CHAPTER V

Result

1. Identification of uridine phosphorylase gene

From the bioinformatics underlying NCBI resources, we had selected model organisms to study on uridine phosphorylase nucleotide and amino acid sequences. The enzymes, including *M. musculus* uridine phosphorylase (GenBank accession number : A57501, 311 amino acids) and *E. coli* uridine phosphorylase (GenBank accession number : P12758, 253 amino acids), were identified for their amino acid sequences (Figures 5-1,5-2).

Each amino acid sequence was analyzed for sequence homology by the BLAST program. Only *E. coli* uridine phosphorylase was found to be similar to *P. falciparum* sequence with about 46 % homology, but *M. musculus* uridine phosphorylase had no significant similarity in the genome database (Figure 5-3).

The similar sequence of *P. falciparum* was identified on chromosome 7 at the position 47423 bp to 49214 bp with 28 % identity and chromosome 5 at the position 569899 bp to 569198 bp with 28 % identity to the *E. coli* amino acid sequence (Figure 5-4). These two sequences were then identified for the open reading frame (ORF). The open reading frame was consisted of 735 bp, no intron, start at position 983 bp and stop at position 1720 bp of the fragment on the chromosome 5 (Figure 5-5). The deduced

amino acid sequence of the *P. falciparum* uridine phosphorylase contained 245 amino acids and had a molecular mass of 28 kDa.

Figure 5-1 Amino acid sequence of *M. musculus* uridine phosphorylase

```
A57501. uridine phosphory...
[gi:1363252]
LOCUS       A57501                    311 aa            linear   ROD 20-JUN-2000
DEFINITION  uridine phosphorylase (EC 2.4.2.3) I - mouse.
ACCESSION   A57501
VERSION     A57501  GI:1363252
DBSOURCE    pir: locus A57501;
            summary: #length 311 #molecular-weight 34056 #checksum 2858;
            PIR dates: 08-Dec-1995 #sequence_revision 08-Dec-1995 #text_change
            20-Jun-2000.
KEYWORDS    glycosyltransferase; pentosyltransferase.
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 311)
  AUTHORS   Watanabe,S., Hino,A., Wada,K., Eliason,J.F. and Uchida,T.
  TITLE     Purification, cloning, and expression of murine uridine
            phosphorylase
  JOURNAL   J. Biol. Chem. 270 (20), 12191-12196 (1995)
  MEDLINE   95263571
   PUBMED   7744869
FEATURES             Location/Qualifiers
     source          1..311
                     /organism="Mus musculus"
                     /db_xref="taxon:10090"
     Protein         1..311
                     /product="uridine phosphorylase I"
                     /EC_number="2.4.2.3"
ORIGIN
        1 maatgteakd lenhhndcfi qlsnpniaam kedvlyhfnl ststhdfpam fgdvkfvcvg
       61 gsssrmntfi kyvaaelgld hpgkeypnic agtdryamyk agpvlsvshg mgipsigiml
      121 helikmlyha rcsnitiiri gtsggiglep gsvvitqqav necfkpefeq ivlgkrvirn
      181 tnldaqlvqe lvqcssdlne fpmvvgntmc tldfyegqgr ldgalcsyte kdkqsylraa
      241 haagvrniem essvfatmcg acglkaavvc vtlldrlqgd qintphdvlv eyqqrpqrlv
      301 ghfikkslgr a
```

Figure 5-2 Amino acid sequence of *E. coli* uridine phosphorylase.

```
LOCUS       UDP_ECOLI                253 aa            linear   BCT 15-JUN-2002
DEFINITION  Uridine phosphorylase (UDRPase).
ACCESSION   P12758
VERSION     P12758  GI:136740
DBSOURCE    swissprot: locus UDP_ECOLI, accession P12758;
            class: standard.
            created: Oct 1, 1989.
            sequence updated: Oct 1, 1993.
            annotation updated: Jun 15, 2002.
            xrefs: gi: 43241, gi: 43242, gi: 836656, gi: 148229, gi: 2367306,
            gi: 1790265, gi: 78925, gi: 421186
            xrefs (non-sequence databases): SWISS-2DPAGE P12758, EcoGene
            EG11045, InterPro IPR000845, Pfam PF01048, ProDom PD003928, PROSITE
            PS01232
KEYWORDS    Transferase; Glycosyltransferase; Complete proteome.
SOURCE      Escherichia coli.
  ORGANISM  Escherichia coli
            Bacteria; Proteobacteria; gamma subdivision; Enterobacteriaceae;
            Escherichia.
FEATURES             Location/Qualifiers
     source          1..253
                     /organism="Escherichia coli"
                     /db_xref="taxon:562"
     gene            1..253
                     /gene="UDP"
                     /note="B3831"
     Protein         1..253
                     /gene="UDP"
                     /product="Uridine phosphorylase"
                     /EC_number="2.4.2.3"
ORIGIN
 >E.coli
      MSKSDVFHLG LTKNDLQGAT LAIVPGDPDR VEKIAALMDK PVKLASHREF TTWRAELDGK
      PVIVCSTGIG GPSTSIAVEE LAQLGIRTFL RIGTTGAIQP HINVGDVLVT TASVRLDGAS
      LHFAPLEFPA VADFECTTAL VEAAKSIGAT THVGVTASSD TFYPGQERYD TYSGRVVRHF
      KGSMEEWQAM GVMNYEMESA TLLTMCASQG LRAGMVAGVI VNRTQQEIPN AETMKQTESH
      AVKIVVEAAR RLL
```

Figure 5-3 BLAST result of *M. musculus* uridine phosphorylase

Query= M.musculus , 311 bases, B2A checksum.
(311 letters)

Database: closure status sequence from chromosome 1 (Sanger) 20011015; Complete sequence from chromosome 2 (TIGR submission in Genbank); P. falciparum 3D7 chromosome 3 complete sequence (Sanger Centre submission in GenBank); closure status sequence from chromosome 4 (Sanger) 20011015; finished status sequence from chromosome 5 (Sanger) 20011015; closure status sequence from chromosome 6 (Sanger) 20011015; closure status sequence from chromosome 7 (Sanger) 20011015; closure status sequence from chromosome 8 (Sanger) 20011015; closure status sequence from chromosome 9 (Sanger) 20011015; P. falciparum June 04-2001 data freeze assembled sequence for chromosome 10 (TIGR) 010601; P. falciparum June 04-2001 data freeze assembled sequence for chromosome 11 (TIGR) 010601; P. falciparum closure status sequence for chromosome 12 (Stanford) 010524; closure status sequence from chromosome 13 (Sanger) 20011015; P. falciparum June 04-2001 data freeze assembled sequence for chromosome 14 (TIGR) 010601; closure status UNASSIGNED sequence from chromosome 6,7,8 (BLOB) (Sanger) 20011015
1262 sequences; 26,748,290 total letters

No significant similarity found. For reasons why, **click here**.

Figure 5-4 BLAST result of *E. coli* uridine phosphorylase

```
                                                                    Score     E
Sequences producing significant alignments:                         (bits) Value

gnl|pf7|Sanger_BLOB3.0.000068  (63626 bp, 1981 reads) Plasmo...     100    1e-21
gnl|pf5|Sanger_new  Plasmodium falciparum 3D7 finished statu...     100    1e-21
```

Alignments

```
>gnl|pf7|Sanger_BLOB3.0.000068 (63626 bp, 1981 reads) Plasmodium falciparum 3D7
closure status
            sequence from chromosome 7
            ftp://ftp.sanger.ac.uk/pub/pathogens/malaria2/unfinished_c
            ontigs/MAL7.fasta.151001.gz
          Length = 59147

 Score = 99.8 bits (247), Expect = 1e-21
 Identities = 70/249 (28%), Positives = 116/249 (46%), Gaps = 4/249 (1%)
 Frame = +3

Query: 8     HLGLTKNDLQGATLAIVPGDPDRVEKIAALMDKPVKLASHREFTTWRAELDGKPVIVCST 67
              HL ++K  +    L +  GDP RV+KI  + D  V LA +RE+ +      G+  +  S
Sbjct: 48423 HLKISKEQITPVVLVV--GDPGRVDKIKVVCDSYVDLAYNREYKSVECHYKGQKFLCVSH 48596

Query: 68    GIGGPSTSIAVEELAQLGIRTFLRIGTTGAIQPH-INVGDVLVTTASVRLDGASLHFAPL 126
              G+G    ++  EEL Q G +  +R G+ G++QP  I  GD+ +  A+VR D  S
Sbjct: 48597 GVGSAGCAVCFEELCQNGAKVIIRAGSCGSLQPDLIKRGDICICNAAVREDRVSHLLIHG 48776

Query: 127   EFPAVADFECTTALVEAAKSIGATTHVGVTASSDTFYPGQERYDTYSGRVVRHFKGSMEE 186
              +FPAV DF+    L + A+ +      G++ SSD +YP             +     +E+
Sbjct: 48777 DFPAVGDFDVYDTLNKCAQELNVPVFNGISVSSDMYYPN------------KIIPSRLED 48920

Query: 187   WQAMGVMNYEMESATLLTMCASQGLRAG---MVAGVIVNRTQQEIPNAETMKQTESHAVK 243
              +        EME ATL+ +   + ++ G   +V G      + +  N     Q E + +K
Sbjct: 48921 YSKANAAVVEMELATLMVIGTLRKVKTGGILIVDGCPFKWDEGDFDNNLVPHQLE-NMIK 49097

Query: 244   IVVEAARRL 252
              I + A  +L
Sbjct: 49098 IALGACAKL 49124
```

Figure 5-4 BLAST result of *E. coli* uridine phosphorylase (cont.)

