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APPENDIX I Media and solutions or Reagents

SMM buffer consists of : 0.5 M sucrose

0.02 M Meleate

0.02 M $MgCl_2.6H_2O$

pH 6.5 adjusted with NaOH

PEG solution (40%w/v) contains : 40 g PEG (M.W.6000)

50 ml 2x strength SMM buffer in 100 ml

SMMP medium is prepared by mixing equal volumes of 4x strength penassay broth and 2x strength SMM buffer.

DM3 regeneration medium consists of following sterile solutions per liter:

4% agar	200 ml
1 M sodium succinate (pH7.3)	500 ml
3.5% K_2HPO_4 and 1.5% KH_2PO_4	100 ml
20% glucose	25 ml
1M $MgCl_2.6H_2O$	20 ml
2% filter-sterilized bovine serum albumin (added to the mixture when the temperature is about 55°c)	5 ml

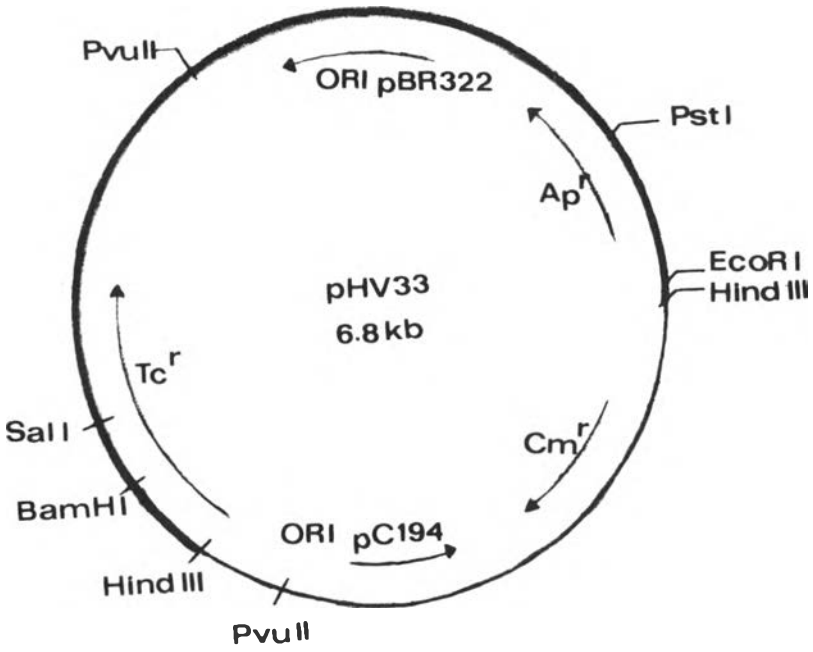
PM medium consists of following:

Bactotryptone	10 g
Yeast extract	5 g
NaCl	10 g
Soluble starch	10 g
Phenol red	0.1 g
Methyl orange	0.3 g
Water to	1 l

