REFERENCES

Ajikumar, P.K. et al., 2008. Terpenoids: opportunities for biosynthesis of natural product drugs using engineered microorganisms. Molecular pharmaceutics, 5(2): 167-90.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J., 1990. Basic local alignment search tool. Journal of molecular biology, 215(3): 403-10.

Altschul, S.F. and Lipman, D.J., 1990. Protein database searches for multiple alignments. Proceedings of the National Academy of Sciences of the United States of America, 87(14): 5509-13.

Altschul, S.F. et al., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research, 25(17): 3389-402.

Bak, S. et al., 2011. Cytochromes p450. The Arabidopsis book / American Society of Plant Biologists, 9: e0144.

Benveniste, I., Lesot, A., Hasenfratz, M.P., Kochs, G. and Durst, F., 1991. Multiple forms of NADPH-cytochrome P450 reductase in higher plants. Biochemical and biophysical research communications, 177(1): 105-12.

Bohlmann, J. and Keeling, C.I., 2008. Terpenoid biomaterials. The Plant journal : for cell and molecular biology, 54(4): 656-69.

Bohlmann, J., Meyer-Gauen, G. and Croteau, R., 1998. Plant terpenoid synthases: molecular biology and phylogenetic analysis. Proceedings of the National Academy of Sciences of the United States of America, 95(8): 4126-33.

Bolwell, G.P., Bozak, K. and Zimmerlin, A., 1994. Plant cytochrome P450. Phytochemistry, 37(6): 1491-506.

Chanama, M., Wunnakup, T., De-Eknamkul, W. and Chanama, S., 2009. Improvement of Thin-Layer Chromatography for Enzyme Assay of Geranylgeraniol 18-Hydroxylase from Croton stellatopilosus Ohba. Journal of Planar Chromatography-Modern TLC, 22(1): 49-53.

Chang, M.C., Eachus, R.A., Trieu, W., Ro, D.K. and Keasling, J.D., 2007. Engineering Escherichia coli for production of functionalized terpenoids using plant P450s. Nature chemical biology, 3(5): 274-7.

Chapple, C., 1998. Molecular-Genetic Analysis of Plant Cytochrome P450-Dependent Monooxygenases. Annual review of plant physiology and plant molecular biology, 49: 311-343.

Chau, M. and Croteau, R., 2004. Molecular cloning and characterization of a cytochrome P450 taxoid 2alpha-hydroxylase involved in Taxol biosynthesis. Arch Biochem Biophys, 427(1): 48-57.

Chau, M., Jennewein, S., Walker, K. and Croteau, R., 2004. Taxol biosynthesis: Molecular cloning and characterization of a cytochrome P450 taxoid 7 beta-hydroxylase. Chemistry & biology, 11(5): 663-72.

Collu, G. et al., 2001. Geraniol 10-hydroxylase, a cytochrome P450 enzyme involved in terpenoid indole alkaloid biosynthesis. FEBS letters, 508(2): 215-20.

Coon, M.J., 2002. Enzyme ingenuity in biological oxidations: a trail leading to cytochrome p450. The Journal of biological chemistry, 277(32): 28351-63.

Coon, M.J., 2003. Multiple oxidants and multiple mechanisms in cytochrome P450 catalysis. Biochemical and biophysical research communications, 312(1): 163-8.

Coon, M.J., 2005a. Cytochrome P450: nature's most versatile biological catalyst. Annual review of pharmacology and toxicology, 45: 1-25.

Coon, M.J., 2005b. Omega oxygenases: nonheme-iron enzymes and P450 cytochromes. Biochemical and biophysical research communications, 338(1): 378-85.

Coon, M.J., Ding, X.X., Pernecky, S.J. and Vaz, A.D., 1992. Cytochrome P450: progress and predictions. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 6(2): 669-73.

Coon, M.J., Vaz, A.D. and Bestervelt, L.L., 1996. Cytochrome P450 2: peroxidative reactions of diversozymes. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 10(4): 428-34.