Alignments

```
>gnl|pf5|Sanger_new Plasmodium falciparum 3D7 finished status sequence from chromosome
5
             ftp://ftp.sanger.ac.uk/pub/pathogens/malaria2/unfinished_co
             ntigs/MAL5.fin.151001.gz
          Length = 1343552

 Score = 99.8 bits (247), Expect = 1e-21
 Identities = 70/249 (28%), Positives = 116/249 (46%), Gaps = 4/249 (1%)
 Frame = -2

Query: 8      HLGLTKNDLQGATLAIVPGDPDRVEKIAALMDKPVKLASHREFTTWRAELDGKPVIVCST 67
               HL ++K  +    L +  GDP RV+KI  + D  V LA +RE+ +      G+  +  S
Sbjct: 569899 HLKISKEQITPVVLVV--GDPGRVDKIKVVCDSYVDLAYNREYKSVECHYKGQKFLCVSH 569726

Query: 68     GIGGPSTSIAVEELAQLGIRTFLRIGTTGAIQPH-INVGDVLVTTASVRLDGASLHFAPL 126
               G+G    ++  EEL Q G +  +R G+ G++QP  I  GD+ +  A+VR D  S
Sbjct: 569725 GVGSAGCAVCFEELCQNGAKVIIRAGSCGSLQPDLIKRGDICICNAAVREDRVSHLLIHG 569546

Query: 127    EFPAVADFECTTALVEAAKSIGATTHVGVTASSDTFYPGQERYDTYSGRVVRHFKGSMEE 186
               +FPAV DF+    L + A+ +      G++ SSD +YP              +     +E+
Sbjct: 569545 DFPAVGDFDVYDTLNKCAQELNVPVFNGISVSSDMYYPN------------KIIPSRLED 569402

Query: 187    WQAMGVMNYEMESATLLTMCASQGLRAG---MVAGVIVNRTQQEIPNAETMKQTESHAVK 243
               +        EME ATL+ +   + ++ G   +V G      + +  N     Q E + +K
Sbjct: 569401 YSKANAAVVEMELATLMVIGTLRKVKTGGILIVDGCPFKWDEGDFDNNLVPHQLE-NMIK 569225

Query: 244    IVVEAARRL 252
               I + A  +L
Sbjct: 569224 IALGACAKL 569198
```

Figure 5-5 Open reading frame of the candidate uridine phosphorylase gene in *P. falciparum*

```
ORF Finder (Open Reading Frame Finder)
Length: 245 aa

    983 atggataatcttttacgccatttaaaaataagcaaggaacaaata
        M  D  N  L  L  R  H  L  K  I  S  K  E  Q  I
   1028 acaccagttgttttagttgtaggagatccaggaagagtcgacaag
        T  P  V  V  L  V  V  G  D  P  G  R  V  D  K
   1073 ataaaagtggtatgtgattcatatgttgatttagcatacaacaga
        I  K  V  V  C  D  S  Y  V  D  L  A  Y  N  R
   1118 gaatacaaaagtgtagaatgtcattataagggtcagaaatttttta
        E  Y  K  S  V  E  C  H  Y  K  G  Q  K  F  L
   1163 tgtgttagtcacggtgtaggttcagcaggatgtgctgtatgtttt
        C  V  S  H  G  V  G  S  A  G  C  A  V  C  F
   1208 gaagaattatgtcaaaatggagctaaagtaattattcgtgcaggt
        E  E  L  C  Q  N  G  A  K  V  I  I  R  A  G
   1253 tcatgtggatctcttcaaccagatttaataaaaagaggtgacata
        S  C  G  S  L  Q  P  D  L  I  K  R  G  D  I
   1298 tgtatatgtaatgcagctgttagggaagatagagtatctcattta
        C  I  C  N  A  A  V  R  E  D  R  V  S  H  L
   1343 ttaattcatggagatttcccagctgttggtgattttgatgtttat
        L  I  H  G  D  F  P  A  V  G  D  F  D  V  Y
   1388 gatactttaaataaatgtgcacaagaattgaatgtgccagttttt
        D  T  L  N  K  C  A  Q  E  L  N  V  P  V  F
   1433 aatggtatcagtgtttcatcagatatgtattatcccaataaaatt
        N  G  I  S  V  S  S  D  M  Y  Y  P  N  K  I
   1478 attccttcaagattagaagattattctaaagctaatgctgctgtt
        I  P  S  R  L  E  D  Y  S  K  A  N  A  A  V
   1523 gttgaaatggaactagccactcttatggttattggaaccttaaga
        V  E  M  E  L  A  T  L  M  V  I  G  T  L  R
   1568 aaagttaaaacaggtggtattcttattgttgatggatgtccattc
        K  V  K  T  G  G  I  L  I  V  D  G  C  P  F
   1613 aaatgggacgaaggggatttcgacaacaatttagttcctcaccaa
        K  W  D  E  G  D  F  D  N  N  L  V  P  H  Q
   1658 ttagaaaatatgattaaaatagccttaggagcatgtgcaaaatta
        L  E  N  M  I  K  I  A  L  G  A  C  A  K  L
   1703 gcaaccaaatatgcctaa 1720
        A  T  K  Y  A
```

2. Synthesis of candidate DNA by PCR.

Primers for DNA amplification by PCR were designed by using the data from the open reading frame and addition of restriction sites of restriction enzymes, *Bam*HI and *Sac*I, into the forward and reverse primers, respectively (Figure 5-6).

SK13 forward primer ;

5' TCT GGA TCC* ATG GAT AAT CTT TTA CGC CAT 3'

SK14 reverse primer ;

5' TTC GAG CTC** GGC ATA TTT GGT TGC TAA TTT 3'

Figure 5-6 Primer design for DNA amplification by PCR

* is the restriction site for *Bam*HI

** is the restriction site for *Sac*I

The DNA fragment of *P. falciparum* uridine phosphorylase gene was generated by PCR using primer, SK13 and SK14, to prime the reaction. The PCR product was approximately 735 bp (Figure 5-7).

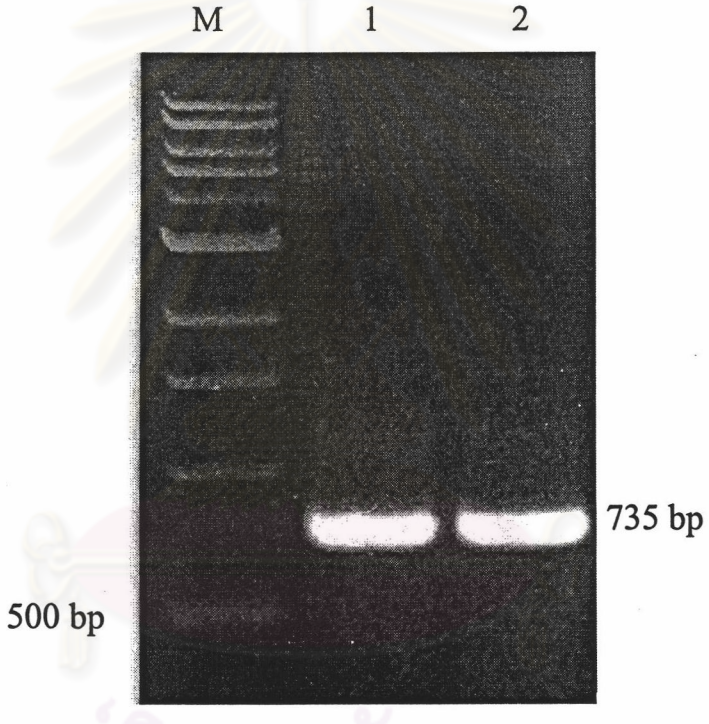

Figure 5-7 PCR amplification of fragment of the candidate gene (*P. falciparum* uridine phosphorylase gene). Numbers on the left and the right sides of the figure are molecular sizes. Lane M is molecular size marker. Lanes 1-2 are PCR products of the reaction.

3. Cloning of the PCR product into the plasmid pDrive

The PCR fragment was cloned into the plasmid pDrive cloning vector (Figure 5-8). Seven colonies were selected for restriction analysis. The plasmids prepared by rapid alkaline minipreparation were digested with *Bam*HI and *Sac*I. Six clones carrying the insert of about 735 bp were identified, as clones 2-7 (Figure 5-9). The nucleotide sequence of clone 2 was determined.

