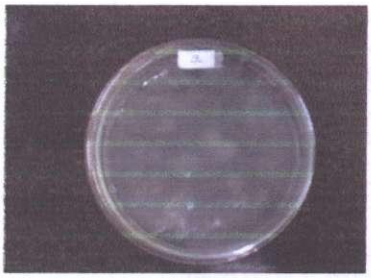
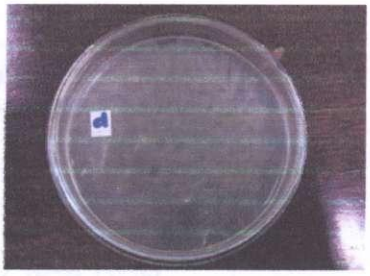
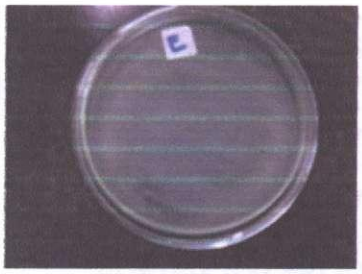
Lampiran 1. Isolat *Vibrio* pada medium TSA



Isolat A

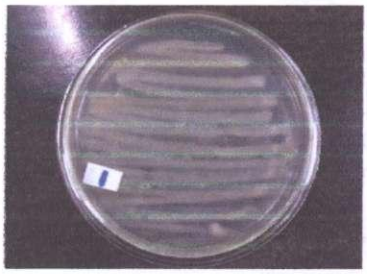


Isolat D



Isolat E

Isolat H



Isolat I

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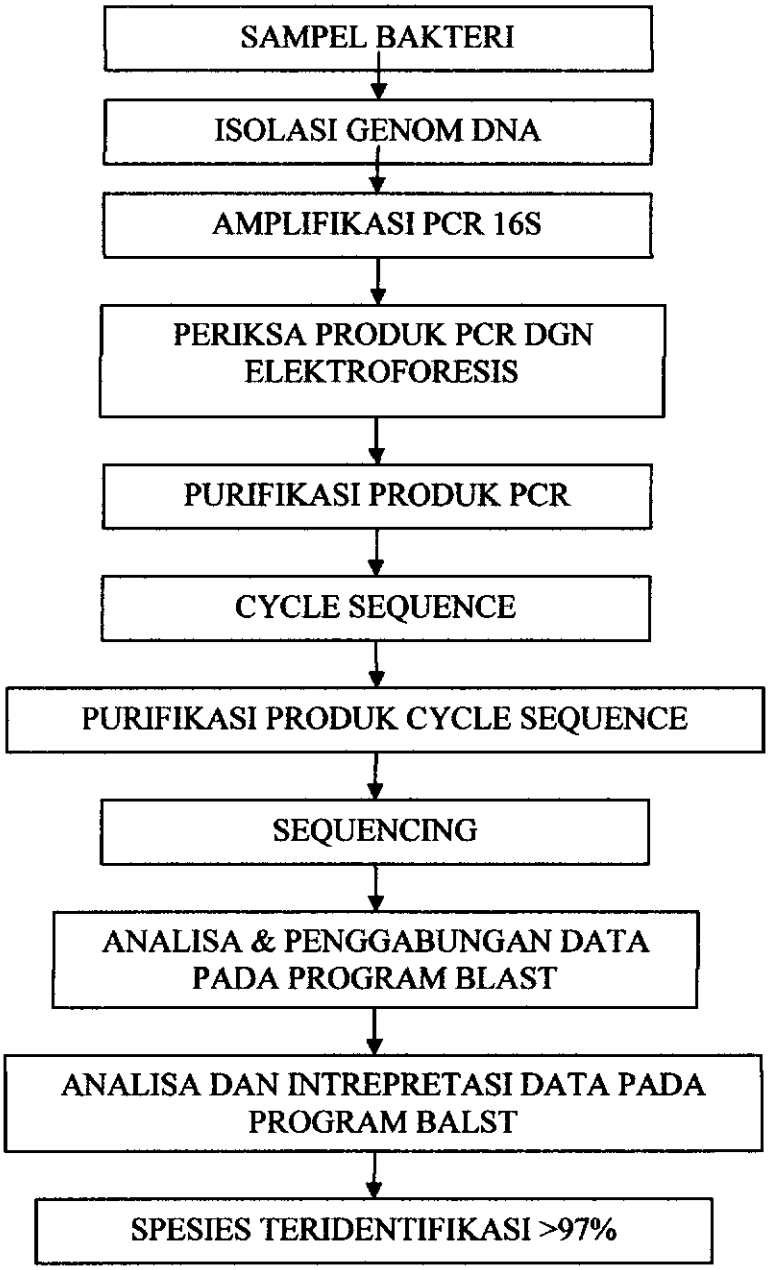
http://repository.unri.ac.id/

Lampiran 2. Alur Kerja Identifikasi Bakteri 16S rDNA



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http://repository.unri.ac.id/

Lampiran 3. Proses Sekuensing

Hidupkan Komputer

↓

Hidupkan **AB 3130**
(tunggu sampai lampu hijau menyala)

↓

Klik **RUN DATA COLLECTION**
(tunggu sampai semua kotak hijau)

