

REFERENCES

1. Twelve major cancers. 1996. Sci. Am. 275 : 92-98.
2. National Cancer Institute. Annual report 1995. Department of Medical Services, Ministry of Public Health, Bangkok, Thailand.
3. Cum, C.P. 1994. Female genital tract. In R.S. Cotran; V. Kumar; and S.L. Robbins (eds.), Robbins Pathologic basis of disease, (5th ed.), pp. 1033-1088. USA : Saunders Company.
4. Robboy, S. J., Duggan, M.A., and Kurman, R.J. 1995. The female reproductive system. In E. Rubin; and J.L. Farber (eds.), Pathology, (2nd ed.), pp. 909-971. Philadelphia : J.B. Lippincott.
5. Lowe, D.G. 1997. Female genital pathology. In I.A. Cree (ed.), Pathology, (1st ed.), pp. 343-366. England : Chapman & Hall Medical.
6. Walsh, C.B., Kay, E.W., and Leader, M.B. 1995. The pathology of cervical cancer. Clin. Obstet. Gynecol. 38:653-661.
7. Herrington, C.S. 1994. Human papillomaviruses and cervical neoplasia I. Classification, virology , pathology, and epidemiology. J.Clin.Pathol. 47 : 1066-1072.
8. Rows, W.E., et al. 1986. Herpes simplex virus type 2: association with carcinoma of the cervix. Science 161:1255-1256.
9. Sclacter, J., et al. 1982. *Chlamydia trachomatis* and cervical neoplasia. JAMA. 248:2134-2139.

10. Doorbar, J., et al. 1991. Specific interaction between HPV-16 E1-E4 and cytokeratins results collapse of the epithelial cell intermediate filament network. Nature 352 : 824-827.
11. de Villers, E.M., et al. 1987. Human papillomavirus infection in women with and without abnormal cervical cytology. Lancet ii : 703-705.
12. Kalantari, M., Karlsen, F., Johansson, B., Sigurjonsson, T., Warleby, B., and Hagmar, B. 1997. Human papillomavirus findings in relation to cervical intraepithelial neoplasia grade : A study on 476 Stockholm women, using PCR for detection and typing of HPV. Hum. Pathol. 28 : 899-904.
13. Liaw, K.L., et al. 1995. Human papillomavirus and cervical neoplasia : A case-control study in Taiwan. Int. J. Cancer 62: 565-571.
14. Cornelissen, M.T., et al. 1992. Detection of human papillomavirus types by the polymerase chain reaction and differentiation between high-risk and low-risk cervical lesion. Vichows Arch B Cell. Pathol.Incl. Mol Pathol. 62 : 167-171.
15. Olesen, A.O., et al. 1995. Human papillomavirus and cervical intraepithelial neoplasia grade II-III : A population-base case-control study. Int. J. Cancer 61: 321-315.
16. Wu, C.H., Lee, M.F., Chang, M.C., and Ho, S.C. 1994. Detection of human papillomavirus types in cervical lesion of patients from Taiwan by the polymerase chain reaction. Sex. Trans. Dis. 21:309-314.

17. Bosch, F.X., et al. 1995. Prevalence of human papillomavirus in cervical cancer : A worldwide perspective. J. Natl. Cancer Inst. 87: 796-802.
18. Bergeron, C., Barrasso, R., Beaudenon, S., Flamant, P., Croissant, O., and Orth, G. 1992. Human papillomaviruses associated with cervical intraepithelial neoplasia. Am. J. Surg. Pathol. 16: 641-649.
19. Williamson, A.L., Brink, N.C., Dehaeck, C.M.C., Ovens, S., Soeters, R., and Rybicki, E. 1994. Typing of human papillomaviruses in cervical carcinoma biopsies from Cape Town. J. Med. Virol. 43: 231-237.
20. Chen, S.L., Han, C.P., Tsao, Y.P., Lee, J.W., and Yin, C.S. 1993. Identification and typing of human papillomavirus in cervical cancers in Taiwan. Cancer 72: 1939-1945.
21. Iv asawa, A., Niemien, P., Lehtinen, M., and Paavonen, J. 1996. Human papillomavirus DNA in uterine cervix squamous cell carcinoma and adenocarcinoma detected by polymerase chain reaction. Cancer 77: 2275-2279.
22. Bhattarakosol,P., Poonnaniti, A., and Niruthisard, S. 1996. Detection and typing of human papillomavirus in cervical cancer in Thai. J. Med. Associ. Thai. 79 (suppl): s56-s64.
23. Morris, M., et al. 1996. Cervical intraepithelial neoplasia and cervical cancer. Obstet. Gynecol. Clin. Nort. Am. 23 : 347-410.
24. Briton, L.A., et al. 1986. Cigarette smoking and invasive cervical cancer. JAMA. 255:3265-3269.

