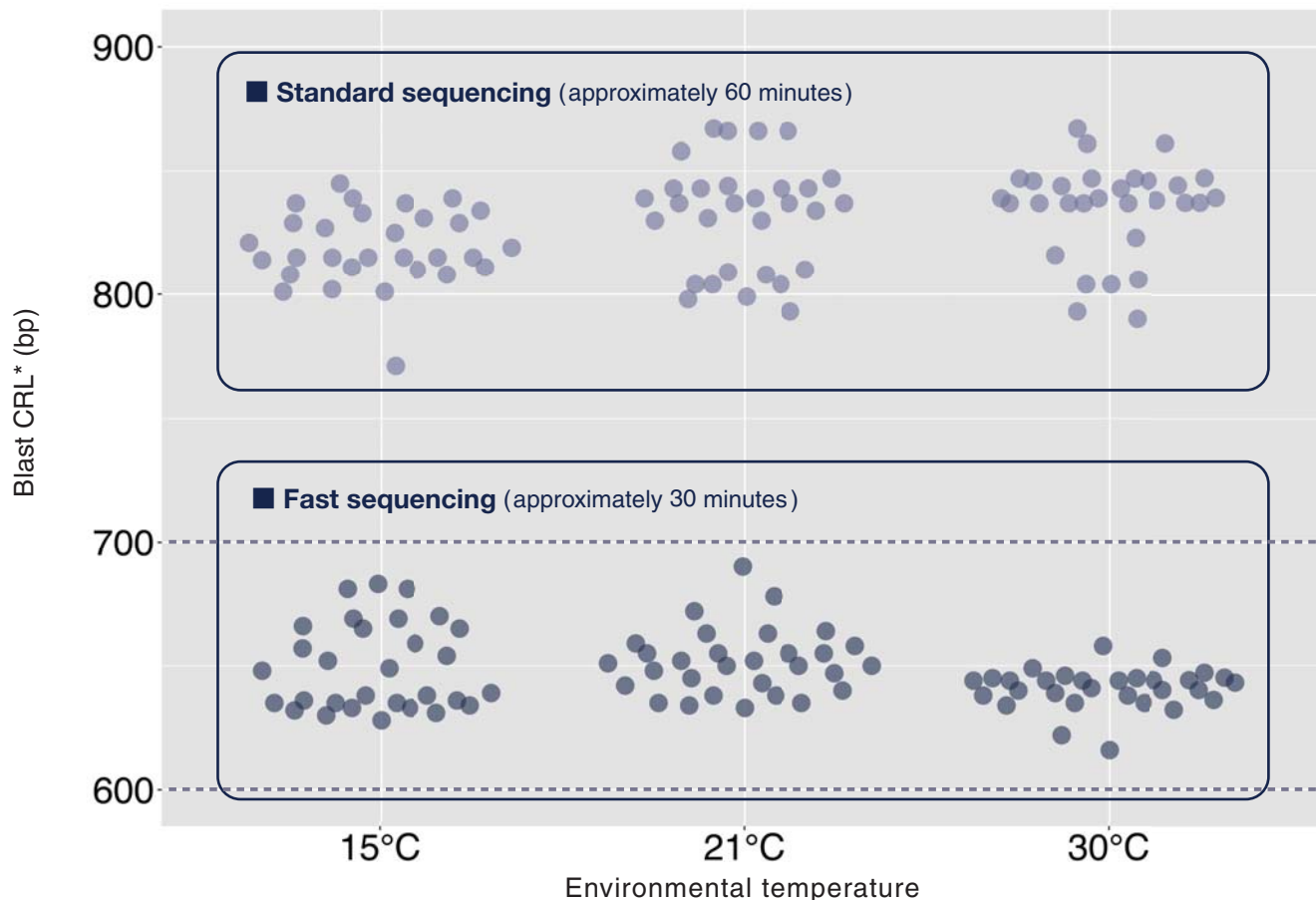


Figure 2: Contiguous read length under the guaranteed ambient temperatures.

*Blast CRL is the longest length of an uninterrupted section of bases allowing a maximum of 2 base mismatches from the reference sequence.

[Accuracy]

In all experiments, a sequencing accuracy of 99.99% or higher was achieved.

Table 1: Sequencing accuracy at guaranteed environmental temperatures

Sequencing mode	Environmental temperature	Number of trials	Analytical range (bp)	Approximate run time (min)	Reading accuracy
Fast Sequencing	15 °C	100	40-600	30	≥99.99%
	21 °C	100	40-600	30	≥99.99%
	30 °C	100	40-600	30	≥99.99%
Standard Sequencing	15 °C	100	40-700	60	≥99.99%
	21 °C	100	40-700	60	≥99.99%
	30 °C	100	40-700	60	≥99.99%

Fragment analysis

[Resolution]

Short tandem repeat (STR) analysis is currently a gold standard for DNA profiling and, thus, one of the most representative applications of fragment analysis on the capillary electrophoresis. In the analysis the repetitions of STR are analyzed, therefore data quality relies on the robust resolution and the sizing accuracy. By running PowerPlex™ ES17 fast allelic ladder, Figure 3 demonstrated that all the peaks with minimum 2 bp intervals spanning 70 – 460 bp are properly separated at both 15 and 30 C of ambient temperature. In addition, all the alleles were called as expected (data not shown). Sizing accuracy was 0.065 bp or less at 21 °C.

