









### C. PCR Cloning Product Confirmation

Confirmation of recombinant pSB1C3 vector by PCR cloning method has been successfully carried out. The PCR cloned electropherogram was visualized on 1 % agarose gel (Fig. 5).

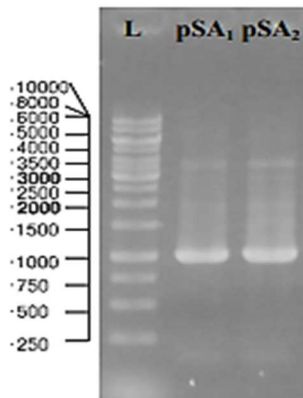


Fig. 5 The electropherogram of the PCR cloning pSB1C3 recombinant vector. L: ladder 1kb, pSA: pSB1C3 recombinant vector (str-AmilCP, 1000 bp)

The sequencing product of the str promoter and AmilCP gene was analyzed using ClustalW and BLASTN software. The results showed that the homology level was 100 % with the str promoter of *E. coli* and 99 % with the AmilCP gene of *Acropora millepora*. The ability of the str promoter to express the reporting protein AmilCP (purplish-blue color) [12] can be seen in Fig. 6.



Fig. 6 The success of the PCR cloning construct

## IV. CONCLUSION

A good mega-primer design is critical to a successful OEPC reaction. The use of web-based bioinformatic technology has been proven to save time and trial costs in the success of PCR Cloning. Mega-primer promoter str characterized by ClustalW, Net-Primer, and BLAST-NCBI software has been shown to increase the success of PCR cloning. The success of the construct is evidenced by the results of 100 % homologous sequencing and the ability of *E. coli* bacteria to express the purplish-blue protein (AmilCP).

## REFERENCES

[1] P. Moezi, M. Kargar, A. Doosti, and M. Khoshneviszadeh, "Multiplex touchdown PCR assay to enhance specificity and sensitivity for concurrent detection of four foodborne pathogens in raw milk," *J. of appl. microbiol.*, vol. 127, pp. 262-273, 2019.

[2] R. Nyaruaba, C. Mwaliko, K. K. Kering, and H. Wei, "Droplet digital PCR applications in the tuberculosis world," *Tuberculosis*, vol. 117, pp. 85-92, 2019.

[3] F. Feliatra, M. Mardalisa, J. Setiadi, I. Lukistyowaty, and A. Hutasoit, "Potential of Secondary Metabolite from Marine Heterotrophic Bacteria against Pathogenic Bacteria in Aquaculture," in *J. of Phys.: Conf. Series*, 2020, p. 012044.

[4] A. Bryksin and I. Matsumura, "Overlap extension PCR cloning," in *Synthetic Biology*, ed: Springer, 2013, pp. 31-42.

[5] Y. Lu, S. Xiao, M. Yuan, Y. Gao, J. Sun, and C. Xue, "Using overlap-extension PCR technique to fusing genes for constructing recombinant plasmids," *J. of basic microbiol.*, vol. 58, pp. 273-276, 2018.

[6] P.-T. Tran, C. F. Zhang, and V. Citovsky, "Rapid generation of inoculum of a plant RNA virus using overlap PCR," *Virology*, vol. 553, pp. 46-50, 2020.

[7] K. M. Goh, K. J. Liew, K. P. Chai, and R. M. Illias, "Use of mega-primer and overlapping extension PCR (OE-PCR) to mutagenize and enhance cyclodextrin glucosyltransferase (CGTase) function," in *In Vitro Mutagen.*, ed: Springer, 2017, pp. 385-396.

[8] S. Chojnacki, A. Cowley, J. Lee, A. Foix, and R. Lopez, "Programmatic access to bioinformatics tools from EMBL-EBI update: 2017," *Nucl. acids research*, vol. 45, pp. W550-W553, 2017.

