APPENDICES

Appendix A: Culture Media

1. Sabouraud Dextrose Agar (SDA)

Dehydrated SDA agar	65.00	g
Deionized H_2O up to	1000.00	ml

Suspension 65 g of the power in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil for 1 min to completely dissolve the powder. Autoclave at 121 ^{o}C 15 lbs for 15 min.

2. Yeast extract peptone dextrose (YEPD) medium

Yeast extract	1.00	g
Peptone	2.00	g
Dextrose	2.00	g
Deionized H_2O up to	100.00	ml

Shake until the solutes have dissolved. Sterilize by autoclaving for 20 min at 15 lbs on liquid cycle.

3. Potato dextrose agar

Potato dextrose agar	39.00	g
Deionized H_2O up to	100.00	ml

Shake until the solutes have dissolved. Sterilize by autoclaving for 20 min at 15 lbs on liquid cycle.

4. Assimilation and fermentation

Basal medium

Bromcresol purple (1.6%)	0.20	ml
0.1 N Sodium hydroxide	1.00	ml
Agar	2.00	gm
Distilled water	90.00	ml

Heat to dissolve

Stock carbohydrate solution

Carbohydrate	1.00	gm
If using raffinose	2.00	gm
Yeast nitrogen base	0.67	gm
Distilled water	10.00	gm

Mix to dissolve, gently heat if necessary

Add the stock carbohydrate solution to melted agar base. Mix well. Adjust to pH 7.0. dispense in 5 ml amount in 16x125 mm. screw cap tubes. Sterilize by autoclaving at 121^{o}C 15 lbs for 10 min. Allow to solidity in a slanted position. Store in refrigerator at 4^{o}C.

5. Urea agar

Solution A

Urea agar base	29.00	gm
Distilled water	100.00	ml

Dissolve powder in water and sterilize by filtration

Solution B

Agar	15.00	gm
Distilled water	900.00	gm

Dissolve agar in water and sterilize by autoclaving at 121 ^{o}C 15 lbs for 15 min. Cool agar to approximately 50^{o}C. Add the 100 ml of sterilize urea agar base. Mix well; dispense aseptically into sterilize tubes. Allow to cool in slanted position to form butt about 1 inch deep and slant approximately 1.5 inches long.

6. RPMI 1640 medium buffered with 0.165 mol/L MOPS, 1L

powdered RPMI 1640 medium (with glutamine and phenol red, without bicarbonate)	10.40	g
MOPS (3-[N-morpholio] propanesulfonic acid) buffer	34.53	g

Dissolve powered medium in 900 ml distilled water. Add MOPS (final concentration of 0.165 mol/L) and stir until dissolved. While stirring, adjust the pH to 7.0 at 25^{o}C using 1 mol/L sodium hydroxide. Add additional water to bring medium to a final volume of 1 L. Filter sterilize and store at 4^{o}C until use.

Appedix B: Reagents and buffers

1. 500 mM Ethylene diaminetetra acetic acid (EDTA)

EDTA (Sodium salt, dehydrate)	18.61	gm
Distilled water	100.00	ml

2. 0.85% Normal saline solution

Sodium chloride	0.85	gm
Distilled water	100.00	ml

Autoclave for 15 min at 121 ^{o}C. and keep in room's temperature

3. 10% Sodium dodecyl sulfate (SDS)

SDS	1.0	gm
Distilled water	10.00	ml

Stores at room's temperature and should be prepared fresh weekly.

4. 50X Tris-Acetate- EDTA (TAE) electrophoresis buffer

2M Tris base	243.00	gm
1M Acetic acid	60.00	gm
0.1 M EDTA	37.20	ml
Distilled water	1000.00	ml

Dissolve and adjust the pH to 8.0. Store at 4^{o}C.

5. 10X Tris EDTA (TE) buffer

1 M Tris chloride (pH 8.0)	100.00	ml
0.5 M EDTA	20.00	ml
Distilled water	1000.00	ml

6. 1 M Tris chloride

Tris chloride (pH 8.0)	121.10	gm
Distilled water	1000.00	ml

Dissolve 121.1 gm of Tris base in 800 ml of distilled water. Adjust the pH to 8.0. Adjust the volume of the solution to 1 liter with distilled water. Sterilize by autoclaving at 121 ^{o}C 15 lbs for 15 min.