Croteau, R., Ketchum, R.E., Long, R.M., Kaspera, R. and Wildung, M.R., 2006. Taxol biosynthesis and molecular genetics. Phytochemistry reviews : proceedings of the Phytochemical Society of Europe, 5(1): 75-97.

Durst, F., Benveniste, I., Salaun, J.P. and Werck-Reichhart, D., 1992. Function, mechanism and regulation of cytochrome P-450 enzymes in plants. Biochemical Society transactions, 20(2): 353-7.

Durst, F. and Nelson, D.R., 1995. Diversity and evolution of plant P450 and P450-reductases. Drug metabolism and drug interactions, 12(3-4): 189-206.

Emanuelsson, O., Brunak, S., von Heijne, G. and Nielsen, H., 2007. Locating proteins in the cell using TargetP, SignalP and related tools. Nature protocols, 2(4): 953-71.

Esser, H.J. and Chayamarit, K., 2001. Two new species and a new name in Thai Croton (Euphorbiaceae). Thai For. Bull. (Bot), 29: 51-57.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39: 783-791.

Felsenstein, J., 1992. Estimating effective population size from samples of sequences: a bootstrap Monte Carlo integration method. Genetical research, 60(3): 209-20.

Froehlich, J.E., Itoh, A. and Howe, G.A., 2001. Tomato allene oxide synthase and fatty acid hydroperoxide lyase, two cytochrome P450s involved in oxylipin metabolism, are targeted to different membranes of chloroplast envelope. Plant physiology, 125(1): 306-17.

Guengerich, F.P., 2002a. Cytochrome P450 enzymes in the generation of commercial products. Nature reviews. Drug discovery, 1(5): 359-66.

Guengerich, F.P., 2002b. Rate-limiting steps in cytochrome P450 catalysis. Biological chemistry, 383(10): 1553-64.

Guengerich, F.P., Martin, M.V., Sohl, C.D. and Cheng, Q., 2009. Measurement of cytochrome P450 and NADPH-cytochrome P450 reductase. Nature protocols, 4(9): 1245-51.

Guengerich, F.P., Miller, G.P., Hanna, I.H., Sato, H. and Martin, M.V., 2002. Oxidation of methoxyphenethylamines by cytochrome P450 2D6. Analysis of rate-limiting steps. The Journal of biological chemistry, 277(37): 33711-9.

Hamann, T. and Moller, B.L., 2007. Improved cloning and expression of cytochrome P450s and cytochrome P450 reductase in yeast. Protein expression and purification, 56(1): 121-7.

Hamberger, B. and Bak, S., 2013. Plant P450s as versatile drivers for evolution of species-specific chemical diversity. Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 368(1612): 20120426.

Hamberger, B. and Bohlmann, J., 2006. Cytochrome P450 mono-oxygenases in conifer genomes: discovery of members of the terpenoid oxygenase superfamily in spruce and pine. Biochemical Society transactions, 34(Pt 6): 1209-14.

Harada, H. and Misawa, N., 2009. Novel approaches and achievements in biosynthesis of functional isoprenoids in Escherichia coli. Applied microbiology and biotechnology, 84(6): 1021-31.

Harada, H. et al., 2009. Efficient synthesis of functional isoprenoids from acetoacetate through metabolic pathway-engineered Escherichia coli. Applied microbiology and biotechnology, 81(5): 915-25.

5691866879

Hofer, R. et al., 2013. Geraniol hydroxylase and hydroxygeraniol oxidase activities of the CYP76 family of cytochrome P450 enzymes and potential for engineering the early steps of the (seco)iridoid pathway. Metabolic engineering, 20: 221-32.

Hull, A.K. and Celenza, J.L., 2000. Bacterial expression and purification of the Arabidopsis NADPH-cytochrome P450 reductase ATR2. Protein expression and purification, 18(3): 310-5.

