ISOLASI DAN IDENTIFIKASI *HOUSEKEEPING GENE* PADA IKAN SIDAT *(Anguilla bicolor)*

SKRIPSI

diajukan untuk memenuhi sebagian syarat untuk memperoleh gelar Sarjana Sains
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ABSTRAK

Housekeeping gene adalah gen kosntitutif yang diperlukan dalam pemeliharaan fungsi sel dan sering digunakan sebagai kontrol internal pada normalisasi ekspresi gen. Akan tetapi informasi genetik dari *housekeeping gene* khususnya pada ikan sidat (*Anguilla bicolor*) masih minim dan banyak yang belum diketahui. Berhubungan dengan hal tersebut maka penelitian ini bertujuan untuk mensikuensing hasil isolasi *housekeeping gene* pada ikan sidat (*Anguilla bicolor*), guna mendapatkan sikuen spesifik dengan cara melakukan perancangan primer *degenerate* pada *housekeeping gene 18s ribosomal RNA* (18S rRNA), *Beta Actin (*ACTB), *Elongation Factor 1-Alpha* (EF1A) dan *Glyceraldehyde-3-Phosphate Dehydrogenase* (GAPDH). Primer *degenerate* yang terdiri dari dua set primer (*outer* dan *inner*) dirancang dengan cara: (1) Pengumpulan sikuen *housekeeping gene* pada ikan class Actinopterygii; (2) Pensejajaran sikuen; (3) Pengunggahan sikuen ke laman *Primaclade*; (4) Pemilihan pasangan primer. Primer yang terpilih selanjutnya digunakan untuk mengamplifikasi *housekeeping gene* DNA *Anguilla bicolor* dengan menggunakan metode *Nested* PCR. Adapun DNA *Anguilla bicolor* didapat dari hasil isolasi DNA menggunakan protokol Sambrook. Kemudian hasil amplifikasi *housekeeping gene* dilakukan sikuensing secara langsung (*direct* PCR) untuk menentukan urutan nukleotida dari sikuen gen. Selanjutnya dilakukan *contig* dan analisis BLAST pada sikuen gen yang didapat untuk memastikan bahwa sikuen tersebut sesuai dengan gen target. Hasil analisis BLAST menunjukkan dari keempat sikuen gen, diperoleh tiga sikuen yang sesuai dengan gen target yaitu gen *18s ribosomal RNA* (18S rRNA), *Beta Actin (*ACTB) dan *Elongation Factor 1-Alpha* (EF1A) dan identifikasi pengelompokan menggunakan dendrogram menunjukkan bahwa ketiga sikuen gen tersebut berkelompok dengan gen target. Kesimpulan dari penelitian ini adalah diperoleh tiga sikuen spesifik pada tiga *housekeeping gene* target yang dapat digunakan sebagai sumber untuk merancang primer spesifik pada penelitian selanjutnya.

Kata Kunci : *Anguilla bicolor*, *Housekeeping gene*, Primer *degenerate*, *Nested* PCR, Primer spesifik.

ISOLATION AND IDENTIFICATION OF *HOUSEKEEPING GENE* IN EEL FISH *(Anguilla bicolor)*

ABSTRACT

Housekeeping gene are effective genes needed in cell maintenance and often used as internal controls in gene expression normalization because of their stable nature. However, genetic information from housekeeping genes, especially in *Anguilla bicolor* is still minimal and much is unknown. In relation to that, this study aims to sequencing the isolation results of housekeeping gene in *Anguilla bicolor* in order to get specific sequences by designing degenerate primer on 18s ribosomal RNA (18S rRNA), Beta Actin (ACTB), Elongation Factor 1-Alpha (ef1a) and *Glyceraldehyde-3-Phosphate Dehydrogenase* (GAPDH). The degenerate primer consists of two primer sets (outer and inner) were designed by: (1) Collection fish sequences of Actinopterygii class; (2) Aligment sequences; (3) Uploading sequences to the Primaclade web; (4) Selection of primary pairs. The selected primers were then used to amplify housekeeping gene of *Anguilla bicolor* using the *Nested* PCR method. DNA of *Anguilla bicolor* was obtained from DNA isolation using the Sambrook protocol. The result of housekeeping gene amplification was then sequenced directly to determine the nucleotide sequences of the gene. Next, contig and BLAST analysis were performed on the gene sequences obtained to ensure that the sequences match the target genes. The results of BLAST analysis showed that the three of four genes sequences were corresponding to the target gene, which are ribosome RNA 18s (18S rRNA), Beta Actin (ACTB) and Elongation Factor 1-Alpha (ef1a). The general grouping of dendrograms showed that the three gene sequences were in groups with gene target. The conclusion of this study is the discovery of three specific sequences in three housekeeping gene targets which can be used as a source for designing specific primers in subsequent studies.