25. Beral, V., Hannaford, P and Kay, C. 1988. Oral contraceptive use and malignancies of the genital tract. Results from the Royal College of General practitioners oral contraceptive study. Lancet 2 :1331-1334.
26. Cox, J.T. 1995. Epidemiology of cervical intraepithelial neoplasia: The role of human papillomavirus. Baillieres Clin. Obstet.Gynaecol. 9 : 1-37.
27. Buckley, J.D., Harris, R.W., Doll, R., Vessey, M.P., and Williams, P.T.1981. Case-control study of the husbands of women with dysplasia or carcinoma of the cervix uteri. Lancet 2 : 1010-1015.
28. de Villers, E.M. 1989. Heterogeneity of the human papillomavirus group. J. Virol. 63: 4898-4903.
29. Howley, P.M. 1996. Papillomavirinae : The viruses and their replication. In B.N., fields. et al. (eds) Field Virology, (3rd ed), pp. 2045-2067. Philadelphia : Lippincot-Raven.
30. Lorincz, A. T. 1987. Detection of human papillomavirus infection by nucleic acid hybridization. Obstet. Gynecol. Clin. North. Am. 14 : 451-469.
31. Namkoong, S.E. 1995. Clinical application of HPV typing in cervical cancer. Int. J. Gynecol. Obstet. 49 (suppl) : s59-s67.
32. Poonnanti, A., Bhattarakosol, P. 1996. Improvement of PCR detection of HPV-DNA using enhanced chemiluminescence system and dot hybridization. J. Med. Associ. Thai. 79 (suppl) : s96-s101.

33. Galloway, D.A. 1994. Human papillomavirus vaccines : a warty problem. Infect. Agent. Dis. 3 : 187-139.
34. Shah, K.V., and Howley, P.M. 1996. Papillomaviruses. In N.B. Fields; et al (eds.), Fields Virology, (3rd ed.), pp. 2077-2109. Philadelphia : Lippincott-Raven Publishers.
35. Galloway, D. A., and McDougall, J.K.1989. Human papillomaviruses and carcinomas. Adv. Virus. Res. 37 : 125-171.
36. zur Hausen, H. 1987. Papillomaviruses in human cancer. Cancer 59 : 1692-1696.
37. Strauss, M.J., Bunting, H., Melnick, J.L. 1949. Virus-like particles and inclusion bodies in skin papillomas. J. Invest. Dermatol. 15 : 433-444.
38. Almeida, J.D., Howatson, A.F., and Williams, M.G. 1962. Electron microscope study of human warts : site of virus production and nature of the inclusion bodies. J.Invest.Dermatol. 38 : 337-345.
39. Melnick, J.L. 1962. Papovavirus group. Science 135 : 1128-1130.
40. Roden, R.B.S., Hubbert, N.L., Kirnbauer, R., Breitburd, F., Lowy, D.R., and Schiller, J.T. 1995. Papillomavirus L1 capsid agglutinate mouse erythrocytes through a proteinaceous receptor. J. Virol. 69 : 5147-5151.
41. Roden, R.B.S., et al. 1994. Interaction of papillomaviruses with the cell surface. J. Virol. 68: 7260-7266.

42. Bonnez, W. 1997. Papillomavirus. In D.D. Richman; R. J. Whitley; and F.G. Hayden (eds.), Clinical virology, (1st ed.), pp. 569-610. USA : Churchill Livingstone.
43. Chen, E.Y., et al. 1982. The primary structure and genetic organization of the bovine papillomavirus type 1 genome. Nature 299 : 529-534.
44. Lambert, P.F. 1991. Papillomavirus DNA replication. J.Virol. 65 : 3417-3420.
45. Liu, J.S., Kuo, S.R., Broker, T.R., and Chow, L.T. 1995. The functions of human papillomavirus type 11 E1, E2 and E2C proteins in cell-free DNA replication. J. Bio. Chem. 270 : 27283-27291.
46. Gopalakrishnan, V., Walker, S., and Khan, S.A. 1995. Stimulation of human papillomavirus type 1a DNA replication by a multimerized AT-rich palindromic sequence. Virology 214 : 301-306.
47. Frattini, M.G., and Laimins, L.A. 1994. Binding of the human papillomavirus E1 origin-recognition with the E2 enhancer-binding protein. Proc. Natl. Acad. Sci. USA. 91 : 12398-12402.
48. Bornstein, J., Rahat, M.A., and Abramovici, H. 1995. Etiology of cervical cancer : current concepts. Obstet. Gynecol. Surve. 50 : 146-154.
49. Roberts, S., et al. 1993. Cutaneous and mucosal human papillomavirus E4 proteins form intermediate filament-like structures in epithelial cells. Virology 197 : 176- 187.

