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Improved Ion PGM® Chip loading reproducibility with the Eppendorf Xplorer® plus electronic pipette and ep Dualfilter T.I.P.S.® LoRetention

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Abstract

This Application Note illustrates how using the Eppendorf Xplorer® plus electronic pipette can significantly improve the reproducibility of the Ion Torrent® Next Generation Sequencing (NGS) workflow. More specifically, for the loading of the Ion 314™ Chip v2, used in the Ion Torrent Personal Genome Machine® (Ion PGM, Thermo Fisher Scientific®, USA). The programmable and reproducible speed settings of the Xplorer plus in combi-nation with the ep Dualfilter T.I.P.S. LoRetention proved to facilitate this delicate process. This improvement negated the influence of the operator's manual pipetting variability to the sequencing output and produced reproducible results with a higher mean value and lower standard deviation.

Introduction

Next Generation Sequencing (NGS) has caused a paradigm shift in many fields of basic and applied sciences. This powerful technique allows researchers to obtain large amounts of data from each experiment, consequently replacing established technologies such as Sanger sequencing, quantitative Polymerase Chain Reaction (qPCR) or Microarrays for many tasks. This data can not only be used for research purposes, but also for the detection of genetic abnormalities like chromosomal aneuploidies or single gene disorders in a fast and reliable manner. [1, 2, 3, 4] In the final step of the sample preparation for sequencing on the Ion PGM System (Thermo Fisher Scientific, USA), the Ion Chip needs to be loaded with the Ion Sphere® Particle (ISP) sequencing reaction mixture. The Ion 314 Chip comprises 1,262,519 addressable wells, which can be loaded with the reaction mixture. Only the wells loaded with the ISP sequencing reaction mixture will carry out the sequencing reaction, thus one of the main success factors is the ISP loading (calculated as a proportion of the number of wells having received ISP to the total number of addressable wells). Loading success is shown as a percent of total available wells. One percent of ISP loading resembles 0.6 to 5 Megabases in total base output, depending on the read length of the run. To gain sufficient coverage and depth of sequencing on the target regions it's crucial to achieve the highest possible total output. Failure in efficient chip loading can lead to decreasing quality of results or may impair successful data interpretation (e.g. the sequence of interest may not be covered sufficiently or mutation calling may not be reliable).

There are several crucial factors that increase the performance of loading the chip such as:
> Constant dispensing speed during loading
> Uninterrupted delivery of the sequencing reaction mix from the pipette tips
> Low initial acceleration of liquid dispensing
> Absence of air bubbles
> The linear dispensing of the liquid.

These factors are hard to control if performed manually with 7 µL of sequencing reaction mix. As a result, the chip loading step is difficult and challenging in terms of reproducibility and ISP loading value. In this keystone step, the importance of proper delivery can place additional pressure on the user increasing the risk of human error, which of course can again impair the successful loading of the chip.

Materials and Methods

> Eppendorf Xplorer plus single channel 0.5-10 µL
> Competitor pipette as stated in method's protocol [5]
> Eppendorf ep Dualfilter T.I.P.S. LoRetention 0.1-10 µL
> Ion PGM System, Thermo Fisher Scientific, USA
> Ion 314 Chip v2, Thermo Fisher Scientific, USA
> 7 µL of a sample containing ISP sequencing reaction mixture prepared according to the method's protocol [5]

Results and Discussion

According to the standard manufacturer's protocol the Ion 314 Chip v2 used in Ion Personal Genome Machine (PGM) System is recommended to be loaded manually with a liquid dispensing speed of approximately 1 µL per second. The working speed of the Eppendorf Xplorer plus 0.5-10 µL pipette can be adjusted to meet these specifications.

Here we present the data collected from 20 separate chip loading procedures performed with both a mechanical pipette and using the electronic Eppendorf Xplorer plus 0.5-10 µL pipette both with the ep Dualfilter T.I.P.S. LoRetention.

Table 1: Comparison of chip loading density [%] between pipetting with a mechanical pipette and Eppendorf Xplorer plus. Both have been used with ep Dualfilter T.I.P.S. LoRetention.

Run no.	Operator no.	Loading density in % Using manual pipette	Run no.	Operator no.	Loading density in % Using Xplorer Plus pipette
1	2	83	**11**	1	88
2	1	58	**12**	2	88
3	2	65	**13**	1	89
4	1	81	**14**	1	87
5	2	41	**15**	2	92
6	1	93	**16**	2	85
7	1	76	**17**	1	81
8	2	78	**18**	2	93
9	2	72	**19**	2	89
10	1	45	**20**	1	85
Mean value in %		69.20			87.70
Stand. Dev.		16.84			3.50
% CV		24.33			3.99

The average value of the chip loading following the standard protocol was 69.20 % and with the use of the electronic pipette this value increased to 87.70 % with a more than 4 times lower standard deviation.

The chips' ISP loading value is calculated by the sequencer and loading density is displayed in a graphic manner (fig. 1 and 2).

