Journal of Tropical Pharmacy and Chemistry

Journal homepage: https://jtpc.farmasi.unmul.ac.id

Primer Design and Analysis for Detection of *mecA* gene

Armini Syamsidi*, Nuur Aanisah, Reyhan Fiqram, Imanuel Al Jultri

Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu, Indonesia
Author for coresponding: armini.syamsidi@gmail.com

Abstract

mecA is a gene that causes antibiotic resistance and it contained in *Staphylococcus aureus*. The gene can be detected using pairs of primer (forward and reverse). Primes is short nucleotide that are used as attachment point for DNA polymerase and as a barrier for the fragment DNA target to be amplified with Polymerase Chain Reaction (PCR). The aims of this study were to design and analysis the nucleotide primer sequences of *mecA*. This research using in silico method of NCBI (National Center of Biotechnology Information) application, clone manager10, oligoanalyzer3.1, perlprimer and primer3plus. The results of design and candidate primer analysis showed that the first candidate of forward and reverse primer that falls within the criteria with base sequences 18-30, 40-60 GC%, Tm 50-60°C, 3'dimer ≤3, stability ≥1,2, secondary structure >-66,944 J/mole, runs ≤5, repeats ≤4, hairpins>-12,552 J/mole. The conclusion is the first candidate of forward primer with 19 base pair (5'GTGAAGCAACCATCGTTAC'3), GC% 47, Tm 58°C, 3'dimer 2, stability 1.6, secondary structure -1,95 and -15,104.24 J/mole, runs 2, hairpins -0,1 start 53,844 and the first candidate of reverse primer with 21 base pair (5'CCTTCTACACCTCCATATCAC'3), GC% 47, Tm 58°C, 3'dimer 0, stability 1.3, secondary structure -4.74 dan -22,509.92 J/mole, runs 2, hairpins -2.5 dan start 55,852. Both of primer can be used for identification of *mecA* gene by PCR method.

Keywords: *mecA* gene, PCR, Primer, Amplification

Submitted: 29 November 2020 **Accepted**: 30 June 2021 **DOI**: https://doi.org/10.25026/jtpc.v5i3.297

1. Introduction

Resistance caused by antimicrobials has become a major problem for global public health, including Indonesia [1]. According to [2] the case of antimicrobial resistance experienced by large countries such as Europe and the United States has a significant increase resulting

in the death toll each year reaches 50 thousand people, while the global figure has reached 700 thousand every year. Antimicrobial resistance occurs due to inactivated bacteria (changes in drug activity) resulting in reduced accumulation of intracellular drugs and loss of drug affinity to the bacterial cell receptors, the penicillin-binding protein (PBPs = penicillin-binding proteins) [3].

The *mecA* gene's role is to expresses PBP2a or penicillin that binds to PBP so that the bacteria are eliminated by activating its autolytic enzymes. The *mecA* gene then encodes PBP2a, which is located in a transposon. Transposons have a DNA structure that can migrate through the genome of an organism. This structure will be part of the plasmid but can also be derived from bacterial chromosomes. If the transposon contains resistant genes, it will enter the plasmid and move to another cell. In such a way, if the plasmid is able to replicate itself in a new host or transposon, the cell becomes resistant to antibiotics [4].

Identifying antibiotic resistance to target gene fragments can be done by using polymerase chain reaction (PCR) techniques. The PCR method required inspection techniques with the principle of amplification of DNA. In the amplification of the target gene fragments, a component called a primer is needed. This primer will later act as a barrier to the target DNA fragment to be amplified [5]. The target fragment will then require a pair of primers that match the target DNA. The primer must meet the criteria to amplify the target gene fragment, specifically *mecA*. Those parameters for primer design consist of primer length, % GC (Guanine-Cytosine), Tm (melting temperature), primer interactions (dimers and hairpins), primer stability, repeats, runs, and false priming. It is better if there are no dimers or hairpins in the primary and forward sections [6].