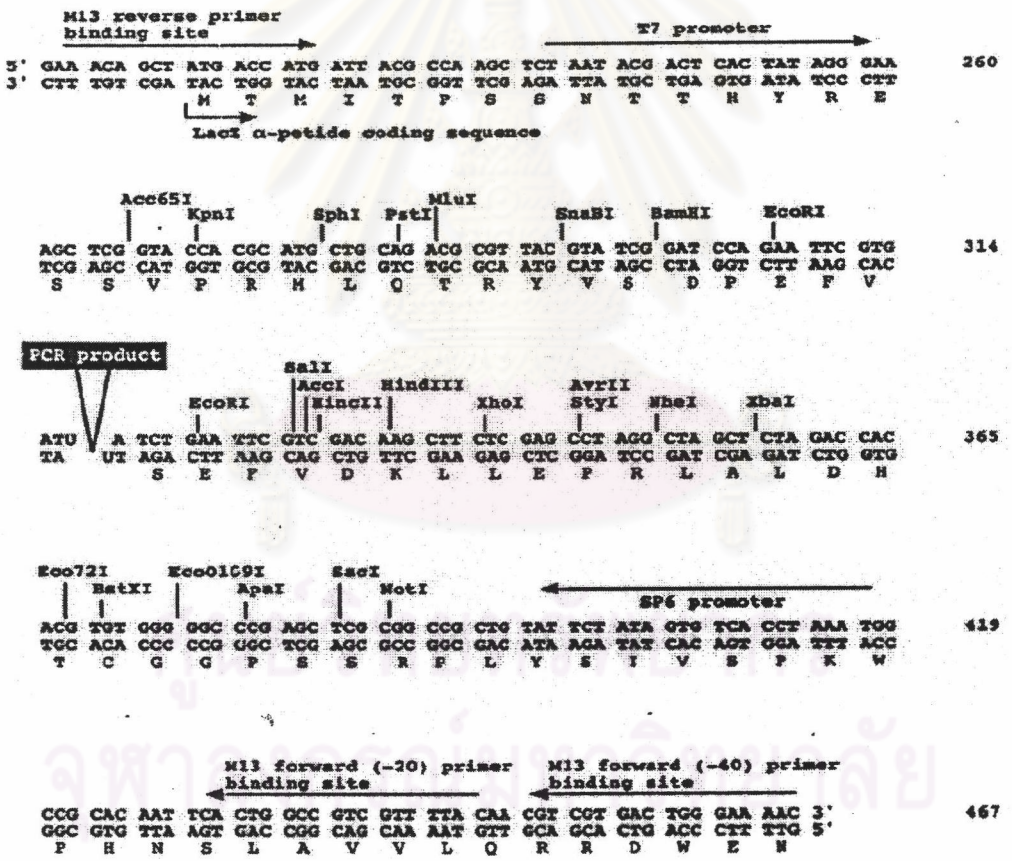

Figure 5-8 Construction of pDrive cloning vector inserted with the PCR fragment of *P. falciparum* uridine phosphorylase gene.

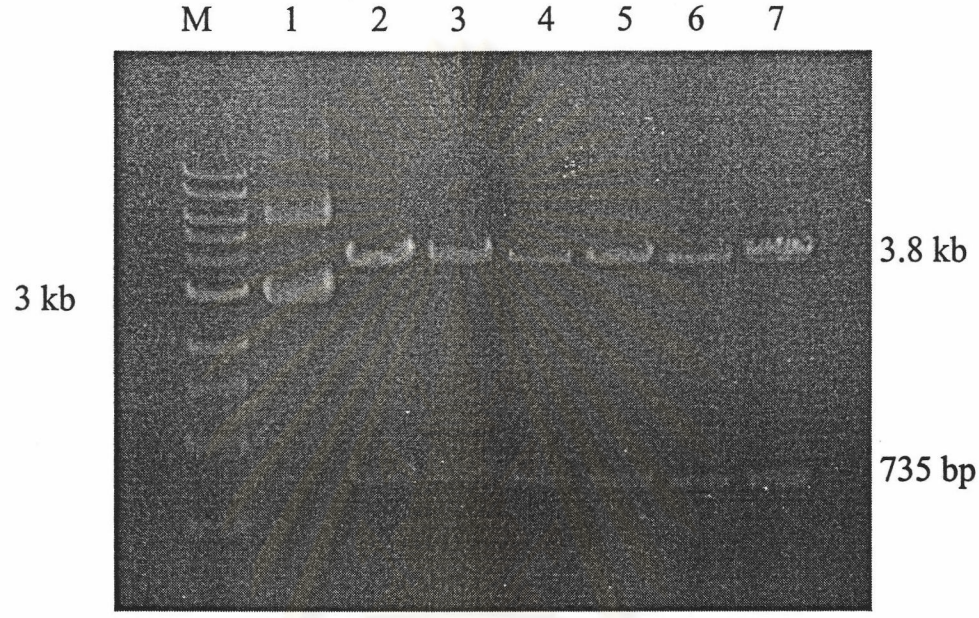

Figure 5-9 Identification of recombinant plasmids, pDrive carrying PCR fragment of *P. falciparum* uridine phosphorylase gene. Numbers on the left and the right sides of the figure are molecular sizes. Lane M is molecular size marker. Lane 1 is uncut construct plasmid. Lanes 2-7 are construct plasmids cut with *Bam*HI and *Sac*I.

4. Analysis of the DNA sequence by the BLAST program

Plasmid DNA from clone 2 was sequenced using an automated DNA sequencer (Figure 5-10). The putative *P. falciparum* uridine phosphorylase sequence was analyzed for sequence homology by the BLAST program. Our query sequence was identical to the open reading frame of the candidate gene (*P. falciparum* uridine phosphorylase gene) with 99% identity (Figure 5-11). It was found that only one base (A) was substituted by (G) at position 130 in our gene homolog.

5. Expression of the *P. falciparum* uridine phosphorylase gene in the bacterial system

5.1 Subcloning of *P. falciparum* uridine phosphorylase gene into a pQE30 expression vector and appropriated host cells.

The DNA insert of uridine phosphorylase gene in clone 2 was subcloned into a pQE30 expression vector (Figure 5-12). The recombinant plasmid carrying the DNA of *P. falciparum* uridine phosphorylase homolog was transformed into DH-5α competent cells. The pQE40 plasmid was transformed as a positive control (data not shown). Ten colonies were selected for minipreparation of plasmid DNA (data not shown), then five colonies from ten were analyzed by the restriction analysis. The plasmids were digested with *Bam*HI and *Sac*I. It was found that one positive clone was identified to contain the DNA insert of *P. falciparum* uridine phosphorylase homolog, as demonstrated by the agrose gel electrophoresis (Lane 5, Figure 5-13).

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer

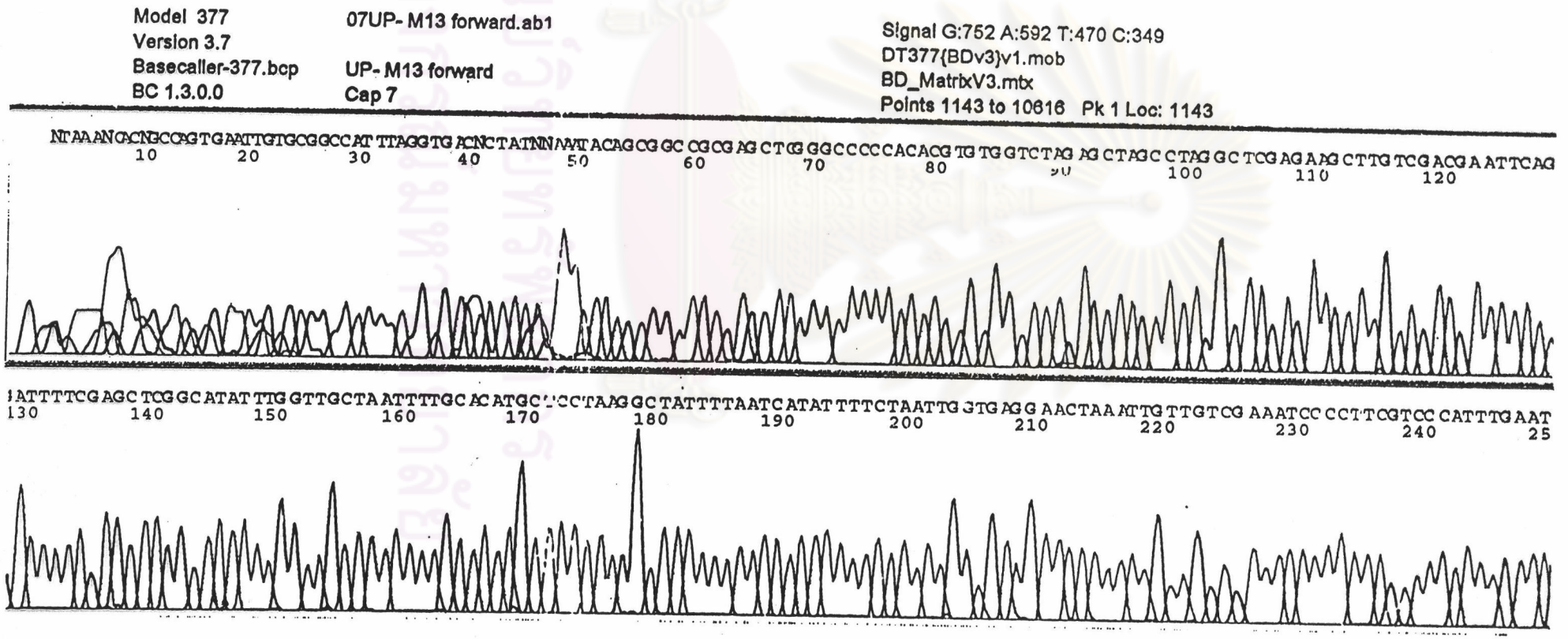

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer (cont.)

