DAFTAR PUSTAKA

Agus, R., Massi, M. N., and Tanoerahardjo, F. S., 2016. Cloning Open Reading Frame (ORF) of Rv2430c Mycobacterium tuberculosis Indonesian Isolate in Escherichia coli JM 109. *American Journal of Biomedical Research*. Vol.4**(4)**. 99-101.

Ahmed, N. dan Hasnain, S. E., 2004. Genomics of *Mycobacterium tuberculosis*: Old Threats & New Trends. . *Indian J Med Res.* Vol. 120: 207-212.

Andersen, P., and Doherty, T. M., 2005. The Succes and Failure of BCG-Implication for A Novel Tuberculosis Vaccine. *Research Gate.* Vol. 3: 656-662.

Brown, T. 2006. *Gene Cloning and DNA Analysis an Introduction, 5th Edition.* Blackwell Publishing Asia Pty Ltd. Australia.

Brown, T. 1987. *Gene Cloning an Introduction.* Van Nostrand Reinhold Co. Ltd. Wokingham.

Centres for Disease Control and Prevention (CDC). 2014. Untreatable: Today's Drug-Resistant Health Threats.

Cole. S.T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M.-A. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor, S. Whitehead, B. G. Barrell. 1998. Dechipering the Biology of Mycobacterium tuberculosis from The Complete Genome Sequence. *Nature Publshing Group*. Vol. 393: 537-544.

Depkes RI, 2005. *Pharmaceutical Care* untuk Penyakit Tuberkulosis. Departemen Kesehatan RI.

Duan, Z., Li, D., Jia, Q., Xu, J., Chen, X., Xu, Z., Liu, H., Chen, B., Wen, J., 2015. The Diagnostic Potential of MPT63-Derived HLA-A*0201-Restricted $CD8^{+}$T-Cell Epotipes For Active Pulmonary Tuberculosis. *Microbiol Immunol*. Vol.59: 705-715.

no, D., and Rudiretna, A., 2001. General Principles and Implementation Polymerase Chain Reaction. *Jurnal Unitas.* Vol.9**(1)**: 17-29.

M.A., B.-W. E. Lee, B. J. Dillon, G. Harth. 1995. Protective Immunity gainst Tuberculosis Induced by Vaccination with Major Extracellular

Proteins of *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA.* Vol. 92: 1530-1534.

Kuhn, H., and Kamenetskii, M.D.F., 2005. Template-Independent Ligation of Single –Stranded DNA by T4 DNA Ligase. *FEBS Journal*. Vol. 272(**2005**): 5991-6000.

Kusumaningsih, P. 2010. Subkloning dan Ekspresi Gen Penyandi Protein Rophtry 1 (ROP1) *Toxoplasma gondii* Isolat WTA. Universitas Gadjah Mada: Yogyakarta.

Madigan, M.T. dan Martinko J.M., 2005. *Brock Biology of Microorganisms 11th ed*. Prentice Hall. New Jersey.

Manalu, H.S.P., 2010. Faktor-faktor yang mempengaruhi Kejadian TB Paru dan Upaya Penanggulangannya. *Jurnal Ekologi Kesehatan.* Vol. 9**(4)**: 1340-1346.

Manca, C., K. Lyashchenko, H. G. Wiker, D. Usai, R. Colangeli, M.L. Gennaro. 1997. Molecular Cloning, Purification, and Serological Characterization of MPT63, a Novel Antigen Secreted by *Mycobacterium tuberculosis*. *Infection and Immunology*. Vol. 65 (**1**): 16-23.

McDonough, K. A., Kress, Y., And Bloom, B. R., 1993. Pathogenesis of Tuberculosis: Interaction of Mycobacterium tuberculosis with Macrophages. *Infection and Immunity.* Vol.61(**7**): 2763-2773.

Mustafa, A. S., 2009. Th1 Cell Reactivity and HLA-DR Binding Prediction for Promiscuous Recognition of MPT63 (Rv1926c), a Major Secreted Protein of Mycobacterium tuberculosis. *Scandinavian Journal of Immunology.* Vol. 69: 213-222.

Philips, J. A., and Ernst, J. D., 2012. *Tuberculosis Pathogenesis and Immunity. Annu. Rev. Pathol. Mech. D.* Vol. 7: 353-384.

