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LAMPIRAN

Lampiran 1 Perhitungan Konsentrasi Isolat DNA *Alcaligenes* sp. JG3

Diketahui:

Absorbansi pada λ 260 nm = 0,590
Absorbansi pada λ 280 nm = 0,435

Maka,

Konsentasi DNA = (A_{260} x 50 x faktor pengenceran) µg/mL
= (0,590 x 50 x 100) µg/mL
= 2950 µg/mL
= 2,95 mg/mL

Kemurnian DNA = A_{260}/A_{280}
= 0,590/0,435
= 1,356

Lampiran 2 Perhitungan Efisiensi Transformasi

Diketahui:

Konsentrasi DNA insert = 0,1 ng
Volume media SOC = 1000 µL
Volume media terkultur = 200 µL
Koloni putih terbentuk = 154

Maka,

$$\text{Efifiensi transformasi} = \frac{koloni\ yang\ terbentuk}{konsentrasi\ DNA\ akhir\ yang\ ditanam}$$

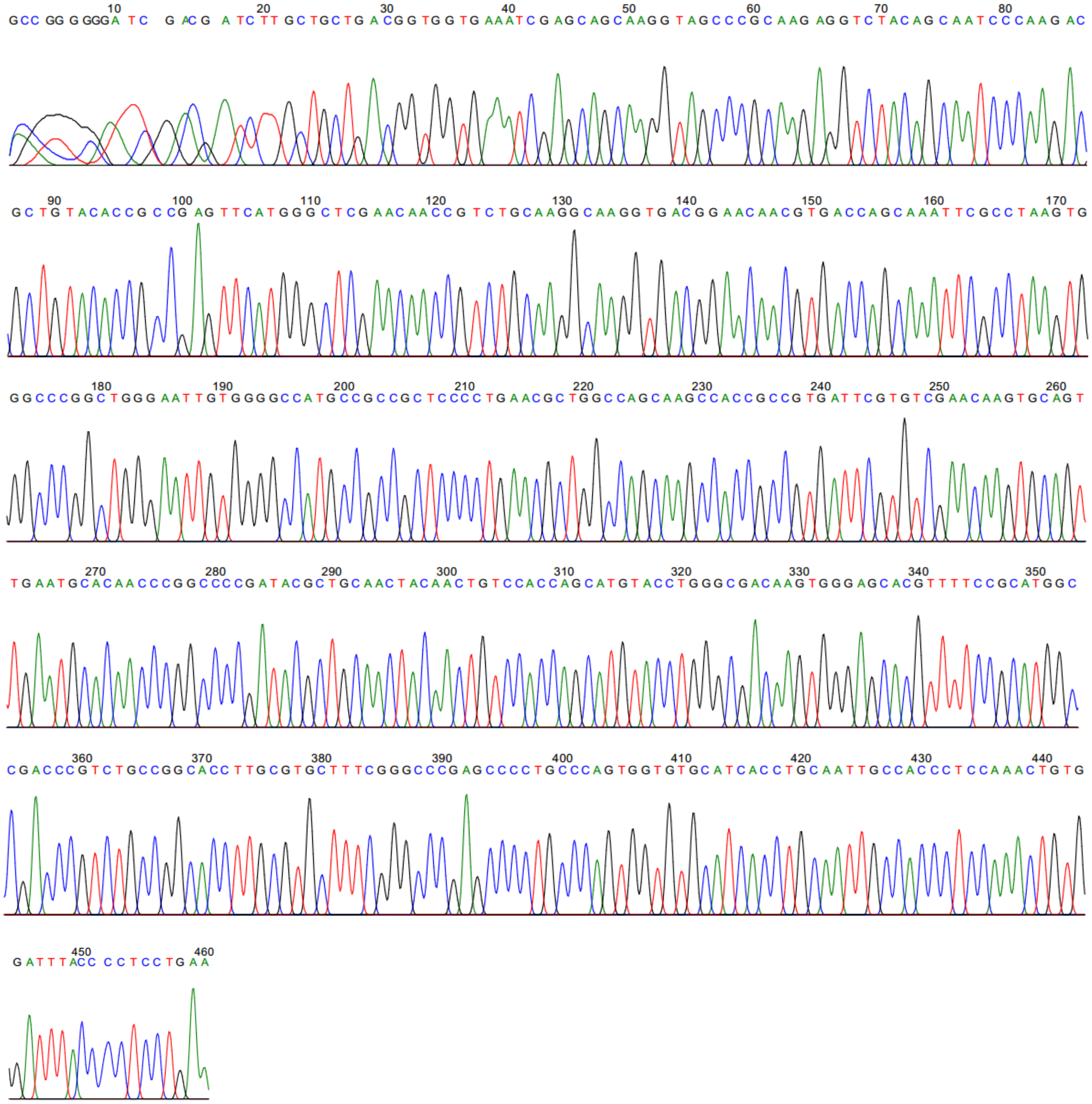
$$= \frac{154}{0{,}1}$$

$$= 1{,}54 \times 10^{3}\ \text{cfu/ng}$$

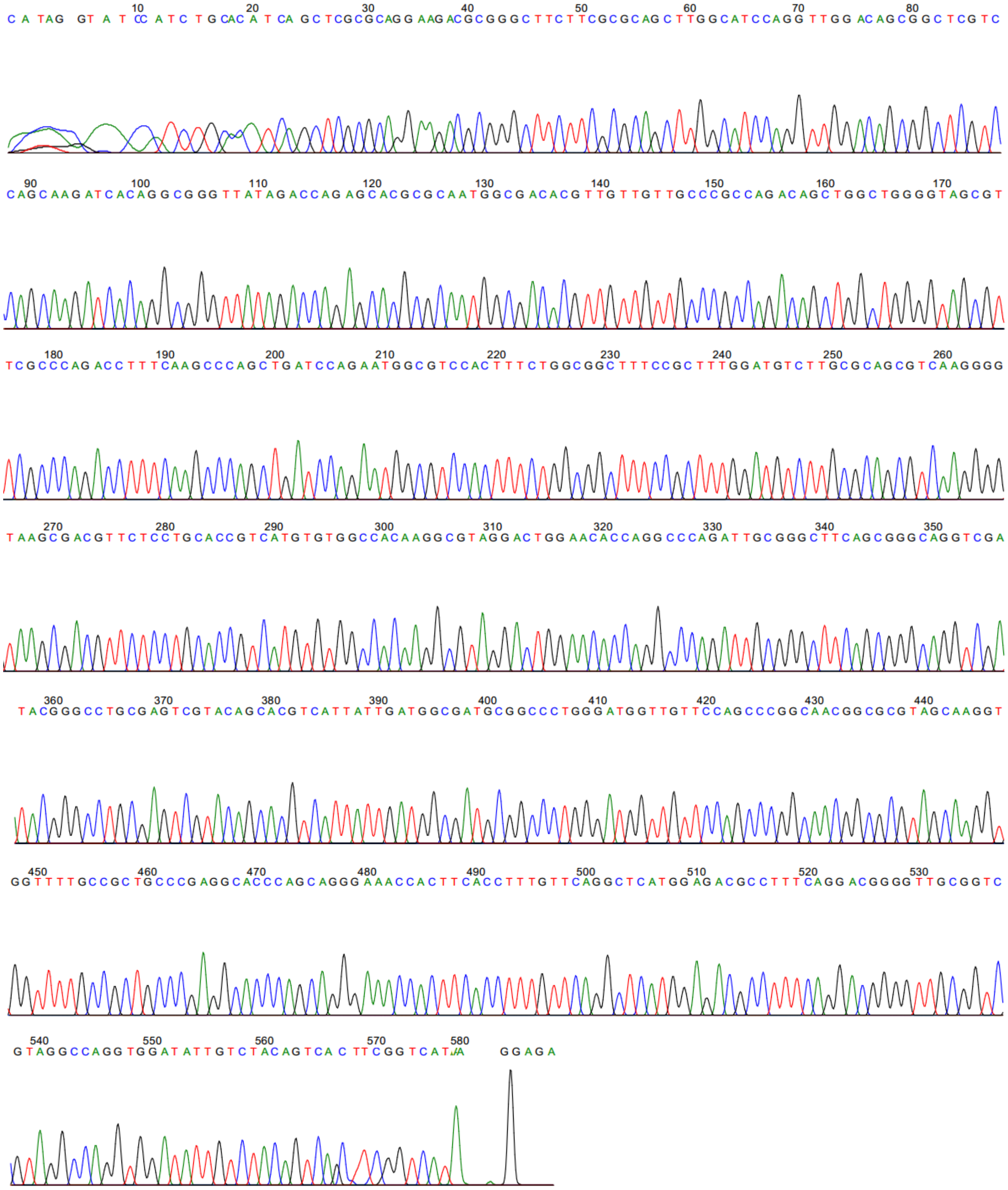
Lampiran 3 Elektroferogram Hasil Analisis Sekuensing