↓

Klik **PROTOCOL MANAGER**
Klik **NEW**, Isi nama, **Type** (reguler), **Run Module**(Fastseq 50_pop_1/standrt50_POP_1)**Dye Set** (Z-BigDye V.3.1), klik **OK**

↓

Klik **PLATE MANAGER**
Klik NEW, Isi Plate Dialog, Klik **OK**
Isi Plate Record **SEQ ANALYSIS PLATE EDITOR**

↓

Klik **RUN SCHEDULER**
Klik **FIND ALL**, Pilih plate name dari daftar,
klik **POSISI PLATE** untuk **LINK**

↓

Pada **START DIALOG BOX**
Klik tanda panah warna **HIJAU**, Klik **OK**

↓

RUNNING

↓

Didapatkan basa nucleotide

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Lampiran 4. Analisis BLAST

Edit urutan DNA hasil sequensing dengan menterjemahkan N menjadi basa sesuai elektroferogram

↓

Copy urutan DNA ke program NotePad

↓

Buka website http://**www.ncbi.nih.nlm.gov**

↓

Klik icon **BLAST**

↓

Klik icon nucleotide-nucleotide blast (blast-n)

↓

Copy urutan DNA yang akan di-blast, kemudian paste di kolom tempat urutan DNA di window blast

↓

Klik **BLAST**, Klik **Format ID**

↓

Dilayar monitor akan muncul window hasil blast yang berisi diagram similarity sequens kita dengan sequens yang ada di NCBI

↓

Pilih sequen yang memiliki **TOP simlarity**

↓

*Tentukan identifikasi contoh sesuai dengan **TOP similarity***

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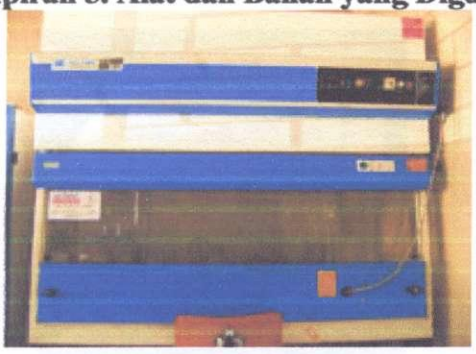
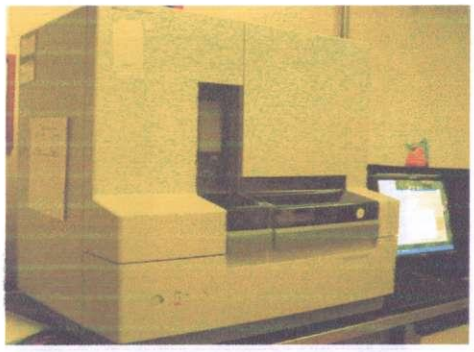
Lampiran 5. Alat dan Bahan yang Digunakan dalam Penelitian



Laminar Air Flow

PCR Thermalcycle *Dice Takara*



AB 3130 *Genetic Analyzer*

Bak Gel Elektroforesis

Spectrophotometer *ND-1000*

Binding Matriks, CLS-TC, SEWS M, DES

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Sentrifuse *Tomy*

Dokumentasi Gel

FastDNA Kit (USA)

Micro Smash *Tomy*

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Lampiran 6. Sekuens Gen 16S rDNA

Isolat	Sekuen Gen 16S rDNA
A	**5'** TGGAGAGTTTGATCCTGGCTCAGATTGAACGCT GGCGGCAGGCCTAACACATGCAAGTCGAGCGGAAACGAGTTATCTGAAC CTTCGGGGAACGATAACGGCGTCGAGCGGCGGACGGGTGAGTAATGCCT AGGAAATTGCCCTGATGTGGGGGATAACCATTGGAAACGATGGCTAATAC CGCATGATGCCTACGGGCCAAAGAGGGGGACCTTCGGGCCTCTCGCGTC AGGATATGCCTAGGTGGGATTAGCTAGTTGGTGAGGTAAGGGCTCACCAA GGCGACGATCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACT GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTT CGGGTTGTAAAGCACTTTCAGTCGTGAGGAAGGTGTTAATAGCATTTGAC GTTAGCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA **3'**
D	**5'** AGAGTTTGATCCTGGCTCAGATTGAACGCTGGC GGCAGGCCTAAGACATGCAAGTCGAGCGGAAACGAGTTATCTTAACCTTC GGGGAACGATAACGCGTCGAGCGGCGGACGGGTGAGTAATGCCTAGGAA ATTGCCCTGATGTGGGGGATAACCATTGGAAACGATGGCTAATACCGCAT GATGCCTACGGGCCAAAGAGGGGGACCTTCGGGCCTCTCGCGTCAGGAT ATGCCTAGGTGGGATTAGCTAGTTGGTGAGGTAAGGGCTCACCAAGGCGA CGATCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACA CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG CGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTT GTAAAGCACTTTCAGTCGTGAGGAAGGTAGTGTAGTTAATAGCTGCATTAT TTGACGTTAGCGACAGAAGAAGCACCGCTAACTCCGTGCCAGCAGCCGC GGTAATACGGAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCG CATGCAGGTGGTTTGTTAAGTCAGATGTGAAAGCCCGGGGCTCAACCTCG GAATAGCATTTGAAACCTGCAGACTAGAGTACTGTAGAGGGGGGTAGAAT TTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGAAGGAAT **3'**
E	**5'** TTTGGAGAGTTTGATCCTGGCTCAGATTGAAC GCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGAAACGAGTTATCTG AACCTTCGGGGAACGATAACGGCGTCGAGCGGCGGACGGGTGAGTAATG CCTAGGAAATTGCCCTGATGTGGGGGATAACCATTGGAAACGATGGCTAA TACCGCATAATGCCTACGGGCCAAAGAGGGGGACCTTCGGGCCTCTCGC GTCAGGATATGCCTAGGTGGGATTAGCTAGTTGGTGAGGTAATGGCTCAC CAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAA CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGC ACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAA **3'**