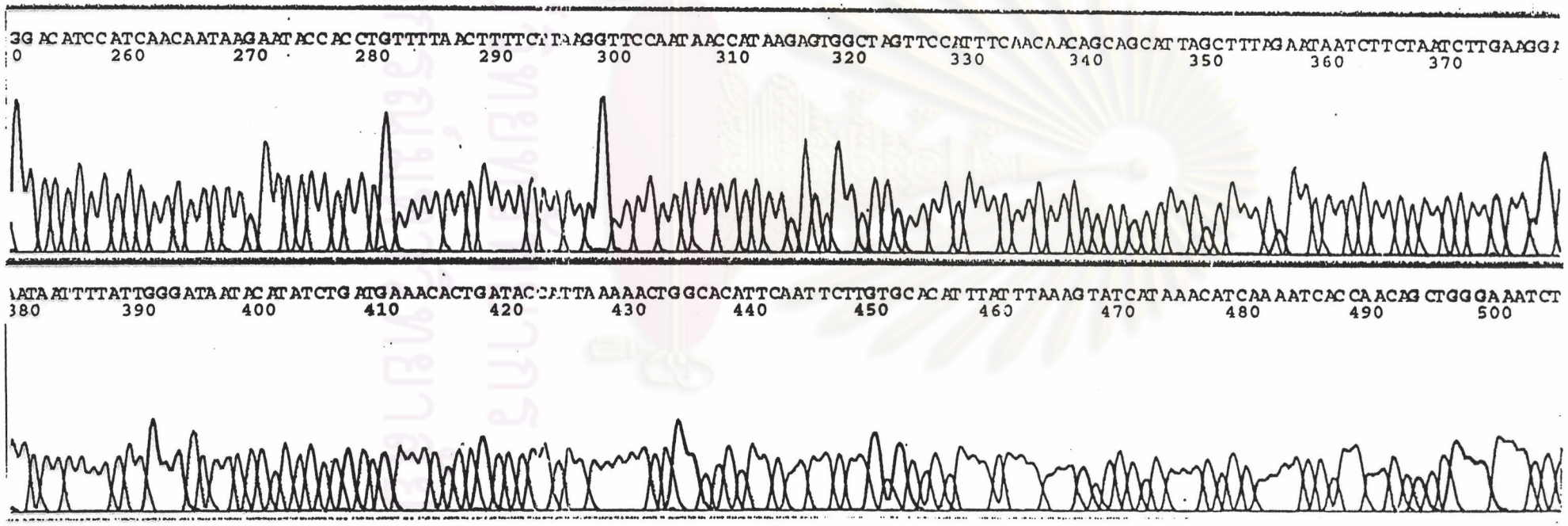

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer (cont.)

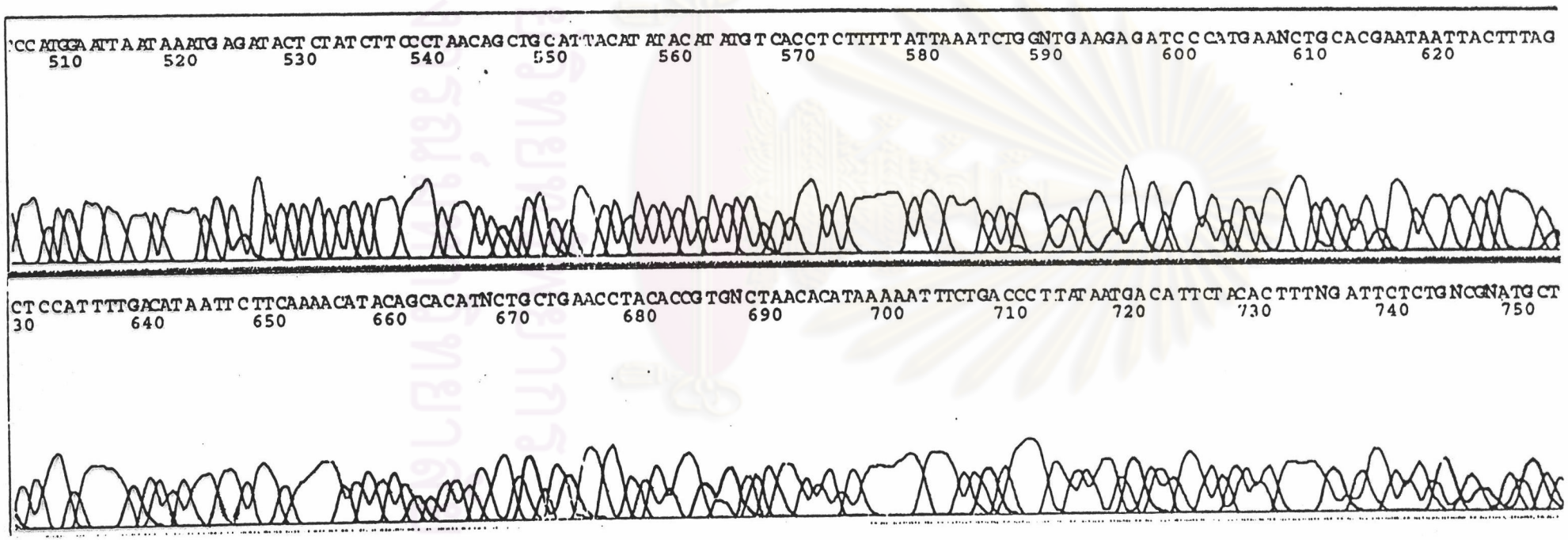

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer (cont.)

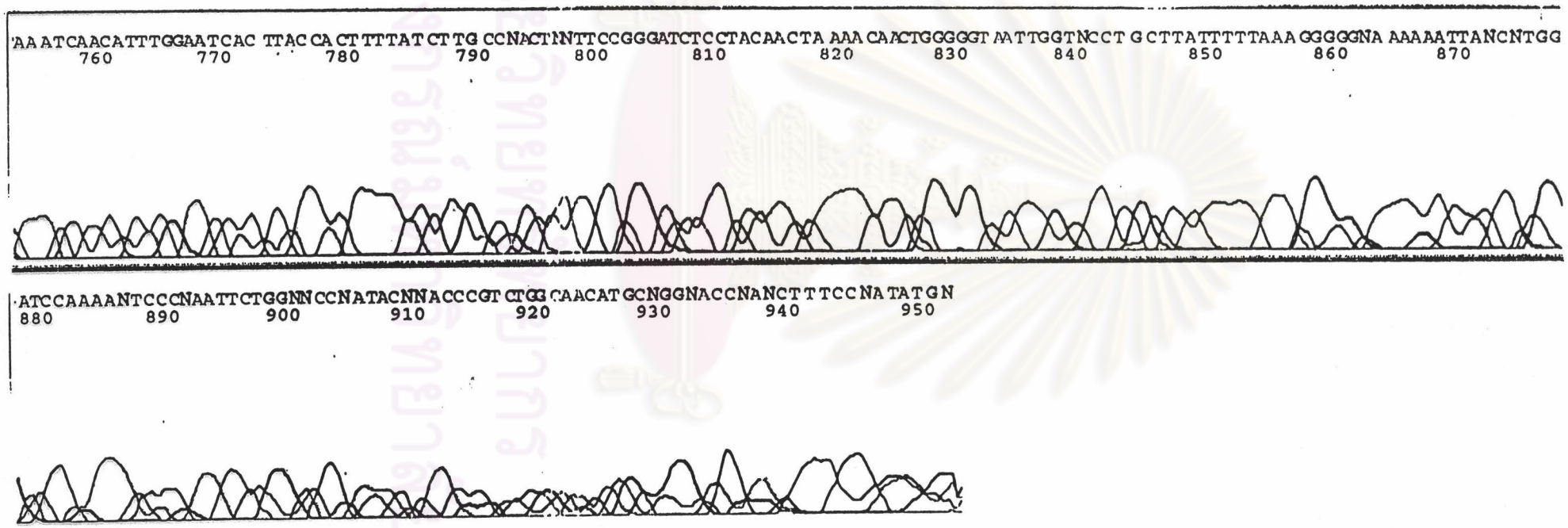

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer (cont.)