50. Straight, S.W., et al. 1993. The E5 oncoprotein of human papillomavirus fibroblasts and effects down regulation of the epidermal growth factor in keratinocytes. J. Virol. 67 : 4521-4532.
51. Howley, P.M. 1991. Role of the human papillomaviruses in human cancer. Cancer Res. (suppl) : s5019-s5022.
52. Bodell, M.A., Jones, K.H., and Laimins, L.A. 1983. The E6-E7 region of papillomavirus type 18 is sufficient for transformation of NTH 3T3 and rat-1 cells. J. Virol. 61 : 3635-3640.
53. Giep, A.E., Herber, R., Jeon, S., Lohse, J.K., Dubielzig, R.R., and Lambert, P.F., 1993. Tumorigenicity by human papillomavirus type 16 E6 and E7 in transgenic mice correlates with alterations in epithelial cell growth and differentiation. J. Virol. 67 : 1373-1384.
54. Scheffner, M., Huibregtse, J.M., Vierstra, R.D., and Howley, P.M. 1993. The HPV-16 E6 and E6-AP complex function as a ubiquitin-protein ligase in the ubiquitination of p53. Cell 75 : 495-505.
55. Scheffner, M., Werness, B.A., Huibregtse, J.M., Levine, A.J., and Howley, P.M. 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 63 : 1129-1136.
56. Evander, M., Frazer, I.H., Payne, E., Qi, Y.M., Hengst, K., and McMillan, N.A.J. 1997. Identification of the α_6 Integrin as a candidate receptor for papillomaviruses. J. Virol. 71 : 2449-2456.

57. Schneider, A., et al. 1987. Distribution of human papillomavirus 16 genome in cervical neoplasia by molecular in situ hybridization of tissues section. Int. J. Cancer 39 : 717-712.
58. Stoler, M.H., and Broker, T.R. 1986. In Situ hybridization detection of human papillomavirus DNA and messenger RNA in genital condylomas and a cervical carcinomas. Hum. Pathol. 17 : 1250-258.
59. Ho, G.Y.F., et al. 1995. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J. Natl. Cancer Inst. 87 : 1365-1371.
60. Coggin, J.R., and zur Hausen, H. 1979. Workshop on papillomavirus and cancer. Cancer Res. 39 : 545-546.
61. Fisher, S.G. 1994. Epidemiology : A tool for study of human papillomavirus-related carcinogenesis. Intervirology 37:215-225.
62. Melchers, W., de Mare, S., Kuitert, E., Galama, J., Walboomers, J., and van den Brule, A.J. 1993. Human papillomavirus and cutaneous warts in meat handlers, J. Clin. Microbiol. 31: 2547-2549.
63. Kato, S., Inoue, M., Koyama, M., Fukita, M., Tanizawa, O., and Hakura, A. 1994. Detection of high-risk human papillomavirus in the cervix and semen of sex partners, J. Infect. Dis. 170 : 682-685.
64. Luy, C., et al. 1991. Determinants of genital human papillomavirus infection in young women. J. Natl. Cancer Inst. 83 : 997-1003.