Keywords: *Anguilla bicolor*, *Housekeeping gene*, *Degenerate* Primer, *Nested* PCR, *Specific* Primer.

DAFTAR ISI

DAFTAR PUSTAKA

Alato, E., Capoccioni, F., Terradez M.J., Schiavina, M., Leone, C., De Leo, G. & Ciccotti, E. (2016). Quantifying 60 Years of Declining European Eel (*Anguilla Anguilla* L.,1758) Fishery Yields in Mediterranean Coastal Lagoons. *ICES Journal of Marine Science*, 73(1): 101-110. doi: https://doi.org/10.1093/icesjms/fsv084.

Alfandary, R. (2015). *4 Tips for Efficient Primer Design*. [*Online*]. Diakses dari http://www.genomecompiler.com/tips-for-efficient-primer-design/.

Amelio, A & Andrea . (2019). *Encyclopedia of Bioinformatics and Computational Biology*. Maryland : Elsevier Inc.

Biosystem. (2010). *GeneAmp® PCR System 9700 Base Module.* Amerika Serikat : Biosystem.

Bock,, T. (2018). What is a Dendogram. [Online]. Diakses dari https://www.displayr.com/what-is-dendrogram/.

Borriss, R., Christian R., Jochen B., Oliver B., Oleg R., & Hans-Peter K. (2011). Whole Genome Sequence Comparisons in Taxonomy. *Methode in Microbiologu*. 38(18): 409-436. doi: https://doi.org/10.1016/B978-0-12-387730-7.00018-8.

Bowden, C. (1985). *Nomenclature Committee of the International Union of Biochemistry*. [*Online*]. Diakses dari http://www.insdc.org/documents/feature_table.html#7.4.1.

Butler, J.M. (2012). *Advanced Topics in Forensic DNA Typing: Methodology*. Maryland : Elsevier Inc.

Campbell, N. A. & J. B. Reece. (2008). *Biologi*, Edisi Kedelapan Jilid 3.Terjemahan: Damaring Tyas Wulandari. Jakarta: Erlangga.

Campbell, N. A. & J. B. Reece. (2010). 3. *Biologi*, Edisi Kedelapan Jilid 3 *Terjemahan: Damaring Tyas Wulandari*. Jakarta: Erlangga.

Can, T. (2014). Introduction to Bioinformatics. *Methods in Molecular Biology*. 1107, 51-71. doi: 10.1007/978-1-62703-748-8_4.

Carr, J., Donna G. W. & Randall T. H. (2010). *Molecular Detection of Multiple Respiratory Viruses*. Maryland : Elsevier Inc.

Caudrec, M, J. M, Zalouk-vergnoux A., Kamari A., Moreau B., Blanchet L., ... L. Poirier. (2016). Thyroid endocrine status of wild European eels (*Anguilla anguilla*) in the Loire (France) Relationships with Organic Contaminant Body

Burdens. *Science of the Total Environment*, 550: 391-405. doi: https://doi.org/10.1016/j.scitotenv.2015.12.136.

Chen, J., Alan M.M.E. & Donna J.P. (2009). Constructing Overview + Detail Dendrogram-Matrix Views. *IEEE Transactions on Visualization and Computer Graphics*, 15(6): 889-896. doi: 10.1109/TVCG.2009.130.

Choudhuri, S. (2014). *Bioinformatics for Begininners*. Maryland: Elsevier Inc.

