

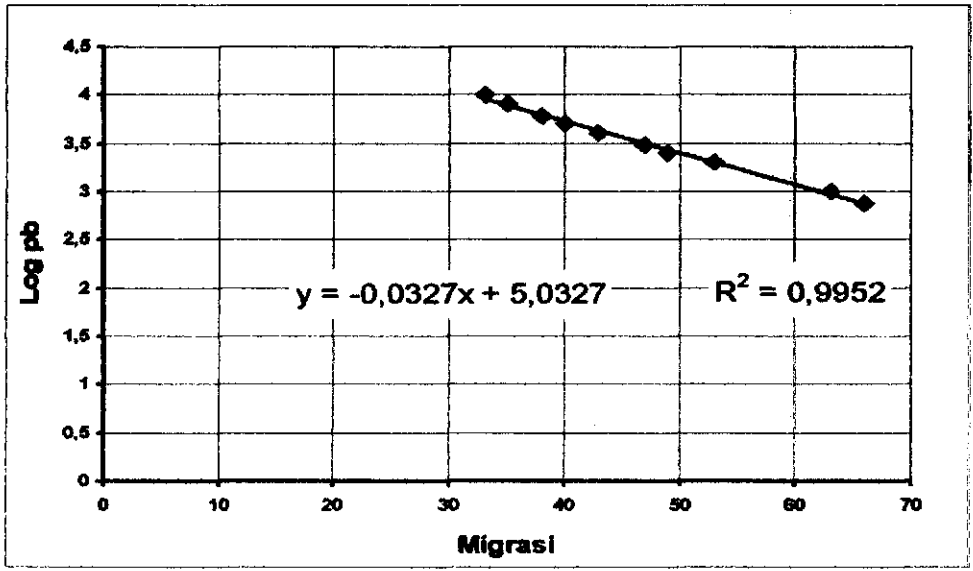
Lampiran 1. Pembuatan Bahan

1. **Bufér tris-base 0,5 M pH 8 (Mr: 121,144)**
Sebanyak 1,5143 gram tris-base dilarutkan dengan 20 mL aquades, dan diatur pH menjadi 8 dengan penambahan HCl atau NaOH. Kemudian diencerkan hingga 25 mL.
2. **Larutan EDTA 0,5 M (Mr: 292,504)**
Sebanyak 3,6563 gram Na-EDTA dilarutkan dengan 25 mL aquades
3. **Bufér TAE 50 x kuat sebagai stok**
Sebanyak 24,2 gram tris-base + 5,71 mL CH₃COOH glasial + 10 mL EDTA 0,5 M pH 8 dicampurkan dengan 80 mL aquades, kemudian diencerkan hingga 100 mL.
4. **Loading bufer**
Dicampurkan 500 µL gliserol 10 % + 333 µL TAE 10 x kuat + 100 µL *bromophenol blue* 0,1 % (0,001 gram dilarutkan dengan 1 mL aquades).
5. **Gel agarosa 0,8 % dalam TAE 1 x kuat**
Sebanyak 0,24 gram agarosa dilarutkan dengan 30 mL TAE 1 x kuat.
6. **Gel agarosa 1,2 % dalam TAE 1 x kuat**
Sebanyak 0,36 gram agarosa dilarutkan dengan 30 mL TAE 1 x kuat.
7. **Bufér 0,1 M Na-fosfat pH 7**
Larutan A : 1 M larutan Na₂HPO₄ (17,79 gram dalam 100 mL, Mr: 177,9)
Larutan B : 1 M larutan NaH₂PO₄ (13,79 gram dalam 100 mL, Mr: 137,9)
Untuk membuat larutan bufer pH 7 sebanyak 100 mL, maka perbandingan larutan yang dicampurkan sesuai tabel berikut :

