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September 2008

COMPARISON OF ABI 3730XL CAPILLARY SEQUENCER AND MEGABACE4500 CAPILLARY SEQUENCER: THE TWO MACHINES ARE EVALUATED FOR THEIR COMPLEXITY, ACCURACY, REPRODUCIBILITY, PRODUCTIVITY AND COST EFFECTIVENESS.

by

Jacques P. Thimote

A Thesis

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of Lehigh University

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in

Chemistry

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Date

(Thesis Advisor)

Table of Contents

Table of Contents

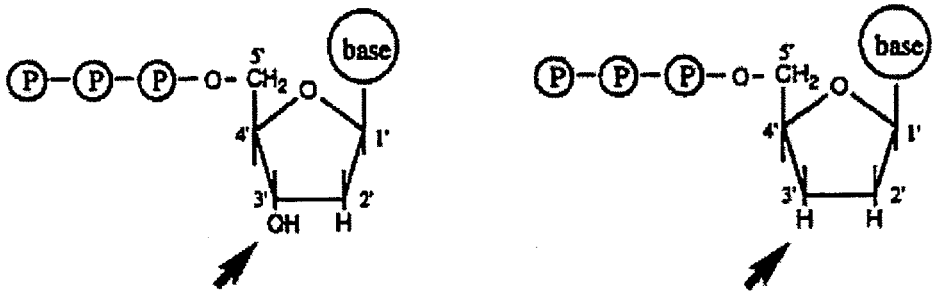
Abstract

The purpose of this research is to compare the ABI 3730xl sequencer to the Megabace 4500 sequencer by evaluating the sequence data obtained from both machines with DNA sample that were cloned into plasmid or M13 phase. The machines were evaluated for their complexity, accuracy, reproducibility, productivity and cost effectiveness. The Megabace 4500 is more complex to operate than the ABI 3730xL. The Megabace4500 required manual interaction with a technician to load each run. More than seven steps were required to load one 384-well plate. However, the ABI 3730xL is more user friendly and required only one step to load a 384-well plate. Even though the accuracy and reproducibility of each capillary sequencer were similar, the productivity was different. The Megabace4500 is more suited for high throughput DNA analysis. It generated about 1 million bases in an 8-hour period when compared to the ABI, which produced about 340,000 bases per 8-hour period. Furthermore, the Megabace 4500 uses an efficiently high powered solid-state laser that couples together with a scanning confocal optics system. This innovation has significantly increased detection sensitivity for longer reads and also provides a much more operational lifetime when compared to the array and the argon laser of the ABI 3730xL.

Introduction

DNA Sequencing is the process of determining the order of nucleotide bases, adenine, guanine, cytosine and thymine in a strand of DNA. The Sanger method is one of the best sequencing technique used by scientists today. This method involves synthesis of a complementary DNA template using 2'-deoxynucleotides and termination of the synthesis using 2', 3'-deoxynucleotides (see fig.1 below). This process is performed with a DNA sequencer. Over the last two decades, DNA sequencers have become significantly important due to large genomic projects and the need to increase accuracy, reproducibility, and cost effectiveness while decreasing complexity.

Fig. 1



Deoxynucleotide Dideoxynucleotide

DNA sequencers employ electrophoresis techniques to accomplish DNA analysis by applying an electric field across the gel matrix to separate fluorescent-labeled DNA molecules that differ in size by one base. Smaller DNA molecules move rapidly and larger DNA molecules move more slowly. The DNA molecule moves through the gel, and reach at a point where a laser is focused on it. The laser emits

at a wavelength that is absorbed by the fluorescent dye specific to the base at the end of the molecule. That causes a base-specific signal that can be automatically recorded by an optical detector. The intensity of the fluorescent signal is directly proportional to the number of strands of DNA that are in the reaction. One can obtain a weak signal because the initial amount of DNA is small. However, increasing the number of cycles in the PCR program allows one to increase the intensity of the fluorescent signal.

