APPENDICES

APPENDIX A

CHEMICALS AND REAGENTS

Chemical or Reagent	Source
1 Kb Plus DNA Ladder™	Gibco BRL, NY, USA
Acetic acid, glacial	Merck, Darmstadt, Germany
Agar granulated	BBL, MI, USA
Ammonium acetate	Fluka, Buchs, Switzerland
Ampicillin	Sigma, MO, USA
Boric acid	Merck, Darmstadt, Germany
Bromophenol blue	Merck, Darmstadt, Germany
D (+) Glucose	Fluka, Buchs, Switzerland
Disodium Ethylenediaminetetra acetate·$2H_2O$	Amresco, OH, USA
Ethidium bromide	Sigma, MO, USA
Ethyl alcohol	Merck, Darmstadt, Germany
FACS Lysing Solution	Becton Dickinson, CA, USA
Glycerol	Sigma, MO, USA
High Pure PCR Template Preparation Kit	Roche Molecular Biochemicals, Mannheim, Germany
High Pure Plasmid Isolation Kit	Roche Molecular Biochemicals Mannheim, Germany
*Hinc*II restriction endonuclease	Promega, USA
Hydrochloric acid	Merck, Darmstadt, Germany
Isoprep	Robbins Scientific co., CA, USA
Isopropanol	Merck, Darmstadt, Germany

LE agarose	Seakem-BMA, ME, USA
Magnesium chloride hexahydrate	Merck, Darmstadt, Germany
Magnesium sulfate heptahydrate	Merck, Darmstadt, Germany
MinElute Gel Extraction Kit	Qiagen, Germany
pGEM®-T Easy Vector Kit	Promega, USA
Platinum® *Taq* DNA Polymerase High Fidelity	Invitrogen, USA
Potassium acetate	Merck, Darmstadt, Germany
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Sigma, MO, USA
Sodium hydrogen phosphate	Merck, Darmstadt, Germany
Sodium hydroxide	Merck, Darmstadt, Germany
SureFill™ 6% Sequencing gel	Visible Genetic, USA
Taq DNA polymerase	Promega, USA
Thermo Sequenase Cy5 Dye Terminator Cycle Sequencing Kit	Amersham Bioscience, England
Tris base	Sigma, MO, USA
Tryptone	Difco Laboratories, MI, USA
Yeast extract	Gibco, NY, USA

APPENDIX B

INSTRUMENTS

Instrument-model	Source
Analytical balance	Sartorius, Germany
Biological safety cabinet class II	Gelman Sciences, Australia
Dry Block Heater	Biosan Laboratory Inc., USA
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
Gel Toaster™ unit	Visible Genetic, USA
GeneObjects™ software	Visible Genetic, USA
Hemacytometer counting chamber	Improved neubauer, USA
Horizontal Gel Electrophoresis System	BRL, USA
Hot Air Oven	Sheldon, USA
Incubator shaker	Amerex Instruments Inc., USA
Light microscope	Olympus, Japan
Long-Read Tower™ sequencer	Visible Genetic, USA
MicroCel 700 Cassette	Visible Genetic, USA
Refrigerated centrifuge	Sorvall, Germany
Refrigerated microcentrifuge	Sorvall, Germany
Refrigerator (4°C)	Sanyo, Thailand
Refrigerator (-20°C)	Sanyo, Thailand
Refrigerator (-70°C)	Kelvinator Scientific, USA
pH Meter	Pierce, USA
Photo Documentation	Fotodyne, USA