Qiagen, 2008. *A Passion For Innovation.* Annual Report 2008. USA.

Rostinawati, 2008. Aktivitas Bakteri Ekstrak Etanol dan Ekstrak Air Kelopak Bunga Rosela (*Hibiscus sabdariffa* L.*)* Terhadap *Micobacteryum tuberculosis* Galur Labkes -026 (*Multi Drug Resisten)* dan L.) dan *Mycobacterium tuberculosis* Galur H37Rv secara In Vitro. Universitas Padjajaran. Jatinagor.

S[illegible]A., 2012. *Evauation of Diagnostic Potential of Recombinant MPT63 [illegible]rotein in Bovine Tuberculosis*. Deemed University.

[illegible]k, J. dan D. W. Russell. 2001. *Molecular cloning: A laboratory manual [illegible]olume 1-3 3rd Ed*. Cold Spring Harbor Laboratory Press. New York.

Sampson, S., R. Warren, M. Richardson, P. V. Helden. 2001. IS6110 Insertion in *Mycobacterium tuberculosis*: Preedominantly into Coding Regions. *Journal of Clinical Microbiollogy*. Vol. 39 (**9**) : 3423-3424.

Snustad, D. P., and Simmons, M. J., 2003. *Principle of Genetics. 3rd ed.* John Wiley & Sons, Inc. Hoboken.

Suharyo, 2013. Determinasi Penyakit Tuberkulosis di Daerah Pedesaan. *Jurnal Kesehatan Masyarakat.* Vol. 9 (**1):** 85-91.

Suharti, N., dan Putra, A. E., 2011. Pengaruh Pemberian Vaksin BCG secara Oral dan Subkutan terhadap Komponen Seluler dan Humoral pada *Rattus norvegicus* Galur Wistar. *Majalah Kedokteran Andalas.* Vol. 35(2): 117-125.

Vasanthakumari, R., 2007. *Textbook of Microbiology*. BI Publication Pvt Ltd. New Delhi.

Wang, B.L., Y. Xu, Z.M. Li, Y.M. Xu, X.H. Wengand, H.H. Wang. 2005. Antibody Response to Four Secretory Proteins from *Mycobacterium Tuberculosis* and Their Complex Antigen In TB Patients. *Int. J. Tuberc. Lung Dis*. Vol. 9 (**12**): 1327-1334.

Wangko, S., Kristanto, E., 2010. Kloning Manfaat Versus Masalah. *Jurnal Biomedik*. Vol. 2.(**2**): 88-94.

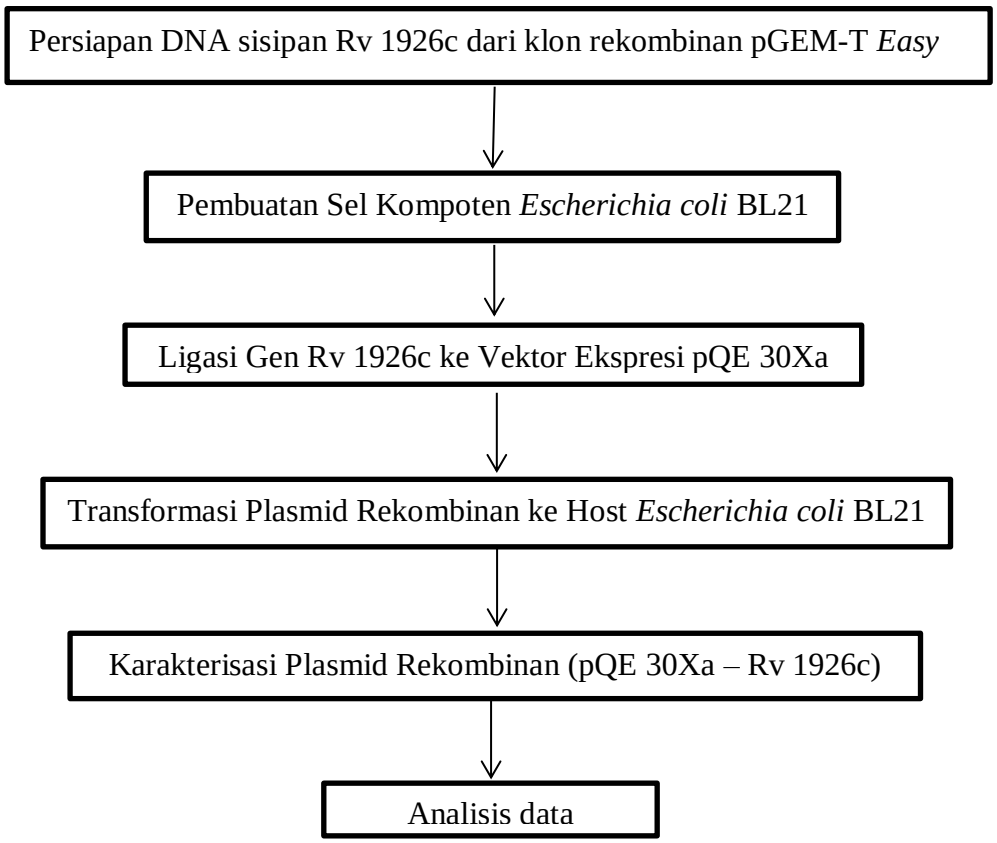
Werninghaus, C. R., K. Magdorf, K. Stark, K. Lyashchenko, , M. L. Gennaro, R. Colangeli, T. M. Doherty, P. Andersen, G. Plum, U. Herz, H. Renz, U. Wahn. 2003. The Potential of Recombinant Antigens ESAT-6, MPT63 For Specific Discrimination of *Mycobacterium Tuberculosis* And *M. Avium* Infection. Eur. J. Pediatr. Vol. 162:534-536.