65. K irlsson, R., et al. 1995. Lifetime number of partners as the only independent risk factor for human papillomavirus infection : A population-based study. Sex. Trans. Dis. 22 : 119-127.
66. Wheeler, C.M., et al. 1993. Determinants of genital papillomavirus infection among cytologically normal women attending the University of New Mexico Student Health Center. Sex. Trans.Dis. 20 : 286-289.
67. Moraes, E.A., Ioshimoto, L.M., Leao, E., and Zugaib, M. 1994. Presence of human papillomavirus DNA in amniotic fluids of pregnant women with cervical lesion. Gynecol. Oncol. 54 : 152-158.
68. D ew, W.L. 1990. Papovaviruses. In P.R. Murray; W.L. Drew; and G.S. Kobayashi (eds.), Medical microbiology. pp. 539-546. USA : Wolfe Medical Publication.
69. Glssmann, L., et al. 1982. Molecular cloning and characterization of human papillomavirus DNA derived from laryngeal papilloma. J.Virol. 44 : 393-400.
70. Schadendorf, D., Tiedemann, K.H., Haas, N., and Czarnetzki, B.M. 1991. Detection of human papillomaviruses in paraffin-embedded condylomata accuminata-comparison of immunohistochemistry, *In situ* hybridization, and polymerase chain reaction. J. Invest.Dermatol. 97 ; 549-554.
71. Greer, C.E., et al. 1995. Human papillomavirus (HPV) type distribution and serologic response to HPV type 6 virus-like particles in patients with genital warts. J. Clin. Microbiol. 33 : 2058-2063.

72. Wood worth, C.D., Doniger, J., and DiPaolo, J.A. 1989. Immortalization of human foreskin keratinocytes by virions human papillomavirus DNAs corresponds to their association with cervical carcinoma. J.Virol. 63 : 156-164.
73. Richart, R.M. 1968. Natural history of cervical intraepithelial neoplasia. Clin. Obstet.Gynecol. 10 : 748-784.
74. Campion, M.J., et al. 1986. Progressive potential of mild cervical atypia : Prospective, cytological, colposcopic, and virology study. Lancet 2 : 237-240.
75. Koutsky, L.A., et al. 1992. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N. Eng. J. Med. 327 : 1272-1278.
76. Cox, J.T., Lorincz, A.T., Schiffman, M.H., Sherman M.E., Cullen, A., and Kurman, R.J. 1995. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of ASUCS. Am. J. Obstet. Gynecol. 172 : 946-954.
77. Cruzick, J., et al. 1990. Type-specific HPV DNA as a predictor of high grade cervical intraepithelial neoplasia in young women. Eu. J.Cancer 26 : 684-690.
78. Maxwell, G.L., and Carlson, J.W. 1996. Oncogenes in gynecologic oncology. Obstet. Gynecol. Sur. 51 : 710-717.

79. Androphy, E.J., Lawy, D.R., and Schiller, J.T. 1987. Bovine papillomavirus E2 transaction gene product binds to specific sites in papillomavirus DNA. Nature 325 : 70-73.
80. Chang, F., Syrjanen, S., Tervahauta, A., and Syrjanen, K. 1993. Tumorigenesis associated with the p53 tumor suppressor gene. Br. J. Cancer 68 : 653-661.
81. Marx, J. 1993. How p53 suppresses cell growth. Science 262: 1644-1645.
82. Frazer, I.H. 1996. Immunology of papillomavirus infection. Cur.Opin.Immunol. 8 : 484-491.
83. Kirnbauer, R. 1996. Papillomavirus-like particles for serology and vaccine development. Intervirology 39 :54-61.
84. Sapp, M., Volpers, C., and Streeck, R.E. 1996. Synthesis, properties and applications of papillomavirus-like particles. Intervirology 39 : 49-53.
85. Potry, K.U., et al. 1996. Human papillomavirus is associated with the frequent detection warty and basaloid high-grade neoplasia of the vulva and cervical neoplasia among immunocompromised women. Gynecol.Oncol. 60 : 30-34.
86. Potry, K.U., et al. 1994. Cellular immunodeficiency enhances the progression of human papillomavirus-associated cervical lesion. Int. J. Cancer 57 : 836-840.

87. Porreco, R., Penn, I., Droegemueller, W., Greer, B., and Makowski, E. 1975. Gynecologic malignancies in immunosuppressed organ homograft recipients. Obstet. Gynecol. 45 : 359-364.
88. Halpert, R., et al. 1986. Human papillomavirus and lower genital neoplasia in renal transplant patients. Obstet. Gynecol. 68 : 251-258.
89. Feltkamp, M.P., et al. 1993. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protect against tumor induce by human papillomavirus type 16- transformed cells. Eu. J. Immunol. 23 : 2242-2249.
90. Connor, M.O., Apt, D., and Bernard, H.U. 1997. DNA tumor viruses : Papilloma. In J.R. Bertino (ed.), Encyclopedia of cancer, (vol.I), pp 520-531, USA : Academic Press.
91. Kienzler, J.L., et al. 1993. Humoral and cell mediated immunity to human papillomavirus type-1 (HPV-1) in human warts. Br. J.Dermatol. 108: 665-672.
92. Fisher, S.G., et al. 1996. The association of human papillomavirus type E6 and E7 antibodies with stage of cervical cancer. Gynecol.Oncol. 61 : 73-78.
93. Southern, E.M. 1975. Detection of specific sequence among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98 : 503-517.