Appendix C: Properties of amino acid

1. Abbreviations for amino acid

Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

2. The codon dictionary.

First Position	Second Position: U	Second Position: C	Second Position: A	Second Position: G	Third Position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met (start)	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

The third nucleotide is less specific than the first two, and codon are read in 5’ to 3’ direction.

Appendix D: Chromatogram of nucleotide sequence

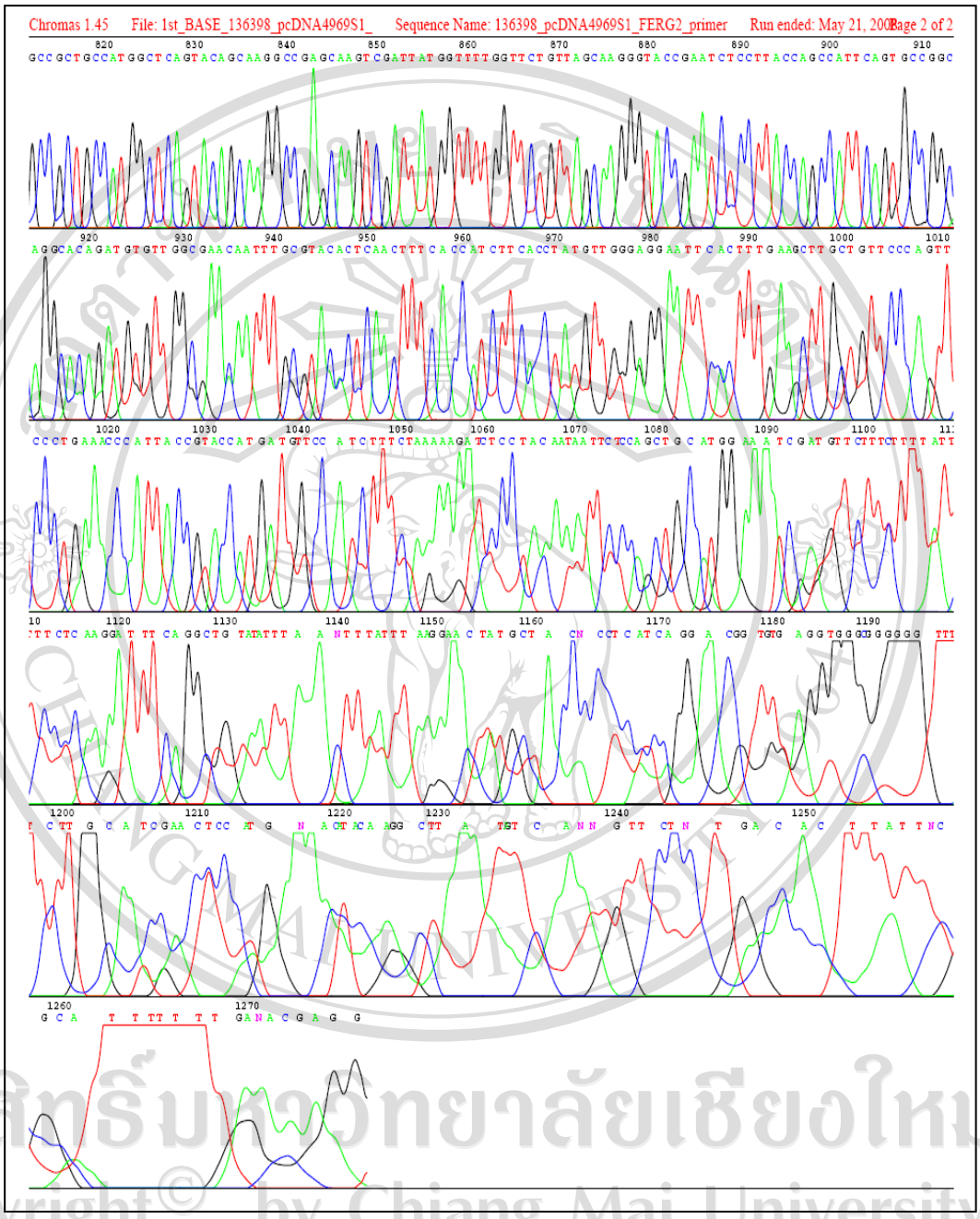

Figure 27. A cDNA entire sequence of the ERG11 protein coding region of *C. neoformans* CN4969S isolate by FERG2 primer.