Jensen, K., Jensen, P.E. and Moller, B.L., 2011. Light-driven cytochrome p450 hydroxylations. ACS chemical biology, 6(6): 533-9.

Jensen, K. and Moller, B.L., 2010. Plant NADPH-cytochrome P450 oxidoreductases. Phytochemistry, 71(2-3): 132-41.

Jones, D.T., Taylor, W.R. and Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. Computer applications in the biosciences : CABIOS, 8(3): 275-82.

Kakinuma, K. et al., 2001. New approach to multiply deuterated isoprenoids using triply engineered Escherichia coli and its potential as a tool for mechanistic enzymology. Journal of the American Chemical Society, 123(6): 1238-9.

Kaspera, R. and Croteau, R., 2006. Cytochrome P450 oxygenases of Taxol biosynthesis. Phytochemistry reviews : proceedings of the Phytochemical Society of Europe, 5(2-3): 433-444.

Kirby, J. and Keasling, J.D., 2009. Biosynthesis of plant isoprenoids: perspectives for microbial engineering. Annual review of plant biology, 60: 335-55.

Koga, T. et al., 1996a. Bactericidal effect of plaunotol, a cytoprotective antiulcer agent, against Helicobacter pylori. J Antimicrob Chemother, 33(3): 387-97.

Koga, T. et al., 1996b. In-vitro and in-vivo antibacterial activity of plaunotol, a cytoprotective antiulcer agent, against Helicobacter pylori. The Journal of antimicrobial chemotherapy, 37(5): 919-29.

Koopmann, E. and Hahlbrock, K., 1997. Differentially regulated NADPH:cytochrome P450 oxidoreductases in parsley. Proceedings of the National Academy of Sciences of the United States of America, 94(26): 14954-9.

Kuhner, M.K. and Felsenstein, J., 1994. A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. Molecular biology and evolution, 11(3): 459-68.

Kuroda, H., Oshima, T., Kaneda, H. and Takashio, M., 2005. Identification and functional analyses of two cDNAs that encode fatty acid 9-/13-hydroperoxide

5691868 79

lyase (CYP74C) in rice. Bioscience, biotechnology, and biochemistry, 69(8): 1545-54.

Laursen, T., Jensen, K. and Moller, B.L., 2011. Conformational changes of the NADPH-dependent cytochrome P450 reductase in the course of electron transfer to cytochromes P450. Biochimica et biophysica acta, 1814(1): 132-8.

Mansuy, D., 1998. The great diversity of reactions catalyzed by cytochromes P450. Comparative biochemistry and physiology. Part C, Pharmacology, toxicology & endocrinology, 121(1-3): 5-14.

Meijer, A.H. et al., 1993. Isolation and characterization of a cDNA clone from Catharanthus roseus encoding NADPH:cytochrome P-450 reductase, an enzyme essential for reactions catalysed by cytochrome P-450 mono-oxygenases in plants. The Plant journal : for cell and molecular biology, 4(1): 47-60.

Misawa, N., 2011. Pathway engineering for functional isoprenoids. Current opinion in biotechnology, 22(5): 627-33.

Mizutani, M., 2012. Impacts of diversification of cytochrome P450 on plant metabolism. Biological & pharmaceutical bulletin, 35(6): 824-32.

Mizutani, M. and Ohta, D., 2010. Diversification of P450 genes during land plant evolution. Annual review of plant biology, 61: 291-315.

Mizutani, M. and Sato, F., 2011. Unusual P450 reactions in plant secondary metabolism. Archives of biochemistry and biophysics, 507(1): 194-203.

Murataliev, M.B. and Feyereisen, R., 1999. Mechanism of cytochrome P450 reductase from the house fly: evidence for an FMN semiquinone as electron donor. FEBS letters, 453(1-2): 201-4.

Nadler, S.G. and Strobel, H.W., 1988. Role of electrostatic interactions in the reaction of NADPH-cytochrome P-450 reductase with cytochromes P-450. Archives of biochemistry and biophysics, 261(2): 418-29.