94. Morrison, E.A.B., Goldberg, G.L., Kodish, A.S., and Burk, R. D. 1992. Polymerase chain reaction detection of human papillomavirus : Quantitation may improve clinical utility. J. Clin. Microbiol. 30 : 39-43.
95. Saiki, R. L., et al. 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239 : 487-491.
96. Kuypers, J.M., et al. 1993. Comparison of dot filter hybridization, Southern transfer hybridization, and polymerase chain amplification for diagnosis of anal human papillomavirus infection. J. Clin. Microbiol. 31 : 1003-1006.
97. Wu, A.U., Ben-Ezra, J., Winberg, C., Colombero, A.M., and Rappaport, H. 1990. Analysis of antigen receptor gene rearrangements in ethanol and formaldehyde-fixed, paraffin-embedded specimens Lab. Invest. 63 : 107-114.
98. Bauer, H.M., et al. 1991. Genital human papillomavirus infection in female university students as determined by PCR-based method. JAMA. 265 : 472-477.
99. Plasmid DNA miniprep. 1994. In L. Davis; M. Kuehl; and J. Battey (eds.), Basic methods in molecular biology. (2nd ed.), pp. 245-259. USA: Prentice-Hall International.
100. MacMahon, B., Pugh, T.F. 1970. Epidemiology-principles and method. Boston: Little Brown.

101. Jenkins, A., et al. 1991. Detection of genital papillomavirus types by polymerase chain reaction using common primers. APMIS. 99 : 667-673.
102. Ting, Y., and Manos, M.M. 1990. Detection and typing of human papillomavirus. In M.A. Innis ; D. H. Gelfand ; J.J. Sninsky and T.J. Whites. (eds.) PCR protocols : A guide to methods and applications, pp. 357-367. Sandiago : Academmic Press.
103. Yoshikawa, H., Kawana, T., Kitagawa, K., Yoshikura, H., and Iwamoto, A. 1991. Detection and typing of multiple genital human papillomaviruses by DNA amplification with consensus primers. Jpn. J. Cancer Res. 82 : 524-531.
104. Schiffman, M.H. 1992. Commentary : recent progress in defining the epidemiology of human papillomavirus infection and cervical neoplasia. J.Natl.Cancer Inst. 84 : 394-398.
105. de Villiers, E.M., et al. 1992. Human papillomavirus DNA in women with and without cytological abnormalities : results of five year follow-up study. Gynecol. Oncol. 44 : 33-39.
106. Walker, J., Bloss, J.D., Liao, S.Y., Berman, M., Bergen, S., and Wilczynski, S.P. 1989. Human papillomavirus genotypes as a prognostic indicator in carcinoma of the uterine cervix. Obstet. Gynecol. 75 : 781-785.
107. Arends, M., et al. 1993. Human papillomavirus type 18 associates with more advanced cervical neoplasia than human papillomavirus type 16. Hum. Pathol. 24 : 432-437.

108. Nimmanahaeminda, K., Thamprasert, K., and Chantratita, W. et al. 1994. Detection of various types of human papillomaviruses in premalignant and malignant cervical lesion using DNA-DNA *in situ* hybridization. J. Med. Assoc. Thai. 77 : 120-125.
109. Siripanyaphinyo, u., Auewarakul, P., Siritantikorn, S., Laiwejpithaya, S., and Thakernpol, K. 1996. Detection of human papillomavirus DNA in patients with cervical intraepithelial neoplasia and cervical cancer. New frontiers in medical technology : The 20th Medical Technology conference. 20 : 123-124.
110. Si, J.Y., et al. 1991. A research for the relationship between human papillomavirus and human uterine cervical carcinoma. I. The identification of viral genome and subgenomic sequences in biopsies of Chinese patients. J. Cancer Res. Clin. Oncol. 117 : 454-459.
111. Crook, T., Wrede, D., Tidy, J.A., Mason, W.P., Evans, D.I., and Vousden, K.H. 1992. Clonal p53 mutation in primary cervical cancer : association with human papillomavirus negative tumors. Lancet 339 : 1070-1073.
112. Kurvinen, K., Tervahauta, A., Syrjanen, S., Chang, F., and Syrjanen, K. 1994. The state of the p53 gene in human papillomavirus (HPV)-positive and HPV-negative genital precancer lesions and carcinomas as determined by single-strand conformation polymorphism analysis and sequencing. Anticancer Res. 14 : 177-181.

