

## รายการอ้างอิง

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ภาคผนวก ก.

น้ำยา สารเคมี และวัสดุอุปกรณ์

1. น้ำยาและสารเคมี

Absolute ethanol	( Merck, Germany )
Agarose ultrapure	( BRL, U.S.A. )
Bromophenol blue	( Sigma, U.S.A. )
Chloroform	( Merck, Germany )
Deoxynucleotide triphosphate ( dNTPs )	( Pronega, U.S.A. )
Ethidium bromide	( Sigma, U.S.A. )
Ethylenediamine tetraacetic acid ( EDTA )	( Sigma, U.S.A. )
Ficoll ( 400 - DL )	( Sigma, U.S.A. )
Glacial acetic acid	( Merck, Germany )
Isoamyl alcohol	( Merck, Germany )
Lambda DNA / Hind III	( BRL, U.S.A. )
2 - Mercaptoethanol	( Sigma, U.S.A. )
Mineral oil	( BRL, U.S.A. )
Phenol ultrapure	( BRL, U.S.A. )
Phi 174 / Hae III	( BRL, U.S.A. )
Primer I 5'-CTT TGT ACT AGG AGG CTG TAG GC-3'	( Synthetic genetics, U.S.A. )



Primer II	5'-GGA GGA GGG CGA AGC CAC ACT-3'	(Synthetic genetics, U.S.A.)
Primer III	5'-GCT TTG GGG CAT GGA CAT TGA-3'	(Synthetic genetics, U.S.A.)
Primer IV	5'-CTA CTA ATT CCC TGG ATG CTG G-3'	(Synthetic genetics, U.S.A.)
Proteinase K		( Amresco, U.S.A. )
Sodium acetate		( Sigma, U.S.A. )
Sodium chloride		( Merck, Germany )
Sodium dodecyl sulfate		( Amresco, U.S.A. )
Sodium hydroxide		( Merck, Germany )
Tag DNA polymerase with 10x buffer		( Promega, U.S.A. )
Tris base		( Sigma, U.S.A. )
Xylene cyanol FF		( Sigma, U.S.A. )
123 bp ladder DNA		( BRL, U.S.A. )



2. วัสดุพลาสติก และเครื่องแก้ว

Beaker	( Pyrex, U.S.A. )
Centrifuge tube 50 ml with screwcap	( Nunc, Denmark )
Cylinder	( Pyrex, U.S.A. )
Erlenmeyer flask	( Pyrex, U.S.A. )
Micro - centrifuge tube 1.5 ml, 0.6 ml	( Elkay, U.S.A. )
Pipette tip 0-200 ul, 200 - 1000 ul	( Elkay, U.S.A. )
Pipette tip with filter 0-200 ul	( BRL, U.S.A. )
Polaroid film type 667	( Berlijucker, U.S.A. )
Serological pipettes	( Pyrex, U.S.A. )

3. เครื่องมือวิทยาศาสตร์

Automatic pipettes	( Gilson, France )
DNA Thermal Cycler 480	( Perkin Elmer Cetus, U.S.A. )
FRC - 10 camera system	( Fotodyne, U.S.A. )
Horizon 58 horizontal gel electrophoresis system	(BRL, U.S.A.)
Microcentrifuge	( Fotodyne, U.S.A. )
Power supply	( BRL, U.S.A. )
UV transilluminator	( Fotodyne, U.S.A. )
Mixer vortex	( Scientific industries, U.S.A.)

ภาคผนวก ข.

สารเคมี และ วิธีการเตรียม

1. 1M Tris pH 7.4, 7.6, 8.0

Tris base	121.1	กรัม
เติมน้ำกลั่น	800	มล.

ปรับ pH เท่าที่ต้องการด้วย conc. HCl ปริมาตร

pH 7.4 เติม conc. HCl 70 มล.

pH 7.6 เติม conc. HCl 60 มล.

pH 8.0 เติม conc. HCl 42 มล.

นำไปทำให้ปราศจากเชื้อด้วยวิธี autoclave ที่อุณหภูมิ 121° ซ. 15 นาที

2. 10% SDS pH 7.2

Sodium dodecyl sulfate	10	กรัม
เติมน้ำกลั่นปลอดเชื้อให้ได้ปริมาตร	100	มล.