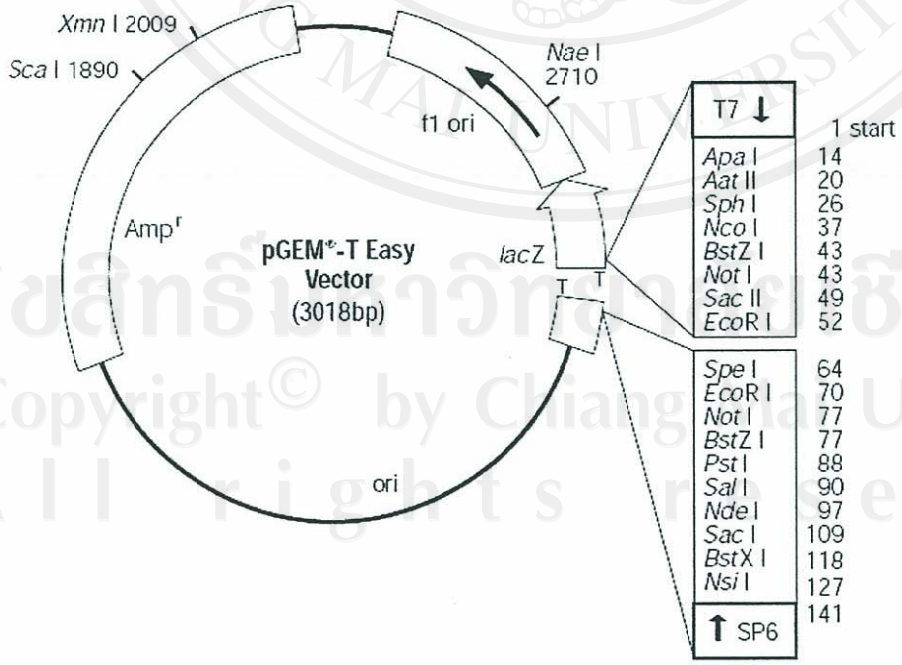
Primer Premier version 5.00 software	PREMIER Biosoft International, CA, USA
Sero-fuge centrifuge	Beckton Dickinson, USA
Spectrophotometer UV1201	Shimadzu Co., Japan
SureFill™ Injector	Visible Genetic, USA
Thermal Cycler GeneAmp® PCR system 2700	Applied Biosystems, USA
Ultraviolet transilluminator	Vilber Lourmat, France
Vortex Mixer	Scientific Industries, USA
Water bath	Sheldon, USA

APPENDIX C

pGEM®-T Easy Vector

The pGEM®-T Easy Vector System is a convenient system for the cloning of PCR products. The vector is prepared by cutting pGEM®-T Easy Vectors with *EcoR* V at base 60 of the sequence and adding a 3′ terminal thymidine to both ends. These single 3′-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product into the plasmids by preventing recircularization of the vector and providing a compatible overhang for PCR products generated by certain thermostable polymerases. These polymerases often add a single deoxyadenosine, in a template-independent fashion, to the 3′-ends of the amplified fragments.

1. pGEM®-T Easy Vector sequence circle map



2. pGEM®-T Easy Vector Sequence reference points

T7 RNA Polymerase transcription initiation site	1
SP6 RNA Polymerase transcription initiation site	141
T7 RNA Polymerase promoter	3002-6
SP6 RNA Polymerase promoter	136-158
multiple cloning site	10-128
lacZ start codon	180
lacoperon sequences	2839-2999, 166-395
lacoperator	100-216
b-lactamase coding region	1337-2197
phage f1 region	2383-2838
binding site of pUC/M13 Forward Sequencing Primer	2959-2975
binding site of pUC/M13 Reverse Sequencing Primer	176-192