a. Primer pUC/M13

b. Primer internal *forward* (Fi)



c. Primer internal *reverse* (Ri)



Lampiran 4 Urutan Nukleotida Hasil Analisis Sekuensing

a. Primer pUC/M13

```
1     AGCTACGACG GCAGTGATTG TATACGACTC ACTATAGGGC GAATTGGGCC  50
51    CGACGTCGCA TGCTCCCGGC CGCCATGGCG GCCGCGGGAA TTCGATTATG 100
101   ACCGAGCTGA CTGTAGACAA TATCCACCTG GCCTACGACC GCAACCCCGT 150
151   CCTGAAAGGC GTCTCCATGA GCCTGAACAA AGGTGAAGTG GTTTCCCTGC 200
201   TGGGTGCCTC GGGCAGCGGC AAAACCACCT TGCTACGCGC CGTTGCCGGG 250
251   CTGGAACAAC CATCCCAGGG CCGCATCGCC ATCAATAATG ACGTGCTGTA 300
301   CGACTCGCAG GCCCGTATCG ACCTGCCCGC TGAAGCCCGC AATCTGGGCC 350
351   TGGTGTTCCA GTCCTACGCC TTGTGGCCAC ACATGACGGT GCAGGAGAAC 400
401  GTCGCTTACC CCTTGACGCT GCGCAAGACA TCCAAAGCGG AAAGCCGCCA  450
451  GAAAGTGGAC GCCATTCTGG ATCAGCTGGG CTTGAAAGGT CTGGGCGAAC  500
501  GCTACCCCAG CCAGCTGTCT GGCGGGCAAC AACAACGTGT CGCCATTGCG  550
551  CGTGCTCTGG TCTATAACCC GCCTGTGATC TTGCTGGACG AGCCGCTGTC  600
601  CAACCTGGAT GCCAAGCTGC GCGAAGAAGC CCGCGTCTTC CTGCGCGAGC  650
651  TGATTGTGCA GATGGGTTTG TCGGCTTTGA TGGTGACCCA TGACCAGGCG  700
701  GAAGCCATGG CGATTTCGGA CCGAATCTTG CTGCTGAACG GTGGTGAAAT  750
751  CGAGCAGCAA GGTAGCCCGC AAGAGGTCTA CAGCAATCCC AAGACGCTGT  800
801  ACACCGCCGA GTTCATGGGC TCGAACAACC GTCTGCAAGG CAAGGTGACG  850
851  GAACAACGTG ACCAGCAAAT TCGCCTAAGT GGGCCCGGCT GGGAATTGTG  900
901  GGGCCATGCC GCCGCTCCCC TGAACGCTGG CCAGCAAGCC ACCGCCGTGA  950
951  TTCGTGTCGA ACAAGTGCAG TTGAATGCAC AACCCGGCCC CGATACGCTG 1000
1001 CAACTACAAC TGTCCACCAG CATGTACCTG GGCGACAAGT GGGGGCACGT 1050
1051 TTTCCGCATG GCCGACCCGT CTGCCGGCAC CTTGCGTGCT TTCGGGCCCG 1100
1101 AGCCCCTGCC CAGTGGTGTG CATCACCTGC AATTGCCACC CTCCAAACTG 1150
1151 TGGATTTACC CCTCCTGAAT CACTAGTGAA TTCCCGGCCG CCTGCAGGTC 1200
1121 GACCTAATGG GAAAGCTCCC AACCCGTTGG ATGCAAACCT GGATATTCTA 1250
1251 TGGTCCCCAA AAAGCTTGCG AATCTGGTAG CTTTTTTTCT TGTTATTAAA 1300
1301 AATTCGCCAA GGGGGTGGCG GGGAG 1325
```

b. Primer Fi

```
1    GCCGGGGGGA TCGACGATCT TGCTGCTGAC  GGTGGTGAAA TCGAGCAGCA 50
51   AGGTAGCCCG CAAGAGGTCT ACAGCAATCC CAAGACGCTG TACACCGCCG 100
101 AGTTCATGGG CTCGAACAAC CGTCTGCAAG GCAAGGTGAC GGAACAACGT 150
151 GACCAGCAAA TTCGCCTAAG TGGGCCCGGC TGGGAATTGT GGGGCCATGC 200
201 CGCCGCTCCC CTGAACGCTG GCCAGCAAGC CACCGCCGTG ATTCGTGTCG 250
251 AACAAGTGCA GTTGAATGCA CAACCCGGCC CCGATACGCT GCAACTACAA 300
301 CTGTCCACCA GCATGTACCT GGGCGACAAG TGGGAGCACG TTTTCCGCAT 350
351 GGCCGACCCG TCTGCCGGCA CCTTGCGTGC TTTCGGGCCC GAGCCCCTGC 400
401 CCAGTGGTGT GCATCACCTG CAATTGCCAC CCTCCAAACT GTGGATTTAC 450
451 CCCTCCTGAA 460
```

c. Primer Ri

```
1    CATAGGTATC CATCTGCACA TCAGCTCGCG  CAGGAAGACG CGGGCTTCTT 50
51   CGCGCAGCTT GGCATCCAGG TTGGACAGCG GCTCGTCCAG CAAGATCACA 100
151 GGCGGGTTAT AGACCAGAGC ACGCGCAATG GCGACACGTT GTTGTTGCCC 200
201 GCCAGACAGC TGGCTGGGGT AGCGTTCGCC CAGACCTTTC AAGCCCAGCT 250
```

```
300 GATCCAGAAT GGCGTCCACT TTCTGGCGGC TTTCCGCTTT GGATGTCTTG 350
351 CGCAGCGTCA AGGGGTAAGC GACGTTCTCC TGCACCGTCA TGTGTGGCCA 400
401 CAAGGCGTAG GACTGGAACA CCAGGCCCAG ATTGCGGGCT TCAGCGGGCA 450
500 GGTCGATACG GGCCTGCGAG TCGTACAGCA CGTCATTATT GATGGCGATG 550
551 CGGCCCTGGG ATGGTTGTTC CAGCCCGGCA ACGGCGCGTA GCAAGGTGGT 600
601 TTTGCCGCTG CCCGAGGCAC CCAGCAGGGA AACCACTTCA CCTTTGTTCA 650
651 GGCTCATGGA GACGCCTTTC AGGACGGGGT TGCGGTCGTA GGCCAGGTGG 700
701 ATATTGTCTA CAGTCACTTC GGTCATAAAA  GGAGA 735
```