Naur, P. et al., 2003. CYP83A1 and CYP83B1, two nonredundant cytochrome P450 enzymes metabolizing oximes in the biosynthesis of glucosinolates in Arabidopsis. Plant Physiol, 133(1): 63-72.

Nebert, D.W. and Nelson, D.R., 1991. P450 gene nomenclature based on evolution. Methods in enzymology, 206: 3-11.

Nebert, D.W. et al., 1989. The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. DNA, 8(1): 1-13.

Nelson, D. and Werck-Reichhart, D., 2011. A P450-centric view of plant evolution. The Plant journal : for cell and molecular biology, 66(1): 194-211.

Nelson, D.R., 1998. Cytochrome P450 nomenclature. Methods in molecular biology, 107: 15-24.

Nelson, D.R., 1999. Cytochrome P450 and the individuality of species. Archives of biochemistry and biophysics, 369(1): 1-10.

Nelson, D.R., 2002. Mining databases for cytochrome P450 genes. Methods in enzymology, 357: 3-15.

Nelson, D.R., 2006. Cytochrome P450 nomenclature, 2004. Methods in molecular biology, 320: 1-10.

Nelson, D.R., 2009. The cytochrome p450 homepage. Human genomics, 4(1): 59-65.

Nelson, D.R. et al., 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics, 6(1): 1-42.

Nelson, D.R., Schuler, M.A., Paquette, S.M., Werck-Reichhart, D. and Bak, S., 2004. Comparative genomics of rice and Arabidopsis. Analysis of 727 cytochrome P450 genes and pseudogenes from a monocot and a dicot. Plant physiology, 135(2): 756-72.

Newcomb, M. et al., 2003a. Kinetic isotope effects implicate two electrophilic oxidants in cytochrome p450-catalyzed hydroxylations. Journal of the American Chemical Society, 125(20): 6064-5.

Newcomb, M., Hollenberg, P.F. and Coon, M.J., 2003b. Multiple mechanisms and multiple oxidants in P450-catalyzed hydroxylations. Archives of biochemistry and biophysics, 409(1): 72-9.

Nicholas, K., Nicholas, H. and Deerfield, D., 1997. GeneDoc: Analysis and Visualization of Genetic Variation 4. EMBNEW.

Nualkaew, N., De-Eknamkul, W., Kutchan, T.M. and Zenk, M.H., 2006. Membrane-bound geranylgeranyl diphosphate phosphatases: purification and characterization from Croton stellatopilosus leaves. Phytochemistry, 67(15): 1613-20.

Nualkaew, N. et al., 2013. Molecular cloning and catalytic activity of a membrane-bound prenyl diphosphate phosphatase from Croton stellatopilosus Ohba. Phytochemistry, 91: 140-7.

Omura, T. and Sato, R., 1963. Fractional solubilization of haemoproteins and partial purification of carbon monoxide-binding cytochrome from liver microsomes. Biochimica et biophysica acta, 71: 224-6.

Omura, T. and Sato, R., 1964a. The Carbon Monoxide-Binding Pigment of Liver Microsomes. I. Evidence for Its Hemoprotein Nature. The Journal of biological chemistry, 239: 2370-8.

Omura, T. and Sato, R., 1964b. The Carbon Monoxide-Binding Pigment of Liver Microsomes. Ii. Solubilization, Purification, and Properties. The Journal of biological chemistry, 239: 2379-85.

Paquette, S.M., Jensen, K. and Bak, S., 2009. A web-based resource for the Arabidopsis P450, cytochromes b5, NADPH-cytochrome P450 reductases, and family 1 glycosyltransferases (http://www.P450.kvl.dk). Phytochemistry, 70(17-18): 1940-7.

Poulos, T.L., 2005. Intermediates in P450 catalysis. Philosophical transactions. Series A, Mathematical, physical, and engineering sciences, 363(1829): 793-806; discussion 1035-40.