To be able to amplified, the *mecA* gene must involve a pair of primer sequences (forward and backward) [7]. Primer is a short sequence of nucleotides with 18-30 bases used as a barrier to target DNA fragments and as an attachment for DNA polymerase enzymes to detect and amplify DNA from specific genes. Forward primers synthesize in the forward direction (DNA target 5 '- 3') and reverse the primer then synthsize in the opposite direction (DNA target 3 '- 5') [8].

As information technology develops, the software has been designed to help design and analyze primers that are NCBI (National Center for Biotechnology Information), OligoAnalyze 3.1, Perprimer, Clone manager10, Primer3 plus primary. These designs in silico make it easier to organize good primer candidates for the process of amplification of gene fragments [5].

Based on the background described above, the authors were interested in conducting research on the primer design of the *mecA* gene by the in silico method.

2. Experimental section

2.1 Materials

The software used in this study were the NCBI sites, Clone Manager 10 software, Oligoanalyze 3.1 sites, perlprimer sites, primer3plus, and Primary-Blast program, in which installed in laptop (Acer aspire3 A314-32-C3X0 Intel® Celeron® N4000 processor, 4GB DDR4 memory) by using WiFi networks. The materials used in this study were DNA sequence data of *Staphylococcus aureus* SC640 DNA, and *Staphylococcal* cassette chromosome islands

2.2 Methods

mecA gene nucleotide sequence data was obtained from the National Center for Biotechnology Information (NCBI) database at the site http://www.ncbi.nlm.nih.gov using the "nucleotide" in resources's menu. The keyword used was the gen to be searched for [9].

2.3 Design of primer candidates using Clone Manager10 software

The primer design carried out on the "Molecule List" included gene sequences that had been stored as the Fasta format on the NCBI website. Then the primer criteria and desired

target area were typed in the "Default Primer Criteria" menu and further providing several primer choices [10].

2.4 Analysis of forward and reverse primer using Clone Manager10 software

The results of the analysis were seen as the "Primer Report" and "Analyze Mix Wizard", with the sequences of each primer (forward and reverse) and the template sequence. Meanwhile, to analyze each primer, primer names and primer sequences were analyzed using "Direct Entry" wizard in the software [11].

2.5 Analysis of forward and reverse primer using Oligoanalyzer 3.1 software

The primer candidates obtained were analyzed by considering the primer criteria in the integrated DNA technologies' (IDT) oligoanalyzer 3.1 program https://www.idtdna.com/pages/tools/oligoanalyzer. The primer sequence was entered into the sequence box to get the hairpin, Cross-Dimer and Self-Dimer information then choose analyze button to look at the major physical properties [9].

2.6 Analysis of forward and reverse primer using perlprimer software

The primaer candidates obtained were analyzed by using the the perlprimer to calculate the melting temperature (Tm), the amplicon size and the stability of the primer-dimer [12].

2.7 Analysis of forward and reverse primer using primer3plus software

The results of the primary candidates obtained were analyzed using the primer check tab http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi, which is a tab provided by primer3plus and can be used to obtain certain primer criteria such as GC%, melting temperature, and self-complementarity [13].

2.8 Data Analysis

A good primer candidate base sequence was used to see similarities with other organisms. It was done by entering into the "nucleotide blast" page, in the "enter accession" box, the selected primer (forward or reverse) was filled in with the appropriate job title then click "blast". A new display appeared, then took to the next stage. The results appeared to indicate any organisms that can be used with the design results primer [5].

3. Results and Discussion

The cause of resistance is due to the influence of the *mecA* gene on *Staphylococcus aureus*. This gene has generally been studied and can cause antibiotic resistance in *Staphylococcus aureus* [14]. The presence of this gene is seen in the SCCmec chromosome of *Staphylococcus aureus*, which encodes a specific transpeptidase enzyme that causes bacteria to be resistant to the use of methicillin and produce penicillin-binding protein (PBP) 2A [15]. In this study, *mecA* gene nucleotide sequence data was used to design primer candidates by in silico.