pH to 7.4 adjusted with 10 N NaOH

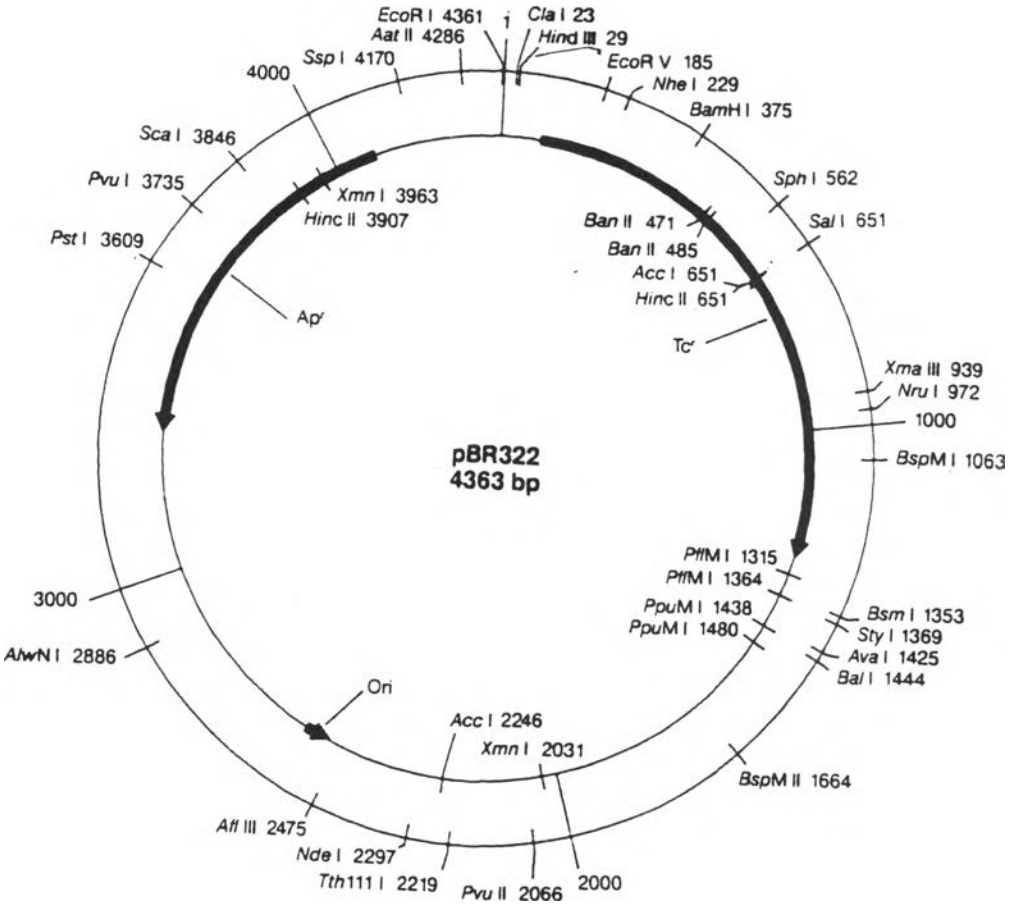
PM-agar has 15 g Bactoagar added per liter.

APPENDIX II Restriction map of plasmid shuttle vector pHV33 : the thin line indicates pC194 and the thick line indicates pBR322 (Primrose and Ehrlich, 1981; Horinouchi and Weisblum, 1982)



APPENDIX III Restriction map of plasmid pBR322

(Balbas *et al.*, 1986)