ปรับ pH ให้ได้ 7.2



3. 0.5M EDTA pH 8.0

disodium ethylene diamine tetraacetate  $2H_2O_2$       186.1      กรัม  
 ละลายด้วยน้ำกลั่นและปรับปริมาตรให้เป็น      1000      มล.  
 ปรับ pH ให้ได้ 8.0 ด้วย NaOH  
 นำไปทำให้ปราศจากเชื้อด้วยวิธี autoclave ที่อุณหภูมิ 121°C. 15 นาที

4. 3M Sodium acetate pH 5.2

Sodium acetate  $3H_2O$       40.81      กรัม  
 น้ำกลั่น      80      มล.  
 ปรับ pH ด้วย glacial acetic acid  
 เติมน้ำกลั่นให้ได้ปริมาตร      100      มล.  
 นำไปทำให้ปราศจากเชื้อด้วย วิธี autoclave ที่อุณหภูมิ 121°C. 15 นาที

5. 5M NaCl

Sodium chloride      29.22      กรัม  
 ละลายด้วยน้ำกลั่นและปรับปริมาตรให้ได้      100      มล.  
 นำไปทำให้ปราศจากเชื้อด้วย วิธี autoclave ที่อุณหภูมิ 121°C. 15 นาที

6. Saturated phenol in Tris pH 8.0

ละลาย phenol ( nucleic acid grade ) ที่ 65°C.  
 สกัดด้วย 1.0M Tris pH 8.0 หลาย ๆ ครั้ง  
 สกัดด้วย 0.1M Tris pH 8.0 จนกระทั่ง pH ของชั้น aqueous phase > 7.6  
 เก็บในขวดสีน้ำตาล หรือห่อด้วยกระดาษปอนด์

7. 50X Tris - acetate buffer ( TAE )

Trisma base	242	กรัม
Glacial acetic acid	57.1	มล.
0.5M EDTA pH 8.0	100	มล.
เติมน้ำกลั่นครบ	1000	มล.

นำไปทำให้ปราศจากเชื้อด้วย วิธี autoclave ที่อุณหภูมิ 121°C. 15 นาที

8. 10% beta - mercaptoethanol

beta - mercaptoethanol	2	มล.
น้ำกลั่น	18	มล.

9. 10 mg/ml Ethidium bromide

Ethidium bromide	0.1	กรัม
เติมน้ำกลั่นครบ	10	มล.

10. 10X Gel loading buffer Type II

0.25% Bromophenol blue

0.25 Xylene cyanol

25% Ficoll ( type 400 ) in H<sub>2</sub>O

เก็บไว้ที่อุณหภูมิห้อง

11. Chloroform : isoamylalcohol ( 24 : 1 )

Isoamylalcohol	4	มล.
Chloroform	96	มล.

12. TE buffer pH 7.6 ( 10 mM Tris HCl pH 7.6 , 1mM EDTA

1M Tris HCl pH 7.6	1	มล.
0.5M EDTA	0.2	กรัม
น้ำกลั่นที่ปราศจากเชื้อ	98.8	มล.

13. 20 mg/ml Proteinase K

Proteinase K	0.2	กรัม
น้ำกลั่นที่ปราศจากเชื้อ	10	มล.
เก็บไว้ที่ - 20°ซ.		

## ประวัติผู้เขียน

นาย บรรยง คันธวะ เกิดวันที่ 5 กันยายน พ.ศ. 2505 อำเภอเกาะคา จังหวัดลำปาง สำเร็จการศึกษาปริญญาวิทยาศาสตรบัณฑิต (เทคนิคการแพทย์) คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่ ในปีการศึกษา 2528 และเข้าศึกษาต่อในหลักสูตรวิทยาศาสตรมหาบัณฑิต ที่จุฬาลงกรณ์มหาวิทยาลัย เมื่อ พ.ศ. 2533 ปัจจุบันรับราชการที่หน่วยปฏิบัติการกลาง และชันสูตรโรค โรงพยาบาลมหาราชนครเชียงใหม่ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