Lampiran 5 Urutan Nukleotida Gen Pengkode Lipase dari Bakteri *Alcaligenes faecalis* subsp. *faecalis* NCIB 8687

```
LOCUS       NZ_AKMR01000005 1071 bp DNA linear CON 07-MAY-2013
DEFINITION  Alcaligenes faecalis subsp. faecalis NCIB 8687 Contig_6, whole
            genome shotgun sequence.
ACCESSION   NZ_AKMR01000005    REGION: 11239..12309
VERSION     NZ_AKMR01000005.1  GI:393758538
DBLINK      Project: 170035
            BioProject: PRJNA170035
KEYWORDS    WGS; RefSeq.
SOURCE      Alcaligenes faecalis subsp. faecalis NCIB 8687
ORGANISM    Alcaligenes faecalis subsp. faecalis NCIB 8687
            Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales;
            Alcaligenaceae; Alcaligenes.
REFERENCE   1  (bases 1 to 1071)
AUTHORS     Phung le,T., Trimble,W.L., Meyer,F., Gilbert,J.A. and Silver,S.
TITLE       Draft Genome Sequence of Alcaligenes faecalis subsp. faecalis
            NCIB 8687 (CCUG 2071)
JOURNAL     J. Bacteriol. 194 (18), 5153 (2012) PUBMED   22933773
REFERENCE   2  (bases 1 to 1071)
AUTHORS     Phung,L.T., Trimble,W.L., Meyer,F., Gilbert,J.A. and Silver,S.
TITLE       Direct Submission
JOURNAL     Submitted (12-JUN-2012) Department of Microbiology and
            Immunology, University of Illinois, M/C 790 835 S. Wolcott,
            Chicago, IL 60612,USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to
            final NCBI review. The reference sequence was derived from
            AKMR01000005. Annotation was added by the NCBI Prokaryotic
            Genomes Automatic Annotation Pipeline Group. Information about
            the Pipeline can be
            found here:
            http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html. Please
            beaware that the annotation is done automatically with little or
            no manual curation. Bacteria available from culture collections:
            NCIB 8156, ATCC 8750 and others.
```

```
            ##Genome-Assembly-Data-START##
            Assembly Method       :: MIRA v. 3.4.0
            Assembly Name         :: Gretchen3
            Genome Coverage       :: 171.0x
            Sequencing Technology :: Illumina HiSeq
            ##Genome-Assembly-Data-END##
FEATURES    Location/Qualifiers
     gene            1..1071/locus_tag="QWA_05490"
     CDS             1..1071/locus_tag="QWA_05490"/note="COG3842 ABC-type
                     spermidine/putrescine transport systems, ATPase
                     components"/codon_start=1
                     /transl_table=11/product="lipase"/protein_id="WP_003801
                     170.1"/db_xref="GI:489897727"
/translation="MTELTVDNIHLAYDRNPVLKGLSMSLNKGEVVSLLGASGSGKTT
LLRAVAGLEQPSQGRIAINENVLYDSQNRIDLPAEARNLGLVFQSYALWPHMTVQDNV
AYPLTLRKTSKAQSRQRVDAILDQLGLKGLGERYPSQLSGGQQQRVAIARALIYNPPV
ILLDEPLSNLDAKLREEARVFLRELIVKMGLSALMVTHDQAEAMAISDRILLLNGGEI
EQQGTPQEVYSNPKTLYTAEFMGSNNRLQGKVTEQRDQQTRLSGPGWELWGHAAAPLS
AGQHATAVIRVEQVQLNAQPGPDTLPLQLSTSMYLGDKWEHVFRMADPTAGTLRAFGP
EPLPSGVHHLQLPPSKLWIYPS"
ORIGIN

1    atgaccgagc tgactgtaga caacattcat ctggcctacg accgcaaccc ggttctgaaa
61   ggcctgtcca tgagcctgaa caaaggtgag gtggtttccc tgctgggggc ctcgggtagt
121  ggcaaaacca ccttgctgcg tgcggttgcc gggctcgaac agccctccca agggcgtatc
181  gccatcaatg agaacgtgct gtacgactcg caaaaccgta tcgacctgcc cgccgaggcg
241  cgcaatctgg gcctggtttt ccagtcctat gccttgtggc cacacatgac cgtgcaggac
301  aatgtggctt atccgctcac gctgcgcaaa acctccaagg cgcaaagccg ccaaagggtg
361  gacgccattt tggatcaatt ggggttgaaa ggcctgggcg agcgctaccc cagccaactg
421  tctggagggc aacagcagcg tgtggccatt gcccgtgcgc tgatttacaa ccccccggtc
481  atcttgctgg atgagccact gtccaacctg gatgccaagc tgcgtgaaga ggcccgcgtg
541  ttcctgcgcg aactgattgt gaaaatgggc ctgtccgctt tgatggtgac ccatgaccag
601  gcggaggcca tggcgatttc ggaccggatc ctgttgctga acggtggcga aatcgagcaa
661  caaggcaccc cgcaagaggt ctacagcaat cccaagaccc tgtacaccgc cgagttcatg
721  ggctcgaaca atcgtctgca aggcaaggtt accgagcaac gcgatcaaca aactcggctt
781  agcggccctg gttgggagtt atggggccat gccgccgctc ccctgagcgc cggccagcac
841  gccaccgccg tgattcgcgt cgaacaagtg caactgaatg cgcagcccgg ccccgatacg
901  ctgccactgc agctatccac cagcatgtat ctgggcgaca agtgggagca cgttttccgc
961  atggccgacc cgactgccgg cacactgcga gctttcggac ctgaaccact gcccagtggc
1021 gtgcatcatc tgcaattgcc gccctccaaa ctgtggattt acccctcctg a
```

Lampiran 6 Hasil Translasi Urutan Nukleotida Lipase JG3 dengan ExPAsy

Keterangan: *Open Reading Frame* ditandai dengan warna biru.