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H	5' AGAGTTTGATCCTGGCTCAGATTGAACGCTGG CGGCAGGCCTAACACATGCAAGTCGAGCGGAAACGAGTTAACTGACCCT TCGGGTGACGTTAACGGCGTCGAGCGGCGGACGGGTGAGTAATGCCTG GGAAATTGCCCTGATGTGGGGGATAACCATTGGAAACGATGGCTAATAC CGCATAATGCCTTCGTGCCAAAGAGTGGGACCTTAGGGCCTCTCGCGTC AGGAGATGCCCAGGTGGGATTAGCTAGTTGGTGAGGTAATGGCTCACCA AGGCGACGATACCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAAG TGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGC ACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGATGGC CTTCGGGTTGTAAAGCACTTTCAGCAGTGAGGAAGGCGGGTACGTTAAT AAGTGCTCGTTTGACGTTAGCTGCAGAAGAAGCACCGGCTAACTCCGTG CCAGCAGCCGCGGTAATACGGAGGGTGCGAGCGTTAATCGGAATTACTG GGCGTAAAGCGCATGCAGGTGGTTCGTTAAGTCAGATGTGAAAGCCCGG GGCTCAACCTCGGAACTGCATTTGAAACTGGCGGACTAGCGTACTGTAG AGGGGGGTAGAATTTCAGGTGTAGCGGTGCAATGCGTAGAGATCTGAAG GAATAC 3'
I	5' CAGGCCTAACACATGTAAGTCGAGCGGCAGC ACAGAGAAACTTGTTTCTCGGGTGTCGAGCGGCGGACGGGTGAGTAATG CCTGGGAAATTGCCCTGATGTGGGGGATCACCATTGGAAACGATGGCTA ATACCGCATGATGCCCTTGATTATAATGAACAGGAGCCAAAGAGGGGGA CCTTCGGGCCTCTCGCGTCAGGAAATGCCCAGGCGGGATTAGCTAGTTG GTGAGGTAAGGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGG ATGATCAGCCACACTGGAAGTGAGACACGGTCCAGACTCCTACGGGAGG CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC CGCGTGTGTGAAGAGGGCCTTCGGGTTGTAAAGCACTTTCAGTTGTGAG GAAGGGGGTGTTGTGAATAGCAGCATCATTTGACGTTAGCAACAGAAGA AGCACCGGCGAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCG AACGTTAATCGGAATTACTGGGCGTAAAGCGCATGCAGGTGGTTTGTTAA GTCACATGTGAAAGCCCCGGGCTCAACCTGGGAATTGCATTTGAGACTG GCAAACTAGAGTACTGTAGAGGGGGGTAGAATTTCAGGTGTAGCGGTGT AATGCGTAGAGATCTGAAGGAATACCGGTGGCGAAGGCGTCCCCCTGG ACAGATACTGACACTCAGATGCGAAAGCGTGGGGAGCAAACAGGTTTGC GAAGACGCAGGTGTGCCTTCGGGAGCTCTGAGACAG 3'

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Lampiran 7. GenBank Flat File