Christensen, K.D., Kathryn A.P., Robert C.G., & Dmitry D. (2018). Cost Analyses of Genomic Sequencing: Lessons Learned from the MedSeq Project.*Value Health*, 21(9): 1054-1061. doi: https://doi.org/10.1016/j.jval.2018.06.013.

Clark, W. & Kimberley C. (2000). "An introduction to DNA: Apectrophotometry, degradation, and the "Frankengel' experiment". Dalam S. J. Karcher (Penyunting), Proceedings of the 22nd Workshop/Conference of the Association for Biology Laboratory Education (ABLE) (hlm. 81-99). Canada: University of Alberta.

Cortes, D.C.C & Lyn R.G. (2014). Methods for extracting genomic DNA from whole blood samples: current perspectives. *Journal of Biorepository Science for Applied Medicine*, 2: 1-9. doi: http://dx.doi.org/10.2147/BSAM.S46573.

Cseke, L.J. & Joseph R.H. (2012). *Methods in Cell Biology*. Amsterdam: Elsevier Inc.

Dharmayanti, N.L.P.I. (2011). *Filogenetika Molekuler: Metode Taksonomi Organisme Berdasarkan Sejarah Evolusi*. Bogor: Balai Penelitian Veteriner.

Edwards, D.R & Denhardt D.T. (1985). A Study of Mitochondrial and Nuclear Transcription With Cloned cDNA Probes Changes in the Relative Abundance of Mitochondrial Transcripts After Stimulation of Quiescent Mouse Fibroblasts. *Exp Cell Res*, 157(1): 127–143. doi: 10.1016/0014-4827(85)90157-0.

Efron, B., Elizabeth H. & Susan H. (1996). Bootstrap Confidence Levels for Phylogenetic Trees, *Proceding of the National Academic Sciences*, 93(23): 13429. doi: 10.1073/pnas.93.23.13429.

Elsa. (2015). *Sikuensing DNA*. [*Online*]. Diakses dari https://news.labsatu.com/sikuensing-dna/.

Fahmi, M. R. (2015). "Short Communication: Conservation Genetic Of Tropical Eel In Indonesian Waters Based On Population Genetic Study". Prosiding *Seminar Nasional Masyarakat Biodiversitas Indonesia* (hlm. 38-43). Depok: Balai Penelitian dan Pengembangan Budidaya Ikan Hias.

Fahmi, M.R. & Hirnawati, R. (2010). *Keragaman Ikan Sidat Tropis (Anguilla sp.) Di Perairan Sungai Cimandiri, Pelabuhan Ratu, Sukabumi. Prosiding Forum Inovasi Teknologi Akuakutur*. Depok: Balai Riset Budidaya Ikan Hias.

Fashier, J. & Petter C. (2011). *BLAST Glossary*. United States: The national library of medicine.

Fatchiyah, E.L.A, Sri W & Sri R. (2011). *Biologi Molekuler Prinsip Dasar Analitis*. Jakarta: Penerbit Erlangga.

Filby, A.L & Tyler, C.R. (2007). Appropriate 'Housekeeping' Genes for Use in Expression Profiling the Effects of Environmental Estrogens in Fish.BioMed *Central Molecular Biology,* 8: 1-13. doi: 10.1186/1471-2199-8-10.

Geneious. (2018). *Primer Design Tutorial*. [*Online*]. Diakses dari https://www.geneious.com/web-tutorials/primer-design-tutorial/#Exercise_4.

Genetic Home Reference. (2013). *ACTB Gene*. [*Online*]. Diakses dari https://ghr.nlm.nih.gov/gene/ACTB#sourcesforpage.

Gorron, E., Fausto R., Diana B, Luis M.R.R., Andriana B., Silvia R & Joe T. (2010). A New Method for Deigning Degenerate Primers and its Use in the Identification of Sequences in Brachiaria Showing Similarity to Apomixis-Associated Genes. *Bioinformatics Aplications Note*, 26(16): 2053-2054. doi: 10.1093/bioinformatics/btq312.

Green, M. R. & Joseph S. (2019). Analysis Of DNA By Agarose Gel Electrophoresis. *Cold Spring Harb Protoc*. doi: 10.1101/pdb.top100388.