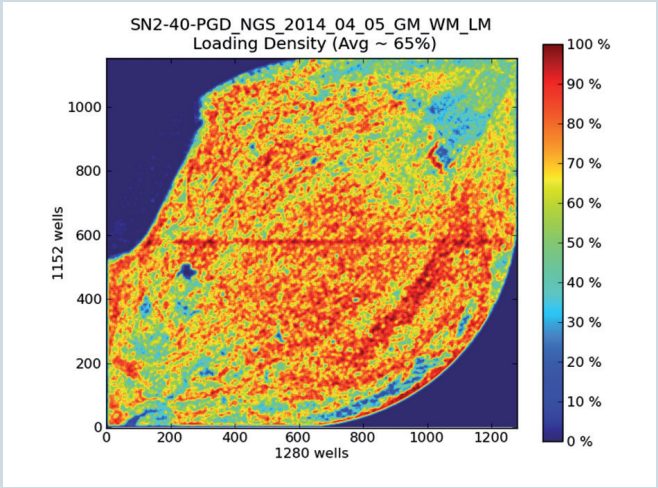

Figure 1: Chip loading density after manual ISP sequencing reaction mixture. Loading density was ~65 %.

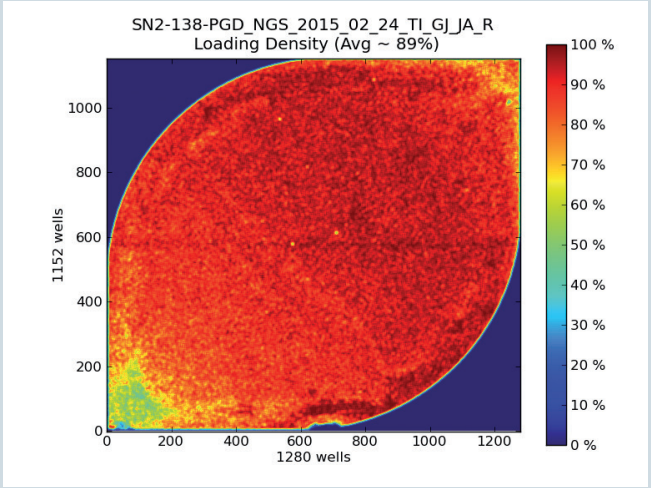

Figure 2: Chip loading density after loading with the Eppendorf Xplorer plus electonic pipette. Loading density was ~89 %.

Another parameter relevant to the method's evaluation is the reproducibility of the loading level. During the operators' manual pipette handling, the coefficient of variability was reaching more than 24 %, making it difficult to ensure efficient loading level in every run. Switching to the electronic Eppendorf pipette increased both minimal loading level for the Ion 314 Chip v2 and the reproducibility of this process. Using the Eppendorf electronic pipette decreased the influence of the operators' pipetting skills on the data collection yield and quality in the NGS procedure.

Conclusion

We have shown that using the Eppendorf Xplorer plus electronic pipette in combination with the ep Dualfilter T.I.P.S. LoRetention significantly increases the quality and reproducibility of the sensitive process of loading the Ion 314 Chip v2. This is mainly due to the programmable and constant dispensing speed that results in the continuous dispensing of the sequencing reaction mixture without introducing air bubbles. The influence of the individual user's manual pipetting skills was significantly reduced in the sensitive, but important step of the Ion Torrent NGS workflow. Taken together, the loading density and loading level of the Chip is increased, resulting in a greater sequencing output in usable bases, directly meaning more successful and reliable sequencing runs.
This again illustrates that Eppendorf's high precision laboratory equipment not only supports day-to-day routine experiments but also facilitates procedures' optimization supporting the most demanding applications.

Literature

[1] Łukaszuk K, Pukszta S, Wells D, Cybulska C, Liss J, Płóciennik Ł, Kuczyński W, Zabielska J. Routine use of next-generation sequencing for preimplantation genetic diagnosis of blastomeres obtained from embryos on day 3 in fresh in vitro fertilization cycles. Fertil Steril. 2015, 103(4):1031-6.
[2] Treff NR, Fedick A, Tao X, Devkota B, Taylor D, Scott RT Jr. Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease. Fertil Steril. 2013, 99(5):1377-1384.
[3] Gagan J, Van Allen EM. Next-generation sequencing to guide cancer therapy. Genome Med. 2015, 29;7(1):80.
[4] Hosomichi K, Shiina T, Tajima A, Inoue I. The impact of next-generation sequencing technologies on HLA research. J Hum Genet. 2015, Aug 27. doi: 10.1038/jhg.2015.102. [Epub ahead of print]
[5] User Guide: Ion PGM™ Sequencing 200 Kit v2, Thermo Fisher Scientific®.

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Xplorer® Plus 0.5-10 µL	4861 000.708	4861000708
Eppendorf ep Dualfilter T.I.P.S.® LoRetention 0.1-10 µL	0030 077.610	0030077610

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