ORF terpilih dalam studi ini adalah **5'3' Frame 2.**

5'3' Frame 1

S Y D G S D C I R L T I G R I G P D V A C S R P P W R P R E F D
Y D R A D C R Q Y P P G L R P Q P R P E R R L H E P E Q R Stop
S G F P A G C L G Q R Q N H L A T R R C R A G T T I P G P H R H
Q Stop Stop R A V R L A G P Y R P A R Stop S P Q S G P G V P V
L R L V A T H D G A G E R R L P L D A A Q D I Q S G K P P E S G
R H S G S A G L E R S G R T L P Q P A V W R A T T T C R H C A C
S G L Stop P A C D L A G R A A V Q P G C Q A A R R S P R L P A
R A D C A D G F V G F D G D P Stop P G G S H G D F G P N L A A
E R W Stop N R A A R Stop P A R G L Q Q S Q D A V H R R V H G L
E Q P S A R Q G D G T T Stop P A N S P K W A R L G I V G P C R
R S P E R W P A S H R R D S C R T S A V E C T T R P R Y A A T T
T V H Q H V P G R Q V G A R F P H G R P V C R H L A C F R A R A
P A Q W C A S P A I A T L Q T V D L P L L N H Stop Stop I P G R
L Q V D L Met G K L P T R W Met Q T W I F Y G P Q K A C E S G S
F F S C Y Stop K F A K G V A G

5'3' Frame 2

A T T A V I V Y D S L Stop G E L G P T S H A P G R H G G R G N
S I Met T E L T V D N I H L A Y D R N P V L K G V S Met S L N K
G E V V S L L G A S G S G K T T L L R A V A G L E Q P S Q G R I
A I N N D V L Y D S Q A R I D L P A E A R N L G L V F Q S Y A L
W P H Met T V Q E N V A Y P L T L R K T S K A E S R Q K V D A I
L D Q L G L K G L G E R Y P S Q L S G G Q Q Q R V A I A R A L V
Y N P P V I L L D E P L S N L D A K L R E E A R V F L R E L I V
Q Met G L S A L Met V T H D Q A E A Met A I S D R I L L L N G G
E I E Q Q G S P Q E V Y S N P K T L Y T A E F Met G S N N R L Q
G K V T E Q R D Q Q I R L S G P G W E L W G H A A A P L N A G Q
Q A T A V I R V E Q V Q L N A Q P G P D T L Q L Q L S T S Met Y
L G D K W G H V F R Met A D P S A G T L R A F G P E P L P S G V
H H L Q L P P S K L W I Y P S Stop I T S E F P A A C R S T Stop

W E S S Q P V G C K P G Y S Met V P K K L A N L V A F F L V I K
N S P R G W R G

5’3’ Frame 3

L R R Q Stop L Y T T H Y R A N W A R R R Met L P A A Met A A A
G I R L Stop P S Stop L Stop T I S T W P T T A T P S Stop K A S
P Stop A Stop T K V K W F P C W V P R A A A K P P C Y A P L P G
W N N H P R A A S P S I Met T C C T T R R P V S T C P L K P A I
W A W C S S P T P C G H T Stop R C R R T S L T P Stop R C A R H
P K R K A A R K W T P F W I S W A Stop K V W A N A T P A S C L
A G N N N V S P L R V L W S I T R L Stop S C W T S R C P T W Met
P S C A K K P A S S C A S Stop L C R W V C R L Stop W Stop P Met
T R R K P W R F R T E S C C Stop T V V K S S S K V A R K R S T
A I P R R C T P P S S W A R T T V C K A R Stop R N N V T S K F
A Stop V G P A G N C G A Met P P L P Stop T L A S K P P P Stop
F V S N K C S Stop Met H N P A P I R C N Y N C P P A C T W A T
S G G T F S A W P T R L P A P C V L S G P S P C P V V C I T C N
C H P P N C G F T P P E S L V N S R P P A G R P N G K A P N P L
D A N L D I L W S P K S L R I W Stop L F F L L L K I R Q G G G
G E

3’5’ Frame 1

L P A T P L A N F Stop Stop Q E K K L P D S Q A F W G P Stop N
I Q V C I Q R V G S F P I R S T C R R P G I H Stop Stop F R R G
K S T V W R V A I A G D A H H W A G A R A R K H A R C R Q T G R
P C G K R A P T C R P G T C W W T V V V A A Y R G R V V H S T A
L V R H E S R R W L A G Q R S G E R R H G P T I P S R A H L G E
F A G H V V P S P C L A D G C S S P Stop T R R C T A S W D C C
R P L A G Y L A A R F H H R S A A R F G P K S P W L P P G H G S
P S K P T N P S A Q S A R A G R R G L L R A A W H P G W T A A R
P A R S Q A G Y R P E H A Q W R H V V V A R Q T A G W G S V R P

D L S S P A D P E W R P L S G G F P L W Met S C A A S R G K R R
S P A P S C V A T R R R T G T P G P D C G L Q R A G R Y G P A S
R T A R H Y Stop W R C G P G Met V V P A R Q R R V A R W F C R
C P R H P A G K P L H L C S G S W R R L S G R G C G R R P G G Y
C L Q S A R S Stop S N S R G R H G G R E H A T S G P I R P I V
S R I Q S L P S Stop

3’5’ Frame 2

S P P P P W R I F N N K K K S Y Q I R K L F G D H R I S R F A S
N G L G A F P L G R P A G G R E F T S D S G G V N P Q F G G W Q
L Q V Met H T T G Q G L G P E S T Q G A G R R V G H A E N V P P
L V A Q V H A G G Q L Stop L Q R I G A G L C I Q L H L F D T N
H G G G L L A S V Q G S G G Met A P Q F P A G P T Stop A N L L
V T L F R H L A L Q T V V R A H E L G G V Q R L G I A V D L L R
A T L L L D F T T V Q Q Q D S V R N R H G F R L V Met G H H Q S
R Q T H L H N Q L A Q E D A G F F A Q L G I Q V G Q R L V Q Q D
H R R V I D Q S T R N G D T L L L P A R Q L A G V A F A Q T F Q
A Q L I Q N G V H F L A A F R F G C L A Q R Q G V S D V L L H R
H V W P Q G V G L E H Q A Q I A G F S G Q V D T G L R V V Q H V
I I D G D A A L G W L F Q P G N G A Stop Q G G F A A A R G T Q
Q G N H F T F V Q A H G D A F Q D G V A V V G Q V D I V Y S Q L
G H N R I P A A A Met A A G S Met R R R A Q F A L Stop Stop V V
Y N H C R R S

3’5’ Frame 3

P R H P L G E F L I T R K K A T R F A S F L G T I E Y P G L H P
T G W E L S H Stop V D L Q A A G N S L V I Q E G Stop I H S L E
G G N C R Stop C T P L G R G S G P K A R K V P A D G S A Met R
K T C P H L S P R Y Met L V D S C S C S V S G P G C A F N C T C
S T R I T A V A C W P A F R G A A A W P H N S Q P G P L R R I C
W S R C S V T L P C R R L F E P Met N S A V Y S V L G L L Stop
T S C G L P C C S I S P P F S S K I R S E I A Met A S A W S W V

```
T I K A D K P I C T I S S R R K T R A S S R S L A S R L D S G S
S S K I T G G L Stop T R A R A Met A T R C C C P P D S W L G
Stop R S P R P F K P S Stop S R Met A S T F W R L S A L D V L R
S V K G Stop A T F S C T V Met C G H K A Stop D W N T R P R L R
A S A G R S I R A C E S Y S T S L L Met A Met R P W D G C S S P
A T A R S K V V L P L P E A P S R E T T S P L F R L Met E T P F
R T G L R S Stop A R W I L S T V S S V I I E F P R P P W R P G
A C D V G P N S P Y S E S Y T I T A V V A
```

Lampiran 7 Hasil Pemotongan Enzim Restriksi Nukleotida Lipase JG3 dengan *Restriction Mapper*

Name: Lip.JG3

Overhang: five_prime, three_prime, blunt

Minimum Site Length: 5 bases

Maximum Number of Cuts: all

Included: all commercial, prototypes only

Noncutters: AatII, AbsI, AclI, AflII, AgeI, AjuI, AlfI, AloI, AlwNI, ApaLI, ArsI, AscI, AsuII, AvrII, BaeI, BamHI, BarI, BbvCI, BciVI, BclI, BdaI, BglI, BglII, BplI, Bpu10I, BsaAI, BsaBI, BsaXI, BseMII, BsePI, BseRI, BsmI, BspHI, BsrBI, BtsI, ClaI, CspCI, DraII, DraIII, DrdI, Eam1105I, Eco31I, Eco47III, EcoNI, EcoRI, EcoRV, FalI, FseI, FspAI, GsuI, HaeII, HaeIV, HindII, HindIII, HpaI, KpnI, MauBI, MluI, NarI, NdeI, NheI, NotI, NruI, OliI, PacI, PfoI, PmaCI, PmeI, PpiI, PpuMI, PshAI, PsiI, PI-PspI, PspXI, PstI, PvuI, SacI, SacII, SalI, SanDI, SapI, ScaI, PI-SceI, SexAI, SfiI, SgfI, SgrAI, SgrDI, SmaI, SmlI, SnaBI, SpeI, SphI, SrfI, Sse8387I, SspI, StuI, SwaI, TsoI, TstI, Tth111I, VspI, XbaI, XhoI, XhoII, XmnI.