3. pGEM®-T Easy Vector Sequence

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   1 GGGCGAATTG GGCCCGACGT CGCATGCTCC CGGCCGCCAT GGCGGCCGCG
  51 GGAATTCGAT*ATCACTAGTG AATTCGCGGC CGCCTGCAGG TCGACCATAT
 101 GGGAGAGCTC CCAACGCGTT GGATGCATAG CTTGAGTATT CTATAGTGTC
 151 ACCTAAATAG CTTGGCGTAA TCATGGTCAT AGCTGTTTCC TGTGTGAAAT
 201 TGTTATCCGC TCACAATTCC ACACAACATA CGAGCCGGAA GCATAAAGTG
 251 TAAAGCCTGG GGTGCCTAAT GAGTGAGCTA ACTCACATTA ATTGCGTTGC
 301 GCTCACTGCC CGCTTTCCAG TCGGGAAACC TGTCGTGCCA GCTGCATTAA
 351 TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GGCGCTCTTC
 401 CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCGG CTGCGGCGAG
 451 CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG
 501 GATAACGCAG GAAAGAACAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA
 551 CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC CGCCCCCCTG
 601 ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA
 651 GGACTATAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC
 701 TCCTGTTCCG ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT
 751 CGGGAAGCGT GGCGCTTTCT CATAGCTCAC GCTGTAGGTA TCTCAGTTCG
 801 GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA
 851 GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG
 901 TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC
 951 AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA
1001 CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC
1051 CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC
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1101 ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG
1151 AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG
1201 CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA
1251 AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC
1301 AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA
1351 TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT
1401 GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC
1451 TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG
1501 ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT
1551 CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC
1601 TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGGCATTG
1651 CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC
1701 TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA
1751 AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG
1801 CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT
1851 GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA
1901 GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT
1951 CAATACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC
2001 ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT
2051 GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT
2101 CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT
2151 GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT
2201 CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA
2251 GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG
2301 CGCACATTTC CCCGAAAAGT GCCACCTGTA TGCGGTGTGA AATACCGCAC
2351 AGATGCGTAA GGAGAAAATA CCGCATCAGG CGAAATTGTA AACGTTAATA
2401 TTTTGTTAAA ATTCGCGTTA AATATTTGTT AAATCAGCTC ATTTTTTAAC
2451 CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGACCGA
2501 GATAGGGTTG AGTGTTGTTC CAGTTTGGAA CAAGAGTCCA CTATTAAAGA
2551 ACGTGGACTC CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC
2601 CCACTACGTG AACCATCACC CAAATCAAGT TTTTTGCGGT CGAGGTGCCG
2651 TAAAGCTCTA AATCGGAACC CTAAAGGGAG CCCCCGATTT AGAGCTTGAC
2701 GGGGAAAGCC GGCGAACGTG GCGAGAAAGG AAGGGAAGAA AGCGAAAGGA
2751 GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC GCGTAACCAC
2801 CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC ATTCGCCATT
2851 CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT
2901 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA
2951 ACGCCAGGGT TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT
3001 GTAATACGAC TCACTATA
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* Cloned insert site indicated by an asterisk

APPENDIX D

NUCLEIC ACID AND AMINO ACID SYMBOL

1. Nucleic acid symbol

Nucleic acid	Symbol
Adenine	A
Cytocine	C
Guanine	G
Inosine	I
Thymine	T
Uracil	U
A + C	M
A + G	R
A + T	W
C + G	S
C + T	Y
G + T	K
A + C + G	V
A + C + T	H
A + G + T	D
C + G + T	B
A + C + G + T	N

2. Amino acid symbol

Amino acid	Three letter abbreviation	One letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
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

APPENDIX E

REAGENTS AND BUFFERS PREPARATION

1. Commonly used stock solutions

1 M Tris-HCl (pH 8.0)

Tris base 121.1 g

- Dissolve in 800 ml distilled water
- Adjust the pH to 8.0 by adding concentrated HCl
- Adjust the volume to 1 liter with distilled water
- Sterilize by autoclaving and store at room temperature

0.5 M EDTA (pH 8.0)

Disodium Ethylenediaminetetra acetate·$2H_2O$ 186.1 g

- Dissolve in 800 ml distilled water
- Adjust the pH to 8.0 by adding NaOH
- Adjust the volume to 1 liter with distilled water
- Sterilize by autoclaving and store at room temperature

5 M Potassium acetate (pH 7.5)

Potassium acetate 49.1 g

- Dissolve in 90 ml distilled water
- Adjust the pH to 7.5 by adding acetic acid
- Adjust the volume to 100 ml with distilled water
- Sterilize by autoclaving and store at 4°C

10 % SDS

Sodium dodecyl sulfate 1 ml

Distilled water 100 ml

2. Reagents for isolation of the PBMCs

Phosphate buffer saline (PBS) pH 7.2

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g

- Dissolve in 800 ml distilled water
- Adjust the pH to 7.2 with HCl and fill up distilled water to 1 liter
- Sterilize by autoclaving and store at room temperature

TE buffer (pH 8.0)