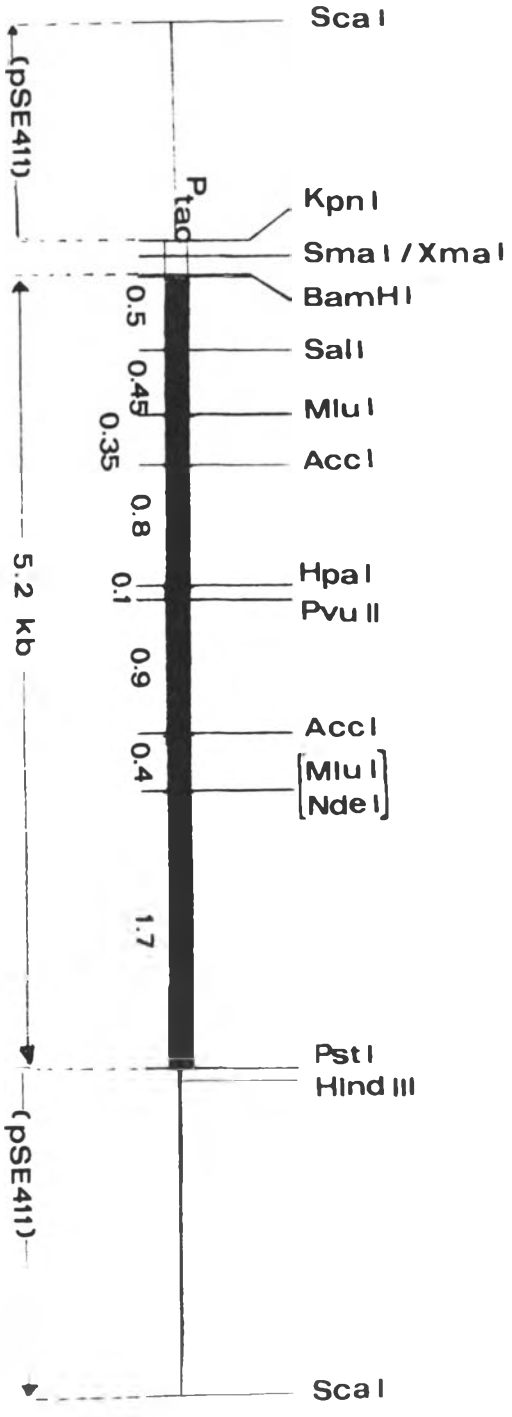


APPENDIX IV Restriction map of plasmid pCSBC8 (สาศิกิ, 1993)

■ : CGTase gene from chromosomal DNA of *Bacillus* sp. A11

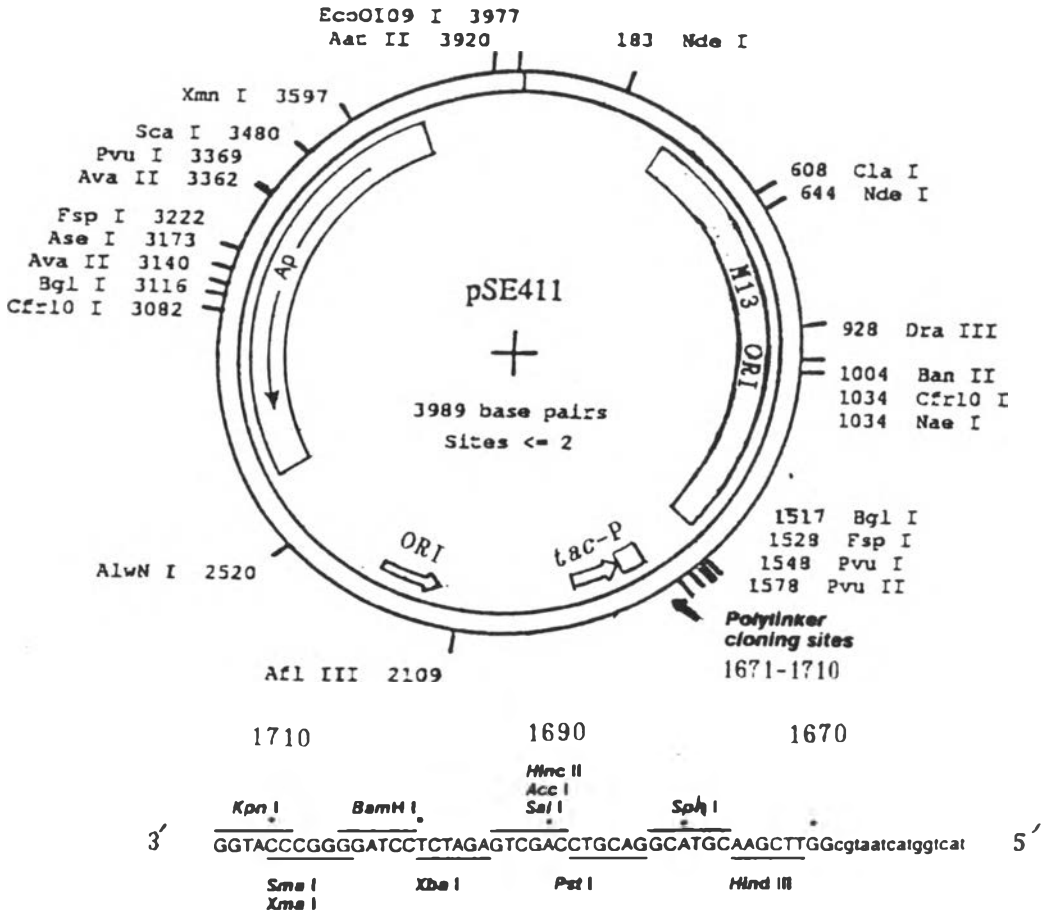
□ : partial multiple cloning sites from pUC18