Nucleotide sequence data available in NCBI (National Center for Biotechnology Information) website. This site has a comprehensive database containing nucleotide sequences of 260,000 organisms that are regularly updated [16]. Based on figure 1 that the *mecA* gene nucleotide sequence data are contained in the DNA sequence data of *Staphylococcus aureus* SC640 DNA, Staphylococcus Cassette Chromosome islands. In this study, the data was used to design primer candidates using Clone Manager10 software.

Primer is a short nucleotide base used as a point of attachment of the DNA polymerase enzyme in the formation or elongation of DNA of a specific gene [6]. Moreover, its function as a barrier to DNA target fragments that will be amplified in the polymerase chain reaction (PCR) process [5]. According to Pradnyaniti, Wirajana, and Yowani (2016), characterization of a good primer parameters included base

length, GC%, Tm, 3'dimer, stability, secondary structure, runs repeats, and hairpins. Based on table 1, the primer candidates obtained were analyzed using clone manager10, oligoanalyzer, perlprimer, and primary3plus program [11].

Table 1. The *mecA* gene primer candidate from the Clone manger10 program

		Primer candidate	Start	Product Length
1	F	GTGAAGCAACCATCGTTAC	53844	2008
	R	CCTTCTACACCTCCATATCAC	55852	2008
2	F	TGAAGCAACCATCGTTAC	53848	2005
	R	CTTCTACACCTCCATATCAC	55850	2005
3	F	TGAAGCAACCATCGTTAC	53808	2040
	R	TCTACACCTCCATATCAC	55848	2040
4	F	TGAAGCAACCATCGTTAC	53808	2041
	R	TTCTACACCTCCATATCAC	55849	2041
5	F	GTGAAGCAACCATCGTTAC	53807	2044
	R	CCTTCTACACCTCCATATC	55851	2044
6	F	GTGAAGCAACCATCGTTAC	53807	2043
	R	CTTCTACACCTCCATATCAC	55850	2043
7	F	TGAAGCAACCATCGTTAC	53808	2042
	R	CCTTCTACACCTCCATATCAC	55850	2042
8	F	TGAAGCAACCATCGTTAC	53708	2243
	R	CCTTCTACACCTCCATATC	55951	2243
9	F	TGAAGCAACCATCGTTAC	53808	2142
	R	CTTCTACACCTCCATATCC	55950	2142
10	F	CAGTGAAGCAACCATCGTTAC	53805	2143
	R	CCTTCTACACCTCCATATC	55951	2143

Table 2. The results of the primer analysis using clone manager10

Primer		Length	GC%	Tm °C	3'Dimer	Stability (J*)	Runs	Repeats	Hairpins
1	F	19	47	58	2	6,694.4	2	-	-
	R	21	47	58	0	5,439.2	2	2	-
2	F	18	44	56	2	6,694.4	2	-	-
	R	20	45	56	0	5,439.2	2	2	-
3	F	18	44	56	2	6,694.4	2	-	-
	R	18	44	53	0	5,439.2	2	2	-
4	F	18	44	56	2	6,694.4	2	-	-
	R	19	42	54	0	5,439.2	2	-	-
5	F	19	47	58	2	6,694.4	2	-	-
	R	19	47	55	0	11,296.8	2	2	-
6	F	19	47	58	2	6,694.4	2	-	-
	R	20	45	56	0	5,439.2	2	2	-
7	F	18	44	56	2	6,694.4	2	-	-
	R	21	47	58	0	5,439.2	2	2	-
8	F	18	44	56	2	6,694.4	2	-	-
	R	19	47	55	0	11,296.8	2	2	-
9	F	18	44	56	2	6,694.4	2	-	-
	R	19	47	55	0	7,112.8	2	2	-
10	F	21	47	61	2	12,552	2	-	-
	R	19	47	55	0	11,296.8	2	2	-

Note- *J: Joule
F: Forward
R: Reverse
GC : Guanine-Cytosine
TM : Temperature melting

