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)

■ 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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Effect of sample injection conditions on performance of DS3000 Compact CE Sequencer

Abstract

With the DS3000 Compact CE Sequencer (hereinafter, the “DS3000”), DNA molecules are injected into a capillary array as a result of the difference in electrical potential between the anode and the negatively charged DNA molecules. The magnitude and duration of the applied voltage (hereinafter , the “injection voltage” and “injection time”, respectively) have a significant effect on the number of molecules injected and the peak resolution, thereby affecting the accuracy of electrophoresis measurements. The run protocols in the DS3000 are preconfigured to optimize the performance of the instrument for a wide variety of samples, and generally do not require further adjustment. However, in some specific cases, such as samples that produce extremely weak signals, higher-quality data may be obtained by tweaking the sample injection conditions. Here, we describe the quality of data obtained under a variety of sample injection conditions. Although the effect is limited, further optimization of the injection conditions allows detection and analysis of electrophoresis peaks for samples that have been diluted up to 100 times.

DS3000
Compact CE Sequencer

Results

Sequencing analysis

Electrophoresis was performed using the 3500/3500xL Sequencing Standards, BigDye® Terminator v3.1 (Applied Biosystems®), employing both Fast and Standard sequencing. The samples were injected under an injection voltage of 1.2 kV for 4 (default), 8, 12, or 24 seconds. Figure 1 shows the correlation between the average signal intensity and the QV20 contiguous read length (CRL). The signal intensity is averaged over all peaks between 100 to 200 bases. The QV20 CRL represents the maximum contiguous read length for which the average QV (basecalling accuracy) for 21 bases in the slide window exceeds 99%. Figure 1 shows that stronger signals can be obtained for longer injection times. However, longer injection times also lead to higher QV variations. For example, some QV20 CRL values are less than 300 for an injection time of 24 seconds.

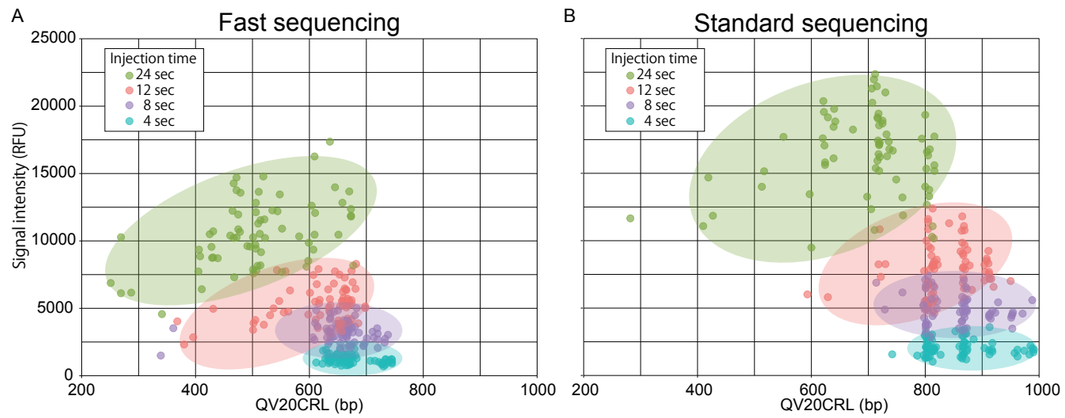


Figure 1:Comparison of injection time, signal intensity and QV20 CRL

The y-axis (signal intensity) represents the average intensity of over all peaks from 100 to 200 bases. The x-axis (QV20 CRL) represents the maximum contiguous read length for which the average QV (basecalling reliability) of the 21 bases in the slide windows exceeds 99%. The test was conducted 72 times for Fast sequencing (A) and Standard sequencing (B).

As shown in Figure 2, a similar trend is observed for the error rate. When the injection is 4 or 8 seconds, the error rate never exceeds 1%. In contrast, for an injection time of 24 seconds, the error rate increases with increasing DNA fragment length. Errors are prominent in the middle to the end of the read. Therefore, although stronger signals can be obtained by increasing the injection time, there is a risk of reducing the accuracy of basecalling for longer DNA fragments.