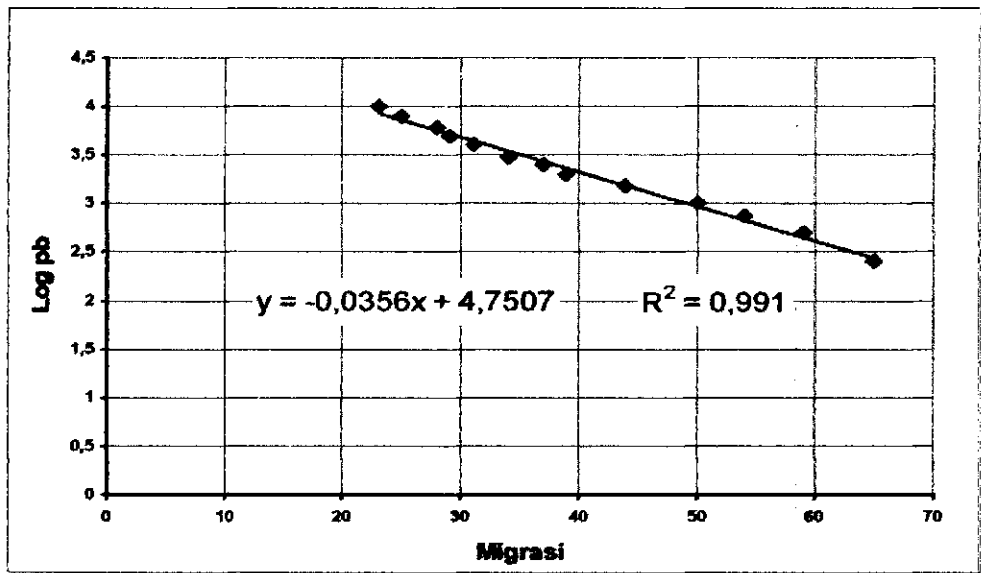
| No. | pH | Na ₂ HPO ₄ 1 M (mL) | NaH ₂ PO ₄ 1 M (mL) |
|-----|-----|---|---|
| 1. | 6,0 | 1,20 | 8,80 |
| 2. | 6,2 | 1,78 | 8,22 |
| 3. | 6,4 | 2,55 | 7,45 |
| 4. | 6,6 | 3,52 | 6,48 |
| 5. | 6,8 | 4,63 | 5,37 |
| 6. | 7,0 | 5,77 | 4,23 |
| 7. | 7,2 | 6,84 | 3,16 |

Lampiran 2. Kurva hubungan jarak migrasi standar DNA terhadap log pb

1. Hasil elektroforesis gambar 9 (penentuan berat molekul DNA hasil isolasi)



2. Hasil elektroforesis gambar 11 (Penentuan berat molekul DNA hasil PCR)



Lampiran 3. Data penentuan titik leleh (*T_m*)

| No. | Temperatur (⁰ C) | Absorbans (<i>A</i> ₂₆₀) | Absorbans Relatif |
|-----|------------------------------|---------------------------------------|-------------------|
| 1. | 30 | 0,163 | 1,000 |
| 2. | 32 | 0,208 | 1,276 |
| 3. | 34 | 0,235 | 1,442 |
| 4. | 36 | 0,610 | 3,742 |
| 5. | 38 | 0,632 | 3,877 |
| 6. | 40 | 0,656 | 4,025 |
| 7. | 42 | 0,679 | 4,166 |
| 8. | 44 | 0,681 | 4,178 |
| 9. | 46 | 0,689 | 4,227 |
| 10. | 48 | 0,700 | 4,294 |
| 11. | 50 | 0,705 | 4,325 |
| 12. | 52 | 0,701 | 4,301 |
| 13. | 54 | 0,700 | 4,294 |
| 14. | 56 | 0,720 | 4,417 |
| 15. | 58 | 0,738 | 4,528 |
| 16. | 60 | 0,762 | 4,675 |
| 17. | 62 | 0,785 | 4,816 |
| 18. | 64 | 0,879 | 5,393 |
| 19. | 66 | 0,935 | 5,736 |
| 20. | 68 | 0,972 | 5,963 |
| 21. | 70 | 0,945 | 5,798 |
| 22. | 72 | 0,965 | 5,920 |
| 23. | 74 | 0,930 | 5,706 |
| 24. | 76 | 0,971 | 5,957 |
| 25. | 78 | 1,061 | 6,509 |
| 26. | 80 | 1,095 | 6,718 |
| 27. | 82 | 1,130 | 6,933 |
| 28. | 84 | 1,143 | 7,012 |
| 29. | 86 | 1,197 | 7,344 |
| 30. | 88 | 0,906 | 5,558 |
| 31. | 90 | 0,958 | 5,877 |