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LOCUS       FJ404761 526 bp DNA linear BCT 29-OCT-2008
DEFINITION  Vibrio alginolyticus strain FNS A08 16S ribosomal RNA
            gene, partial sequence.
ACCESSION   FJ404761
VERSION     FJ404761
KEYWORDS    .
SOURCE      Vibrio alginolyticus
  ORGANISM  Vibrio alginolyticus
            Bacteria; Proteobacteria; Gammaproteobacteria;
            Vibrionales;
            Vibrionaceae; Vibrio.
REFERENCE   1  (bases 1 to 526)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Screening bacteria Vibrio sp. from Indonesia as source
            of shrimp disease, using sequence 16S rDNA technique
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 526)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-SEP-2008) Marine Science, University of
            Riau, HR Soebrantas Km 12,5 Panam, Pekanbaru, Riau
            28293, Indonesia
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="FNS A08"
                     /specific_host="Penaeus monodon"
                     /db_xref="taxon:663"
                     /country="Indonesia: shrimp culture farm in
                     estuarine area
                     of Bantan Air, Bengkalis Island, Riau province"
     rRNA            <1..>526
                     /product="16S ribosomal RNA"
ORIGIN
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       61 agcggaaacg agttatctga accttcgggg aacgataacg gcgtcgagcg
gcggacgggt
      121 gagtaatgcc taggaaattg ccctgatgtg ggggataacc attggaaacg
atggctaata
      181 ccgcatgatg cctacgggcc aaagaggggg accttcgggc ctctcgcgtc
aggatatgcc
      241 taggtgggat tagctagttg gtgaggtaag ggctcaccaa ggcgacgatc
cctagctggt
      301 ctgagaggat gatcagccac actggaactg agacacggtc cagactccta
cgggaggcag
      361 cagtggggaa tattgcacaa tgggcgcaag cctgatgcag ccatgccgcg
tgtgtgaaga
      421 aggccttcgg gttgtaaagc actttcagtc gtgaggaagg tgttaatagc
atttgacgtt
      481 agcgacagaa gaagcaccgg ctaactccgt gccagcagcc gcggta
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LOCUS       FJ404762 420 bp DNA linear   BCT 29-OCT-2008
DEFINITION  Vibrio harveyi strain FNS B08 16S ribosomal RNA gene,
            Partial sequence.