Rana, S. et al., 2013. NADPH-cytochrome P450 reductase: molecular cloning and functional characterization of two paralogs from Withania somnifera (L.) dunal. PloS one, 8(2): e57068.

Rinthong, P.-o., Jindaprasert, A. and De-Eknamkul, W., 2009. Simple Densitometric TLC Analysis of Plunotol for screening if High-Plaunotol-Containing Plants of *Croton stellatopilosus* Ohba. Journal of Planar Chromatography, 22(1): 55-58.

Ro, D.K., Ehlting, J. and Douglas, C.J., 2002. Cloning, functional expression, and subcellular localization of multiple NADPH-cytochrome P450 reductases from hybrid poplar. Plant physiology, 130(4): 1837-51.

Rungrotmongkol, T., Frecer, V., De-Eknamkul, W., Hannongbua, S. and Miertus, S., 2009. Design of oseltamivir analogs inhibiting neuraminidase of avian influenza virus H5N1. Antiviral Res, 82(1): 51-8.

Saitou, N. and Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4): 406-25.

Scherbak, N. et al., 2011. The pea SAD short-chain dehydrogenase/reductase: quinone reduction, tissue distribution, and heterologous expression. Plant physiology, 155(4): 1839-50.

Schuler, M.A. and Werck-Reichhart, D., 2003. Functional genomics of P450s. Annual review of plant biology, 54: 629-67.

Shephard, E.A., Phillips, I.R., Bayney, R.M., Pike, S.F. and Rabin, B.R., 1983. Quantification of NADPH: cytochrome P-450 reductase in liver microsomes by a specific radioimmunoassay technique. The Biochemical journal, 211(2): 333-40.

Sitthithaworn, W. et al., 2009. Cloning and expression of 1-decxy-d-xylulose 5-phosphate synthase cDNA from Croton stellatopilosus and expression of 2C-

methyl-d-erythritol 4-phosphate synthase and geranylgeranyl diphosphate synthase, key enzymes of plaunotol biosynthesis. J Plant Physiol, 167(4): 292-300.

Sitthithaworn, W. et al., 2010. Cloning and expression of 1-deoxy-d-xylulose 5-phosphate synthase cDNA from Croton stellatopilosus and expression of 2C-methyl-d-erythritol 4-phosphate synthase and geranylgeranyl diphosphate synthase, key enzymes of plaunotol biosynthesis. J Plant Physiol, 167(4): 292-300.

Sitthithaworn, W.P., B; De-Eknamkul, W, 2006. Localization of Plaunotol in the Leaf of Croton stellatopilosus Ohba. ScienceAsia, 32: 17-20.

Stigliani, A.L., Giorio, G. and D'Ambrosio, C., 2011. Characterization of P450 carotenoid beta- and epsilon-hydroxylases of tomato and transcriptional regulation of xanthophyll biosynthesis in root, leaf, petal and fruit. Plant & cell physiology, 52(5): 851-65.

Tamaki, K., Imaishi, H., Ohkawa, H., Oono, K. and Sugimoto, M., 2005. Cloning, expression in yeast, and functional characterization of CYP76A4, a novel cytochrome P450 of petunia that catalyzes (omega-1)-hydroxylation of lauric acid. Bioscience, biotechnology, and biochemistry, 69(2): 406-9.

Tamura, K. et al., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular biology and evolution, 28(10): 2731-9.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular biology and evolution, 30(12): 2725-9.

Tansakul, P. and De-Eknamkul, W., 1998. Geranlygeraniol-18-hydroxylase: the last enzyme on the plaunotol biosynthetic pathway in Croton sublyratus. Phytochemistry, 47(7): 1241-1246.

Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G. and Covello, P.S., 2006. Artemisia annua L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. FEBS Lett, 580(5): 1411-6.

Thompson, J.D., Gibson, T.J. and Higgins, D.G., 2002. Multiple sequence alignment using ClustalW and ClustalX. Current protocols in bioinformatics / editoral board, Andreas D. Baxevanis ... [et al.], Chapter 2: Unit 2 3.