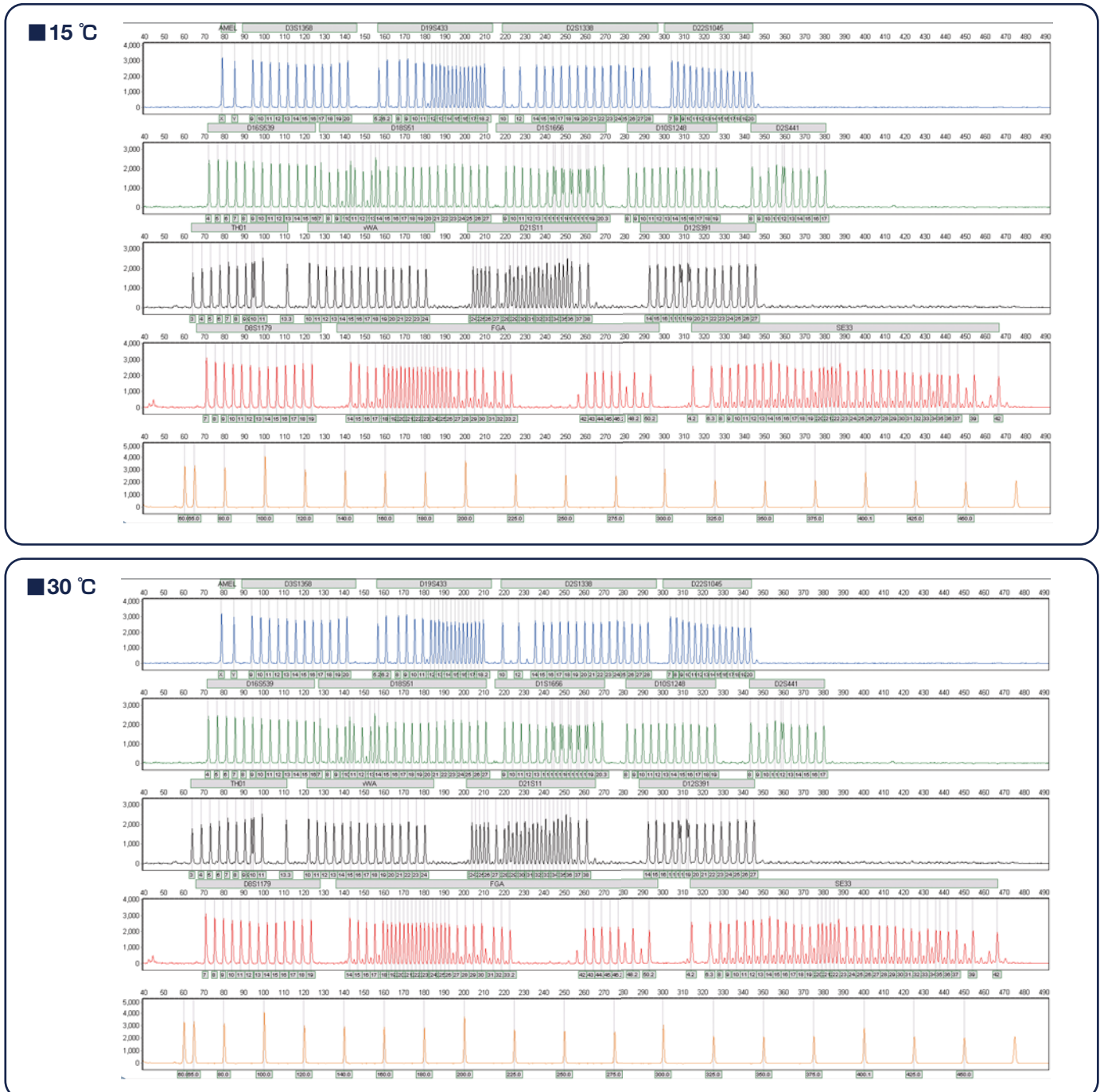


Figure 3: Allelic ladder electrophoretic pattern

The electropherogram view is obtained from GeneMarker HID (SoftGenetics, LLC, sold separately).

DS3000 Primary Specifications

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	<ul style="list-style-type: none"> • 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

*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™ ES17 Fast Allelic Ladder and WEN ILS 500 ESS. (Promega, sold separately)

• BigDye is a registered trademark of Thermo Fisher Scientific Inc.
• Promega, PowerPlex is a registered trademark of Promega Corporation.

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

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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