In the 1990's, slab gel electrophoresis dominated DNA sequencing. This type of sequencer used a thin gel layer between two glass plates to perform electrophoresis. The electrophoresis separation required 12 hours or more and was limited to 24 samples at a time. However, the successive generation of gel-based sequencers has significantly reduced the time and increased the number of sample throughput. One of the limitations of gel-based sequencers is the large amount of labor required. The major labor involved is the time consuming tasks of preparing a new gel for each separation, loading each DNA sample onto the gel and cleaning the system after each separation. This type of sequencer also required lane tracking to determine the location of different lanes of separated samples. Lane tracking is a very important step in the sequence determination process in gel-based sequencers. It can directly affect the accuracy of the base call for an entire DNA sample or several samples. Lane tracking is challenging when there is a wide range of sequence image configurations including: comb sizes and types, sample loading format, gel sizes

sequencing chemistry and gel matrices (11). However, lane-tracking is not required for capillary based sequencers.

Capillary array electrophoresis has emerged as a valuable tool for DNA analysis in the early 2000's. This technology not only increased the number of DNA samples that can be simultaneously analyzed but also reduced the labor and time consumed for each run compared to slab gel electrophoresis. With capillary electrophoresis, each DNA sample is analyzed within a capillary having the diameter of a human hair; the DNA samples are irradiated all at once with laser light. By using a charged couple device (camera) that acts as a simultaneous multichannel detector, the capillary array sequencer is able to measure the fluorescent dyes. More than 100 capillaries are bundled together to process many DNA samples at the same time. Many companies have introduced capillary gel electrophoresis. The ABI 3730XL and the Megabace 4500 are the two best capillary sequencers used by researchers today for DNA analysis. These two sequencers are significantly improved in operational efficiency. The time required for electrophoresis in these two capillary array sequencers is significantly less than that of other current gel based sequencers. Both capillary array sequencers used sheath flow fluorescent detection systems. This innovative technology is very important in preventing the scattering of laser light irradiated across the surface of the array and significantly increased the sensitivity of fluorescence detection by more than one order of magnitude (11). However, the Megabace 4500 uses high powered solid-state laser that couples

together with the scanning confocal optics system. This innovation increased detection sensitivity for longer reads and also provides more operational lifetime when compared to the array and the argon laser of the ABI 3730xl.

I have directly compared the ABI 3730xl and the Megabace 4500 for their accuracy and reproducibility by evaluating the sequence data obtained from both instruments with DNA samples that were cloned into plasmid; and also compared their complexity and cost effectiveness.

Materials Section

GeneAmp PCR System 9700 (PE Applied Biosystems, Serial number N805-0200) was used to perform the cycle sequencing reaction. Abgene Thermo-Sealer (Model number AB-0384/240 and AB-0384/110) was used in conjunction with a heat sealing film to cover 384-Plates before cycle sequencing and denaturation cycle. Hydra II Micro-Dispenser with 2-Position X/Y Plate Stage from Matrix Tech Corp (Catalogue Number 10961XY) was employed to transfer liquid. To perform the RCA reaction the Templiphi DNA Sequencing Template Amplification kit from GE Healthcare Life-sciences (Product Number 25640001, Code 25-6400-01) was used. The Sanger reactions were accomplished by using DYEnamic™ ET Terminator (MegaBase™) Sequencing Premix from GE Healthcare (Product number 93-81096), and BigDye® Terminator v3.1 Readily Reaction Cycle Sequencing Kit from PE Applied Biosystems (Product Number 4337456, Part Number 4337035 Rev. A). ABI 3730xL DNA analyzer from Applied Biosystems

and Megabace 4500 DNA analyzer from Amersham Biosciences were used to perform DNA analysis.