Table 3. The results of the primer analysis using Oligoanalyzer

Primer Candidate		Oligoanalyzer Analysis						
		GC%	Bp	Tm°C	Secondary structure			
					3' Dimer	Hairpin ΔG (J/mole)	Hetero-dimer ΔG (J/mole)	Self-dimer ΔG (J/mole)
1	F	47.4	19	51.5	47.4	-418.4	-8,158.8	-15,104.24
	R	47.6	21	52	2	-10,460	-19,832.16	-22,509.92
2	F	44.4	18	50	1	-418.4	-8,158.8	-15,104.24
	R	45	20	49.4	2	-10,460	-13,807.2	-16,359.44
3	F	44.4	18	50	1	-418.4	-15,104.24	-8,158.8
	R	44.4	18	47.3	1	-10,460	-13,807.2	-16,359.44
4	F	44.4	18	50	1	-418.4	-8,158.8	-15,104.24
	R	42.1	19	48.1	2	-10,460	-13,807.2	-16,359.44
5	F	47.4	19	51,5	1	-418.4	-8,158.8	-15,104.24
	R	47.4	19	48.8	2	-10,460	-8,158.8	-6,150.48
6	F	47.4	19	51.5	1	-418.4	-8,158.8	-15,104.24
	R	45	20	49.4	1	-10,460	-13,807.2	-16,359.44
7	F	44.4	18	50	1	-418.4	-8,158.8	-15,104.24
	R	47.6	21	52	2	-10,460	-19,539.28	-16,359.44
8	F	44.4	19	48.8	1	-418.4	-8,158.8	-15,104.24
	R	47.4	19	48.8	1	-10,460	-19,539.28	-16,359.44
9	F	44.4	18	50	1	-418.4	-8,158.8	-13,137.76
	R	47.4	19	48,8	2	-10,460	-19,413.76	-16,359.44
10	F	47.6	21	54	1	-418.4	-8,158.8	-15,104.24
	R	47.4	19	48.8	3	-10,460	-19,539.28	-16,359.44

Note : GC : Guanine Cytosine
Bp : Base pair
Tm : Temperature melting

Table 4. The results of the primary analysis using perlprimer

	Primer candidate	GC%	Bp	Tm°C	Hetero-dimer ΔG (J/mole)	Self-dimer ΔG (J/mole)
1	F	47	19	59.10	-6,861.76	-2,803.28
	R	47	21	59,10	-6,861.76	-2,803.28
2	F	44	18	57.53	-4,518.72	-2,803.28
	R	45	20	56.78	-4,518.72	-2,803.28
3	F	44	18	57.53	-	-2,803.28
	R	44	18	54,41	-	-2,803.28
4	F	44	18	57.53	-1,380.72	-2,803.28
	R	42	19	55.40	-1,380.72	-2,803.28
5	F	47	19	59.10	-4,518.72	-2,803.28
	R	47	19	59.90	-4,518.72	-2,803.28
6	F	47	19	58.10	-6,861.76	-2,803.28
	R	45	20	56.78	-6,861.76	-2,803.28
7	F	44	18	57.53	-4,518.72	-2,803.28
	R	47	21	59.35	-4,518.72	-2,803.28
8	F	44	19	57.53	-4,518.72	-2,803.28
	R	47	19	55.90	-4,518.72	-2,803.28
9	F	44	18	57.53	-4,518.72	-2,803.28
	R	47	19	55.90	-4,518.72	-2,803.28
10	F	44	21	57.53	-4,518.72	-2,803.28
	R	47	19	55.90	-4,518.72	-2,803.28

Note : GC : Guanine Cytosine
Bp : Base pair
Tm : Temperature melting

Table 5. The results of the primary analysis using primer3plus

Primer		Lenght	GC%	Tm°C	Stability ΔG (J)
1	F	19	47.4	54.0	22,593.6
	R	21	47.6	53.7	26,359.2
2	F	18	44.4	52.8	22,593.6
	R	20	45.0	49.4	26,359.2
3	F	18	44.4	52.8	22,593.6
	R	18	44.4	45.2	26,359.2
4	F	18	44.4	52.8	22,593.6
	R	19	42.1	47.7	26,359.2
5	F	19	47.4	54.0	22,593.6
	R	19	47.4	49.2	23,012
6	F	19	47.4	54.0	22,593.6
	R	20	45.0	49.4	26,359.2
7	F	18	44.4	52.8	22,593.6
	R	21	47.6	53.7	26,359.2
8	F	19	44.4	52.9	22,593.6
	R	19	47.4	49.2	23,012
9	F	18	44.4	52.8	22,593.6
	R	19	47.4	49.2	29,706.4
10	F	21	47.6	58.3	22,593.6
	R	19	47.4	49.2	23,012