Keterangan : Absorbans relatif = $\frac{A_{260} \text{ (pada suhu } T \text{ leleh)}}{A_{260} \text{ (pada suhu kamar)}}$

Contoh perhitungan (30 ⁰C) : Absorbans relatif = $\frac{0,163}{0,163}$

Absorbans relatif = 1,000

Lampiran 4. Hasil printout pembacaan sistem sekuensing sebelum diperbaiki

1. Hasil sekuensing DNA *Trichoderma sp.* TNJ63 yang disekuens menggunakan primer ITS5

5' – NNNNNNNNNNNNNNNNNCGNNNGGNNCATTACCGAGTTTACACTCCNNNNCCAN
TGTGNNNNNNACCAAAGTGTTCCTCGGCGGGGTCACGCCCGGGTGCCTCGCAN
CCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAAGTCTTTCTGTAGTCCCTC
GCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAATGAATCAAAGTCTTCAACA
ACGGATCTCTTGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAAATCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTA
TTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCTCCGGGGGA
TCGGCGTTGGGGATCGGGACCCCTCACACGGGTGNCGGCCCCNAAATACAGNGGC
GGTCTCGCCGCANCCCTCTCTGCGCANTANTTTGCACAACCTCANNACNGGGAGCGC
GGCGCGTCCACNTCCGTAACANCAANTTTCTGAANTGTNNACCTNCNNATCANG
NAAGANNNNNCGCTGAACNTNATCCNNNTCNATNNNNNGNANGANNNNNN - 3'

2. Hasil sekuensing DNA *Trichoderma sp.* TNJ63 yang disekuens menggunakan primer ITS4

5' – NNGNNNNNNNNNN
NNNNNNNNNNCNTNNNNNCNGNCCNNNNNCNCNTTTCNANNNTGGGTGTTTNNNNNN
NNNNNNNNNNNNNNNGNGCGAGNNCCNCNNGCAACNNNNNNNNNNNAGGNNNNN
NNNNNNNNNNNNNNNTNNNNNNNNCNNNNNNNNNNNNNNNGGNNNNNNNNC
NNNNNNCNCNNNNNNNNNNNNNNNNNA - 3'