Experimental Section

A 384-well glycerol plate that contained E. coli with the DNA insert was taken from -80° C to perform Rolling Circle of Amplification (RCA). The purpose of this step is to amplify a circular DNA template for cycle sequencing. An aliquot of 0.6uL from the glycerol plate that contains the DNA insert was transferred to a plate containing 3uL of buffer. This plate was placed in a thermocycler for 5 minutes at 95° C, where the cells were lysed and the plasmid was released. Then 2.4uL of the Templiphi Premix that contains Phi29 Polymerase, dNTP's and random hexamers were added. The plate was then incubated overnight at 30° C where numerous copies of the DNA were produced. After incubation, 6uL of water added to the plate, mixed and the plate was ready for Sanger reaction. The Sanger method produces several number of dye labeled fragments. Before the sequencing reaction, each sample was tested on the agarose gel to verify that the RCA reaction worked properly in each well. Out of 384 samples 356 gave positive bands and 28 samples failed. Each capillary sequencer machine requires a different type of Dye Chemistry. For this purpose two plates were sequenced. One plate was sequenced with Big Dye Chemistry Kit while the other plate was sequenced with ET Terminator Kit. For the Big Dye Chemistry, a mixture of 200uL Big Dye kit, 1.3mL Big Dye buffer, 2mL of DD water, and 16uL of T7 primer (100 Micro

molar) was prepared. Whereas for the ET terminator Chemistry a chemistry cocktail comprised of 1.8mL of ET Terminator Kit, 1.5mL of DD water and 10uL of T7 primer (100 Micro molar) was made. One 384-well plate was labeled Big Dye and another plate was labeled ET Terminator. 9uL of each mixture and 1uL of diluted RCA sample were added to the appropriate labeled plates. And then run for 30 Cycles on the thermocylcer to complete the Sanger reaction. The cycle sequencing was performed using GeneAmp PCR 9700 with the following conditions:

Denaturation	95° C	10 Seconds
Annealing	50° C	5 Seconds
Extention	60° C	4 Minutes

After the sequencing reaction was completed, cell debris, buffers, salts and unincorporated dyes were removed from the sample before the plate can be loaded onto the capillary sequencers. This process was accomplished by using a Millipore MultiScreen filtration system. A reservoir half-filled with buffer containing 0.3mM EDTA, pH 8.0 in DD water was placed into the Hydra II (384-needles) along with the sequencing reaction plate. 20uL of buffer per well were transferred to the reaction plate and mixed. Then the mixed (sequencing reaction + buffer) was transferred to the MultiScreen384-SEQ plate. Then the MultiScreen was placed onto a vacuum pump set at 25" Hg until the wells were completely empty. After 2 repeated cycles, 20uL of buffer were added in each well to the Multiscreen384-SEQ plate and mixed 20 times with the Hydra II. The 20uL solution was then

divided into two 384 well plates with 10uL in each well. One plate was used for sequencing while the other plate was placed in the -20° C freezer. Before loading each plate onto the capillary sequencer, 5uL of formamide was added to each sample. The following steps were taken when loading the samples in the Megabace 4500 sequencer:

1. Filled one labeled translucent water tank with 120ml of Milli-Q water and one labeled yellow buffer tank with 120ml of 1.5X running buffer.
2. Spun six tubes of 2.6% V2E LPA for 2 minutes at 3,000rpm.
3. Loaded the buffer tank into the left (cathode) side and the matrix tubes into the right (anode) side and closed the drawers completely. Then started the pre-run.
4. Verified that the correct values appear in the electrophoresis parameter: Sample Injection Voltage: 2 KV, Run Voltage: 5.9 KV, Sample injection time: 36 sec, Run Time: 215 min.
5. Filled a 384-well NUNC plate with 85ul of 1.5X running buffer per well and placed the plate on the Buffer Plate Adapter.
6. After the pre-run had been completed and started the run protocol, the remaining steps were performed on a subset of instruments. The assigned sample plate was loaded on the adapter into the left (cathode) side of the first instrument in the subset.
7. After the sample injection was completed, the 85ul of 1.5X buffer plate was loaded on the deep-well plate adapter into the left (cathode) side of the

instrument followed by the blue 1.75ml of 1.5X buffer tubes into the right (anode) side of the instrument.

Only one step was performed on the ABI 3730xL sequencer. However, the following items were checked before loading the plate: the instrument's green status light was on, adequate levels of buffer and water were in the appropriate reservoir, and the level of POP-7 polymer in the bottle to ensure sufficient volume for run.