APPENDIX I.

REAGENTS, MATERIALS AND INSTRUMENTS

A. REAGENTS

Absolute ethanol	(Merck, Germany)
Agarose ultrapure	(BRL, U.S.A.)
Ampicillin sodium salt	(Amresco, U.S.A.)
Bovine serum albumin	(Sigma, U.S.A.)
Chloroform	(Sigma, U.S.A.)
dNTPs	(BRL, U.S.A.)
Developer (Agfa G-150)	(Agfa, Belgium)
Dextrose	(Difco, U.S.A.)
ECL 3'-oligolabelling and detection system (RPN 2131)	(Amersham, U.S.A.)
Ethidium bromide	(Amresco, U.S.A.)
Ethylenediamine tetraacetic acid (EDTA)	(Amresco, U.S.A.)
Fetal bovine serum (FBS)	(BRL, U.S.A.)
Glacial acetic acid	(Merck, Germany)
Isoamyl alcohol	(Sigma, U.S.A.)
Kodak rapid fixer with hardener	(Kodak, U.S.A.)
Minimum essential medium (MEM)	(BRL, U.S.A.)
Phenol (saturated phenol)	(Amresco, U.S.A.)
Potassium acetate	(Sigma, U.S.A.)
Proteinase K	(BRL, U.S.A.)
Sodium dodecyl sulfate (SDS)	(Amresco, U.S.A.)
Sodium hydroxide (NaOH)	(Merck, Germany)
Sodium chloride (NaCl)	(Merck, Germany)
Sodium citrate	(Sigma, U.S.A.)

Trypsin	(Grand Island, U.S.A.)
Taq DNA polymerase (with MgCl ₂ and PCR buffer)	(BRL, U.S.A.)
Tris-base	(Sigma, U.S.A.)
Tris-HCl	(Sigma, U.S.A.)
Tryptone	(Lab M, U.S.A.)
ØX 174/ <i>Hae</i> III	(BRL, U.S.A.)

MATERIALS

Chromatography 3 MM paper	(Whatman, England)
Hyperfilm™ X-ray film	(Amersham, U.S.A.)
Hybond™-N+ nylon membrane	(Amersham, U.S.A.)
Hybridization bag	(BRL, U.S.A.)
Polaroid film (no. 667)	(Berlijucker, U.S.A.)
Tissue culture flask	(Nunc, Denmark)

INSTRUMENTS

Autoclave (model S-90N)	(Tomy seiko, Japan)
DNA thermocycle system	(Hybaid, England)
Incubator type 80	(Mettmert, Germany)
Microcentrifuge	(Fotodyne, U.S.A.)
Power supply (model 200/2.0)	(Bio-Rad, U.S.A.)
Sub cell submarine electrophoresis cell	(Bio-Rad, U.S.A.)
Water bath	(Julabo, Germany)

APPENDIX II.

REAGENTS PREPARATION

REAGENTS FOR DNA EXTRACTION

1. **10% SDS (W/V), pH 7.2**

Sodium dodecyl sulfate	10	g
Deionized distilled water (DDW)	100	ml
Adjusted to pH to 7.2		

2. **0.5 M EDTA pH 8.0**

Ethylenediamine tetraacetic acid	186.1	g
DDW	800	ml
Adjusted to pH to 8.0		
Adjusted volume to 1000 ml with DDW and sterilized by autoclaving		

3. **1 M Tris-base, pH 7.4, 7.6 and 8.0**

Tris-base	121.1	g
DDW	800	ml
Adjusted to pH by adding conc. HCl		
pH 7.4	add	conc. HCl 70 ml
pH 7.6	add	conc. HCl 60 ml
pH 8.0	add	conc. HCl 42 ml
Sterilized by autoclaving		