3. Hasil sekuensing DNA *Trichoderma sp.* TNJ63 yang disekuens menggunakan primer ITS3

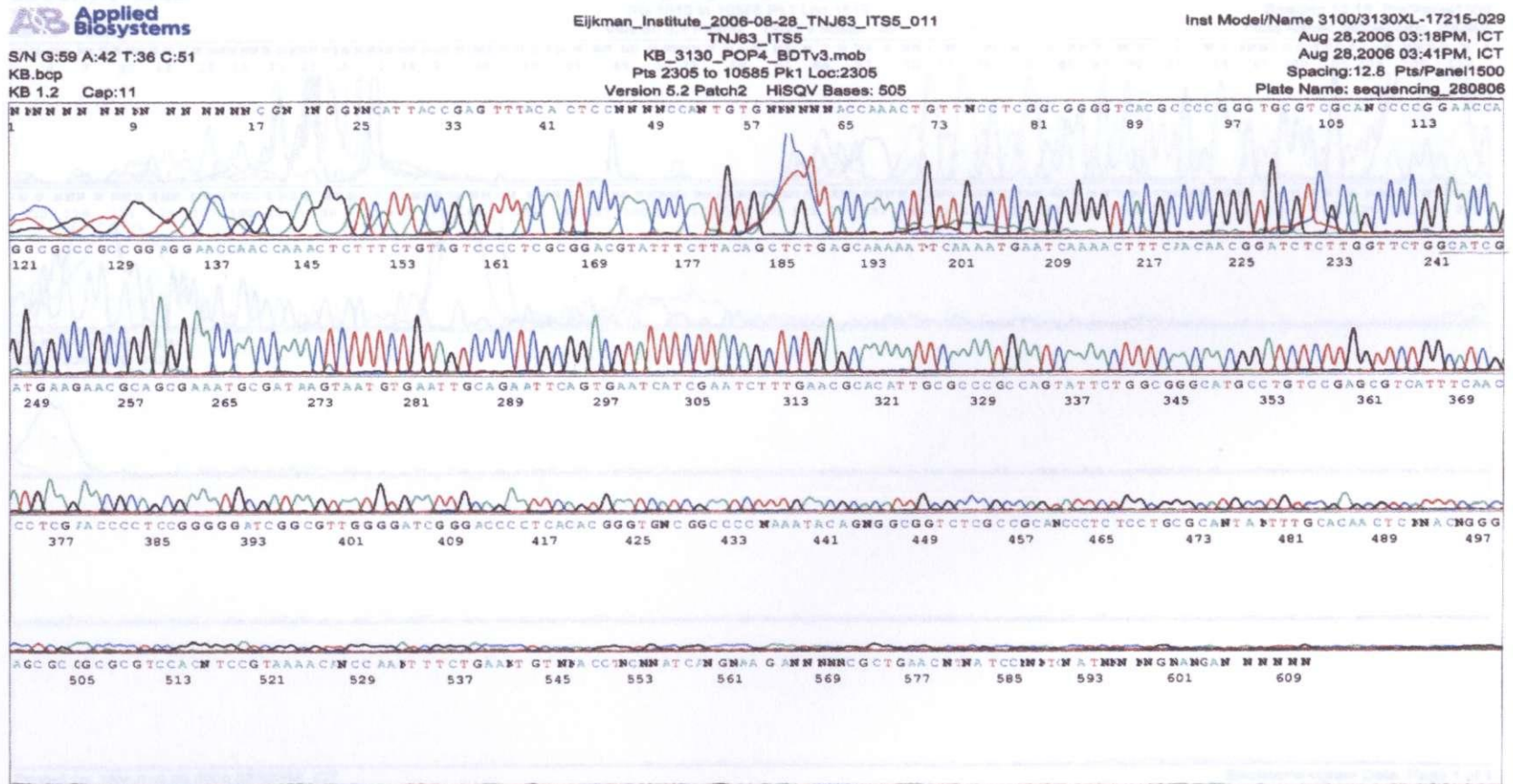
5' – NNNNNNNNNNNNGGNGNNTTGNNAATTCNGTGATCNTCGAATCTTTGACGCAC
ATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTC
GAACCCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGG
CCCCGAAATACAGTGGCGGTCTCGCCGCAGCCTCTCTGCGCAGTAGTTTGCACAA
CTCGCACCAGGAGCGCGGCGCTCCACGTCCGTAACACCCAACTTTCTGAAATG
TTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCNNNNNNNTNAAGCGGA
GNNNNNNCCNNNNNNNNNTNNNNNAANNTTTTNNNNNNNTTTGAANNNNNNNN
NNAANNNNNANAAANNANNNNNNNNNNNNNNNNNNNNNNNNNNNNTNNNNNGGN
NNNNNNNNNGGNNNNNCNGNNNNCNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNANNNNANNNNTTNNNNNNNNNNNNNNNGNNNTNNGNANNNNNNANNNN
NNNNNN - 3'