Guerfali, F.Z., Dhafer L., Abdellatif B. & Fredj T. (2019). Designing and Running an Advanced Bioinformatics and Genome Analyses Course in Tunisia. *Computational Biology*, 15(1): 1-13. doi : https://doi.org/10.1371/journal.pcbi.1006373.

Hakim, A.H., M Mukhlis K., Nurlisa A.B. & Ridwan Affandi. (2015). Komposisi Spesies Ikan Sidat (*Anguilla* Spp.) di Delapan Sungai yang Bermuara ke Teluk Palabuhanratu, Sukabumi, Indonesia. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, 7(2): 573-585.

Hamzah, P. (2014). *Evaluasi Marka Simple Sequence Repeat (SSR) untuk Identifikasi Genotip Klon Karet* (*Hevea brasiliensis*). (Skripsi). Institut Pertanian Bogor.

Harrison, A.J., Walker, A.M., Pinder, A.C., Briand , C. & Aprahamian, M.W. (2014). A Review of Glass Eel Migratory Behaviour, Sampling Techniques and Abundance Estimates in Estuaries: Implications For Assessing Recruitment, Local Production And Exploitation. *Reviews in Fish Biology and Fisheries,* 24: 967-983. doi: https://doi.org/10.1007/s11160-014-9356-8.

Hayashi, R. (2014). Gene Expression and the Impact of Antioxidant Supplements in the Cataractous Lens. *Handbook of Nutririon*: *Diet and the Eye*, 517-524. doi: https://doi.org/10.1016/B978-0-12-401717-7.00052-6.

Hellman, L.M. & Fried M.G. (2007). Electrophoretic Mobility Shift Assay (EMSA) for Detecting Protein-Nucleic Acid Interactions. *Nature Protocols,* 2(8): 1849-1861. doi: 10.1038/nprot.2007.249.

Hidayat, T. (t.t.). *Analisis Filogenetika Menggunakan Program PAUP* Berbasis Window*. Departemen Pendidikan Biologi, FPMIPA, UPI Bandung.

Hogg, K. (2016). *A* Primer *for Designing Degenerate* Primers. [*Online*]. Diakses dari https://bitesizebio.com/18992/a-primer-for-designing-degenerate-primers/.

Hoy, M.A. (2013). *Insect Molecular Genetic.* 3rd Edition. England: Academic Press

Index to Organism Names (ION). (2019). [*Online*]. Diakses dari http://www.organismnames.com/details.htm?lsid=157199.

Iserte, J.A., Stephan, B.I., Goni, S.E., Borio, C.S., Ghiringhelli, P.D. & Lozano, M.E. (2013). Family-specific degenerate Primer design: a tool to design consensus degenerated oligonucleotides. *Biotechnol Research International*, 2013(38364): 1-10. doi: http://dx.doi.org/10.1155/2013/383646.

Jacoby, D., Harrison, I.J. & Gollock, M. (2014). *Anguilla bicolor*: The IUCN Red List of Threatened Species. doi: http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T166894A67015710.en.

Joshi, M. & Deshpande J.D. (2010). Polymerase Chain Reaction: Methods, Principles And Application. International. *Journal of Biomedical Research*, 2(1): 81-97. doi: https://doi.org/10.7439/ijbr.v2i1.83.

Kamaliah. (2017). Perbandingan Metode Ekstraksi Dna Phenol-Chloroform Dan Kit Extraction Pada Sapi Aceh Dan Sapi Madura. *Journal Biotik*, 5(1): 60-66.

Kim, H., Nana K., KyuHyeon A., JaeHyung K. & Min-Soo K. (2016). MRPrimerW: a tool for rapid design of valid high-quality primers for multiple target qPCR experiments. *Nucleid Acid Reesearch*, 44: 259-266. doi: 10.1093/nar/gkw380.

Koroh, P.A & Lumenta, C. (2014). Pakan Suspensi Daging Kekerangan Bagi Pertumbuhan Benih Sidat (*Anguilla bicolor*). *Budidaya Perairan*, 2(1): 7 – 13. doi: https://doi.org/10.35800/bdp.2.1.2014.3787.