[9] F. M. Putra, F. K. Surado, and G. I. Sampurno, "Feature Selection Techniques for Selecting Proteins that Influence Mouse Down Syndrome Using Genetic Algorithms and Random Forests," *JOIV: Int. on Informat. Visualiz.* vol. 4, pp. 162-165, 2020.

[10] M. Mardalisa, F. Feliatra, and N. Nursyirwani, "Multiple Antibiotic Resistance Index of Escherichia coli Isolates from Dumai Sea Waters Riau Province," *Berkala Perikanan Terubuk*, vol. 49, pp. 734-739, 2021.

[11] H. Christensen and J. E. Olsen, "Primer Design," in *Introduction to Bioinfo. in Microbiol.*, ed: Springer, 2018, pp. 81-102.

[12] A. V. Koehler, P. K. Korhonen, R. S. Hall, N. D. Young, T. Wang, S. R. Haydon, and R. B. Gasser, "Use of a bioinformatic-assisted primer design strategy to establish a new nested PCR-based method for Cryptosporidium," *Parasit. & vect.*, vol. 10, p. 509, 2017.

[13] S. Ahsan and D. Summers, "Identification of a toxin coding fragment in pBSSB1, a linear plasmid from Salmonella enterica serovar Typhi that can stabilize a multicopy plasmid," *Asian Pacific J. of Tropical Biomed.*, vol. 8, p. 365, 2018.

[14] L. E. Post, A. E. Arfsten, F. Reusser, and M. Nomura, "DNA sequences of promoter regions for the str and spc ribosomal protein operons in *E. coli*," *Cell*, vol. 15, pp. 215-229, 1978.

[15] C. Yi, J. Sjöberg, and D. Johansson, "Numerical modelling for blast-induced fragmentation in sublevel caving mines," *Tunnell. and Undergr. Space Techn.*, vol. 68, pp. 167-173, 2017.

[16] J. Zuber, B. J. Cabral, I. McFadyen, D. M. Mauer, and D. H. Mathews, "Analysis of RNA nearest neighbor parameters reveals interdependencies and quantifies the uncertainty in RNA secondary structure prediction," *Rna*, vol. 24, pp. 1568-1582, 2018.

[17] J. Guo, D. Starr, and H. Guo, "Classification and review of free PCR primer design software," *Bioinformatics*, 2020.

[18] M. Mardalisa, S. Suhandono, and M. Ramdhani, "Isolation and Characterization of str Promoter from Bacteria Escherichia coli DH5 $\alpha$  using Reporter Gene AmilCP (*Acropora millepora*)," in *IOP Conf. Series: Earth and Environ. Sci.*, 2020, p. 012014.

[19] T.-W. Xue and Z.-Y. Guo, "What Is the Real Clausius Statement of the Second Law of Thermodynamics?," *Entropy*, vol. 21, p. 926, 2019.

[20] L. Du, C. Zhang, Q. Liu, X. Zhang, and B. Yue, "Krait: an ultrafast tool for genome-wide survey of microsatellites and primer design," *Bioinformatics*, vol. 34, pp. 681-683, 2018.

[21] L. Mancabelli, C. Milani, G. A. Lugli, F. Fontana, F. Turrone, D. van Sinderen, and M. Ventura, "The Impact of Primer Design on Amplicon-Based Metagenomic Profiling Accuracy: Detailed Insights into Bifidobacterial Community Structure," *Microorganisms*, vol. 8, p. 131, 2020.

[22] A. C. Panda and M. Gorospe, "Detection and analysis of circular RNAs by RT-PCR," *Bio Protoc*, vol. 8, p. e2775, 2018.

[23] G. A. Hudson, R. J. Bloomingdale, and B. M. Znosko, "Thermodynamic contribution and nearest-neighbor parameters of pseudouridine-adenosine base pairs in oligoribonucleotides," *Rna*, vol. 19, pp. 1474-1482, 2013.

[24] M. F. Sloma, M. Zuker, and D. H. Mathews, "Predictive Methods Using RNA Sequences," *Bioinformatics*, p. 155, 2020.