Widodo, Irianto, A., dan Pramono, H., 2016. Karakteristik Morfologi *Mycobacterium tubercuosis* yang Terpapar Obat Anti TB Isoniazid (INH). *Biosfera*. Vol. 33(**3**). 109-115.

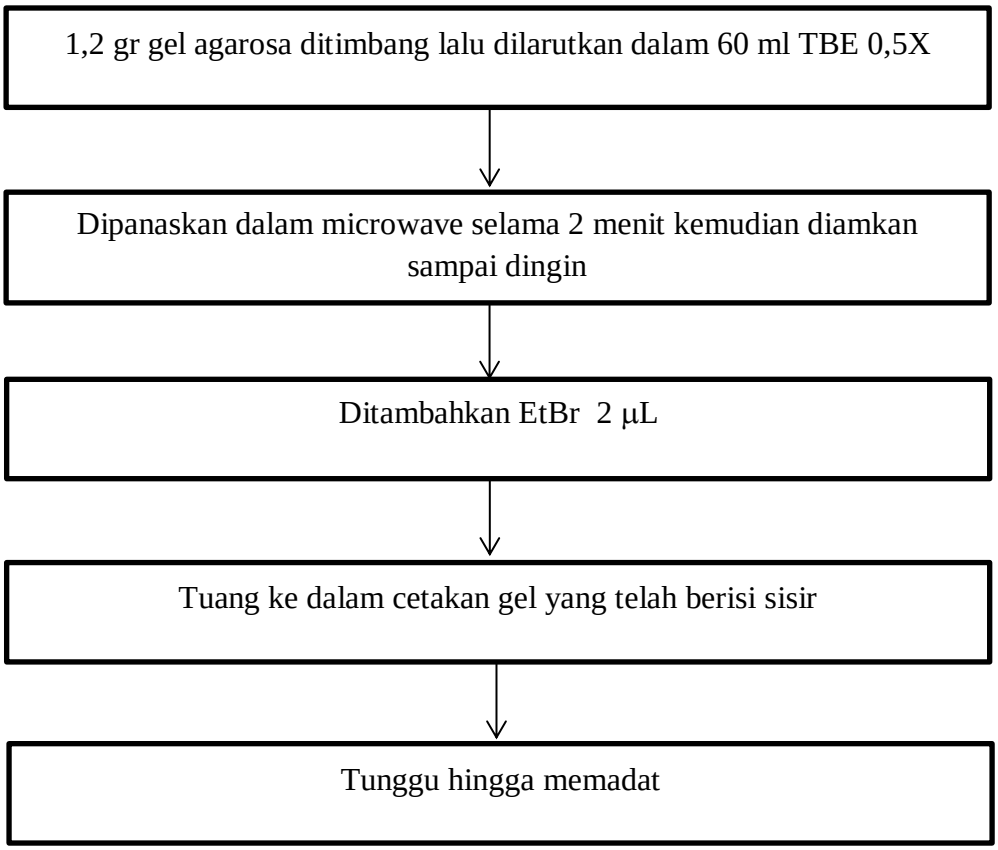
WHO, 2017. *Global Tuberculosis Report 2017*. World Health Organization.