4. Hasil sekuensing DNA *Trichoderma sp.* TNJ63 yang disekuens menggunakan primer ITS2

5' – NNNNNNNNNNNNNNNNNNTTTTGNTTCTTTNNNTTTTGNTCNNAGCTNNNNNN
NACGTCCGCNNGGGACTACAGANNNNNNNNNNNNNTCCTCCGGCGGGCNCNN
NNNTCCGGGGCTGCGACGCACCCGGGGCGTGACCCCGCCNNNNNAACAGTTTGGT
AACGTTACATTGGGTTTGGGAGTTGAAACTCGGTAATGATCCCTCCGCTGGTTCA
CCAACGGAGACCTTNNNNNACTTTTACTNNANNNNNNTNNNNNGATNCNNNNNN
NTCTGNANTNANTTNNNTANCNATTNNNNNGNGNNNNCCNCNANGNNNAAAN
CNNNNNNNNNNNNNNNAANCNTTTNNNNNTTTTNNNTNNNNGNANANNTNNN
ANANNANGNCCNGNANNNNANANTGNNNNNAGTTTGNTTGGTNNNNNCGNNGGG
TCNCNNGGTNCNGGCGCTGCNANNNNNNNGCNCNNTNAANCCNNNAAATNNNNNA
ANTTTNCNAANNGGNNNTGNGGGGTTNGNNANTNNNNN - 3'

Lampiran 5. Spektrogram sekuensing hasil PCR

1. Spektrogram sekuensing DNA *Trichoderma sp.* TNJ63 yang disekuens menggunakan primer ITS5



45

Lampiran 6. Printout Gen Bank dari sekuens *Trichoderma* sp. TNJ63

LOCUS EF467659 632 bp DNA linear PLN 02-APR-2007
 DEFINITION *Trichoderma* sp. TNJ63 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
 ACCESSION EF467659
 VERSION EF467659.1 GI:134143128
 KEYWORDS .
 SOURCE *Trichoderma* sp. TNJ63
 ORGANISM *Trichoderma* sp. TNJ63
 Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic Hypocreales; *Trichoderma*.
 REFERENCE 1 (bases 1 to 632)
 AUTHORS Nugroho, T.T., Restuhadi, F., Ito, T.R., Faisal, F., Saryono, S. and Chainulfiffah, A.M.
 TITLE Molecular identification of Riau biocontrol *Trichoderma* strains
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 632)
 AUTHORS Nugroho, T.T., Restuhadi, F., Ito, T.R., Faisal, F., Saryono, S. and Chainulfiffah, A.M.
 TITLE Direct Submission
 JOURNAL Submitted (02-MAR-2007) Department of Chemistry, FMIPA, University of Riau, Kampus Binawidya Jl. Raya Soebrantas Km 12,5, Pekanbaru, Riau 28293, Indonesia
 FEATURES Location/Qualifiers
 source 1..632
 /organism="Trichoderma sp. TNJ63"
 /mol_type="genomic DNA"
 /strain="TNJ63"
 /isolation_source="Riau citrus grove soil"
 /db_xref="Taxon:434037"
 /country="Indonesia"
 /note="PCR primers=fwd_name: ITS5, rev_name: ITS4;
 PCR primers=fwd_name: ITS3, rev_name: ITS2"
 rRNA <1..60
 /product="18S ribosomal RNA"
 misc RNA 61..242
 /product="internal transcribed spacer 1"
 rRNA 243..400
 /product="5.8S ribosomal RNA"
 misc RNA 401..574
 /product="internal transcribed spacer 2"
 rRNA 575..>632
 /product="28S ribosomal RNA"
 ORIGIN
 1 ttcttgggaag taaaagttcg taacaaggtc tccgttggtg aaccagcggg gggatcatta
 61 cggagtttac aactccaaa cccaatgtga acgttaccaa actgttgcc cggcggggtc
 121 acgccccggg tgcgtgcgag ccccggaacc aggcgcccgc cggaggaacc aaccaaactc
 181 tttctgtagt cccctcgcg acgtatttct tacagctctg agcaaaaatt caaatgaat
 241 caaaactttc aacaacggat ctcttggttc tggcatcgat gaagaacgca gcgaaatgag
 301 ataagtaatg tgaattgcag aattcagtg atcatcgaat cttgaaacgc acattgcgcc
 361 cgccagtatt ctggcgggca tgcctgtccg agcgtcattt caaccctcga acccctccgg
 421 gggatcggcg ttggggatcg ggaccctca cacgggtgcc ggccccgaa tacagtggcg
 481 gtctcgccgc agcctctcct gcgcagtagt ttgcacaact cgcaccggga gcgcggcgcg
 541 tccacgtccg taaaacaccc aactttctga aatgttgacc tcggatcagg taggaatacc
 601 cgctgaactt atccttttct attttccgga ag

//