Keterangan: Pemotongan Lip.JG3 dalam studi ini dilakukan menggunakan enzim BamHI dan HindIII.

Cutters:

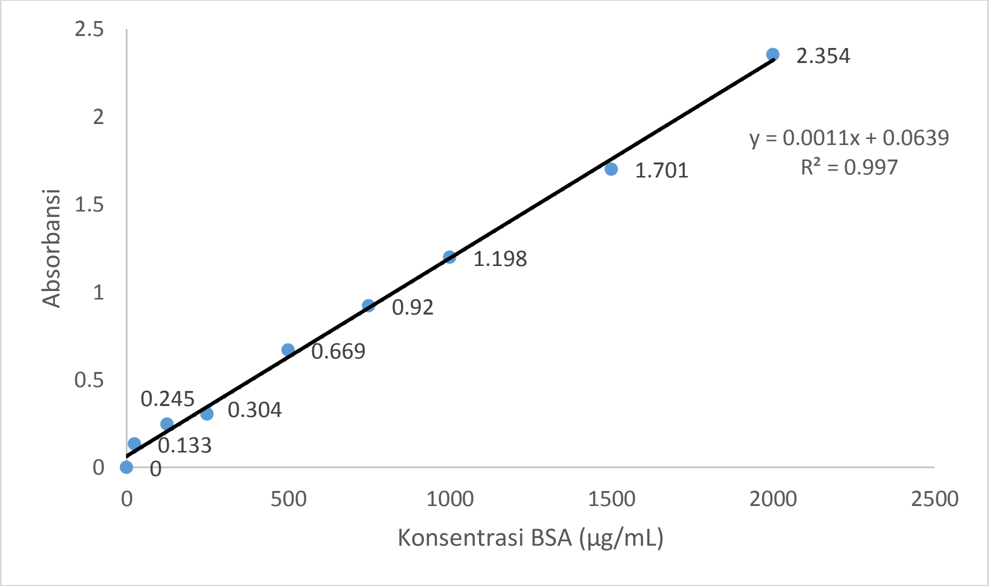
Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BalI	TGGCCA	6	blunt	1	279
BtrI	CACGTC	6	blunt	1	195
NaeI	GCCGGC	6	blunt	1	918
AarI	CACCTGC	7	five_prime	1	977
AflIII	ACRYGT	6	five_prime	1	438
ApoI	RAATTY	6	five_prime	1	770
AvaII	GGWCC	5	five_prime	1	621
BsmAI	GTCTC	5	five_prime	1	69
Bsp1407 I	TGTACA	6	five_prime	1	701
BstEII	GGTNACC	6	five_prime	1	585
BtgZI	GCGATG	6	five_prime	1	162
Cfr10I	RCCGGY	6	five_prime	1	916
Esp3I	CGTCTC	6	five_prime	1	69
MfeI	CAATTG	6	five_prime	1	973
NcoI	CCATGG	6	five_prime	1	608
PasI	CCCWGGG	7	five_prime	1	168
PleI	GAGTC	5	five_prime	1	199
RsrII	CGGWCCG	7	five_prime	1	621
StyI	CCWWGG	6	five_prime	1	608
TatI	WGTACW	6	five_prime	1	701
TfiI	GAWTC	5	five_prime	1	626
AgsI	TTSAA	5	three_prime	1	387
BfiI	ACTGGG	6	three_prime	1	947
BsrDI	GCAATG	6	three_prime	1	446

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BstXI	CCANNNNNNTGG	6	three_prime	1	421
EciI	GGCGGA	6	three_prime	1	616
Eco57I	CTGAAG	6	three_prime	1	254
Eco57MI	CTGRAG	6	three_prime	1	254
Hpy99I	CGWCG	5	three_prime	1	307
MmeI	TCCRAC	6	three_prime	1	527
NmeAIII	GCCGAG	6	three_prime	1	735
NspI	RCATGY	6	three_prime	1	867
TspDTI	ATGAA	5	three_prime	1	705
TspGWI	ACGGA	5	three_prime	1	766
TspRI	CASTG	5	three_prime	1	960
XcmI	CCANNNNNNNNNTGG	6	three_prime	1	867
MslI	CAYNNNNRTG	6	blunt	2	289, 862
PvuII	CAGCTG	6	blunt	2	378, 417
AccI	GTMKAC	6	five_prime	2	17, 680
AcyI	GRCGYC	6	five_prime	2	62, 362
AvaI	CYCGRG	6	five_prime	2	111, 940
BspMI	ACCTGC	6	five_prime	2	233, 977
CfrI	YGGCCR	6	five_prime	2	277, 902
EcoP15I	CAGCAG	6	five_prime	2	72, 606
ApaI	GGGCCC	6	three_prime	2	788, 941
BcgI	CGANNNNNNTGC	6	three_prime	2	895, 929
BsgI	GTGCAG	6	three_prime	2	313, 580
BsrI	ACTGG	5	three_prime	2	261, 953

Name	**Sequence**	**Site Length**	**Overhang**	**Frequency**	**Cut Positions**
Hin4I	GAYNNNNNVTC	6	three_prime	2	484, 516
MboII	GAAGA	5	three_prime	2	531, 538
PflMI	CCANNNNNTGG	6	three_prime	2	357, 992
PsrI	GAACNNNNNNTAC	7	three_prime	2	292, 324
TaqII	GACCGA	6	three_prime	2	612, 638
FokI	GGATG	5	five_prime	3	150, 318, 523
Tsp45I	GTSAC	5	five_prime	3	585, 747, 760
BseSI	GKGCMC	6	three_prime	3	788, 890, 941
BccI	CCATC	5	five_prime	4	171, 190, 558, 576
BseYI	CCCAGC	6	five_prime	4	102, 378, 409, 791
SfaNI	GCATC	5	five_prime	4	185, 501, 807, 972
HgaI	GACGC	5	five_prime	5	51, 327, 370, 525, 705
HphI	GGTGA	5	three_prime	5	97, 597, 658, 759, 958
TauI	GCSGC	5	three_prime	5	122, 176, 350, 498, 814
BbvI	GCAGC	5	five_prime	6	128, 308, 506, 620, 669, 827

Name	**Sequence**	**Site Length**	**Overhang**	**Frequency**	**Cut Positions**
EcoRII	CCWGG	5	five_prime	6	29, 167, 251, 506, 596, 869
FauI	CCCGC	5	five_prime	6	237, 247, 418, 479, 541, 677
TseI	GCWGC	5	five_prime	6	116, 321, 519, 633, 657, 840
SduI	GDGCHC	6	three_prime	6	459, 724, 788, 890, 941, 947

