

Lampiran 1. Pembuatan Bahan

1. Bufer 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. Bufer TAE 50 x kuat sebagai stok

Sebanyak 24,2 gram tris-base + 5,71 mL CH_3COOH 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. Bufer 0,1 M Na-fosfat pH 7

Larutan A : 1 M larutan Na_2HPO_4 (17,79 gram dalam 100 mL, Mr: 177,9)

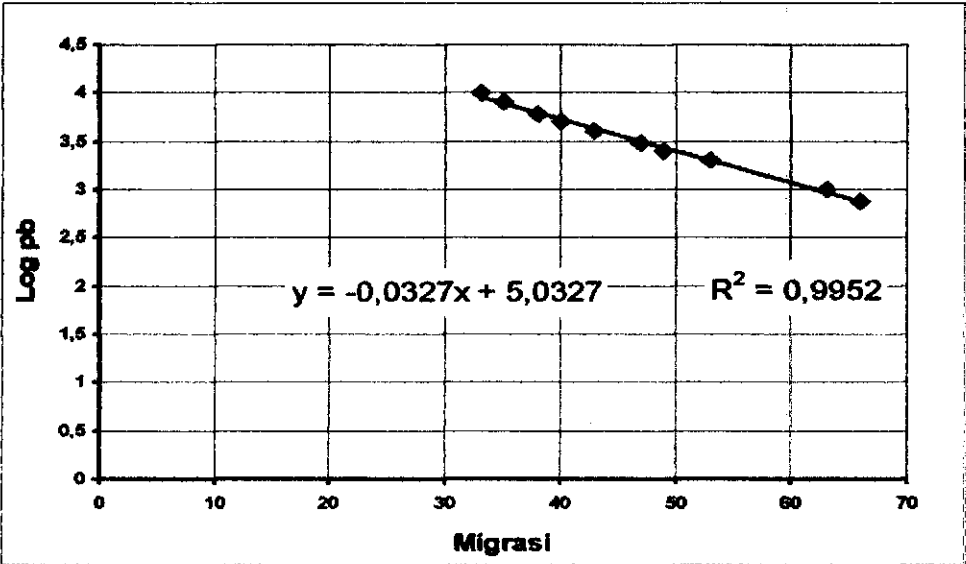
Larutan B : 1 M larutan NaH_2PO_4 (13,79 gram dalam 100 mL, Mr: 137,9)

Untuk membuat larutan bufer pH 7 sebanyak 100 mL, maka perbandingan larutan yang dicampurkan sesuai tabel berikut :

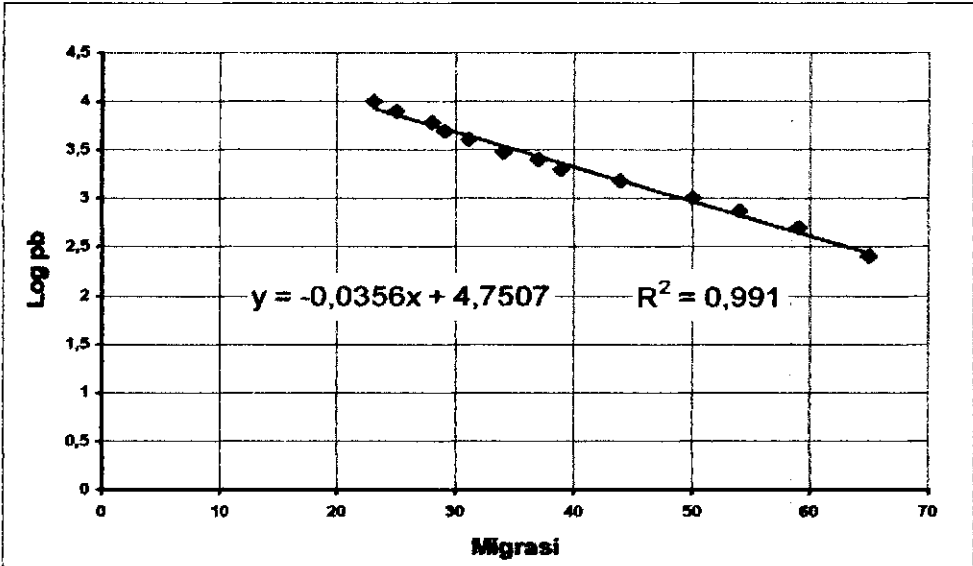
No.	pH	Na_2HPO_4 1 M (mL)	NaH_2PO_4 1 M (mL)
1.	6,0	1,20	8,80
2.	6,2	1,78	8,22
3.	6,4	2,55	7,45
4.	6,6	3,52	6,48
5.	6,8	4,63	5,37
6.	7,0	5,77	4,23
7.	7,2	6,84	3,16