Kozera, B. & Marcia R. (2013). Reference genes in real-time PCR. J *Journal Aplikasi Genetics*, 54(4): 391-406. doi: 10.1007/s13353-013-0173-x.

Kusumawaty, D. (2015). *Pola Distribusi Gen-Gen Virulen pada Aeromonas hydrophila dan Ekspresi Gen-Gen yang Terlibat Dalam Sistem Imunitas Bawaan Ikan Gurame (Osphronemus gouramy) yang Diinfeksi Patogen Paska Pembungkaman dengan ds siRNA MYD88*. (Disertasi). Program Studi Doktor Biologi, Institut Teknologi Bandung, Bandung.

Lee, P.Y., John C., Chih-Yuan H. & Yong H.K. (2012). Agarose Gel Electrophoresis for the Separation of DNA Fragments. *Journal of Visualized Experiments*, 20(62): 1-5. doi: 10.3791/3923.

Li Y.P., Yang B.S., Wang H., Zia R.X., Wang L., ... Liu YQ. (2009). Mitochondrial DNA analysis reveals a low nucleotide diversity of Caligula japonica in China. *African Journal Biotechnology*. 8(12): 2707-2712. doi: 10.5897/AJB09.462.

Lin, Y., Shila G, Dario S, Andy Y.W, Ellis P, Trence P.S., ... Pengyi W. (2017). Housekeeping genes, revisited at the single-cell level. doi: https://doi.org/10.1101/229815.

Linhart C., & Shamir R.(2004). *The Degenerate Primer Design Problem : Theory and Applicatiod*. School of Computer Sciences: Tel Aviv University.

Lorenz, T.C. (2012). Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. *Journal of Visualized Experiments*, 63: 1-15. doi: 10.3791/3998.

Luthfan, W. (2016). *Pengertian dan Jenis-jenis Penelitian Deskriptif*. [*Online*] Diakses dari https://satujam.com/penelitian-deskriptif/.

Macrogen. (2018). Troubleshooting Guide. [*Online*]. Diakses dari https://dna.macrogen.com/eng/support/ces/guide/troubleshooting.jsp

Maddocks, S. & Rowena J. (2016). Quantitative PCR: Things to Consider. *Understanding PCR a Practical Bench-Top Guide*. United Kingdom: Academic Press.

Malcom, J.W. & John H.M. (2014). Analysis of Deep Sequencing Data: Insights and Challenges. *Comprehensive Analytical Chemistry*, 63: 325-354. doi: http://dx.doi.org/10.1016/B978-0-444-62651-6.00015-5.

McCurley, A.T. & Gloria V.C. (2008). Characterization Of Housekeeping Genes In Zebrafish: Male-Female Differences And Effects Of Tissue Type, Developmental Stage And Chemical Treatment. *BMC Molecular Biology*. 9(102): 1-12. doi: 10.1186/1471-2199-9-102.

Meyer, S.K, Mauro D.J., Seal G, Wurzer J., Deriel J.K. & Sirover M.A. (1991). A Human Nuclear Uracil DNA Glycosylase is the 37-Kda Subunit of

Glyceraldehydes 3 Phosphate Dehydrogenase. *Proc. Natl Acad Sci USA*, 88(9): 8460–8464. doi: 10.1073/pnas.88.19.8460.

Mukhopadhyay, C.S., Ratan K.C. & Mir A.I. (2017). *Basic Applied Bioinformatics.* India: Willey Blacwell.

Mulyani Y., Agus P. & Isni N. (2011).Perbandingan Beberapa Metode Isolasi DNA untuk Deteksi Dini *Koi Herpes Virus* (KHV) pada Ikan Mas (*Cyprinus carpio L.). Jurnal Akuatik*, 2(1): 1-16.

Napitupulu, R.J. & Heni B. (2011). Pengolahan Ikan Sidat. *Modul Penyuluhan Perikanan*. pp.1-52.

National Center for Biotechnology Information (NCBI). (2018). *Housekeeping Gene*. [*Online*]. Diakses dari https://www.ncbi.nlm.nih.gov/.

Newell, P.D, Ashwana D.F, Constance A.R, Pete C, & Susan M.M, (2013). A Small-Group Activity Introducing the Use and Interpretation of BLAST. *Journal of Microbiology & Biology Education*. 14(2): 238–243. doi: 10.1128/jmbe.v14i2.637.