Tris-HCl (pH 8.0)	10 mM
EDTA (pH 8.0)	1 mM

- Sterilize by autoclaving and store at room temperature

3. Reagents for Electrophoresis

50X Tris-acetate Buffer (TAE)

Tris base	242 g
Glacial acetic acid	57.1 ml
0.5 M EDTA (pH 8.0)	100 ml

- Dissolve in 1 liter distilled water
- Sterilize by autoclaving and store at room temperature

5X Tris-borate Buffer (TBE)

Tris base	54 g
Boric acid	27.5 g
0.5 M EDTA (pH 8.0)	20 ml

- Dissolve in 1 liter distilled water
- Sterilize by autoclaving and store at room temperature

6X gel loading buffer

Bromophenol blue	0.25 %
Glycerol	30 %

- Mixed thoroughly and keep at -20°C

10 mg/ml Ethidium bromide

Ethidium bromide	1 g

- Dissolve in 100 ml distilled water
- Stir on a magnetic stirrer to ensure that the dye has dissolved
- Wrap the container in aluminum foil and store at room temperature

1% agarose gel

LE agarose	1 g
0.5X TAE	100 ml

2% agarose gel

LE agarose	2 g
0.5X TAE	100 ml

3% agarose gel

LE agarose	3 g
0.5X TAE	100 ml

1 Kb Plus DNA Ladder

1 µg/µl 1 Kb Plus DNA Ladder™ (Gibco BRL, USA)	5 µl
6X gel loading buffer	10 µl
Distilled water	45 µl

4. Reagents for the cloning of PCR products

2 M Mg^{2+} stock

$MgCl_2 \cdot 6H_2O$	20.33 g
$MgSO_4 \cdot 7H_2O$	24.65 g

- Dissolved in 100 ml distilled water
- Sterilize by filtration and store at room temperature

2 M glucose

Glucose	36 g
Distilled water	100 ml

- Sterilize by filtration

SOC medium

Tryptone	2.0 g
Yeast extract	0.5 g
1 M NaCl	1 ml
1 M KCl	0.25 ml
2 M Mg^{2+} stock	1 ml
2 M glucose	1 ml

- Add tryptone, yeast extract, NaCl and KCl to 97 ml distilled water and stir to dissolve
- Autoclave and cool to room temperature
- Add 2M Mg^{2+} stock and 2M glucose, each to a final concentration of 20 mM
- Fill up sterile distilled water to 100 ml
- Sterilize by passage the complete medium through a 0.2 µm filter unit and store at 4°C

LB medium

Tryptone	10 g
Yeast extract	5 g
NaCl	5 g

- Dissolved in 800 ml distilled water
- Adjust pH to 7.0 with NaOH and fill up distilled water to 1 liter
- Sterilize by autoclaving and store at 4°C

LB plates with ampicillin

Agar granulated	15 g
LB medium	1 litter

- Sterilize by autoclaving and allow the medium to cool to 50°C
- Add ampicillin to a final concentration of 100 µg/ml
- Pour 30-35 ml of medium into 85 mm Petri dishes and let the agar harden
- Store at 4°C for up to 1 month

5. Reagent for minipreparations of plasmid DNA

Solution I

Glucose	50 mM
Tris-HCl (pH 8.0)	25 mM
EDTA (pH 8.0)	10 mM

- Sterilize by autoclaving and store at 4°C

Solution II

NaOH (freshly diluted from 10N stock)	0.2 N
SDS	1 %

Solution III

5M Potassium acetate	60 ml
Glacial acetic acid	11.5 ml
Distilled water	28.5 ml

7.5 M Ammonium acetate

Ammonium acetate	77.75 g
Distilled water	100 ml

- Sterilize by filtration and store at 4°C

70 % ethanol

Absolute ethanol	70 ml

- Add distilled water up to 100 ml

CURRICULUM VITAE

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Education

1996 Certificate of senior high school
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Publications

Samleerat, T. Role of Cytokines/Chemokines in AIDS: Disease Progression and Resistance (Review). Bull Chiang Mai Assoc Med Sci 2001; 34(2): 107-117.

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