ACCESSION   FJ404762
VERSION     FJ404762
KEYWORDS    .
SOURCE      Vibrio harveyi
  ORGANISM  Vibrio harveyi
            Bacteria; Proteobacteria; Gammaproteobacteria;
            Vibrionales;
            Vibrionaceae; Vibrio.
REFERENCE   1  (bases 1 to 420)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Screening bacteria Vibrio sp. from Indonesia as source
            of shrimp
            disease, using sequence 16S rDNA technique
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 420)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-OCT-2008) Marine Science, University of
            Riau, HR Soebrantas Km 12,5 Panam, Pekanbaru, Riau \
            28293, Indonesia
FEATURES             Location/Qualifiers
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                     /strain="FNS B08"
                     /specific_host="Penaeus monodon"
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                      estuarine area Bantan Air, Bengkalis Island,
                      Riau province"
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1114R"
     rRNA            <1..>420
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cgatggctaa
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tcaggatatg
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LOCUS       FJ404763 1015 bp DNA linear BCT 29-OCT-2008
DEFINITION  Vibrio parahaemolyticus strain FNS C08 16S ribosomal RNA
            gene, partial sequence.
ACCESSION   FJ404763
VERSION     FJ404763
KEYWORDS    .
SOURCE      Vibrio parahaemolyticus
  ORGANISM  Vibrio parahaemolyticus
            Bacteria; Proteobacteria; Gammaproteobacteria;
            Vibrionales; Vibrionaceae; Vibrio.
REFERENCE   1  (bases 1 to 1015)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Screening bacteria Vibrio sp. from Indonesia as source
            of shrimp disease, using sequence 16S rDNA technique
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1015)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-OCT-2008) Marine Science, University of
            Riau, HR Soebrantas Km 12,5 Panam, Pekanbaru, Riau
            28293, Indonesia
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="FNS C08"