Ningsih, T.Y., Daniel J.W. & Nur S.A.G. (2016). *Deteksi Molekuler Gen Litik Brlf1 Epstein-Barr Virus Pada Penderita Karsinoma Nasofaring*. [*Online*]. Diakses dari https://journal.bio.unsoed.ac.id.

Ojeda, G.N., Amavet, P.S., Rueda, E.C., & Siroski, P.A. (2012). DNA Extraction From Skins of Wild (*Hydrochoerus hydrochaeris* and *Pecari tajacu*) and Domestic (*Sus scrofa domestica*) Species Using a Novel Protocol. *Genet. Mol. Res*, 11(1): 672-678. doi: http://dx.doi.org/10.4238/2012.March.19.1.

Ozturk, A.R & Tolga C. (2017). A Multiplex Primer Design Algorithm For Target Amplification Of Continuous Genomic Regions. *BMC Bioinformatic,* 18(1): 1-9. doi: 10.1186/s12859-017-1716-7.

Pampel, J. (2017). *Housekeeping Genes*. [*Online*]. Diakses dari https://www.genomicsonline.com/resources/16/5049/housekeeping-genes/.

Pavlopoulos, G.A, Theodoros G S., Adriano B.S. & Reinhard S. (2010). A Reference Guide for Tree Analysis and Visualization. *BioData Mining*, 3(1): 1-24. doi: 10.1186/1756-0381-3-1.

Paxon, J. (2008*). Polymerase Chain Reaction Test Interpretation*. [*Online*]. Diakses dari https://www.vetfolio.com/learn/article/polymerase-chain-reaction-test-interpretation.

Pessoa, M.A, Susana P. & Jorge T. (2010). PrimerIdent: A web based tool for conserved primer design. *Bioinformation*, 5(2): 52-54. doi: https://doi.org/10.6026/973206300050.

Pohjanvirta R, Niittynen M, Lind´En J, Boutros P.C., Moffat I.D. & Okey A.B. (2006). Evaluation of Various Housekeeping Genes for Their Applicability for Normalization of mRNA Expression in Dioxin-Treatedrats. *Chem-Bio*l *Interact*, 160(2): 134–149. doi: 10.1016/j.cbi.2006.01.001.

Politis, S.N, David M, Ariana S., Jose-Luis Z.I., Joanna J.M., ... Ian A.E.B. (2017). Temperature Effects on Gene Expression and Morphological Development of European Eel, Anguilla anguilla Larvae. *PLoS ONE*, 12(8): 1-23. doi: https://doi.org/10.1371/journal.

Pratomo, H.B. (2015). *Indonesia Ekspor Ikan Terbaik Sejagat*. [*Online*]. Diakses dari https://www.merdeka.com/uang/indonesia-ekspor-ikan-terbaik-sejagat.html.

Premier Biosoft. (2018). *PCR* Primer *Design Guidelines*. [*Online*]. Diakses dari http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html.

Primer3 Plus. (2019). *Primer 3 Help: Primer Max Self Any*. [*Online*]. Diakses dari http://www.primer3plus.com/web_3.0.0/primer3web_help.htm#PRIMER_MAX_SELF_ANY.

Primyastanto, M. (2016). *Evapro (Evaluasi Proyek)*: *Teori dan Aplikasi pada Usaha Pembesaran Ikan Sidat (Anguilla sp)*. Malang: Universitas Brawijaya Press.

Promega. (2016). *GoTaq® Green Master Mix (M712) Product Information*. [*Online*]. Diakses dari https://worldwide.promega.com/resources/protocols/product-information-sheets/g/gotaq-green-master-mix-m712-protocol/.

Pulungan, S.I. (2013). *The Incredible: Regulasi Molekuler, Housekeeping Genes vs Stress Inducible Genes*. [*Online*]. Diakses dari http://majalah1000guru.net/2013/07/regulasi-molekuler-gen/.

Puspitaningrum, R., Chris A. & Solihin. (2018). *Genetika Molekuler Dan Aplikasinya*. Sleman: Grup penerbitan CV BUDI UTAMA.