Discussion Section

Complexity

Even though ABI 3730xl and the Megabace 4500 used similar technology to perform DNA analysis, they are both different in term of complexity. The complexity comparison was based on: reagents and material preparation, characteristic of operational step, calibration proficiency testing control, troubleshooting and maintenance, and interpretation and judgment of data. The Templiphi Premix is extremely labile at room temperature and should keep on ice for the entire process. The BigDye terminator and ET terminator are more stable at room temperature; however the manufacturer recommended they should be kept on ice when using them. The sequencing reaction and the RCA reaction required precise measurements. From my own experience, the reactions would not work properly if more or less reagents were added. The ABI 3730xl is more user friendly in its operation. Only one step was required to load the samples. This sequencer can load and run up to 8 x 96 or 2 x 384 templates in continuous automated operation

with less than one hour of instrument attendance/labor per 24 hour period. The Megabace 4500 is a 384-capillary array system and required manual interaction with a technician to load each run. More than seven steps were required to load one plate. Both instruments required a barcode for each 384 well plates. I did not have the opportunity to evaluate calibration, proficiency control and troubleshooting because the ABI 3730xl is a fully automated system and required limited human interaction. The only preliminary step to the sequencing reaction is a spectral calibration that ensures all capillaries are functioning properly and the signal appropriate. Many issues may be encountered while operating the Megabace 4500 instrument including skipping a step or opening the wrong software; so troubleshooting is much more complex and sometimes difficult to solve. Maintenance is required for both capillary sequencers on a monthly basis including recording values and initial inspection, checking for disk error, flushing, and calibration. Interpretation and judgment of data in both capillary sequencers are fully automated. A summary of the complexity comparison is shown on table 1.

Table 1

Complexity	Megabace 4500	ABI 3730xL	Comments
Reagents and Material preparation	Reagents are extremely labile and require precise measurement	Reagents are extremely labile and require precise measurement	No difference
Characteristic of Operation steps	Automatically executed	Not fully automatic and require some steps and timing	Different
Calibration and Proficiency Testing Control	Machine is stable, well defined, and readily available	Machine is stable, well defined, and readily available	No difference
Troubleshooting and maintenance	Automatic, self correcting and requires minimal judgment	Require some judgment, technical skill	Different
Interpretation of Data	No interpretation and judgment is needed before releasing results	No interpretation and judgment is needed before releasing results	No difference
Accuracy	99% accurate	99% accurate	No difference
Reproducibility	100% reproducible	100% reproducible	No difference
Productivity	920,000 bases/8-hour	236,000/8-hour	Different
Cost Effectiveness	$370/384-well plate	$644/384-well plate	Different

Accuracy

The ability for each sequencer to report the correct base pair has been evaluated. Out of 356 sequences 341 were successfully analyzed in the Megabace 4500. A similar result was obtained for ABI 3730xL. Both capillary sequencers automatically analyzed raw data by using their own auto-basecaller software. Base-called results can be saved into ABI 3730xl and Megabace4500 formats or third party formats. To evaluate accuracy, the DNA sequence data that has quality value

greater or equal to 20 must be extracted from both capillary sequencers and processed by the base-calling program "Phred". This program calculates a base sequence for the traces and assigns a quality value, Q, to each base. The probability of assigning and erroneous base is $P_{error} = 10^{(-Q/10)}$. It has been shown that Phred's error probabilities are close to100% accurate (9). Based on the PHRED table 2 below the accuracy of each sample was compared to the quality value obtained from both machine.

Table 2

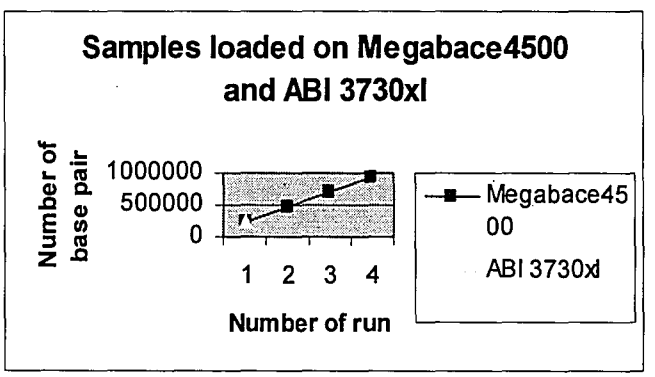
Phred quality score	**The probability the base wrong**	**Accuracy of the base**
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

After Phred analysis a quality value of 20 or better was recorded for each sample greater than 0 base pair which is 99% accurate. The same quality value was recorded before Phred analysis. Both sequencers gave similar results. No difference was shown in either machine. However, for samples that have 0 base pair, a quality value of 3 was recorded in both capillary sequencers. This can be explained by the fact that some capillaries may not work properly and gave false negative results. However, further investigation is needed to examine the reason(s) these capillaries did not work.