                     /specific_host="Penaeus monodon"
                     /db_xref="taxon:670"
                     /country="Indonesia: shrimp culture farm in
                     estuarine area Bantan Air, Bengkalis Island, Riau
                     province"
                     /note="PCR_primers=fwd_name: 9F, rev_name:
                     1114R"
     rRNA            <1..>1015
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121 taatgcctag gaaattgccc tgatgtgggg gataaccatt ggaaacgatg gctaataccg
181 catgatgcct acgggccaaa gagggggacc ttcgggcctc tcgcgtcagg atatgcctag
241 gtgggattag ctagttggtg aggtaagggc tcaccaaggc gacgatccct agctggtctg
301 agaggatgat cagccacact ggaactgaga cacggtccag actcctacgg gaggcagcag
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421 ccttcgggtt gtaaagcact ttcagtcgtg aggaaggtag tgtagttaat agctgcatta
481 tttgacgtta gcgacagaag aagcaccggc taactccgtg ccagcagccg cggtaatacg
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```
LOCUS       FJ404764 1020 bp DNA linear BCT 29-OCT-2008
DEFINITION  Vibrio shiloni strain FNS D08 16S ribosomal RNA gene,
            Partial sequence.
ACCESSION   FJ404764
VERSION     FJ404764
KEYWORDS    .
SOURCE      Vibrio shilonii
  ORGANISM  Vibrio shilonii
            Bacteria; Proteobacteria; Gammaproteobacteria;
            Vibrionales; Vibrionaceae; Vibrio.
REFERENCE   1  (bases 1 to 1020)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Screening bacteria Vibrio sp. from Indonesia as source
            of shrimp disease, using sequence 16S rDNA technique
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1020)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-OCT-2008) Marine Science, University of
            Riau, HR Soebrantas Km 12,5 Panam, Pekanbaru, Riau
            28293, Indonesia
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                     /mol_type="genomic DNA"
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                     /country="Indonesia: shrimp culture farm in
                      estuarine area Bantan Air, Bengkalis Island,
                      Riau province"
                     /note="PCR_primers=fwd_name: 9F, rev_name:
1114R"
     rRNA            <1..>1020
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ORIGIN
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61 ggaaacgagt taactgaccc ttcgggtgac gttaacggcg tcgagcggcg gacgggtgag
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LOCUS       FJ404765 1020 bp DNA linear BCT 29-OCT-2008
DEFINITION  Vibrio vulnificus strain FNS E08 16S ribosomal RNA gene,
            Partial sequence.
ACCESSION   FJ404765
VERSION     FJ404765
KEYWORDS    .
SOURCE      Vibrio vulnificus
  ORGANISM  Vibrio vulnificus
            Bacteria; Proteobacteria; Gammaproteobacteria;
            Vibrionales; Vibrionaceae; Vibrio.
REFERENCE   1  (bases 1 to 1020)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Screening bacteria Vibrio sp. from Indonesia as source
            of shrimp disease, using sequence 16S rDNA technique
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1020)
  AUTHORS   Feliatra, Nugroho,T. and Sazali,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-OCT-2008) Marine Science, University of
            Riau, HR
            Soebrantas Km 12,5 Panam, Pekanbaru, Riau 28293,
            Indonesia
FEATURES             Location/Qualifiers
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                     /isolation_source="sea water"
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                     /country="Indonesia: shrimp culture farm in
                      estuarine area Bantan Air, Bengkalis Island,
                      Riau province"
                     /note="PCR_primers=fwd_name: 8F, rev_name:
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ORIGIN
1   caggcctaac acatgtaagt cgagcggcag cacagagaaa cttgtttctc gggtgtcgag
61  cggcggacgg gtgagtaatg cctgggaaat tgccctgatg tgggggatca ccattggaaa
121 cgatggctaa taccgcatga tgcccttgat tataatgaac aggagccaaa gagggggacc
181 ttcgggcctc tcgcgtcagg aaatgcccag gcgggattag ctagttggtg aggtaagggc
241 tcaccaaggc gacgatccct agctggtctg agaggatgat cagccacact ggaagtgaga
301 cacggtccag actcctacgg gaggcagcag tggggaatat tgcacaatgg gcgcaagcct
361 gatgcagcca tgccgcgtgt gtgaagaggg ccttcgggtt gtaaagcact ttcagttgtg