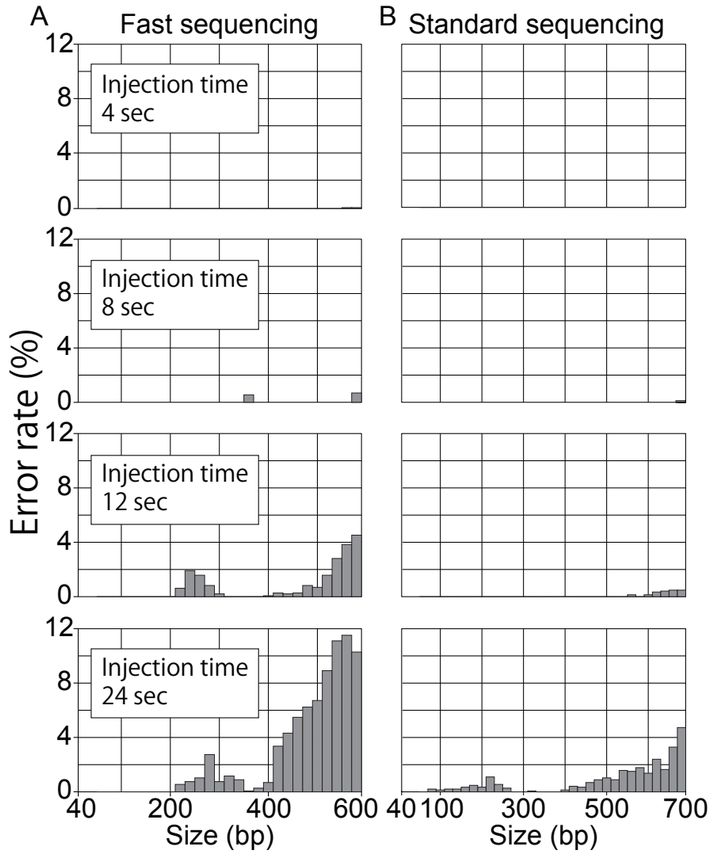
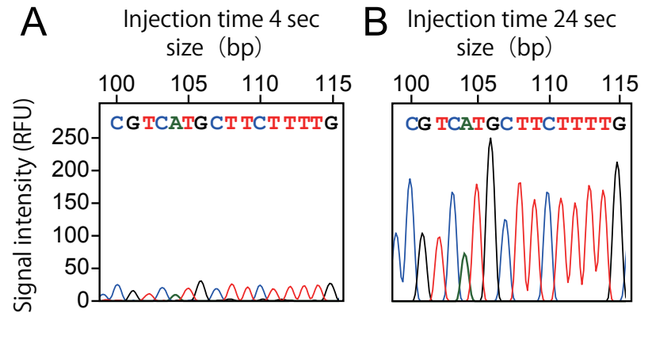


Figure 2: Comparisons of injection time and error rate

The histogram shows the error rate of bases that were called incorrectly in 72 tests for each condition within each bin. The bin size is 20 bases. For both Fast sequencing (A) and Standard sequencing (B), the error rate increases at the end of the reads in proportion to the injection time.

Next, we examined how much the signal intensity could be improved by adjusting the injection time for a diluted sample. Electrophoresis was performed using the 3500/3500xL Sequencing Standards, BigDye® Terminator v3.1 (Applied Biosystems®) on employing Standard sequencing. The samples were diluted to a concentration 100 times less than the manufacturer's instructions. The samples were injected under an injection voltage of 1.2 kV for 4 (default) or 24 seconds, and the results are shown in Figure 3. The signal intensity is low for a 4-seconds injection time, and the average signal intensity between 100 to 200 bases is only 19 RFU. In addition, some peaks have an intensity that is lower than the detection limit, which results in basecall errors (21 bases). On the other hand, for a 24-seconds injection time, the average signal intensity is improved to 125 RFU, and the number of basecall errors is reduced to only two.



C

Run protocol	Injection time (s)	Average signal intensity in 100-200 bases range (RFU)	Number of the erroneously called bases
Standard	4	19	21
Standard	24	125	2

Figure 3: Improve data quality by adjusting the injection time (Sequencing analysis)

The figure shows the electropherogram around 100 bases under the injections for 4 (A) or 24 (B) seconds and the summary of data quality (C). The error bases were counted from 40 to 700 bases.