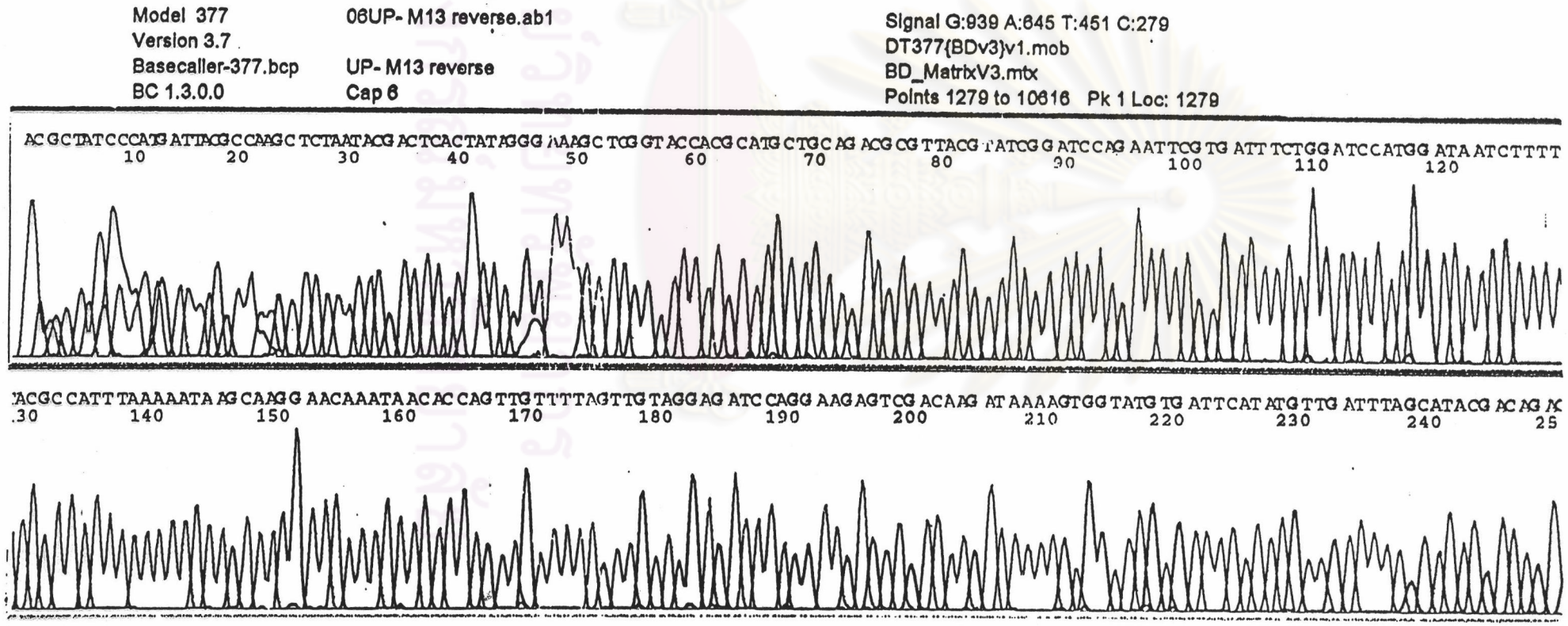

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer (cont.)

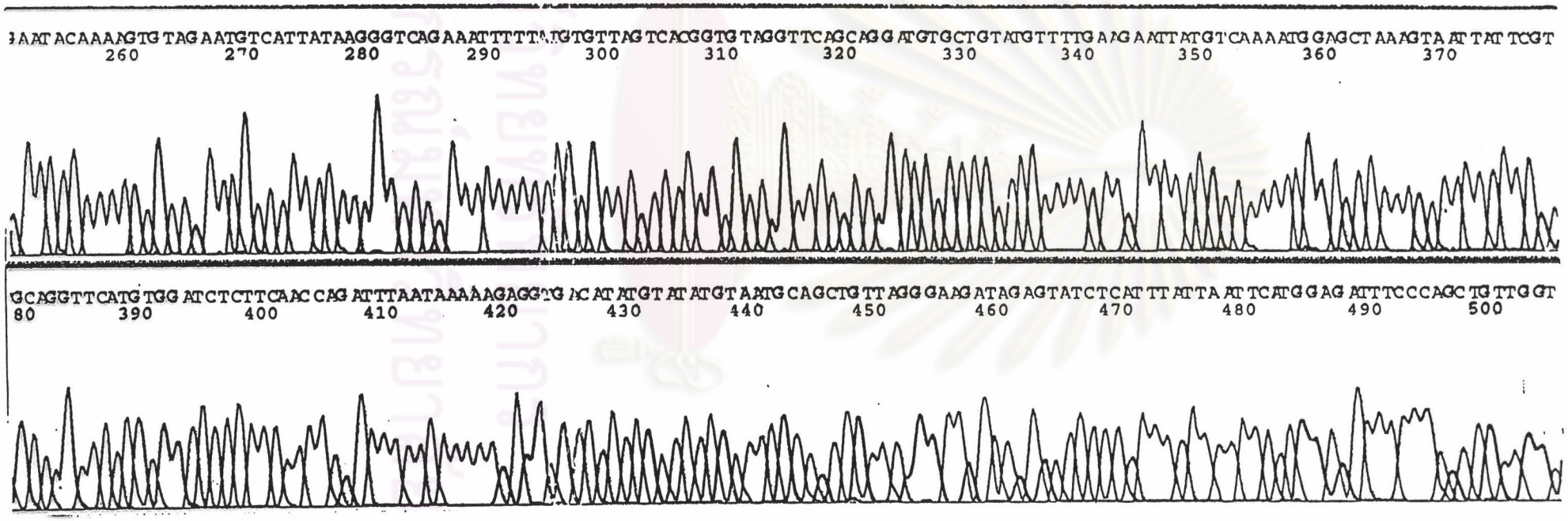

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer (cont.)

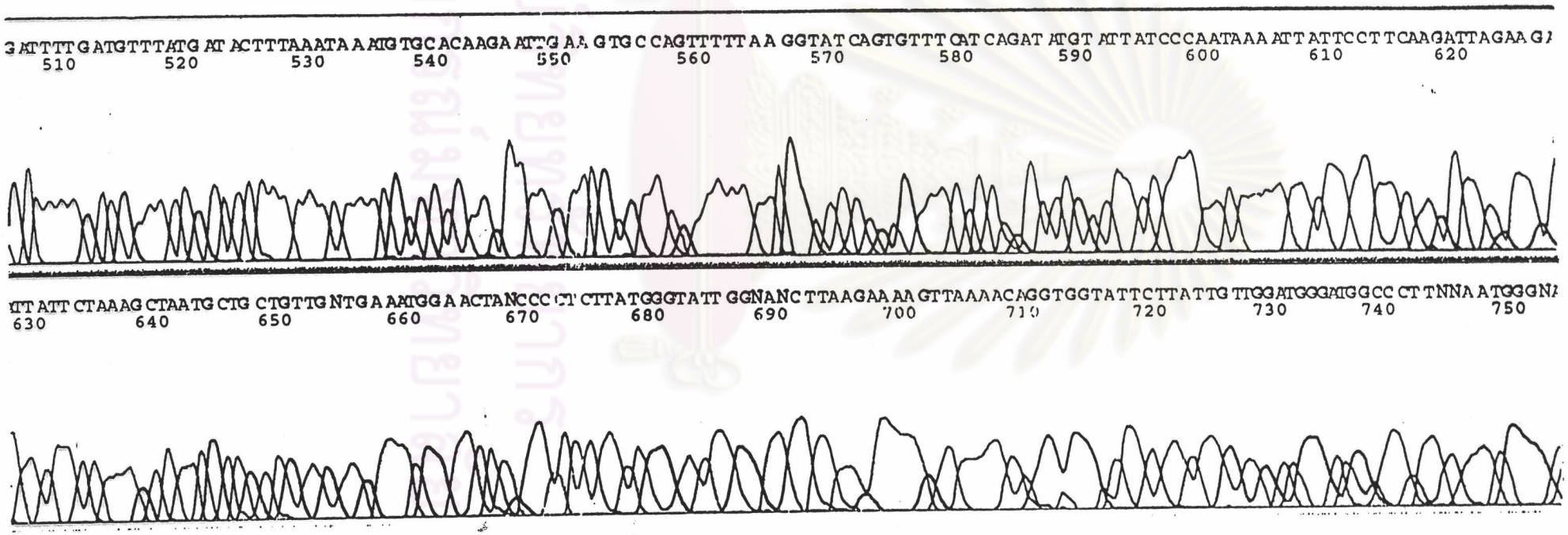

Figure 5-10 Result of DNA sequencing by an automated DNA sequencer (cont.)