421 aggaaggggg tgttgtgaat agcagcatca tttgacgtta gcaacagaag aagcaccggc
481 gaactccgtg ccagcagccg cggtaatacg gagggtgcga acgttaatcg gaattactgg
541 gcgtaaagcg catgcaggtg gtttgttaag tcacatgtga aagccccggg ctcaacctgg
601 gaattgcatt tgagactggc aaactagagt actgtagagg ggggtagaat ttcaggtgta
661 gcggtgtaat gcgtagagat ctgaaggaat accggtggcg aaggcgtccc cctggacaga
721 tactgacact cagatgcgaa agcgtgggga gcaaacaggt ttagataccc tggtagtcca
781 cgccgtaaac gatgtctaca tggaggttgt ggccttgagc cgtggctttc ggagctaacg
841 cgttaagtag acagcctggg gagtacggtc gcaagattaa aactcaaatg aaatgacggg
901 ggcccgcaca agcggtggag catgtggttt aattcgatgc aacgcaaaga accttacct
961 ctcttgacat ccagagaagc cagcgaagac gcaggtgtgc cttcgggagc tctgagacag
//
```

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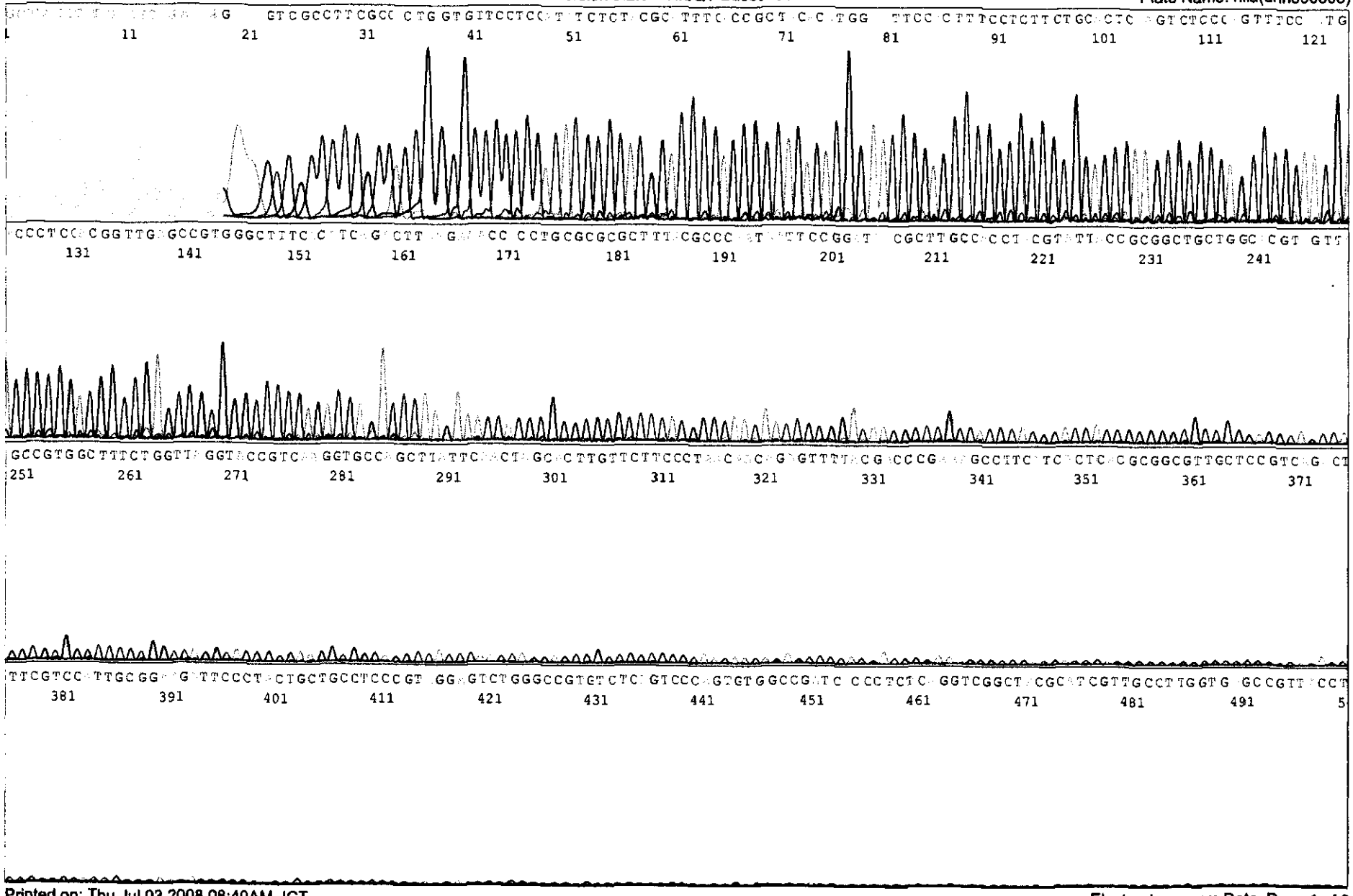
http://repository.unri.ac.id/

Biosystems

S/N G:93 A:36 T:66 C:79
KB.bcp
KB 1.2 Cap:1

2008-06-30_001_A765R
A765R
KB_3130_POP7_BDTv3.mob
Pts 2304 to 13960 Pk1 Loc:2287
Version 5.2.0 HiSQV Bases: 507

Inst Model/Name 3100/3130MERCIAN-17307-036
Jun 30,2008 10:45AM, ICT
Jun 30,2008 11:01AM, ICT
Spacing:19.04 Pts/Panel1500
Plate Name: nila(unri300608)

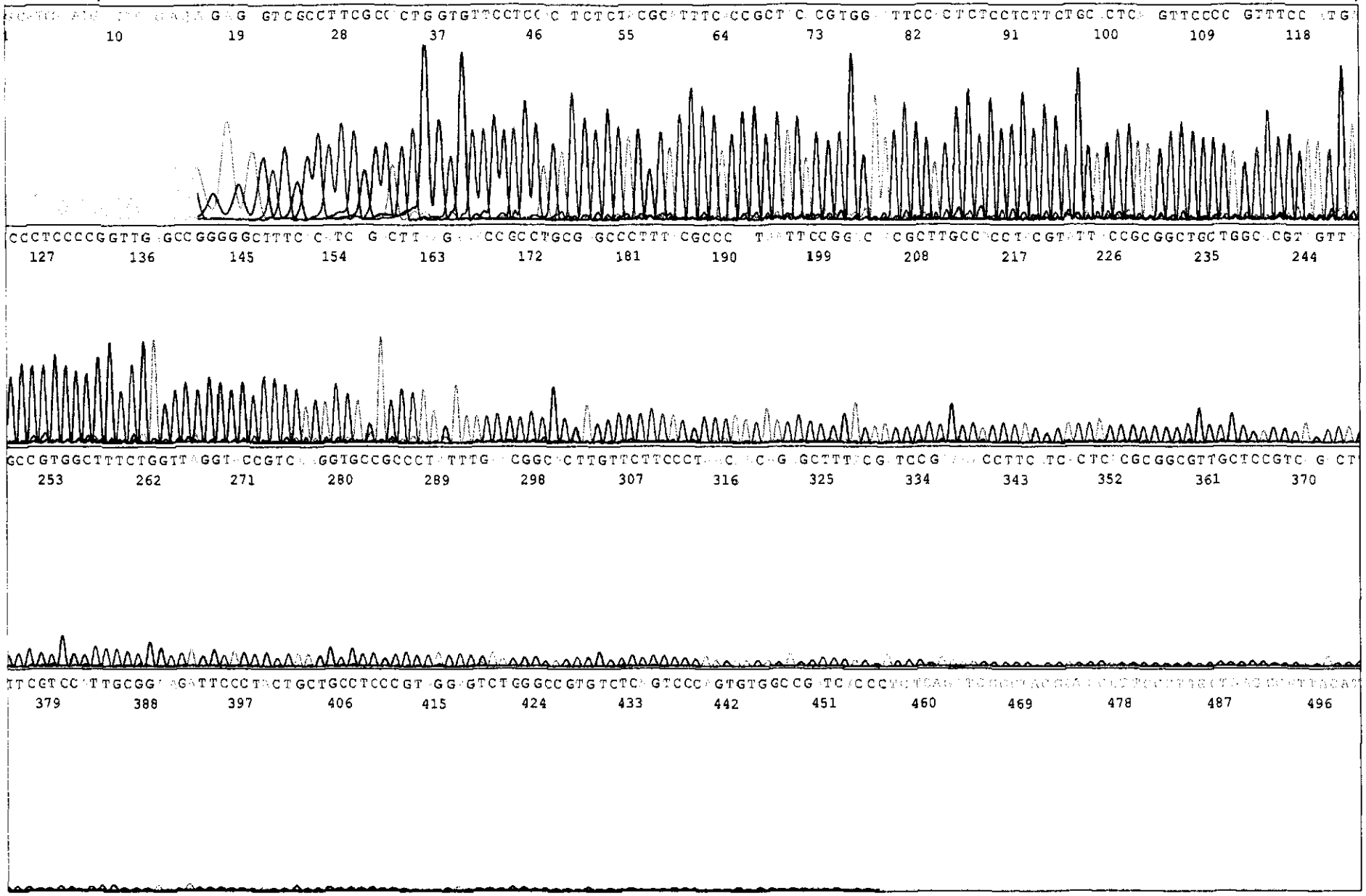


Biosystems

S/N G:68 A:30 T:51 C:64
KB.bcp
KB 1.2 Cap:3

2008-06-30_003_D765R
D765R
KB_3130_POP7_BDTv3.mob
Pts 2304 to 13960 Pk1 Loc:2287
Version 5.2.0 HiSQV Bases: 467

Inst Model/Name 3100/3130MERCIAN-17307-036
Jun 30,2008 10:45AM, ICT
Jun 30,2008 11:01AM, ICT
Spacing:19.12 Pts/Panel1500
Plate Name: nila(unri300608)

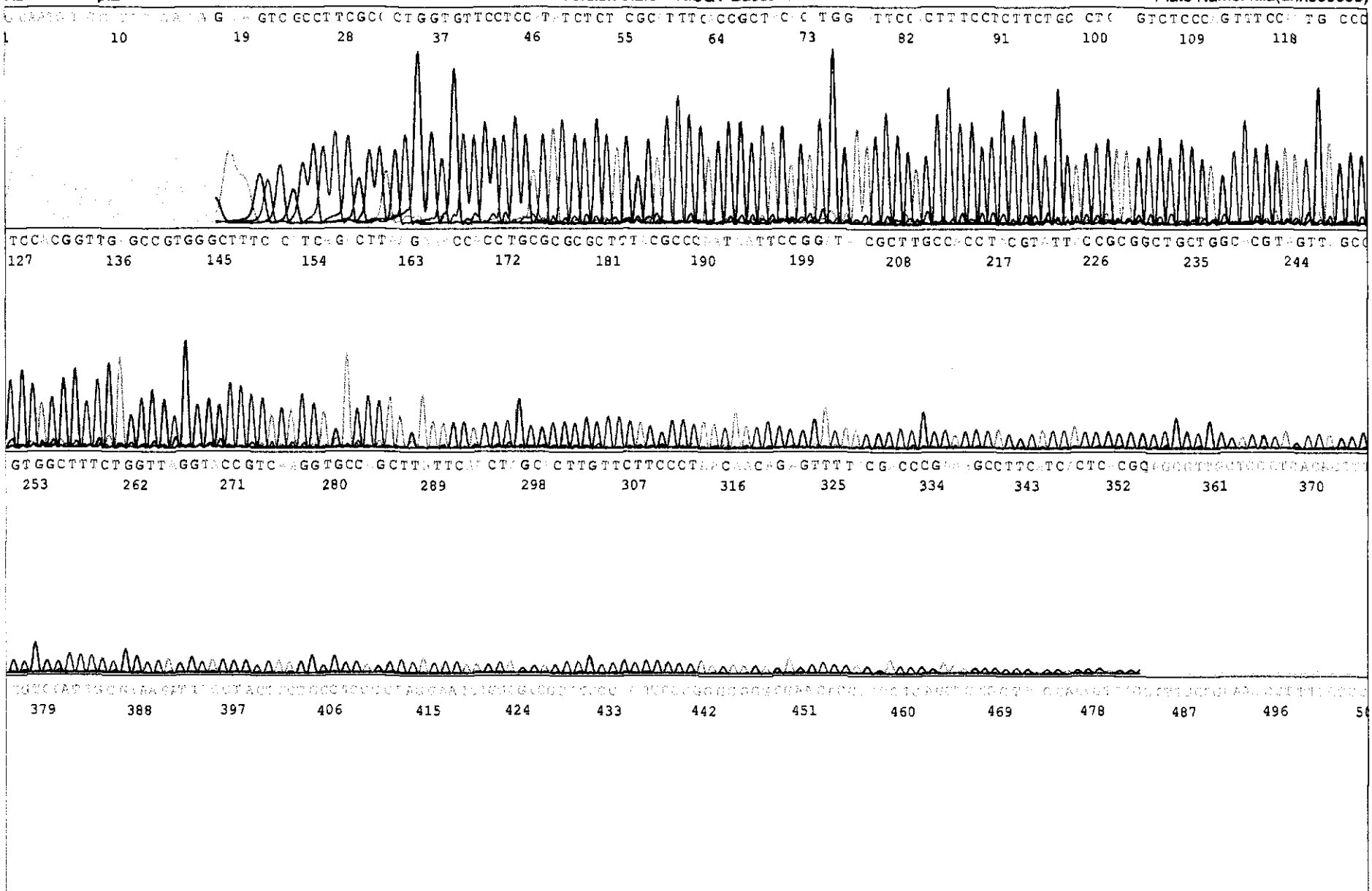


Biosystems

S/N G:128 A:55 T:98 C:118
KB.bcp
KB 1.2 Cap:2

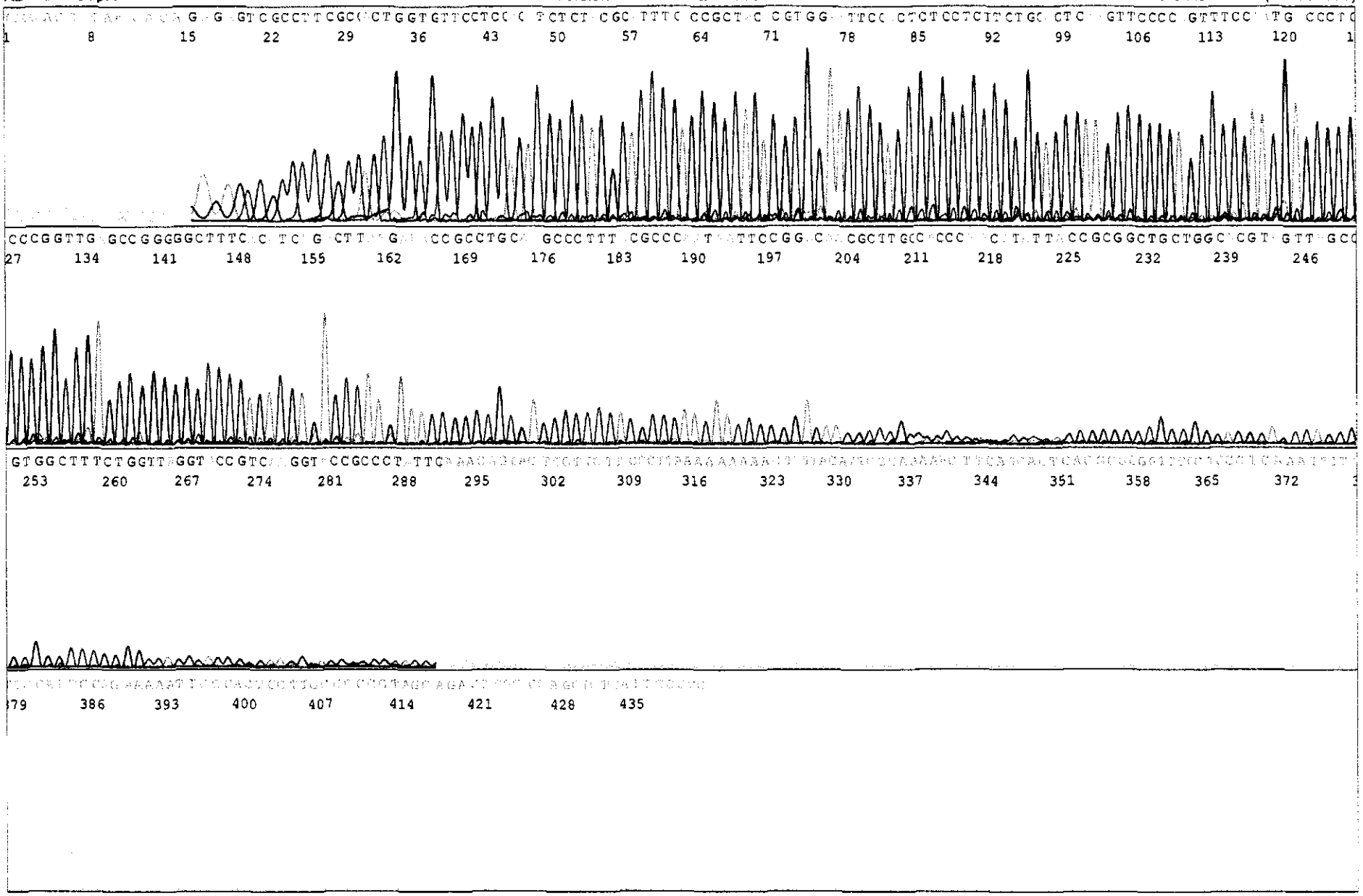
E765R
KB_3130_POP7_BDTv3.mob
Pts 2323 to 13960 Pk1 Loc:2306
Version 5.2.0 HiSQV Bases: 381

Jun 30,2008 10:45AM, ICT
Jun 30,2008 11:01AM, ICT
Spacing:18.84 Pts/Panel1500
Plate Name: nila(unri300608)



Biosystems

2006-06-30_004_H765R
H765R
KB_3130_POP7_BDTv3.mob
Pts 2338 to 13960 Pk1 Loc:2321
Version 5.2.0 HiSQV Bases: 286

Inst Model/Name 3100/3130MERCIAN-17307-036
Jun 30,2008 10:45AM, ICT
Jun 30,2008 11:01AM, ICT
Spacing:17.34 Pts/Panel1500
Plate Name: nila(unri300608)

S/N G:103 A:43 T:65 C:90
KB.bcp
KB 1.2 Cap:4