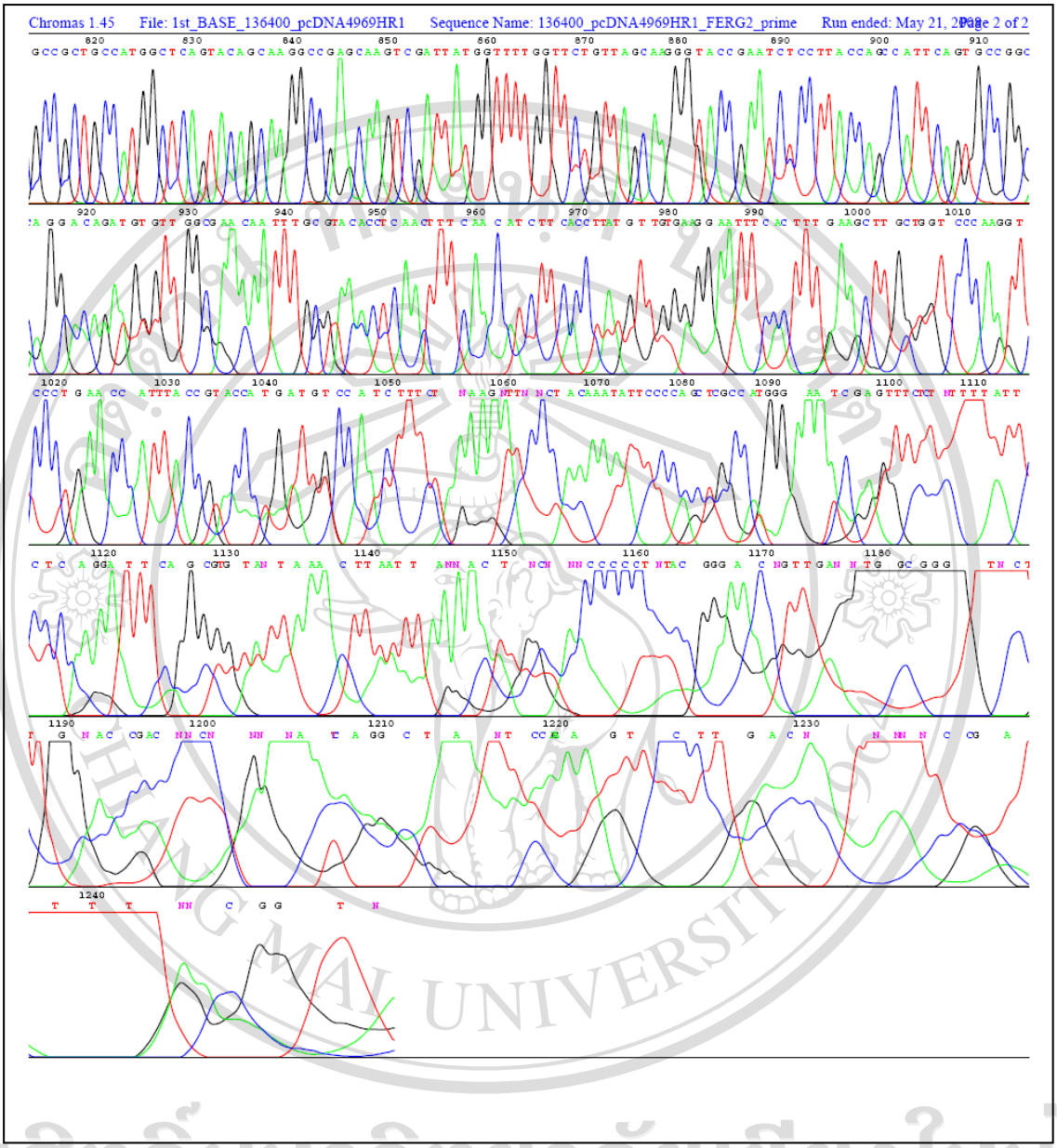

Figure 28. A cDNA entire sequence of the ERG11 protein coding region of *C. neoformans* CN4969HR isolate by FERG2 primer.

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