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Fragment analysis

Electrophoresis was performed using the PowerPlex™ Fusion 6C allelic ladder with size standard sample (WEN Internal Lane Standard 500, Promega®). The samples were injected under an injection voltage of 1.6 kV (default) or 3.2 kV for 4, 9 (default), 18, 27 or 36 seconds. As shown in Figure 4, we first compared the signal intensity and the electrophoresis quality (hereinafter, "EQ"). The EQ is an important indicator of the data quality in fragment analysis. It represents the maximum range over which a difference of a single base length can be distinguished. The EQ is automatically calculated each time a fragment analysis is completed in the DS3000. For an injection voltage of 1.6 kV, the signal intensity increases in proportion to injection time, while keeping EQ high. For an injection voltage of 3.2 kV, the signal intensity is even higher, again maintaining a high EQ, but only for an injection time of 4 or 9 seconds. When the injection time is more than 9 seconds, a stronger signal is obtained, but the EQ decreases.

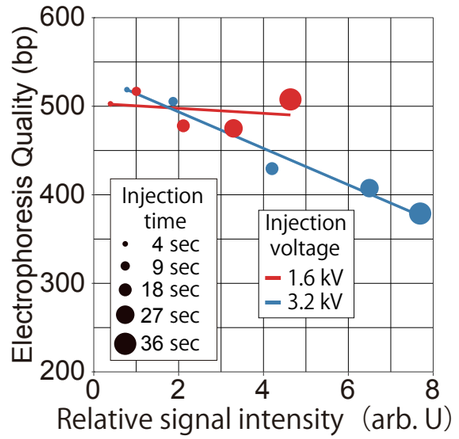


Figure 4: Comparison of signal intensity and Electrophoresis Quality

The x-axis (signal intensity) shows the signal intensity observed at the 100 bases peak of the size standard. The relative signal intensity of each peak of the size standard was plotted with respect to the signal intensity observed under the injection voltage 1.6 kV for 9 seconds. The y-axis (Electrophoresis Quality, EQ) is the indicator of data quality in fragment analysis. Please refer the main text for more detail.

Next, we investigated the dependence of the signal intensity on the DNA fragment size, and the results are show in Figure 5. For an injection voltage of 1.6 kV, the signal intensity is relatively independent of fragment size for short injection times of 4 or 9 seconds. However, for longer injection times, the signal intensity decreases with increasing fragment size. Shorter fragments are injected more than longer fragments. This trend is more pronounced for an injection voltage of 3.2 kV. Thus, for an injection voltage of 1.6 kV, a higher injection time can increase the signal intensity while maintaining a high EQ. For an injection voltage of 3.2 kV, a higher signal intensity and EQ can be obtained for an injection time of 9 seconds or less. However, higher injection times may have an adverse effect on EQ and lead to a dependence of the signal intensity on the fragment length. Please take special care when using applications that require quantitative signal intensity and size accuracy.

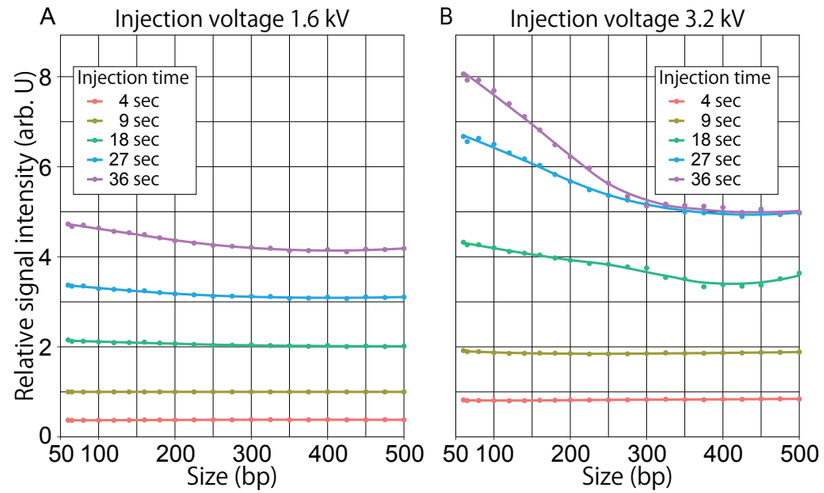
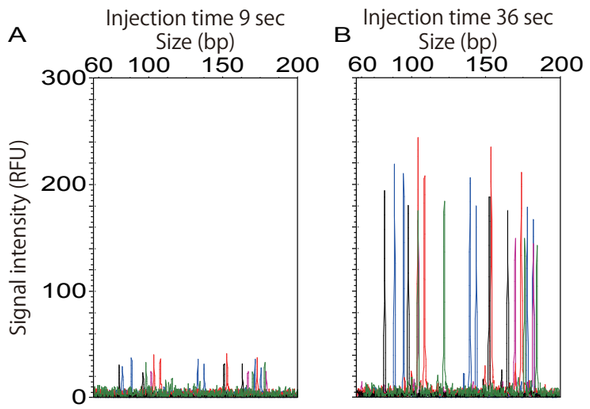