4. Proteinase K (10 mg/ml)

Proteinase K 10 mg/ml in sterile water store at -20°C

5. 3 M Na acetate pH 5.2

Na acetate. $3\text{H}_2\text{O}$ 408.1 g

DDW 800 ml

Adjusted to pH 5.2 by adding glacial acetic

Adjusted volume to 1000 ml with DDW and sterilized by autoclaving

6. 5 M NaCl

NaCl 292.2 g

DDW 800 ml

Adjusted volume to 1000 ml with DDW and sterilized by autoclaving

REAGENTS FOR PLASMID PREPARATION

1. Glucose solution

Tris-HCl pH 8.0 25 mM

Glucose 50 mM

EDTA 10 mM

Filter sterilization and store in aliquots at -20°C

2. Alkaline/SDS solution

NaOH 0.2 M

SDS 1%

Prepared before use

3. KAC solution

Potassium acetate	29.4	g
Glacial acetic acid	11.5	ml
Adjusted volume to 100 ml with DDW		

REAGENTS FOR CELL CULTURE

1. 1X MEM medium

10X MEM	10	ml
1 M HEPES	1	ml
Pen/Strep. Antibiotic (5 unit/ ml)	0.1	ml
10% NaHCO ₃	1	ml
FBS	10	ml
DDW	77	ml

2. 10X PBS (phosphate-buffer saline)

NaCl	40	g
KCl	1	g
NaHPO ₄	5.75	g
KH ₂ PO ₄	1	g
DDW	300	ml

Adjusted to pH 7.4 and adjusted volume to 500 ml with DDW and sterilized by autoclaving

3. Trypsin versene

10X Trypsin	10	ml
1:500 EDTA	10	ml
1X PBS	80	ml
Sterilized by filtration		

MEDIA PREPARATION

1. LB broth

Tryptone	10	g
yeast extract	5	g
NaCl	5	g
1 N NaOH	1	ml

Adjusted volume to 1000 ml with DDW and Sterilized by autoclaving

LB+ Ampicillin medium

LB broth	100	ml
----------	-----	----

Adding ampicillin to final concentration of 50 ug/ml at 45^o after autoclaving

2. LB agar

Tryptone	10	g
Yeast extract	5	g
NaCl	5	g
1 N NaOH	1	ml
Agar	15	g

Adjusted volume to 1000 ml

Adding ampicillin to final concentration of 50 ug/ml at 45^o after autoclaving

REAGENTS FOR PCR AND ELECTROPHORESIS

1. 5X Tris-borate buffer (TBE)

Tris-base	54	g
Boric acid	27.5	g
5 M EDTA, pH 8.0	20	ml
Sterilized by autoclaving		

2. Ethidium bromide (10mg/ml)

Ethidium bromide	1	g
Sterile distilled water	100	ml

3. Loading dye

Bromphenol blue	0.25	g
Xylene cyanol	0.25	g
Ficoll 400	15	g
Sterile distilled water	100	ml

4. 1.2% Agarose gel

Agarose ultrapure	0.75	g
0.5X TBE buffer	50	ml

REAGENTS FOR HYBRIDIZATION**1. 20X SSPE**

NaCl	174	g
NaH ₂ PO ₄	27.6	g
EDTA	74	g
DDW	800	ml

Adjusted to pH 7.4 by adding 1N NaOH and adjusted volume to 1000 ml with DDW and sterilized by autoclaving

2. 20X SSC

3M NaCl	175	g
0.3M Na citrate	88	g
DDW	800	ml

Adjusted to pH 7.0 by adding 1 N NaOH and adjusted volume to 1000 ml with DDW and sterilized by autoclaving

3. Hybridization buffer

Hybridization buffer compound (ECL)	0.1	g
Blocking solution (ECL)	5	ml
10% SDS	0.2	ml

Adding 5X SSC to final volume 100 ml

4. Buffer I

5 M NaCl	30	ml
1 M Tris-base	100	ml
DDW	700	ml

Adjusted to pH 7.5 with conc. HCl and adjusted volume to 1000 ml
with DDW

5. Buffer II

5 M NaCl	80	ml
1 M Tris-base	100	ml
DDW	700	ml

Adjusted to pH 7.5 with conc. HCl and adjusted volume to 1000 ml
with DDW

6. Blocking reagent

Blocking solution (ECL)	5	ml
-------------------------	---	----

Adding Buffer I to final volume 100 ml and store at 4 °C

BIOGRAPHY

Mr. Monthon Lertworapreecha was born on August 15, 1971 in Bangkok, Thailand. He graduated with the Bachelor degree of Science in Animal Health Science from Faculty of Agriculture at Bangphra, Rajamangala Institute of Technology in 1994.