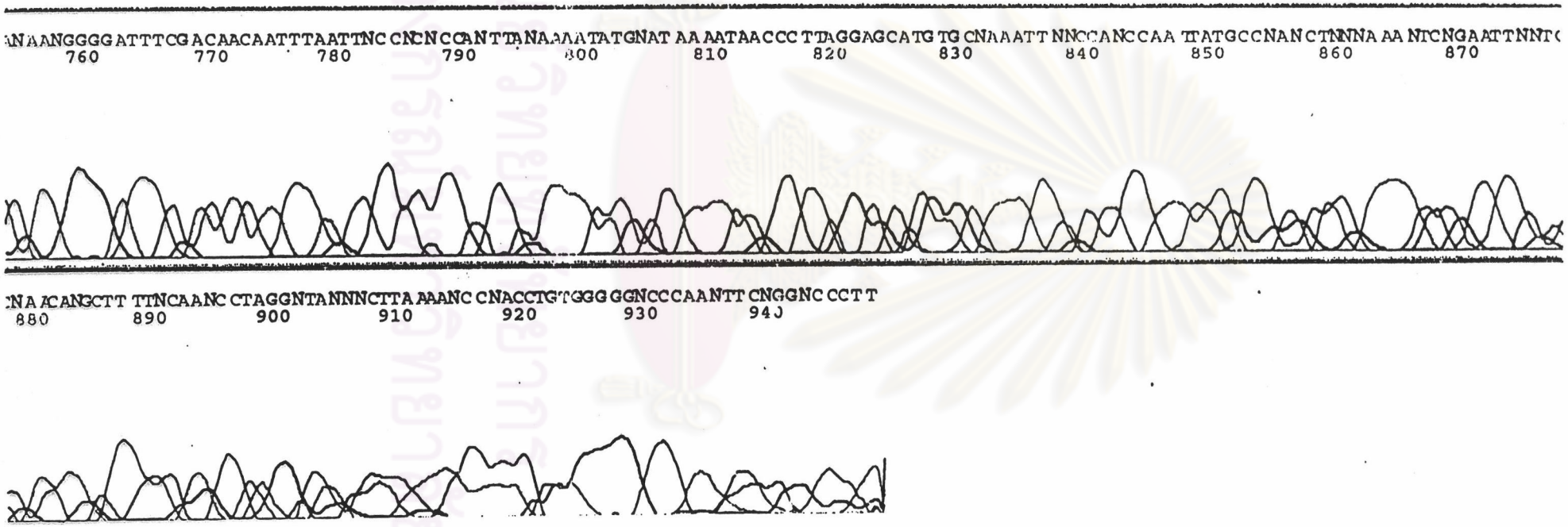

Figure 5-11 Result of DNA sequence analysis by the BLAST program

```
Score = 1408 bits (732), Expect = 0.0
Identities = 734/735 (99%)

Query: 1   atggataatcttttacgccatttaaaaataagcaaggaacaaataacaccagttgtttta 60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1   atggataatcttttacgccatttaaaaataagcaaggaacaaataacaccagttgtttta 60

Query: 61  gttgtaggagatccaggaagagtcgacaagataaaagtggtatgtgattcatatgttgat 120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 61  gttgtaggagatccaggaagagtcgacaagataaaagtggtatgtgattcatatgttgat 120

Query: 121 ttagcatacgacagagaatacaaaagtgtagaatgtcattataagggtcagaaattttta 180
           ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 121 ttagcatacaacagagaatacaaaagtgtagaatgtcattataagggtcagaaattttta 180

Query: 181 tgtgttagtcacggtgtaggttcagcaggatgtgctgtatgttttgaagaattatgtcaa 240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181 tgtgttagtcacggtgtaggttcagcaggatgtgctgtatgttttgaagaattatgtcaa 240

Query: 241 aatggagctaaagtaattattcgtgcaggttcatgtggatctcttcaaccagatttaata 300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 241 aatggagctaaagtaattattcgtgcaggttcatgtggatctcttcaaccagatttaata 300

Query: 301 aaaagaggtgacatatgtatatgtaatgcagctgttagggaagatagagtatctcattta 360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 301 aaaagaggtgacatatgtatatgtaatgcagctgttagggaagatagagtatctcattta 360

Query: 361 ttaattcatggagatttcccagctgttggtgattttgatgtttatgatactttaaataaa 420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 361 ttaattcatggagatttcccagctgttggtgattttgatgtttatgatactttaaataaa 420

Query: 421 tgtgcacaagaattgaatgtgccagtttttaatggtatcagtgtttcatcagatatgtat 480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 421 tgtgcacaagaattgaatgtgccagtttttaatggtatcagtgtttcatcagatatgtat 480

Query: 481 tatcccaataaaattattccttcaagattagaagattattctaaagctaatgctgctgtt 540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 481 tatcccaataaaattattccttcaagattagaagattattctaaagctaatgctgctgtt 540

Query: 541 gttgaaatggaactagccactcttatggttattggaaccttaagaaaagttaaaacaggt 600
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 541 gttgaaatggaactagccactcttatggttattggaaccttaagaaaagttaaaacaggt 600

Query: 601 ggtattcttattgttgatggatgtccattcaaatgggacgaaggggatttcgacaacaat 660
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 601 ggtattcttattgttgatggatgtccattcaaatgggacgaaggggatttcgacaacaat 660

Query: 661 ttagttcctcaccaattagaaaatatgattaaaatagccttaggagcatgtgcaaaatta 720
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 661 ttagttcctcaccaattagaaaatatgattaaaatagccttaggagcatgtgcaaaatta 720

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           |||||||||||||||
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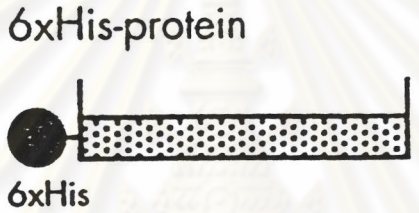


Figure 5-12 Construction of pQE30 expression vector inserted with the DNA fragment of *P. falciparum* uridine phosphorylase gene.