Figure 5: Comparison of sample injection conditions and signal intensity

The fragment analysis data for injection voltages 1.6 kV (A) and 3.2 kV (B) are shown. The relative signal intensity of each peak of the size standard was plotted with respect to the signal intensity observed under the injection voltage 1.6 kV for 9 seconds.

Finally, we tested the recovery of the signal intensity by adjusting the injection time using diluted samples. PCR amplicons were prepared using the PowerPlex™ Fusion 6C System (Promega®) with 1 ng of control DNA (2800M DNA). The amplicons were diluted 3000 times before injection. The samples were injected under a voltage of 1.6 kV (default) for 9 (default) or 36 seconds. GeneMapper™ ID-X ver. 1.6 (Applied Biosystems®) was used for allele calling. The threshold for peak detection was set to 50 RFU. The results are shown in Figure 6. For an injection time of 9 seconds, the average signal intensity for all peaks is 66.3 RFU. Some alleles were missed because the signal intensity was below the threshold. However, by increasing the injection time to 36 seconds, the average signal intensity is improved to 181.8 RFU, and all of the expected alleles are called correctly.



C

Injection time (s)	Average signal intensity (RFU)	Number of alleles detected	Detection ratio
9	66.3	3	6.3%
36	181.8	47	100%

Figure 6: Improve data quality by adjusting the injection time (Fragment analysis)
The figure shows the electropherogram around 100 bases under the injections for 9 (A) or 36 (B) seconds and the comparison of data quality (C).
The average signal intensity is the average value of all amplicon peaks except the size standard.
The number of alleles detected is the number of correctly called alleles among all the observed amplicon peaks.

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Conclusion

This report described practical examples of adjusting the signal intensity and data quality by varying the sample injection conditions. The results showed that increasing the injection time provided a stronger signal intensity but with the risk of lower resolution. By adjusting the injection conditions appropriately, it is possible to analyze samples that produce a low signal intensity, although there are limitations on the quality of the results.

Materials and Methods

Sequencing analysis

3500/3500xL Sequencing Standards, BigDye® Terminator v3.1 (Applied Biosystems®) was dissolved in 300 μL of Hi-Di™ formamide (Applied Biosystems®). For the data shown in Figure 3, the solution was further diluted 100 times with Hi-Di™ formamide. Each sample was analyzed using the DS3000 under the conditions shown in Table 1.

Fragment analysis

WEN Internal Lane Standard 500 (WEN ILS 500) (Promega®) was diluted 20 times with Hi-Di™ formamide. For the data shown in Figure 6, the WEN ILS 500 was diluted 400 times. PCR amplicons were prepared according to the manufacturer's instructions and then diluted 3000 times with Hi-Di™ formamide.Each sample was analyzed using the DS3000 under the conditions shown in Table 1.

Table 1: Electrophoresis conditions

Application	Polymer type	Run protocol	Sample injection condition
Sequencing analysis	Polymer7	Fast sequencing: AB_Seq_36_Fast Standard sequencing: AB_Seq_36_Std	Described in the text
Fragment analysis	Polymer4	Promega_6Dye_WENILS_36_P4	