

ABSTRACT

Amidst a pandemic caused by the SARS-CoV-2 virus, rapid genomic surveillance of the virus is much needed to support epidemiology monitoring of the pathogen. Oxford Nanopore Technologies (ONT) has allowed scalable and high-throughput SARS-CoV-2 sequencing. However, a considerable coverage drop in certain genome regions has been observed in experiments using the ARTIC V3 Classic Protocol (ARTIC V3 primer with SQK-LSK109) and Midnight Protocol (Midnight primer and SQK-RBK110.96). This study aims to optimize the annealing temperature of the primer set and evaluate the performance of each SQK-LSK109 and SQK-RBK109 library preparation kit, as well as the protocol, respectively. Three Delta variant samples with a Ct value of 18, 23, and 25 are used in this study. Gradient PCR tiling was conducted before the sequencing to determine the optimized annealing temperature. The sequencing was done in two batches; batch one using SQK-LSK109 and batch two using SQK-RBK110.96, each with the sample amplified using ARTIC V3 and Midnight primer, respectively. Out of five temperatures, only three (59°C, 61°C, and 63°C) yielded the most consistent and greater viral genome coverage with minimal amplicon drops. While there are no remarkable differences in the sequencing quality of both library preparation kits regardless of the primer, the Midnight protocol demonstrates a greater genome recovery of the SARS-CoV-2 by >98% with fewer amplicon drops in optimized annealing conditions. The Midnight protocol with lower optimized annealing temperature allows rapid and high-quality genomic sequencing to support epidemiology surveillance.

Keywords: SARS-CoV-2, ARTIC V3 primer, Midnight primer, SQK-LSK109 kit, SQK-RBK110.96 kit.

ACKNOWLEDGEMENTS

First and foremost, in the name of Allah, the most gracious and the most merciful. Praise and gratitude I pray to Allah, the Almighty, and to His Messenger, Prophet Muhammad (PBUH), who has given His blessing so that I had the strength to complete my bachelor's thesis entitled "Optimization of Rapid Turnaround SARS-CoV-2 Sequencing Protocol with Multiplexed PCR Tiling". My deepest gratitude goes to my parents, siblings, and best friends for their endless support and motivation from the beginning.

I would like to express my sincere appreciation to Ms. Lidya Kristiani, S.Si, Ph.D., as the thesis advisor who has given her guidance throughout my thesis period, especially during report writing. My highest gratitude to GSI Lab for facilitating and sponsoring my bachelor's thesis research project. Foremost, I would dedicate the biggest honor to dr. Meutia Ayuputeri Kumaheri, M.Res, IBCLC, CIMI as the field supervisor from GSI Lab who has given me lots of opportunities and valuable experience throughout my time there.

Last but not least, my gratitude also tremendously goes to my senior colleagues from the GSI Lab NGS team, especially Ci Vania and Ko Bernard, for always guiding me throughout my thesis project and answering my endless questions every day. I would also like to thank Ci Carissa, Ci Anna, Kak Rei, Kak Dian, Kak Himawan, and Kak Nina for accompanying me during my wet-lab work days.

This report is far from perfect and still has room for improvement. Hence, constructive feedback and input are always welcomed. It is my sincere hope that this study will be beneficial, especially for clinical laboratories that are still fighting against SARS-CoV-2.

Jakarta, 30 June 2022



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TABLE OF CONTENTS

CERTIFICATE OF APPROVAL	i
COPYRIGHT NOTICE	iii
STATEMENT OF ORIGINALITY	iv
ABSTRACT	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES	ix
LIST OF TABLES	x
LIST OF SUPPLEMENTARY MATERIAL	xi
LIST OF ABBREVIATIONS	xiii
CHAPTER 1 INTRODUCTION	1
1.1. Research Background	1
1.2. Research Objective	2
1.3. Scope of Work	3
CHAPTER 2 LITERATURE REVIEW	4
2.1. SARS-CoV-2	4
2.2. Whole Genome Sequencing	6
2.2.1. First Generation Sequencing	6
2.2.2. Second Generation Sequencing	7
2.2.3. Third Generation Sequencing	7
2.3. Oxford Nanopore Technologies (ONT)	8
2.3.1. Principle of nanopore sequencing	9
2.3.2. Principle of library preparation	10
CHAPTER 3 METHODOLOGY	13
3.1. Study Design	13
3.2. Sample Preparation	14
3.3. RNA Extraction	14
3.4. Reverse Transcription	14
3.5. PCR Tiling for Library Preparation	15
3.5.1. ARTIC V3 Primer PCR Master Mix	15
3.5.2. Midnight Primer PCR Master Mix	16
3.5.3. PCR Tiling Thermal Cycler Setup	16

3.6. Agarose Gel Electrophoresis	18
3.7. Library Preparation for Sequencing	18
3.8. Bioinformatics Processing WF-ARTIC Report	19
CHAPTER 4 RESULTS	20
4.1. Agarose Gel Electrophoresis of Gradient PCR Tiling	20
4.2. Sequencing using SQK-LSK109	20
4.3. Sequencing using SQK-RBK110.96	24
CHAPTER 5 DISCUSSION	26
5.1. Annealing Temperature Optimization	26
5.2. ARTIC V3 and Midnight Primer	27
5.3. SQK-LSK109 and SQK-RBK110.96	30
5.4. ARTIC Protocol and Midnight Protocol	31
CHAPTER 6 CONCLUSION	33
REFERENCES	34
APPENDICES	38

LIST OF FIGURES

Figure 2.1. How nanopore works.	8
Figure 2.2. Nanopore and library strand.	10
Figure 2.3. Primer for PCR tiling.	11
Figure 2.4. The mechanism of the library preparation kits.	12
Figure 3.1. Study Design.	13
Figure 4.1. Effect of various annealing temperatures on the coverage plot of Ct 18 ARTIC V3 sequencing using SQK-LSK109.	21
Figure 4.2. Effect of various annealing temperatures on the coverage plot of Ct 23 ARTIC V3 sequencing using SQK-LSK109.	22
Figure 4.3. Effect of various annealing temperatures on the coverage plot of Ct 18 Midnight sequencing using SQK-LSK109.	23
Figure 4.4. Effect of various annealing temperatures on the coverage plot of Ct 23 Midnight sequencing using SQK-LSK109.	23
Figure 4.5. Effect of various annealing temperatures on the coverage plot of Ct 25 ARTIC V3 sequencing using SQK-RBK110.96.	24
Figure 4.6. Effect of various annealing temperatures on the coverage plot of Ct 25 Midnight sequencing using SQK-RBK110.96.	25