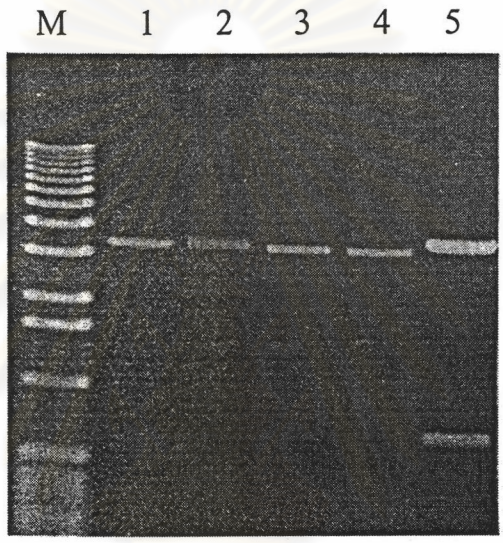


Figure 5-13 Identification of recombinant plasmids, pQE30 carrying DNA fragment of *P. falciparum* uridine phosphorylase gene. Numbers on the right side of the figure is molecular size of the insert. Lane M is molecular size marker. Lanes 1-5 are candidate plasmids cut with *Bam*HI and *Sac*I.

The plasmid DNA from the positive clone was transformed into SG13009 and M15 competent cells, and plasmid pQE40 was also transformed as a positive control. Five colonies were selected for minipreparation of plasmid DNA and restriction analysis. The plasmids were digested with *Bam*HI and *Sac*I. All five colonies selected from SG13009 and M15 cells were positive clones containing the insert, as shown by the agarose gel analysis (Figure 5-14).

5.2 Expression of *P. falciparum* uridine phosphorylase gene in *E. coli*

One from five positive clones was selected for expression by induction with IPTG, and compared with positive control pQE40. The IPTG-induced *E. coli* cells were harvested to detect the recombinant protein expression. The expressed proteins was purified by the Ni-NTA affinity chromatography and then analyzed by 12% SDS-PAGE (Figure 5-15). The major band at approximately 30,000 Da was identified.

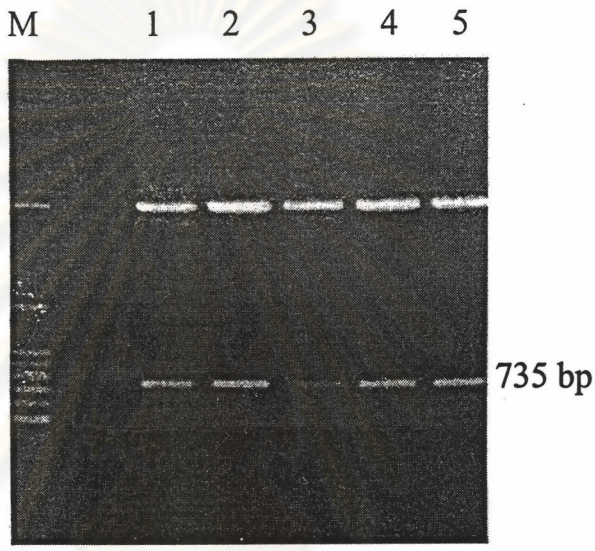


Figure 5-14 Characterization of the transformants. Numbers on the right side of the figure is molecular size of the insert. Lane M is molecular size marker. Lanes 1-3 are positive clones of SG13009. Lanes 4-5 are positive clones of M15.

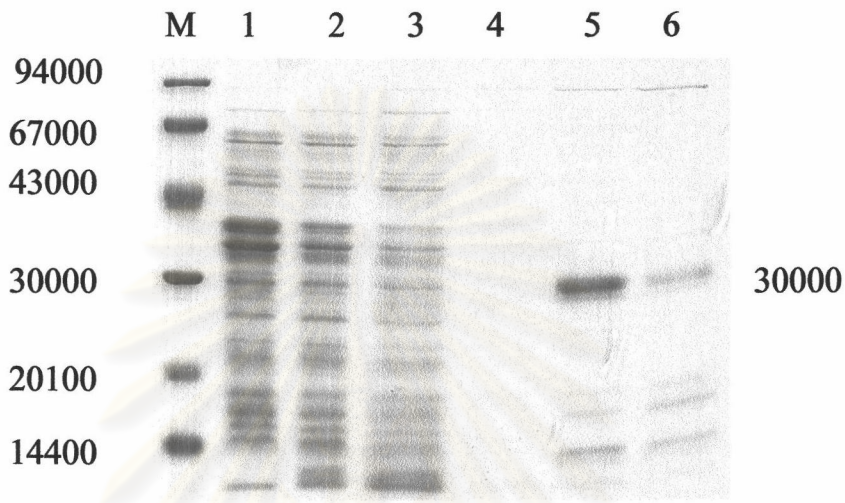


Figure 5-15 SDS-PAGE analysis of recombinantly expressed proteins purified by Ni-NTA affinity chromatography. Numbers on the left and the right sides of the figure are molecular masses in Daltons.

Lane M is standard molecular mass marker proteins

Lane 1 is lysate

Lane 2 is flow-through

Lane 3 is 1st washing fraction

Lane 4 is 2nd washing fraction

Lane 5 is 1st eluate

Lane 6 is 2nd eluate.

6. Study on the enzyme uridine phosphorylase of *P. falciparum* recombinantly expressed in *E. coli*.

The purified expressed proteins were subjected to enzyme assay of uridine phosphorylase activity. The results of uridine phosphorylase activities in lysate and eluate are shown in Tables 5-1 and 5-2. The specific activity of uridine phosphorylase in the lysate was 42.20 ± 3.85 nmol/min/mg protein (n=4) and in eluate was 341.66 ± 82.50 nmol/min/mg protein (n=3). The recombinant enzyme purification was 8-fold and 14 % yield. The k_{cat} value of the purified enzyme was 1.18 min^{-1}.