Productivity

The number of base pairs each capillary sequencer can produce in 8 hours of continuous operation was evaluated. One day before the experiment, a total of 8 x 384-well plates were prepared and ready to load. In 8 hour continuous operation, we successfully sequenced 1536 templates and generated about 920,000 bases pair in Megabace4500 compared to the ABI 3730xl that produced only 236,000 bases (see graph 1). This result is based on one operator per machine.

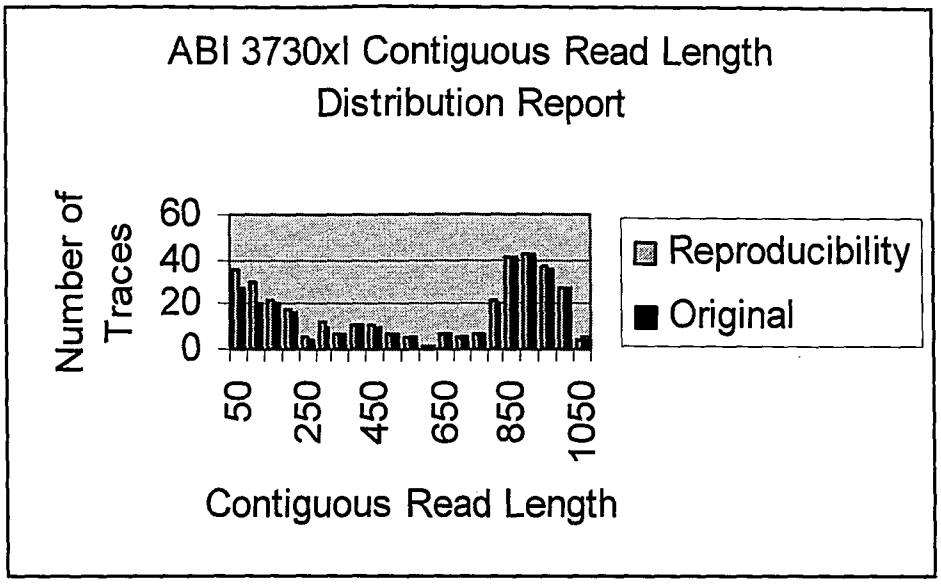
Graph 1



Reproducibility

The glycerol plate that contained E. coli with the DNA insert was sequenced by a different lab. The plate was loaded on the ABI 3730xl only because the Megabace4500 was not available. They repeated the same experiment based on the same procedure and experimental description previously used. The contiguous read length distribution report for the reproducibility test and the plate I sequenced is shown on the data below.

Graph 2



"The Contiguous Read Length is the longest uninterrupted stretch bases with quality higher than a specified limit. In the evaluation of the quality of each base, not only the quality value of that base is used, but also those of adjacent bases within the specific window size". The overall reproducibility of the ABI 3730xl test was 344/344 = 100%. However, a small distribution deviation was observed in the reproducibility test.

Cost Effectiveness

Comparison of cost effectiveness has shown lower reagent and supply cost for MegaBace4500 sequencer than for ABI 3730xL sequencer (see table 3 and 4below). The Megabace4500 uses the DYEnamic ET Dye terminator Kit while the ABI 3730xl uses BigDye terminator reagent. Both dyes are very expensive but they can be relatively cheap when diluted. However, the BigDye Terminator Kit is two times more expensive than the DYEnamic ET Dye Terminator kit. Other components such as Megabace Long Read Matrix used in Megabace4500 and Polymer that is used in ABI 3730xl are also expensive. The average cost to sequence one 384-well bacterial culture plate in ABI 3730xL is $643.97 compare to

MegaBace4500 which is $370.82 (see table 3 and 4). In addition, the Megabace4500 has further reduced per lane cost if labor such as sample preparation cost is included. Furthermore, you can save time and energy cost when using Megabace4500 because this machine took about 2hours to run one 384-well plate compared to the ABI 3730xl that took about 8 hours to complete one 384-well plate.