Wong, D. W. S. 1997. *The ABC of Gen Cloning. International*. Thomson Publishing. New York.

Lampiran 1. Skema Kerja



Lampiran 2. Skema pembuatan gel agarosa 1,2%



Lampiran 3. Komposisi pembuatan media Luria Bertani (LB)

Tabel 1. Komposisi Luria Bertani (LB) Padat

No.	**Komposisi**	**Dosis**
1.	Bacto tryptone	0,5 gr
2.	Bacto yeast extract	0,25 gr
3.	Bacto agar	0,5 gr
4.	NaCl	0,5 gr
	Aquadest	50 ml

Tabel 2. Komposisi Luria Bertani (LB) Cair

No.	Komposisi	Dosis
1.	Bacto tryptone	1 gr
2.	Bacto yeast extract	0,5 gr
3.	NaCl	1 gr
4.	Aquadest	100 ml

Gambar 13. Komposisi LB

Lampiran 4. Komposisi Reagen Purifikasi

Tabel 3. Komposisi Reagen Purifikasi

No.	Komposisi	Volume yang Ditambahkan
1	Produk PCR (sampel)	25 µl
2	Buffer PB	50 µl
	Buffer PE	750 µl
	Buffer EB	50 µl

Gambar 14. Reagen Purifikasi

Lampiran 5. Komposisi Reagen Ligasi

Tabel 4. Komposisi Reagen Ligasi

No.	Komposisi dalam Reaksi Ligasi	Volume yang Ditambahkan
1	Sampel	3 µl
2	pQE 30Xa hasil restriksi	1 µl
3	T4 DNA Ligase	1 µl
4	*Nuclease Free Water*	4 µl
5	*Rapid Ligation Buffer*	1 µl

Lampiran 6. Reagen Transformasi ke Sel *Escherichia coli* BL21

Tabel 5. Reagen Transformasi ke Sel *Escherichia coli*

No.	Komposisi	Volume yang ditambahkan
1.	Ampisilin	20 µl
2.	IPTG	100 µl
3.	X-gal	40 µl
4.	Produk ligasi	10 µl
5.	Sel kompoten	50 µl

Lampiran 7. Reagen PCR Koloni

Tabel 6. Reagen PCR koloni

No.	Komposisi	Volume yang ditambahkan
1.	sampel	3 µl
2.	Go taq green	12 µl
3.	Primer F dan R	1 µl
4.	Nuclease free water	8,5 µl

Lampiran 8. Reagen Isolasi Plasmid

Tabel 7. Reagen Isolasi Plasmid

No.	Komposisi	Volume yang ditambahkan
1.	Buffer P1	250 µl
2.	Buffer P2	250 µl
3.	Buffer N3	350 µl
4.	Buffer PE	700 µl
5.	Buffer EB	40 µl

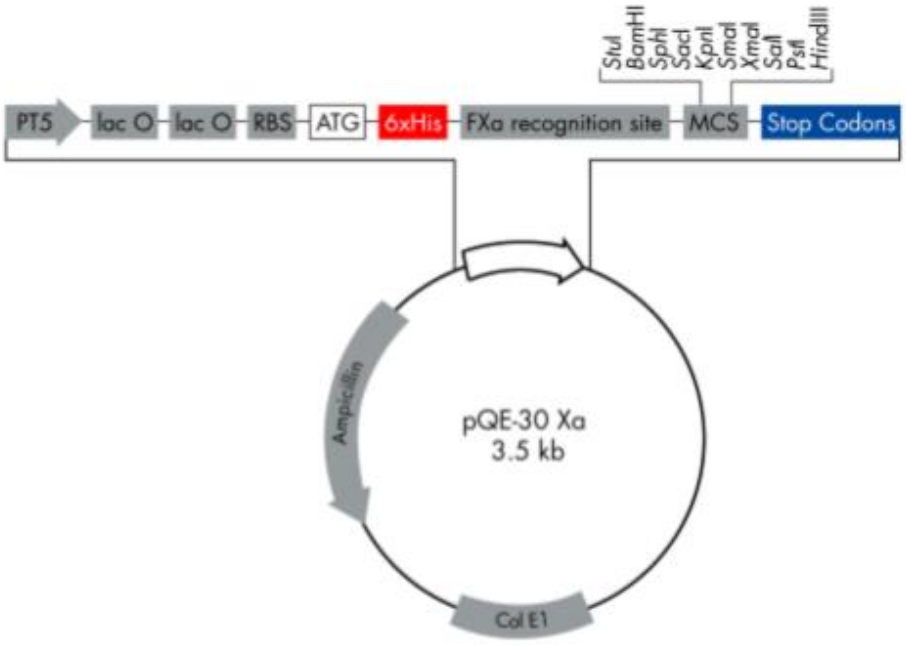
Lampiran 9. Full Genom MPT63 (Rv 1926c) (NCBI Gen Bank: NC_000962.3)