Lampiran 8 Perhitungan Konsentrasi Isolat Protein Hasil Overekspresi



a. Isolat protein kasar

$y = 0{,}0011x + 0{,}0639$ (1)

$y = 2{,}454$ (2)

Substitusi persamaan (2) ke persamaan (1), didapat:

$2{,}454 = 0{,}0011x + 0{,}0639$

$x = 2.172{,}81$

Maka konsentrasi isolat protein kasar adalah 2,172 mg/mL

b. Isolat sisa protein dari penyaringan

$y = 0{,}0011x + 0{,}0639$ (1)

$y = 1{,}293$ (2)

Substitusi persamaan (2) ke persamaan (1), didapat:

$1{,}293 = 0{,}0011x + 0{,}0639$

$x = 1.117{,}36$

Maka konsentrasi ptotein isolat hasil penyaringan adalah 1,117 mg/mL

c. Isolat pencucian pertama

$y = 0{,}0011x + 0{,}0639$ (1)

$y = 0{,}539$ (2)

Substitusi persamaan (2) ke persamaan (1), didapat:

$0{,}539 = 0{,}0011x + 0{,}0639$

x = 431,90
Maka konsentrasi protein isolat pencucian pertama adalah 0,431 mg/mL

d. Isolat pencucian kedua
y = 0,0011x + 0,0639 (1)
y = 0,300 (2)
Substitusi persamaan (2) ke persamaan (1), didapat:
0,300 = 0,0011x + 0,0639
x = 214,63
Maka konsentrasi protein isolat pencucian kedua adalah 0,214 mg/mL

e. Isolat pencucian ketiga
y = 0,0011x + 0,0639 (1)
y = 0,065 (2)
Substitusi persamaan (2) ke persamaan (1), didapat:
0,065 = 0,0011x + 0,0639
x = 1,00
Maka konsentrasi protein isolat pencucian ketiga adalah 0,001 mg/mL

f. Isolat Lip.JG3 murni
y = 0,0011x + 0,0639 (1)
y = 0,120 (2)
Substitusi persamaan (2) ke persamaan (1), didapat:
0,120 = 0,0011x + 0,0639
x = 51,00
Maka konsentrasi isolat Lip.JG3 murni adalah 0,051 mg/mL

Lampiran 9 Perhitungan Unit Aktivitas dan Aktivitas Spesifik dari Hidrolisis oleh Lipase JG3

a. Isolat protein kasar

$$Unit\ Aktivitas\ (U) = \frac{(V\ NaOH\ saampel - V\ NaOH\ standard)\ M\ NaOH}{waktu\ reaksi}$$

$$Unit\ Aktivitas\ (U) = \frac{(240\ \mu L - 150\ \mu L)\ 0{,}0125\ M}{300\ menit}$$

$$Unit\ Aktivitas\ (U) \approx 0{,}004$$

$$Aktivitas\ spesifik\ (U/mg) = \frac{unit\ aktivitas}{massa\ enzim}$$

$$Aktivitas\ spesifik\ (U/mg) = \frac{0{,}004}{1{,}086}$$

$$Aktivitas\ spesifik\ (U/mg) \approx 0{,}004\ \mathrm{U/mg}$$

b. Isolat protein pencucian kedua

$$Unit\ Aktivitas\ (U) = \frac{(V\ NaOH\ saampel - V\ NaOH\ standard)\ M\ NaOH}{waktu\ reaksi}$$

$$Unit\ Aktivitas\ (U) = \frac{(260\ \mu L - 150\ \mu L)\ 0{,}0125\ M}{300\ menit}$$

$$Unit\ Aktivitas\ (U) \approx 0{,}005\ \mathrm{U}$$

$$Aktivitas\ spesifik\ (U/mg) = \frac{unit\ aktivitas}{massa\ enzim}$$

$$Aktivitas\ spesifik\ (U/mg) = \frac{0{,}005\ U}{0{,}043\ mg}$$

$$Aktivitas\ spesifik\ (U/mg) \approx 0{,}116\ \mathrm{U/mg}$$

c. Isolat Lip.JG3 murni

$$Unit\ Aktivitas\ (U) = \frac{(V\ NaOH\ saampel - V\ NaOH\ standard)\ M\ NaOH}{waktu\ reaksi}$$

$$Unit\ Aktivitas\ (U) = \frac{(440\ \mu L - 150\ \mu L)\ 0{,}0125\ M}{300\ menit}$$

$$Unit\ Aktivitas\ (U) \approx 0{,}01175\ \mathrm{U}$$

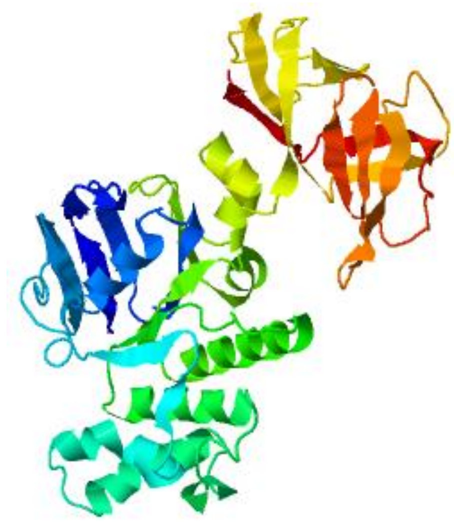
$$Aktivitas\ spesifik\ (U/mg) = \frac{unit\ aktivitas}{massa\ enzim}$$

$$Aktivitas\ spesifik\ (U/mg) = \frac{0{,}01175\ U}{0{,}010\ mg}$$

$$Aktivitas\ spesifik\ (U/mg) \approx 1{,}175\ \mathrm{U/mg}$$

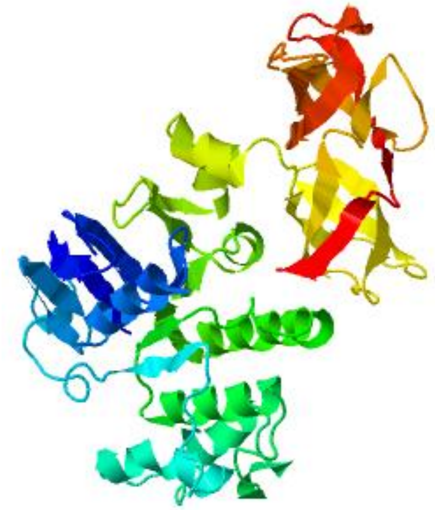
Lampiran 10 Prediksi Model Struktur 3-Dimensi Protein Lipase JG3 oleh I-TASSER