Note : GC : Guanine Cytosine
Tm : Temperaute melting
F: Forward
R: Reverse

Table 2, table 3, table 4, and table 5 show the data analysis results in a row using Clone Manager10 software, OligoAnalyzer program, perlprimer, and primer3plus. The primer length obtained from all primer candidate analysis results ranges from 20-22 base pair (bp). This length has met the ideal primer criteria, which is between 18 to 30 nucleotide bases [6]. For primers with a short below ideal, this will reduce the primer specificity and easily stick to the template with undesirable annealing temperatures. Meanwhile, if the primer is too long, it will not significantly affect the primer specificity [5]. Primers that are more than 30 bases can also trigger secondary structures in the primer.

GC percentage is the amount percentage of guanine and cytosine bases in a primer. The content of good primary GC% is in the range of 40-60% [6]. Primers with a low GC% will reduce efficiency for the PCR process because primers are unable to compete to stick effectively to the template. For a high GC percentage, the bonds between DNA strands become stronger because GC contains more bonds between nucleotides than AT, further it will affect the Tm value and make primers difficult to separate from the template in the PCR process. The guanine base bond with cytosine has three hydrogen bonds. This shows that the pair has a stronger bond than the hydrogen bond, adenine, and thymine, which only has two bonds [17]. Data analysis are shown in table 2, table 3, table 4, and table 5, which is 44-47% of the data were included in a good primary length range.

Melting temperature (Tm) is the temperature at which 50% of the double-stranded DNA is separated and determined from the number and type of bases contained in a primer. The selection for the Tm value of a primer is essential because the primer Tm is influential in the selection of the PCR process annealing temperature. An acceptable Tm primer range from 50-65°C [18]. Primers with too high Tm temperatures produce low PCR products. Otherwise, Tm that is too low tends to stick elsewhere and produce an unspecified amplification product [6]. Primer annealing temperature (Ta) generally ranges from 3-5°C below Tm temperature. Ta temperature define as estimated temperature that primer could be stable attached to the DNA template. If the temperature of Ta is high, then the primer and DNA template are difficult to bind so that it will produce a low (less efficient) PCR product. However, if Ta is too low, it will cause the primer attachment process to be on a non-specific template [19]. Based on the data analysis, all tables indicate that all primary candidates are included in the criteria.

The presence of 3'dimer will indicate hybridization between identical primary bases due to the complementary sequence at the 3' end. Then it will affect the efficiency of the attachment process to the target [5]. According to [20], the dimers at the 3' end can inhibit the amplification process, because the amplification starts at the 3' end so that it can reduce or not form a PCR product. Dimers at the 3' end of the primer have tolerance figures, which should be no more than 3 bases[5]. Based on data analysis, all the primary candidates meet the dimer 3 criteria.

The stability of a primer affects the primer attachment to the template. The range is ≥ 5,020.8 J (Joule); if the stability number is too high, then the primer will also be attached very strong to the template. However, if the stability number is smaller than 1.2, the primer cannot stick well to the template [10]. The data analysis showed that the stability of a primary candidates expressed stability.