Urban, P. et al., 1994. Characterization of recombinant plant cinnamate 4-hydroxylase produced in yeast. Kinetic and spectral properties of the major plant P450 of the phenylpropanoid pathway. European journal of biochemistry / FEBS, 222(3): 843-50.

Urlacher, V.B. and Eiben, S., 2006. Cytochrome P450 monooxygenases: perspectives for synthetic application. Trends in biotechnology, 24(7): 324-30.

Urlacher, V.B. and Girhard, M., 2012. Cytochrome P450 monooxygenases: an update on perspectives for synthetic application. Trends in biotechnology, 30(1): 26-36.

Vongchareonsathit, A. and De-Eknamkul, W., 1998. Rapid TLC-densitometric analysis of plaunotol from Croton sublyratus leaves. Planta Med, 64(3): 279-80.

Watson, C.J. et al., 2001. Localization of CYP86B1 in the outer envelope of chloroplasts. Plant & cell physiology, 42(8): 873-8.

Wei, X., Kuhn, D.N. and Narasimhan, G., 2003. Degenerate primer design via clustering. Proceedings / IEEE Computer Society Bioinformatics Conference. IEEE Computer Society Bioinformatics Conference, 2: 75-83.

Wen, C.C. et al., 2007. Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus. Journal of medicinal chemistry, 50(17): 4087-95.

Werck-Reichhart, D., Bak, S. and Paquette, S., 2002. Cytochromes p450. The Arabidopsis book / American Society of Plant Biologists, 1: e0028.

Werck-Reichhart, D. and Feyereisen, R., 2000. Cytochromes P450: a success story. Genome biology, 1(6): REVIEWS3003.

Werck-Reichhart, D., Jones, O.T. and Durst, F., 1988. Haem synthesis during cytochrome P-450 induction in higher plants. 5-Aminolaevulinic acid synthesis through a five-carbon pathway in Helianthus tuberosus tuber tissues aged in the dark. The Biochemical journal, 249(2): 473-80.

Wungsintaweekul, J. et al., 2008. Transcription profiles analysis of genes encoding 1-deoxy-D-xylulose 5-phosphate synthase and 2C-methyl-D-erythritol 4-phosphate synthase in plaunotol biosynthesis from Croton stellatopilosus. Biol Pharm Bull, 31(5): 852-6.

Wungsintaweekul, J. et al., 2007. Establishment of Croton stellatopilosus suspension culture for geranylgeraniol production and diterpenoid biosynthesis. Z Naturforsch C, 62(5-6): 389-96.

Yamazaki, H. et al., 2002. Roles of NADPH-P450 reductase and apo- and holo-cytochrome b5 on xenobiotic oxidations catalyzed by 12 recombinant human

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cytochrome P450s expressed in membranes of Escherichia coli. Protein expression and purification, 24(3): 329-37.

Yang, C.Q., Lu, S., Mao, Y.B., Wang, L.J. and Chen, X.Y., 2010. Characterization of two NADPH: cytochrome P450 reductases from cotton (Gossypium hirsutum). Phytochemistry, 71(1): 27-35.

Yoshikawa, N. et al., 2009. Plaunotol and geranylgeraniol induce caspase-mediated apoptosis in colon cancer. The Journal of surgical research, 153(2): 246-53.

Zelasko, S., Palaria, A. and Das, A., 2013. Optimizations to achieve high-level expression of cytochrome P450 proteins using Escherichia coli expression systems. Protein expression and purification, 92(1): 77-87.

Zhu, Y. et al., 2006. ELONGATED UPPERMOST INTERNODE encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. The Plant cell, 18(2): 442-56.