– : plasmid vector pSE411



APPENDIX V Restriction map of plasmid vector pSE411

(Dente *et al.*, 1983; Elledge and Davis, 1989)

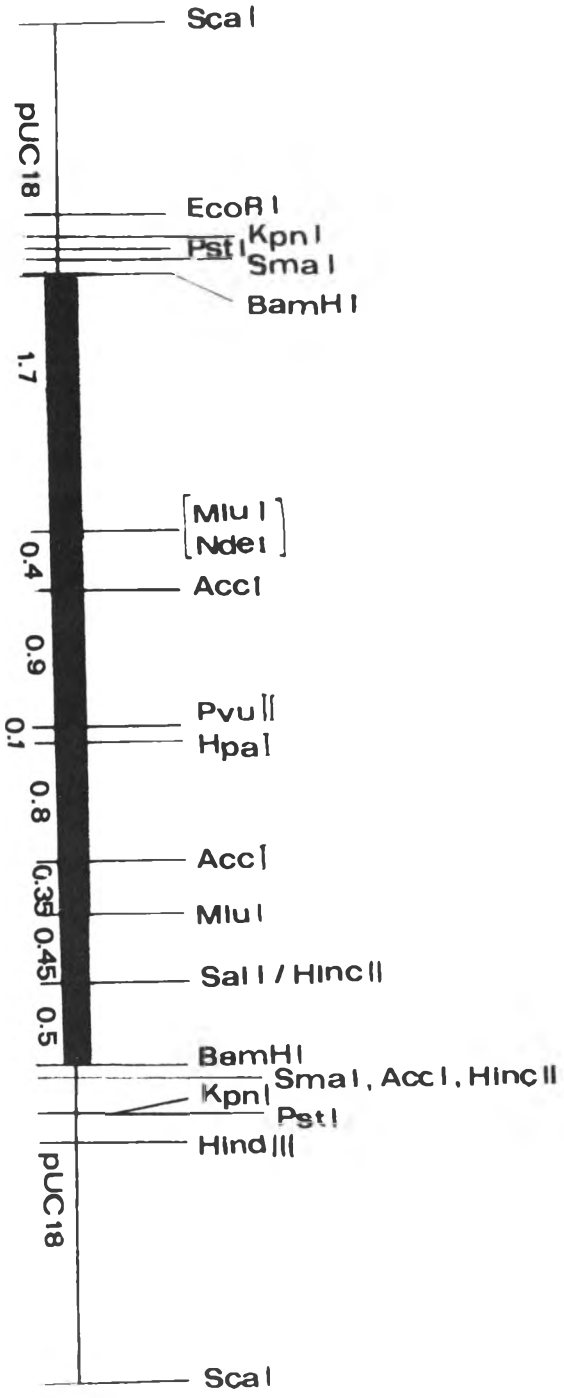


APPENDIX VI Restriction map of plasmid pCSBC5

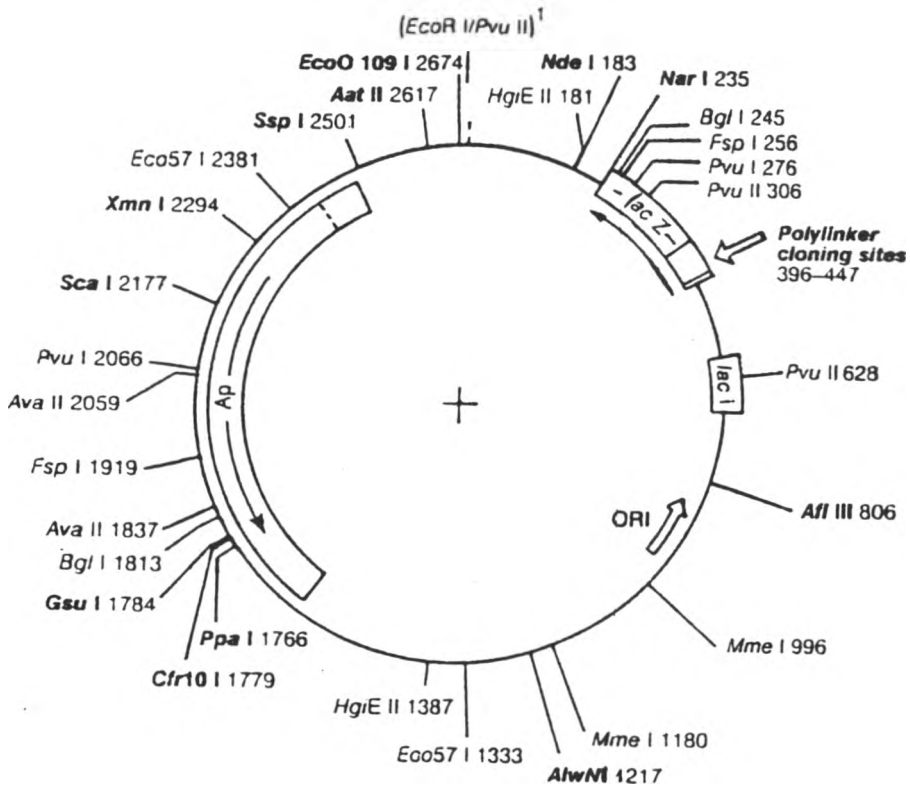
(Personal Communication)