Kinetic parameters were determined by varying concentrations of substrate of the enzyme uridine phosphorylase, uridine, and fixing enzyme concentration. The results are shown in Tables 5-3 and 5-4. The Michaelis-Menten constants (K_m) and catalytic constant (k_{cat}) was calculated from the Lineweaver-Burk plot (Figures 5-16, 5-17, 5-18 and 5-19). The K_m values for non-induced protein and IPTG-induced protein were 28.41 and 121.95 μM, and k_{cat} values were 1.59 and 1.15 min^{-1}, respectively. These results suggested the *E. coli* enzyme in non-induced condition was different from the *P. falciparum* enzyme in the IPTG-induced condition during the heterologous expression.

Table 5-1 Results of uridine phosphorylase activity in the lysate.

Experiment No.	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min/mg)
1	0.0338	1.51	44.68
2	0.0320	1.51	47.19
3	0.0790	3.05	38.61
4	0.0846	3.24	38.30
Mean ± SD		2.33 ± 0.83	42.20 ± 3.85

Table 5-2 Results of uridine phosphorylase activity in the eluate.

Experiment No.	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min/mg)
1	0.0004	0.16	400.00
2	0.0010	0.40	400.00
3	0.0020	0.45	225.00
Mean ± SD		0.33 ± 0.19	341.66 ± 82.50

Table 5-3 Kinetics measurement of uridine phosphorylase activity from non IPTG-induced expression in *E. coli*.

Uridine (μM)	Rate (nmol/min/mg protein)
5	8.61
10	14.56
25	22.53
50	36.96
75	46.71
100	49.75

Table 5-4 Kinetics measurement of *P. falciparum* uridine phosphorylase from IPTG induced expression in *E. coli*.

Uridine (μM)	Rate (nmol/min/mg protein)
5	1.65
10	5.06
25	12.12
50	22.82
75	31.41
100	35.88

Figure 5-16 Michaelis-Menten kinetics of uridine phosphorylase from non IPTG-induced expression in *E. coli*.

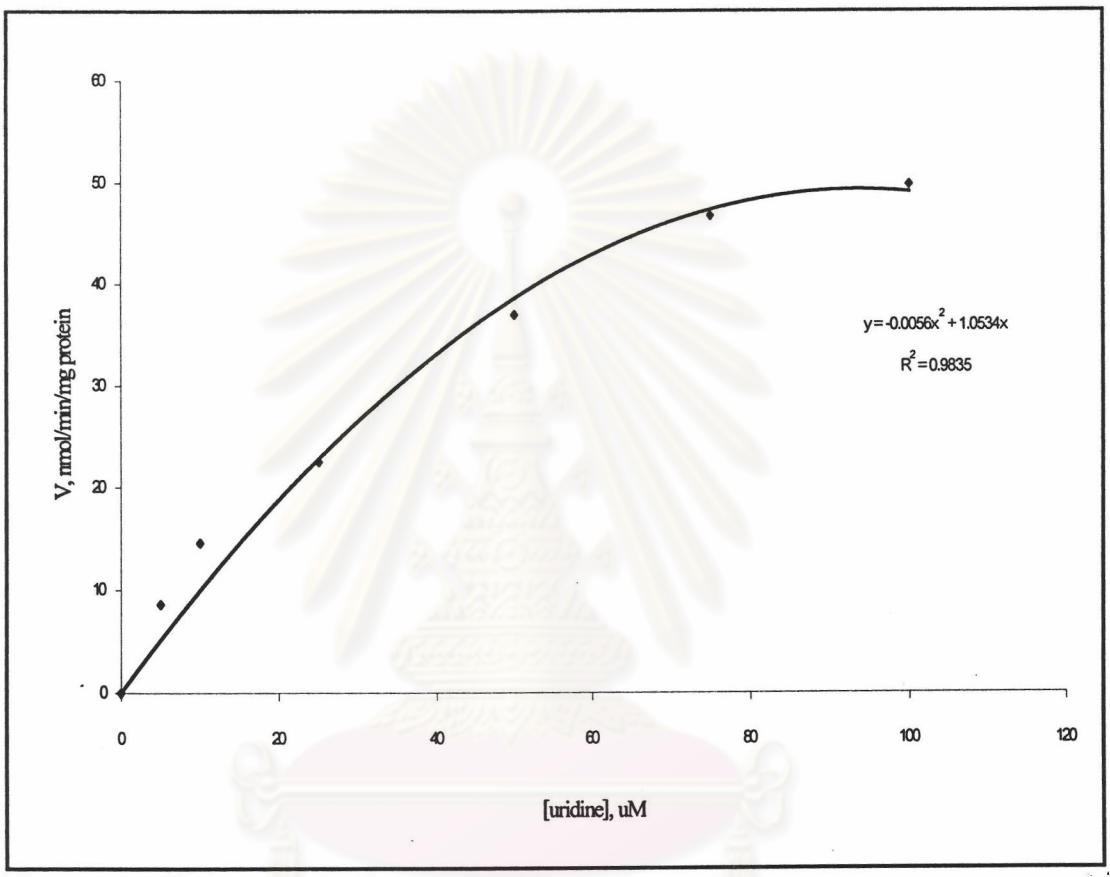


Figure 5-17 Lineweaver-Burk plot of uridine phosphorylase activity from non IPTG-induced expression in *E. coli*. The data were taken from Figure 5-16.

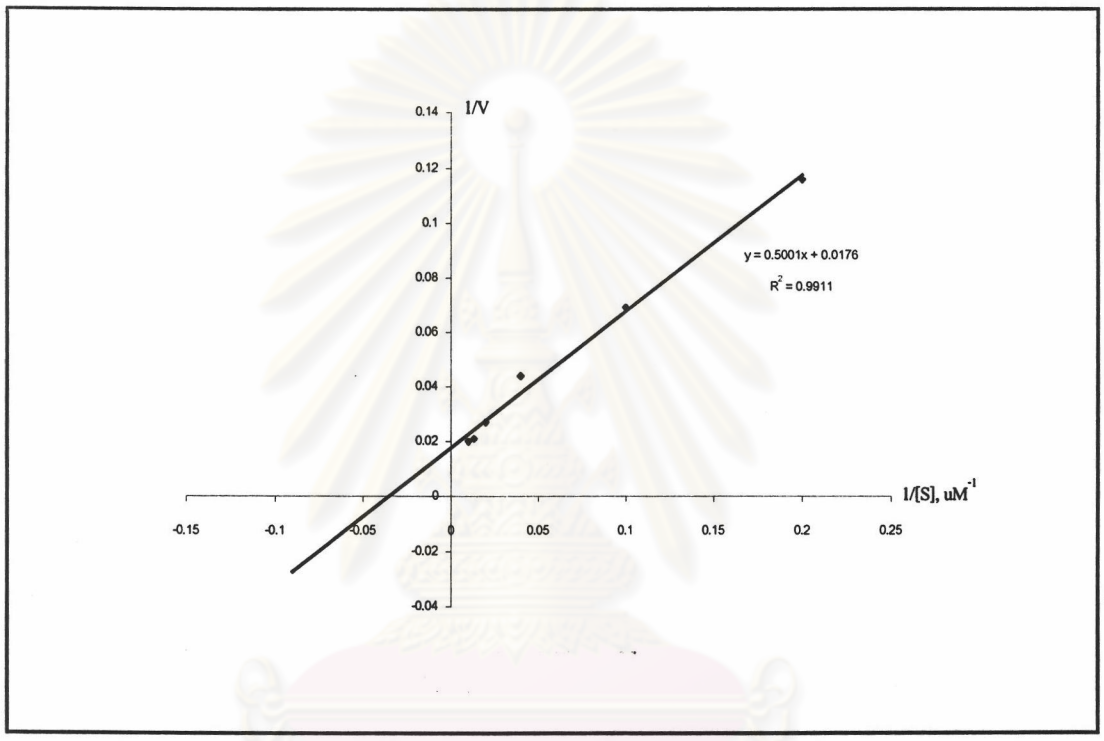


Figure 5-18 Michaelis-Menten kinetics of *P. falciparum* uridine phosphorylase from IPTG- induced expression in *E. coli*.

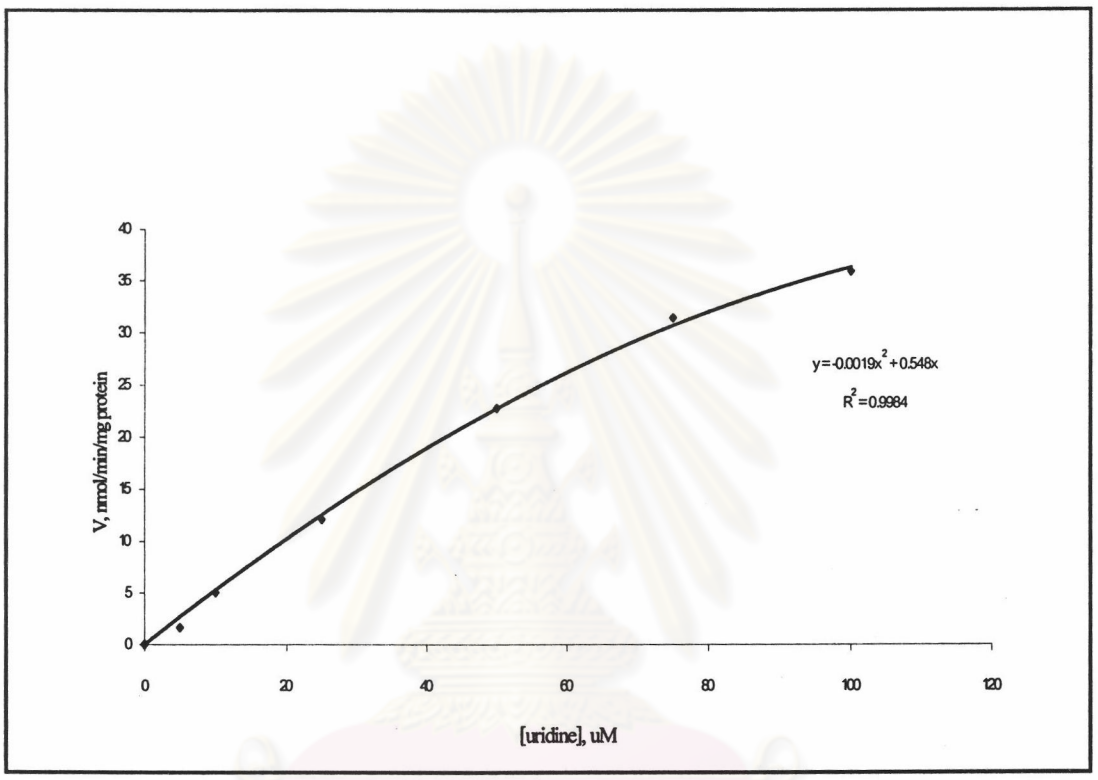
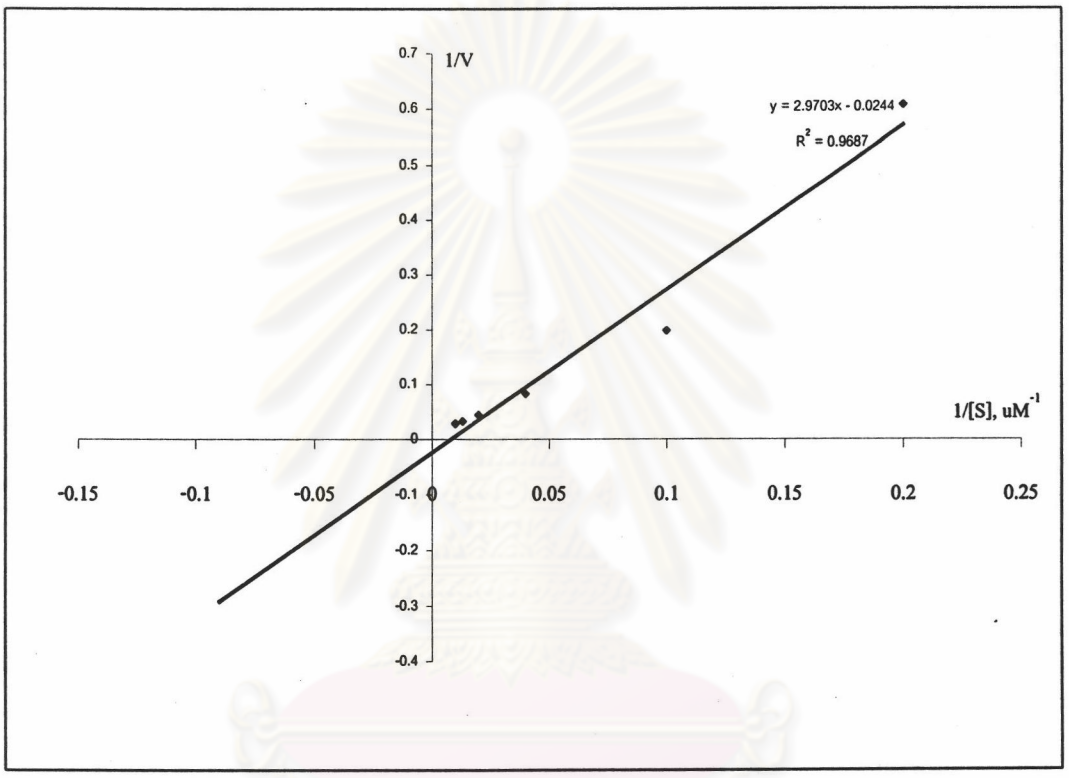


Figure 5-19 Lineweaver-Burk plot of *P. falciparum* uridine phosphorylase from IPTG-induced expression in *E. coli*. The data were taken from Figure 5-18.



Determination of the molecular mass of the *P. falciparum* uridine phosphorylase was performed by using SDS-PAGE analysis. Various molecular mass marker proteins were loaded on a 12% gel of SDS-PAGE, including phosphorylase b (molecular mass = 94 kDa), bovin serum albumin (molecular mass = 67 kDa), ovalbumin (molecular mass = 43 kDa), carbonic anhydrase (molecular mass = 30 kDa), trypsin inhibitor (molecular mass = 20.1 kDa) and α-lactalbumin (molecular mass = 14.4 kDa). The relative mobilities of proteins were plotted against molecular mass on a semi-log graph (Figure 5-20). The molecular mass of *P. falciparum* uridine phosphorylase was then calculated from the standard curve. It was approximately 30 ± 2 kDa (n=3).

Figure 5-20 Standard curve for protein molecular mass determination.

The symbol ● indicates the position of *P. falciparum* uridine phosphorylase