Origin

1 atgaagctca ccacaatgat caagacggca gtagcggtcg tggccatggc ggccatcgcg
61 acctttgcgg caccggtcgc gttggctgcc tatcccatca ccggaaaact tggcagtgag
121 ctaacgatga ccgacaccgt tggccaagtc gtgctcggct ggaaggtcag tgatctcaaa
181 tccagcacgg cagtcatccc cggctatccg gtggccggcc aggtctggga ggccactgcc
241 acggtcaatg cgattcgcgg cagcgtcacg cccgcggtct cgcagttcaa tgcccgcacc
301 gccgacggca tcaactaccg ggtgctgtgg caagccgcgg gccccgacac cattagcgga
361 gccactatcc cccaaggcga acaatcgacc ggcaaaatct acttcgatgt caccggccca
421 tcgccaacca tcgtcgcgat gaacaacggc atggaggatc tgctgatttg ggagccgtag

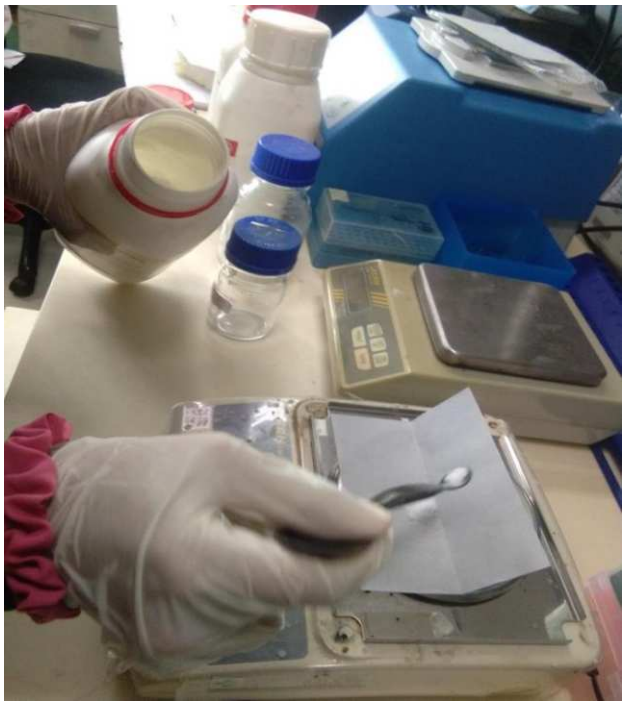
Lampiran 10. Vektor pQE 30Xa

Positions of elements in bases	
Vector size (bp)	3509
Start of numbering at *Xho*I (CTCGAG)	1-6
T5 promoter/lac operator element	7-87
T5 transcription start	61
6xHis tag coding sequence	127-144
Factor Xa Protease recognition site	166-177
Multiple cloning site	175-240
Lambda t_0 transcriptional termination region	256-350
rrnB T1 transcriptional termination region	1112-1210
ColE1 origin of replication	1686
β-lactamase coding sequence	3304-2444



Lampiran 11. Foto Prosedur Kerja

Gambar 15. Persiapan insert Rv 1926c dari pGEM-T Easy



Gambar 16. Pembuatan media Luria Bertani (LB)

Gambar 17. Pembuatan Sel Kompoten

Gambar 18. Transformasi plasmid rekombinan ke *E.coli* BL21

Gambar 19. Karakterisasi Klon Rekombinan

Gambar 20. Elektroforesis