■ : CGTase gene from chromosomal DNA of *Bacillus* sp. A11

– : plasmid vector pUC18



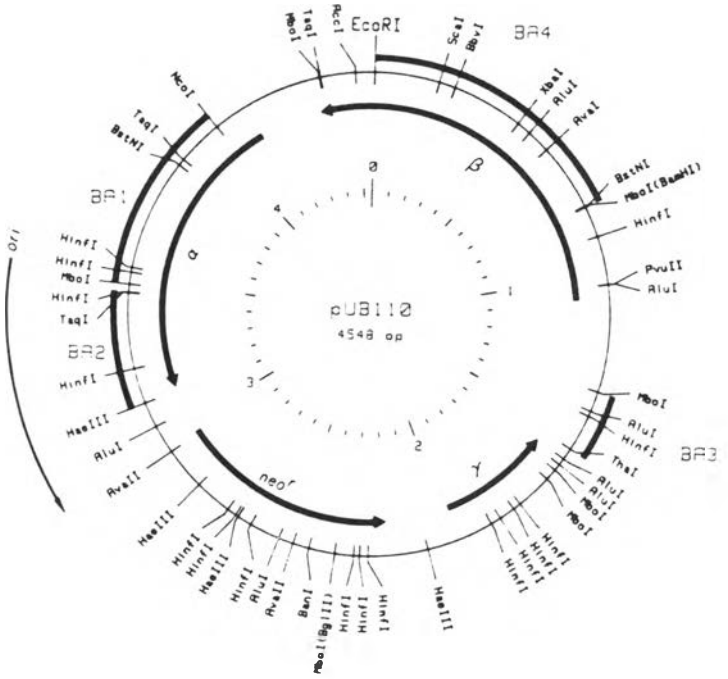
APPENDIX VII Restriction map of plasmid pUC18 (Messing *et al.*, 1981; Yanisch-Perron *et al.*, 1985)



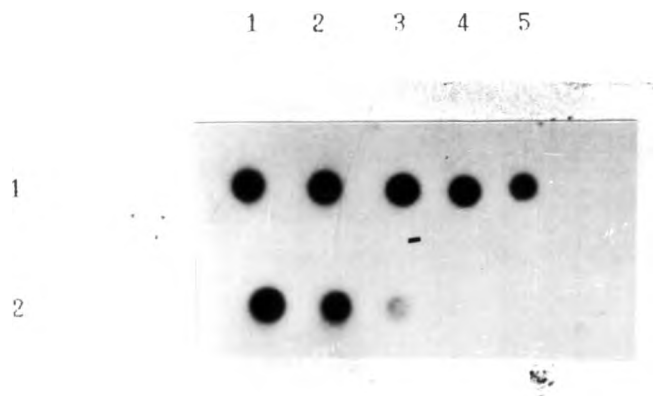
pUC18 multiple cloning site and primer binding region: 371-480