Table 3

Megabace 4500

Materials/ Reagents	Vendors	Stock Number	Pack Size	Total Cost	Quantity/ run	Cost/384
Disposables						
Thermo-Fast Diamond 384	AbGene	AB-111	50/case	$348.00	3	20.88
Reagents						
MegaBACE 4500 2.6% V2E LPA	GE Healthcare	US79676	96/case	975.00	6	60.94
DYEnamic ET Terminator Kits	GE Healthcare	US81050	24ML	5,181.00	1.3ML	281.00
Total						370.82

Table 4

ABI 3730XL

Materials/Reagents	Vendors	Stock Number	Pack Size	Total Cost	Quantity/384	Cost/384
Disposables						
384-well Clear Optical Reaction	Applied Biosystems	4309849	50/case	360	3	21.6
Reagents						
POP-7 Polymer	Applied Biosystems	4331246	28ML	1,020	1.12ML	40.08
BigDye Terminator	Applied	433745	24ML	10,75	1.3ML	582.29

V3.1	Biosystems	6		0		
Total						643.97

Conclusion

Both capillary sequencers are differed in the speed at which samples are run, the number of samples that can be processed in parallel in a single run, reagent and supplies, and cost. The Megabace 4500 is better suited for high throughput DNA analysis because it is able to generate about 1 million bases in an 8-hour period when compared to ABI 3730xl which can produce about 340,000 bases per 8-hour period. However up to 16 x 96-well plates can be loaded on the stacker of the ABI 3730xl for high throughput automated processing. This instrument is completely hands free although small attention is required to ensure adequate buffer, polymer and waste level are maintained.

References

1. Wikipedia.org. http://en.wikipedia.org/wiki/DNA_sequencing (accessed Jan 17, 2008)
2. Dan, B., Christ, D., Eds. ABI 3730xl Analyzer Users Protocol, v2.3; DOE Joint Genome Institute: Walnut Greek, CA, 2005; p 2-21.
3. Christ, D., Eds. MegaBACE 4500 DNA Analysis System Users Protocol, v2.4; DOE Joint Genome Institute Walnut Greek, CA, 2005; p 2-17.
4. Hydra II Microdispenser Manual: Manual Version 1.3, April 2003
5. Applied Biosystems 3730/3730xl DNA Analyzers User Guide: 2002.
6. Biospace.com. http://sec.edgar-online/2001/04/02/0001095811-01-002050/Section2.asp (accessed Nov 20, 2007)
7. Mostafa, R. Pyrosequencing Sheds Light on DNA Sequencing. *Genome Res.* [online] **2001,** pp 1-2.
8. Chris, W., Miranda, H.S., Kathleen, L., Christ, D., Lena, P. JGI.Doe.Org. www.jgi.doe.gov/science/poster/wongJGIUM07.pdf. An Overview of the Joint Genome Institute Production Sequencing Line.
9. Phrad.com. http://www.phrad.com/phred/ (Accessed Jan. 28, 2008)
10. Amersham Bioscience MegaBACE 4000/4500 User Manual: 2003.
11. Christoph, W. S., Eds. *Essentials of Genomics and Bioinformatics*; Wiley-VCH: Calgary, Canada, 2002.

12. James, C.M.; Amanda, A. M. *DNA Sequencing: Sequencing the Genome, Fast* [online] **1999**, *283*, pp 1867-1868.

Biography

I was born on July 24, 1966, in Haiti. My father's name is Clement Thimote and my mother's name is Charles Thimote. I attended Miami Dade Community college in 1992-1994 where I received an Associate of Art degree. I graduated from the University of Florida in 1996 with a Bachelor degree in Microbiology and Cell Sciences. I attended graduated school at Lehigh University in 2005-2008. I received academic honors from The Chancellor's List 2005-2006 and 2006-2007.

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