Primer secondary structures are hairpin, self-dimers, and hetero dimers. Hairpin is an intramolecular interaction in the primer that interferes with the process of primer attachment to the template in the PCR process. Self-dimers are bonds that are formed between similar primers (forward with reverse or reverse with inverted). Cross dimer is a bond formed between forwards and reverse primers [21]. According to [6] that the tolerance value needed for primers to break the hairpin structure by having ΔG greater than -12,552 J/mole while for dimer and hetero-dimer structures have ΔG values greater than -25,104 J/mole. Based on table 2 only shows data on hairpin values where all candidates have no value, in table 3 shows data on secondary structure values all candidates meet tolerance numbers, and in table 4 only shows data on hetero-dimer values and self-dimers where results obtained from all candidates meet the criteria, in table 5 it does not show secondary structure value data.

Nucleotides that are repeated in primers are called repeats. The presence of repeats can cause primer attachment to occur in an undesirable place (mispriming) [6]. Runs are repetitive nucleotides [10]. According to [17] that repeats and runs can also be analyzed manually where the determination of repeats is done by counting repetitions of 2 bases that occur no more than 4 repetitions (example: CTCTCTCT). Moreover, the determination of runs is done by counting the number of repeats of similar bases that occur in each sequence with the condition of repetition not more than 5 bases (example: GGGGG). The result in table show repeats and runs of all primer candidates still in the tolerance range.

Based on the results of the primer analysis from 4 software, all primers meet a variety of the best criteria. But the best primer is a primer that is located right or close to the position before and after the target gene, because if the primer is in the target gene, the results obtained in the extension process (elongation) by the PCR method are partial. All things considered, the best primer is forward and reverse primers in candidate 1 because it is located before the target gene that can be seen in Figure 1 and 2.

This designed primer can precisely cut the target area according to the range of the area designed in silico. This shows that the primer designed is good enough to be used in the PCR process and can produce products according to the desired range of area. A good primer sequence can be used to see similarities with other organisms that can be used as a template for designing candidate map primer genes and amplification processes in the PCR method by using the "Nucleotide-Blast" program provided by the official NCBI website.

The data obtained from results indicate that the map gene primer can be designed in *Staphylococcus aureus* bacteria as mentioned in the database https://www.ncbi.nlm.nih.gov.

Figure 1 . Forward primer A starts from 53844

Figure 2. Reverse primer starts from 55842

4. Conclusion

Based on the results of this study, it can be concluded that the primer design and analysis can be carried out in in silico using the NCBI website (National Center for Biotechnology Information), Clone Manager10, OligoAnalizer, Perlprimer and Primer3plus. Primer sequences that meet good criteria are forward primers on candidate 1 with base length criteria 19, base sequence 5 ' GTGAAGCAACCATCGTTAC'3, start in position 53844 and reverse primer with base length criteria 21, base sequence 5' CCTTCTACACCTCCATATCAC'3, start in position 55852. This designed primer can cut the target area precisely where the location of the primary (start) is very influential in determining a good primer because it can affect the amplification results on the PCR.

Acknowledgement

The authors would like to acknowledge Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University for providing facilities for this work.

Conflict of Interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. AS conceived and designed the study, IJ and RF collected and analysed the data, NA wrote the manuscript. All authors read and approved the manuscript for publication.

References

[1] Yenny, and Herwana, E. 2007. Resistensi dari bakteri enterik: aspek global terhadap antimikroba. *Universa Med.*, **26** (1), 46–56.

[2] WHO. 2014. Antimicrobial resistance. Global report on surveillance. *World Health Organization*.

[3] Wilson, D.N. 2014. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nat. Rev. Microbiol.*, **12** (1), 35–48.

[4] Sudigdoadi, S. 2001. Mekanisme Timbulnya Resistensi Antibiotik Pada Infeksi Bakteri. *Fak. Kedokt. Univeritas Padjadjaran*, 1–14.

[5] Handoyo, D., and Rudiretna, A. 2001. Prinsip umum dan pelaksanaan Polymerase Chain Reaction (PCR). *Unitas*, **9** (1), 17–29.

[6] Borah, P. 2011. Primer designing for PCR. *Sci. Vis.*, **11** (3), 134–136.

[7] Li, Z., Chen, J.H., Hao, Y., and Nair, S.K. 2012. Structures of the PelD cyclic diguanylate effector involved in pellicle formation in *Pseudomonas aeruginosa* PAO1. *J. Biol. Chem.*, **287** (36), 30191–30204.