APPENDIX VIII Restriction map of plasmid pUB110

(McKenzie *et al.*, 1986)



APPENDIX IX Estimation of DNA concentration of nonradioactive DIG-labeled probes by chemiluminescent detection

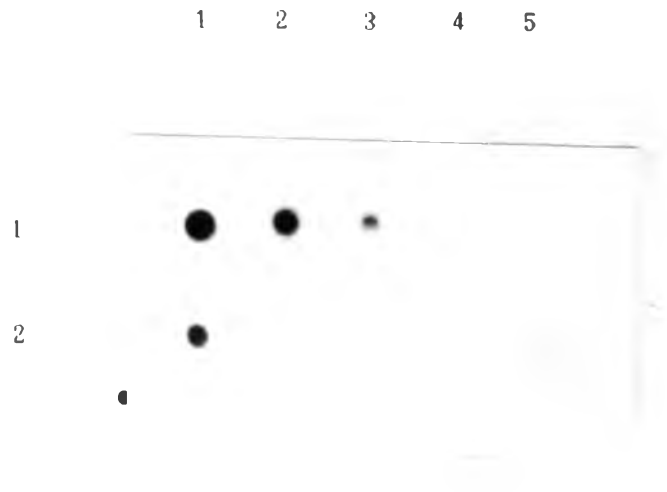


One microliter of each ten-fold serial dilution of Digoxigenated DNA probes was spotted onto the nylon membrane. The signal intensities were visually compared with the labeled control DNA from the kit. The labeled control DNA concentration from 1 ng/μl to 0.1 pg/μl.

row 1 no.1-5 : the labeled control DNA 1 ng/μl to 0.1 pg/μl

row 2 no.1-5 : the labeled *Pst*I-cleaved pCSBC5 inserted CGTase gene fragment 5.2 kb probe

APPENDIX X Estimation of DNA concentration of nonradioactive DIG-labeled probes by chemiluminescent detection

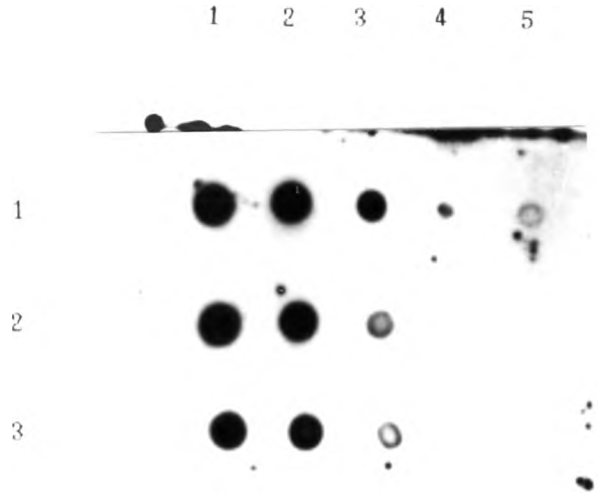


One microliter of each ten-fold serial dilution of Digoxigenated DNA probes was spotted onto the nylon membrane. The signal intensities were visually compared with the labeled control DNA from the kit. The labeled control DNA concentration from 1 ng/μl to 0.1 pg/μl.

row 1 no.1-5 : the labeled control DNA 1 ng/μl to 0.1 pg/μl

row 2 no.1-5 : the labeled 1.7 kb CGTase gene fragment probe

APPENDIX XI Estimation of DNA concentration of nonradioactive DIG-labeled probes by chemiluminescent detection



One microliter of each ten-fold serial dilution of Digoxigenated DNA probes was spotted onto the nylon membrane. The signal intensities were visually compared with the labeled control DNA from the kit. The labeled control DNA concentration from 1 ng/μl to 0.1 pg/μl.

row 1 no.1-5 : the labeled control DNA 1 ng/μl to 0.1 pg/μl

row 2 no.1-5 : the labeled 3 kb CGTase gene fragment probe

row 3 no.1-5 : the labeled 5.2 kb CGTase gene probe

BIOGRAPHY

Miss Vipawan Vitayakritsirikul was born on February 7, 1968 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in MIcrobiology from Chulalongkorn University in 1990