Rahma, S.A. (2018). *Identifikasi Gen Housekeeping Pada Dna Genom Ikan Gurame* (*Osphronemus gouramy*). (Skripsi). Program Studi Biologi, Universitas Pendidikan Indonesia, Bandung.

Reboucas, Emanuela D.L., José J.D.N.C., Maria J.P., José R.D.S.P., Robert V.H. & José R.V.S. (2013). Real Time PCR and Importance of Housekeepings Genes for Normalization and Quantification of mRNA Expression in Different Tissues.

Engineering, Technology And Techniques, 6(1): 143-154. doi: http://dx.doi.org/10.1590/S1516-89132013000100019.

Riviere, J.B., Bregje W. M.V.B., Alexander H., Stanislav S.K., Brian J.O.R, Christian G., ...William B.D. (2012). De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. *Nature Genetic*, 44(4): 440-447. doi: 10.1038/ng.1091.

Roslim, D.I. (2017). Disain Primer Aktin Spesifik Tuntun Angin (Elaeocarpus floribundus). *Jurnal Bioslogos*, 7(1): 1-8. doi: https://doi.org/10.35799/jbl.7.1.2017.16207.

Roy, R. (2013). *Budidaya Sidat*. Jakarta: PT. Agromedia Pustaka.

Sadritdinova, A.F., A. V. Snezhkina, A. A. Dmitriev, G. S. Krasnov, L. N. Astakhova, A. A. Kudryavtsev., ... A. V. Kudryavtseva. (2013). A New Reference Gene, Ef1A, for Quantitative Real Time PCR Assay of the Starfish Asterias rubens Pyloric Ceca. *Cell Biology*, 452(2): 310-312. doi: 10.1134/S0012496613050050.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. 2nd ed. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Sandy, Angga Kly. (2015). *Isolasi DNA Parsial Gen Tyrosinase-Related Protein-1 (Tyrp1) Pada Ikan Gurame (Osphronemus gouramy)*. (Skripsi). Program Studi Biologi, Universitas Pendidikan Indonesia, Bandung.

Saraswathy N. & Ponnusamy R. (2011). *Concepts and Techniques in Genomics and Proteomics*. Cambridge: Woodhead Publishing.

Sarwono, B. (2011). *Budidaya Belut dan Sidat*. Depok: Penebar Swadaya.

Sasmito, D.E.K., Rahadian K. & Izzati M. (2014). "Karakteristik Primer pada Polymerase Chain Reaction (PCR) untuk Sikuensing DNA: Mini Review", *Seminar Nasional Informatika Medis (SNIMed) V* (hlm.1-10). Yogyakarta: Universitas Islam Indonesia *Press*.

Scarcelli, E., Rosa M.P., Ricardo H., Simone M., Flora M.C.F., Febiola R.C. (2005). Molecular Subtyping Of Campylobacter Jejuni Subsp. Jejuni Strains Isolated From Different Animal Species In The State Of São Paulo, Brazil. *Brazilian Journal of Microbiology*, 36: 378-382.

Setiawan, A.N & P Mark L. (2010). *The use of reference gene selection programs to study the silvering transformation in a freshwater eel Anguilla australis: a*

cautionary tale. [*Online*] Diakses dari http://www.biomedcentral.com/1471-2199/11/75.

Sigma-Aldrich. (2010). *OligoArchitect™ Online Glossary of Parameters*. [*Online*]. Diakses dari https://www.gene-quantification.de/oligo_architect_glossary.pdf.

Sinha, P., Saxena, R. K., Singh, V. K., Krishnamurthy, L. & Varshney, R. K. (2015). Selection and validation of housekeeping genes as reference for gene expression studies in pigeonpea (*Cajanus cajan*) under heat and salt stress conditions. *Frontiers in Plant Science*, 6(1071): 1-10. doi: 10.3389/ fpls.2015.01071.

Skutkova, H, Martin V, Sona K, Rene K, & Ivo P. (2013). Preprocessing and Classification of Electrophoresis Gel Images Using Dynamic Time Warping. *International journal of electrochemical science,* 8: 1609-1622. [*Online*]. Diakses dari https://www.researchgate.net.