a. Model 1



Nilai C = 0,56
Nilai TM = 0,79±0,09
RMSD = 5,3±3,4 Å

b. Model 2



Nilai C = - 1,37

c. Model 3

Nilai C = - 1,18

d. Model 4

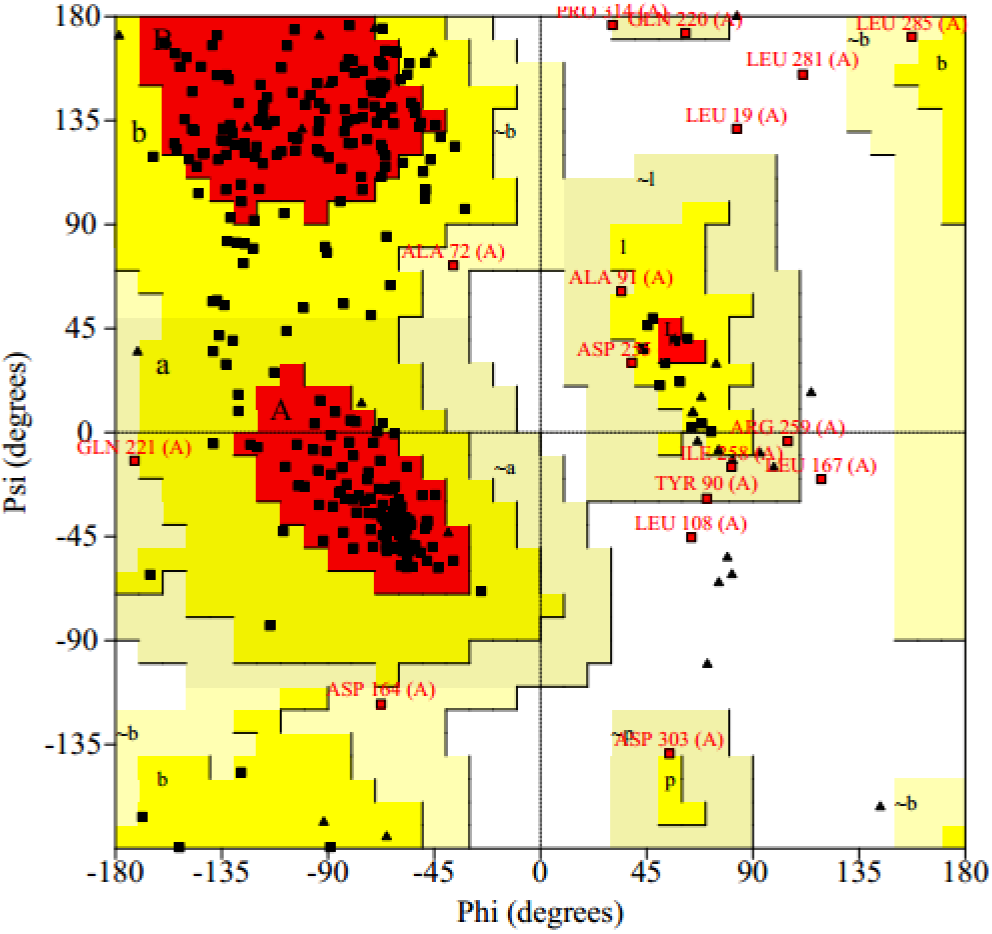
Nilai C = - 0,73

e. Model 5

Nilai C = - 2,07

Lampiran 11 Diagram Ramachandran Hasil Prediksi Model-Model Struktur 3-Dimensi Lipase JG3 dari I-TASSER

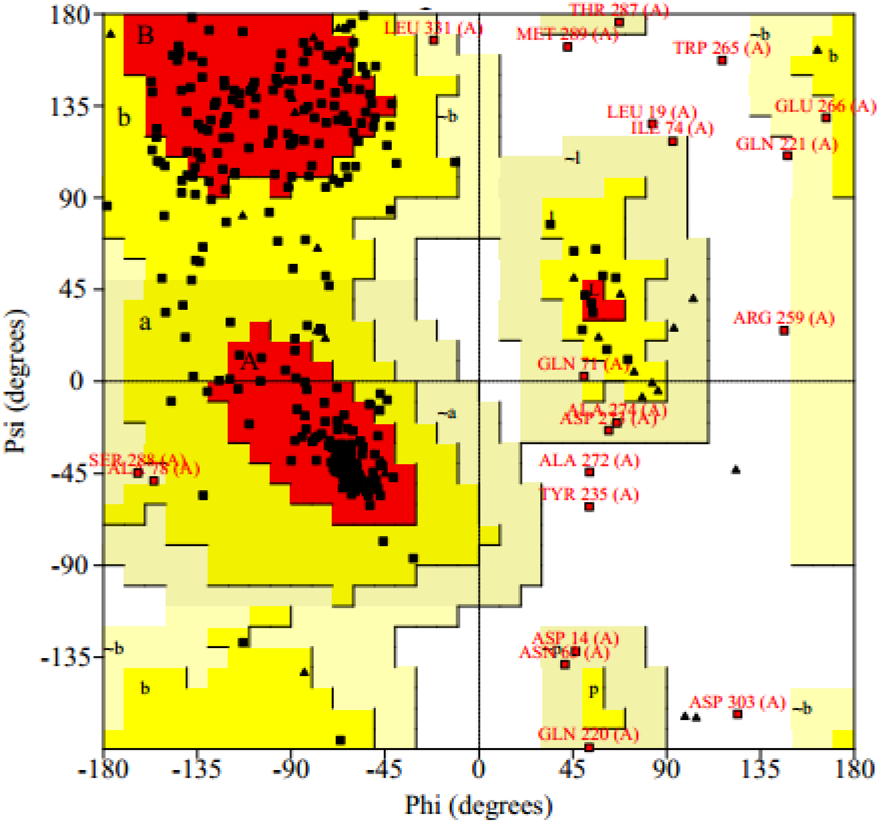
a. Model 1



Keterangan:

Daerah sangat disukai : 73,3%
Daerah tambahan yang diijinkan : 22,4%
Daerah yang diijinkan : 3,1%
Daerah tidak diijinkan : 1,2%

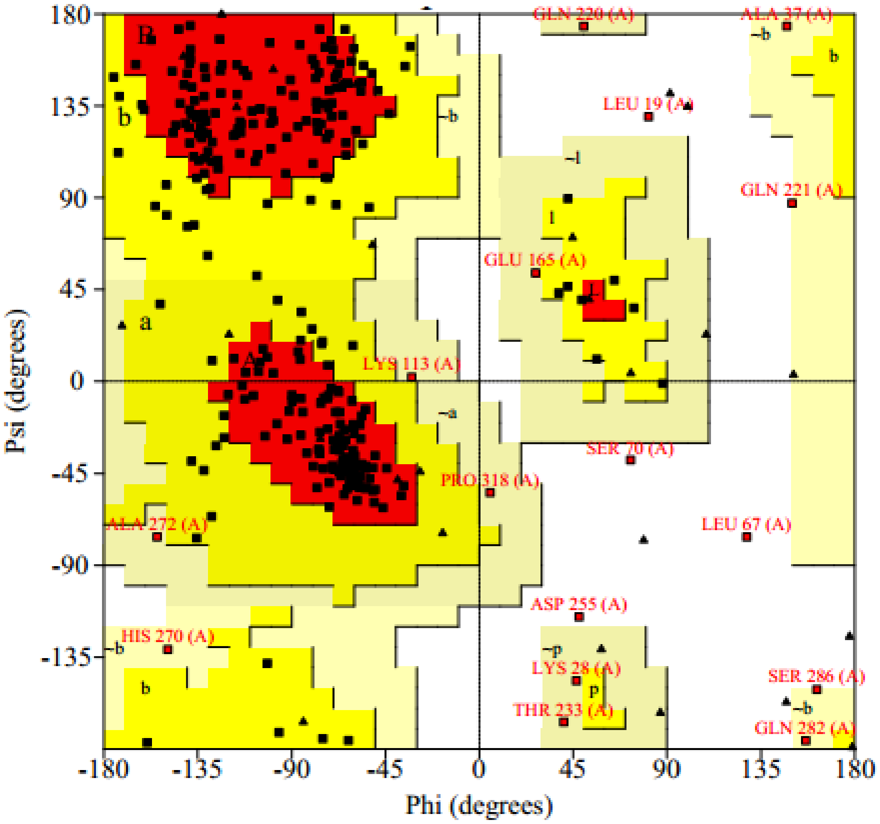
b. Model 2



Keterangan:

Daerah sangat disukai : 71,2%
Daerah tambahan yang diijinkan : 21,8%
Daerah yang diijinkan : 4,2%
Daerah tidak diijinkan : 2,8%

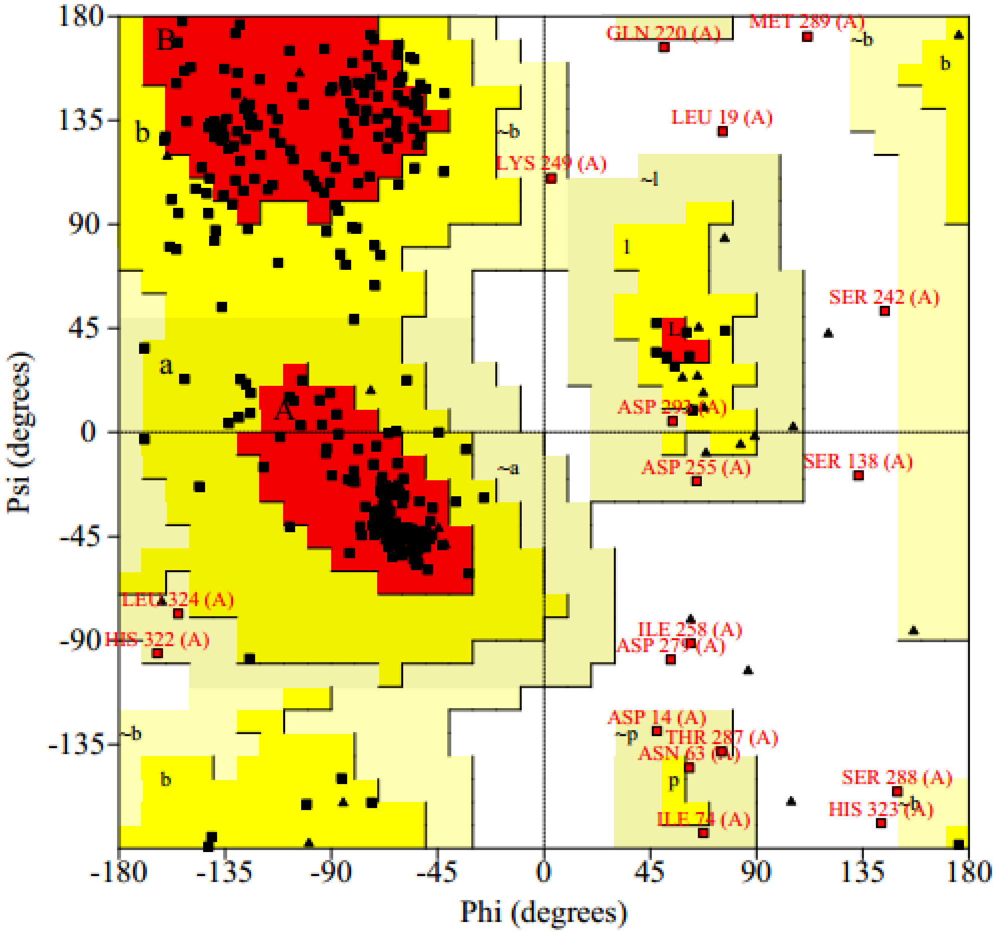
c. Model 3



Keterangan:

Daerah sangat disukai : 76,5%
Daerah tambahan yang diijinkan : 18,2%
Daerah yang diijinkan : 3,5%
Daerah tidak diijinkan : 1,8%

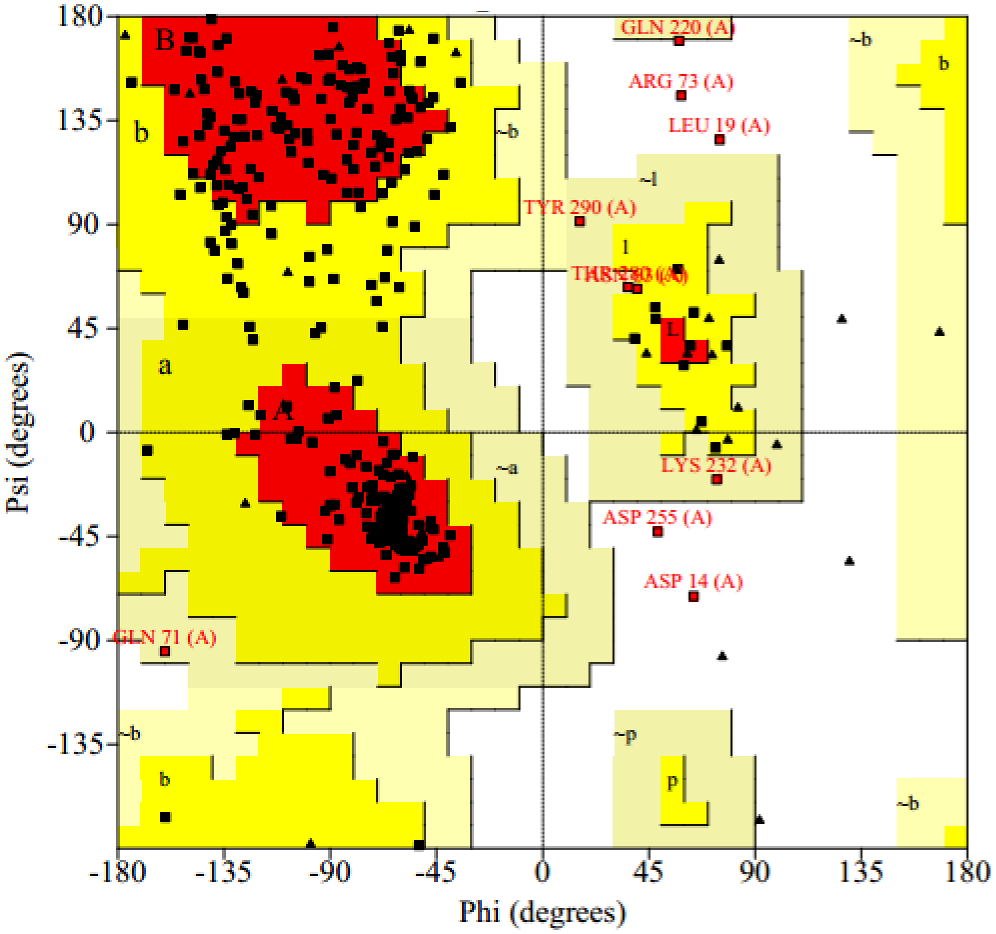
d. Model 4



Keterangan:

Daerah sangat disukai : 74,7 %
Daerah tambahan yang diijinkan : 20,0%
Daerah yang diijinkan : 3,9%
Daerah tidak diijinkan : 1,4 %

e. Model 5



Keterangan:

Daerah sangat disukai : 74,7 %
Daerah tambahan yang diijinkan : 21,8 %
Daerah yang diijinkan : 1,7 %
Daerah tidak diijinkan : 1,8 %