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 ($^{\circ}\text{C}$)	Absorbans (A_{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°C) : 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
TGTGNNNNNNNACCAAAGTGTTCCTCGGCGGGGTACGCCCCGGGTGCGTCGCAN
CCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAAGTCTTTCTGTAGTCCCCTC
GCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACA
ACGGATCTCTTGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAAATCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGCCAGTA
TTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCTCCGGGGGA
TCGGCGTTGGGGATCGGGACCCCTCACACGGGTGNCGGCCCCNAAATACAGNGGC
GGTCTCGCCGCANCCCTCTCCTGCGCANTANTTTGCACAACTCNNACNNGGGAGCGC
GGCGCGTCCACNTCCGTAACCAANTTTCTGAANTGTNNACCTNCNNATCANG
NAAGANNNNCGCTGAACNTNATCCNNNTCNATNNNNNGNANGANNNNNN - 3'

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

5' – NNGNNNNNNNNNN
NNNNNNNNNNCNTNNNNNCNGNCCNNNNNCNCNTTTCNANNNTGGGTGTTTNNNNNN
NNNNNNNNNNNNNNNGNCGAGNNCCNCNNGCAACNNNNNNNNNNNAGGNNNNNN
NNNNNNNNNNNNNNNTNNNNNNNNCNNNNNNNNNNNNNGGNNNNNNNNCNNN
NNNNNNCNCNNNNNNNNNNNNNNNNNNNA - 3'

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

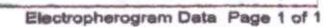
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NNAANNNNNANAAANNANNNNNNNNNNNNNNNNNNNNNNNNNNNNTNNNNNGGN
NNNNNNNNNGGNNNNNCCNGNNNNCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNANNNNANNNNTTNNNNNNNNNNNNNNNGNNNTNNGNANNNNNNNANNNN
NNNNNN - 3'

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

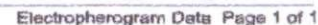
5' – NNNNNNNNNNNNNNNNNNTTTTGNTTCTTTTNNNTTTTGNTCNNAGCTNNNNNN
NACGTCCGCNNGGGGACTACAGANNNNNNNNNNNNNTCCTCCGGCGGGCNCNN
NNNTCCGGGGCTGCGACGCACCCGGGGCGTGACCCCGCCNNNNNAACAGTTTGGT
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CCAACGGAGACCTTNNNNNNACTTTTACTNNANNNNNNTNNNNNGATNCNNNNNN
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CNNNNNNNNNNNNNNNNAANCNTTTNNNNNTTTTNNNTNNNNNGNNANANTNNN
ANANNANGNCCNGNANNNNNANANTGNNNNNAGTTTGNTTGGTNNNNNCGNNGGG
TCNCNNGGTNCNGGCGCTGCNANNNNNNNGCNCNNTNAANCCNNNAAATNNNNNA
ANTTTNCNAANNGGNNNTGNGGGGTTNGNNANTNNNNN - 3'



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



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

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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
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                                   /country="Indonesia"
                                   /note="PCR primers=fwd_name: ITS5, rev_name: ITS4;
                                   PCR primers=fwd_name: ITS3, rev_name: ITS2"
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            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"
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121 acgccccggg tgcgtgcgag ccccggaacc aggcgcccgc cggaggaacc aaccaaactc
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241 caaaactttc aacaacggat ctcttggttc tggcatcgat gaagaacgca gcgaaatgag
301 ataagtaagt tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcgcc
361 cgccagtatt ctggcgggca tgcctgtccg agcgtcattt caaccctcga acccctccgg
421 gggatcggcg ttggggatcg ggaccctca cacgggtgcc ggccccgaaa tacagtggcg
481 gtctcgccgc agcctctcct gcgcagtagt ttgcacaact cgcaccggga gcgcggcgcg
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