Stadhouders, R., Suzan D.P., Jeer A., Jolanda V., Ted H.M.M. & Marthin S. (2010). The Effect of Primer-Template Mismatches on the Detection and Quantification of Nucleic Acids Using the 5′ Nuclease Assay. *The Journal of Molecular Diagnostics*, 12(1): 109-117. doi: 10.2353/jmoldx.2010.090035.

Stubbs, L., (2001). Contig. Dalam Brenner dan Miller (Penyunting), *Encyclopedia of Genetics* (hlm. 463). USA : Academic Press.

Sudargo, F & Hernawati. (2017). Zoologi Vertebrata (Perkuliahan Berbasis Praktikum Superkelas Pisces). Bandung : Jurusan Pendidikan Biologi, FPMIPA, UPI.

Surzycki, S. (2012). *Basic Techniques in Molecular Biology*. New York: Springer.

Tamam, M.H B. (2016). *Pengertian, Prinsip, dan Manfaat Sikuensing DNA.* [*Online*]. Diakses dari https://www.generasibiologi.com/2016/03/sikuensing-dna.html.

Thornton, B & Chhandak B. (2011). Real-Time PCR (qPCR) Primer Design Using Free Online Software. *Biochemistry And Molecular Biology Education,* 39(2): 145-154. doi: 10.1002/bmb.20461.

Topan, M & Nofiandi R. (2015). *Budi Daya Belut & Sidat Gak Pake Masalah*. Jakarta: PT. Agromedia Pustaka.

Trumbo, T.A., Emeric S., Michael G.B., Michael E. & Pugh. (2013). Applied Spectrophotometry: Analysis of a Biochemical Mixture. Laboratory Exercise, 41(4): 2420150. doi: 10.1002/bmb.20694.

Turabelidze, A., Shujuan G., & Luisa A.D. (2010). Importance of Housekeeping gene selection for accurate RT-qPCR in a wound healing model. *Wound Repair Regen*, 18(5): 460-466. doi: 10.1111/j.1524-475X.2010.00611.x

Untergasser, A., Harm N., Xiangyu R., Ton B., Rene G. & Jack A.M.L. (2007). Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research*, 35(2): 1-4. doi:10.1093/nar/gkm306.

Winer J, Jung CK, Shackel I & Williams PM. (1999). Development and Validation Of Real-Time Quantitative Reverse Transcriptase-Polymerase Chain Reaction for Monitoring Gene Expression in Cardiac Myocytes in Vitro. *Anal Biochem*, 270(1), 41–49. doi: 10.1006/abio.1999.4085.

Wronski, A. (2017). *Keeping On Top of Housekeeping genes*. [*Online*]. Diakses dari https://bitesizebio.com/23467/keeping-on-top-of-housekeeping-genes/.

Xia, X., Weiran H., Ruyan W., Xiaopei X., Qiyan D. & Zhongjie C. (2017). Identification of Housekeeping Genes as References for Quantitative Real Time RT-PCR Analysis in *Misgurnus Anguillicaudatus. Journal of Genetics*, 96(6): 1-10. doi*:* 10.1007/s12041-017-0845-0.

Yang, Q., Yin, J., Li, Gao, Qi, L., Yang, F., Wang, R. & Li G. (2014). Reference gene selection for qRT-PCR in Caragana korshinskii Kom. under different stress conditions. *Mol. Biol. Rep.* 41(4): 2325–2334. doi: 10.1007/s11033-014-3086-9.

Ye, J., George C., Irena Z., Iona C., Steve R., & Thomas L.M. (2012). Primer-*BLAST:* A Tool To Design Target-Specific Primers For Polymerase Chain Reaction. *BMC Bioinformatic.* 13(134): 1471-2105. doi: 10.1186/1471-2105-13-134.

Yi,S.W., Myung-Jo Y, Ho-Seong C., Chang-Seop L., Joong-Ki K. & Gee-Wook S. (2013). Molecular characterization of Aeromonas species isolated from farmed eels (*Anguilla japonica*). *Veterinary Microbiology*, 164(2): 195-200. doi: 10.1016/10.1016/j.vetmic.2013.02.006.