[8] Baker, P., Hill, P.J., Snarr, B.D., Alnabelseya, N., Pestrak, M.J., Lee, M.J., Jennings, L.K., Tam, J., Melnyk, R.A., Parsek, M.R., Sheppard, D.C., Wozniak, D.J., and Howell, P.L. 2016. Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms. *Sci. Adv.*, **2** (5), 1–10.

[9] Mawarnursavira, K., Sari., R., Apridamayanti, P. 2014. Optimasi Melting Temperature Primer Degenerate Pada Suhu 60°C Gen ERM(T) (*Erythromycin Ribosome Methylase*) yang

Resistensi Terhadap Bakteri *Streptococcus pyogenes*. *J. Mhs. Farm. Fak. Kedokt. UNTAN*, **59** (9–10).

[10] Maitriani, L.K.B., Wirajana, I.N., and Yowani, S.C. 2015. Desain Primer untuk Amplifikasi Fragmen Gen inhA Isolat134 Multidrug Resistance Tuberculosis (MDR-TB) Dengan Metode Polymerase Chain Reaction. *CAKRA Kim.*, **3** (3), 20–24.

[11] Pradnyaniti, D.., Wirajana, I.., and Yowani, S, C. 2010. Desain Primer secara in silico untuk Amplifikasi Fragmen gen rpoB M*ycobacterium tuberculosis* dengan Polymerase Chain Reaction (PCR). *J. Farm. Udayana*, 124–130.

[12] Marshall, O.J. 2004. PerlPrimer: Cross-platform, graphical primer design for standard, bisulphite and real-time PCR. *Bioinformatics*, **20** (15), 2471–2472.

[13] Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., and Leunissen, J.A.M. 2007. Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Res.*, **35** (SUPPL.2), 71–74.

[14] Oliveira, D.C., and de Lencastre, H. 2011. Methicillin-resistance in *Staphylococcus aureus* is not affected by the overexpression in trans of the *mecA* gene repressor: A surprising observation. *PLoS One*, **6** (8).

[15] Hiramatsu, K. 2004. Elucidation of the Mechanism of Antibiotic Resistance Acquisition of *Methicillin-Resistant Staphylococcus aureus* (MRSA) and Determination of Its Whole Genome Nucleotide Sequence. *Japanese Med. Assoc. J.*, **47** (4), 153–159.

[16] Haryadi, B., Aina, M., and Sadikin, A. 2011. Pelatihan Penggunaan Gen Bank NCBI (National Center for Biotechnology Information) dan Program Mega 4.0 (Molecular Evolutionary Genetics Analysis Version 4.0) Untuk Penelitian. *J. Pengabdi. Pada Masy.*, **12** (52).

[17] Marlina, Ramadhani, P., Eka Putra, A., Rustini, and Aldi, Y. 2015. Desain Primer Multiplex Polymerase Chain Reaction (PCR) Gen E6 HPV Tipe 45 dan HPV Tipe 52. *Pros. Semin. Nas. dan Work. "Perkembangan Terkini Sains Farm. dan Klin.*, **52**, 158–165.

[18] Solanki, G. 2012. Polimerase Chain Reaction. IJPR. Scholar Science Journals Gaurav Solanki Review Article. **2** (2), 81–82.

[19] Yustinadewi, P.D., Yustiantara, P.S., and Narayani, I. 2018. Mdr-1 Gene 1199 Variant Primer Design Techniques in Pediatric Patient Buffy Coat Samples With Lla. *Metamorf. J. Biol. Sci.*, **5** (1), 105.

[20] Judelson, H. 2013. Guidelines for designing primers. Primer Guidelines. 1–5.

[21] Widowati, E.W. 2013. Desain Primer Sitokrom B (Cyt B) Sebagai Salah Satu Komponen PCR (Polymerase Chain Reaction)Untuk Deteksi DNA Babi. *Lap. Penelit. Individ.*, 1–64.