APPENDIX

SDS-PAGE protein separation

Separating 0.375M Tris pH8.8 / 2 small gel

	7.5%	10%	12%	15%
Distilled H2O	4.75 ml	3.92 ml	3.25 ml	2.25 ml
1.5M Tris-HCl pH8.8	2.5 ml	2.5 ml	2.5 ml	2.5 ml
10%SDS	100 µl	100 µl	100 µl	100 µl
Acrylamide/Bis (30%) (BioRad)	2.5 ml	3.33 ml	4 ml	5 ml
Glycerol	100 µl	100 µl	100 µl	100 µl
10%APS	50 µl	50 µl	50 µl	50 µl
TEMED	5 µl	5 µl	5 µl	5 µl

Stacking gel 0.125M Tris pH 6.8

	3.9%	8.3%
Distilled H2O	3 ml	0.9 ml
0.5M Tris-HCl pH6.8	1.25 ml	3.75 ml
10%SDS	50 µl	150 µl
Acrylamide/Bis (30%) (BioRad)	0.65 ml	1.95 ml
Glycerol	50 µl	150 µl
10%APS	25 µl	75 µl
TEMED	5 µl	10 µl

Running buffer

10x stock solution	g/L for 10x stock solution	1x working solution
0.25 M Tris base	30.3 g Tris base	25mM Tris base
1.92M glycine	144.0g Glycine	192 mM Glycine
1.0% SDS	10.0 g SDS	0.1% SDS
	To 1L by dH2O	

2x Tris-Glycine SDS sample buffer

2x concentration	Amount to add for 2x concentration
126 mM Tris-HCl, pH6.8	2.5 ml of 0.5 M Tris-HCl pH6.8
20% glycerol	2 ml glycerol
4% SDS	4 ml of 10%SDS
0.005% Bromophenol blue	0.5 ml of 0.1% Bromophenol blue
Adjust volumn to 10 ml, adding 0.5 ml β-ME before used	

Western blot

Buffer used for western blot

Buffer/ reagent	
Transfer buffer pH8.3	25 mM Tris-HCl pH8.3, 192 mM glycine, 20% (v/v) absolute methanol
TBS buffer	100 mM Tris-HCl pH7.5, 0.9% NaCl
TTBS	100 mM Tris-HCl pH7.7, 0.9% NaCl, 0.1% Tween20
Blocking buffer	5% skim milk in TTBS
Dilute buffer	1% skim milk TTBS; 100 mM Tris-HCl pH7.5, 0.9% NaCl, 0.1% tween20
Conjugate solution (1:2000)	20 µl of AP-conjugate solution in 10 µl of dilution buffer
Alkaline phosphate buffer	100 mM Tris-HCl pH9.5, 100 mM NaCl, 5 mM $MgCl_2$
Substrate solution	66 µl of the NBT stock to 10 ml alkaline phosphatate buffer mix well, then added 33 µl of the BICP stock mix thoroughly

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VITA

Siriluk Sintupachee, raised in Nakhon Si Thammarat province on August 12, 1974. She graduated in 1993 from Benjamarachutit High School. She entered King Mongkut Institute of Technology Ladkrabang in major entomology, Assoc. Prof. Dr. Suvarin Bumroongsook as her advisor. In year 2002 she graduated the master degree in Environmental Biology form Mahidol University with the publication topic as "Closely related Wolbachia strains within the pumpkin arthropod community and the potential for horizontal transmission via the plant" Assit. Prof. Dr. John R Milne was her advisor and fund support provided by the TRF/BIOTEC Special Program for Research and Training in Biodiversity (BRT139026) and the Thailand Research Fund (RTA428001). In the same year she got the position as medical scientist at Medical Biotechnology Center, Department of Medical Science, Ministry of Public Health worked on the recombinant protein production in Research and Development unit. Five years later she got the fund provided by the Office of the Higher Education Commission, the Program Strategic Scholarships Frontier research Network (year 2008) for doctoral degree in Biotechnology. Her thesis focused on CLONING AND CHARATERIZATION OF TERPENOID HYDROXYLASE GENE FROM Croton stellatopilosus Ohba. She was advised by her advisor, Assoc. Prof. Dr. Wanchai De-Eknamkul.

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