LAMPIRAN 1

PERHITUNGAN PEMBUATAN LARUTAN ISOLASI DNA KROMOSOM

***Buffer* TE 50mL pH 8 0,05 M**

Diambil masing-masing 5mL dari stok *buffer* Tris Cl pH 8 0,5M dan stok *buffer* EDTA pH 8 0,5M. Kemudian dicampur dan ditambahkan akuadest hingga 50mL.

- Tris-Cl : $V1 \times M1 \quad = V2 \times M2$

  $V1 \times 0{,}5M = 50mL \times 0{,}05M$

  $V1 \quad = 5mL$

- EDTA : $V1 \times M1 \quad = V2 \times M2$

  $V1 \times 0{,}5M = 50mL \times 0{,}05M$

  $V1 \quad = 5mL$

Larutan Na-Asetat 10mL 3 M

$$M = \frac{x}{BM} \times \frac{1000}{V}$$

$$3M = \frac{x}{82{,}03} \times \frac{1000}{10}$$

$$x = 2{,}4609\ g$$

Ditimbang Na-Asetat 2,4600 g kemudian ditambahkan sedikit akuades dan campur hingga larut sempurna. Setelah itu ditambahkan akuades hingga 10 mL.

Buffer STEP 500µL

- SDS (*Sodium Dodecyl Sulfat)* 0,5% $= \frac{0{,}5g}{100} x \frac{500\ \mu L}{1000\ \mu L}$

  $= 0{,}0025g$

- 0,05 M tris-Cl pH 8 : $V1 \times M1 = V2 \times M2$

$$x \times 0{,}5 = 0{,}05 \times 500\mu L$$

$$x = 50\ \mu L$$

- 0,4 M EDTA pH 8 : $V1 \times M1 = V2 \times M2$

$$x \times 0{,}5 = 0{,}4 \times 500\mu L$$

$$x = 400\ \mu L$$

Larutan TEN 2 mL

- Tris Cl 10 mM

$$V1 \times M1 = V2 \times M2$$

$$x \times 500\ mM = 2000 \times 10\ mM$$

$$x = 40\ \mu L$$

- EDTA 10 mM

$$V1 \times M1 = V2 \times M2$$

$$x \times 500\ mM = 2000 \times 10\ mM$$

$$x = 40\ \mu L$$

- NaCl 150 mM

Stok 0,2 M NaCl dalam 10 mL akuades

$$0{,}2\ M = \frac{x}{58{,}5} \times \frac{1000}{10}$$

$$x = 0{,}117\ g$$

Larutan kerja NaCl 150 mM

$$V1 \times M1 = V2 \times M2$$

$$x \times 200\ M = 2000\ mL \times 150\ mM$$

$$x = 1500\ \mu L$$

- Akuades = 2000 – 40 – 40 – 1500 = 420 µL

Larutan TEN* 2 mL

- Tris Cl 10 mM

$$V1 \times M1 = V2 \times M2$$

$$x \times 500\ mM = 2000 \times 10\ mM$$

$$x = 40\ \mu L$$

- EDTA 1 mM

$$V1 \times M1 = V2 \times M2$$

$$x \times 500\ mM = 2000 \times 1\ mM$$

$$x = 4\ \mu L$$

- NaCl 50 mM

  Stok 0,2 M NaCl dalam 10 mL akuades

$$0{,}2\ M = \frac{x}{58{,}5} \times \frac{1000}{10}$$

$$x = 0{,}117\ g$$

  Larutan kerja NaCl 50 mM

$$V1 \times M1 = V2 \times M2$$

$$x \times 200\ mM = 2000\ mL \times 50\ mM$$

$$x = 500\ \mu L$$

- Akuades = 2000 – 40 – 4 – 500 = 1456 µL

LAMPIRAN 2

PERHITUNGAN LARUTAN ELEKTROFORESIS

Buffer TAE

Stok 50X buffer TAE 200 mL :

- Basa tris : $= \frac{200\ mL}{1000\ mL} \times 242\ g$

  $= 48{,}4\ g$

- Asm asetat glasial $= \frac{200\ mL}{1000\ mL} \times 57{,}1\ g$

  $= 11{,}42\ g$

- 0,5M EDTA pH 8 $= \frac{200\ mL}{1000\ mL} \times 20\ mL$

  $= 20\ mL$

Larutan kerja = 0,5X TAE

dalam 1000 mL akuades

$V1 \times M1 = V2 \times M2$

$V1 \times 50X = 1000\ mL \times 0{,}5X$

$V1 = 10\ mL$

LAMPIRAN 3

KONSENTRASI AGAROSE UNTUK PEMBUATAN GEL ELEKTROFORESIS

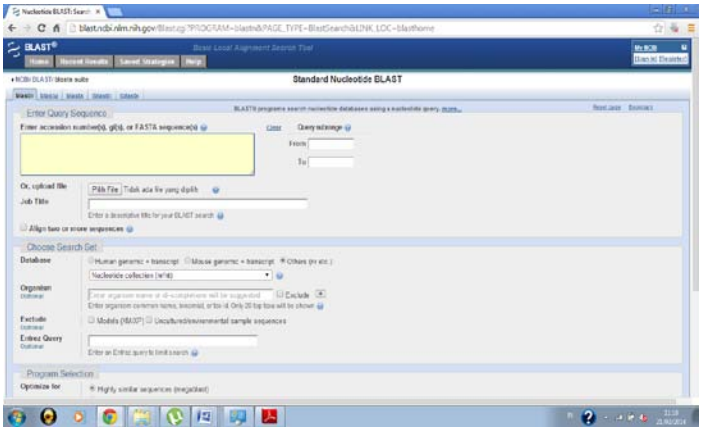
TABLE 5-5 Range of Separation in Gels Containing Different Amounts of Standard Low-EEO Agarose

AGAROSE CONCENTRATION IN GEL (% [W/V])	RANGE OF SEPARATION OF LINEAR DNA MOLECULES (kb)
0.3	5–60
0.6	1–20
0.7	0.8–10
0.9	0.5–7
1.2	0.4–6
1.5	0.2–3
2.0	0.1–2

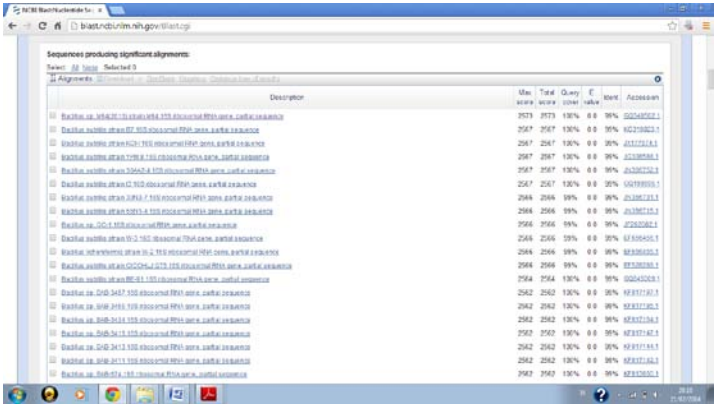
LAMPIRAN 4

PROSES PELACAKAN SEKUEN GEN PENYANDI 16S rRNA PADA *GENBANK*

Tampilan Program BLAST pada http://blast.ncbi.nlm.nih.gov/Blast.cgi



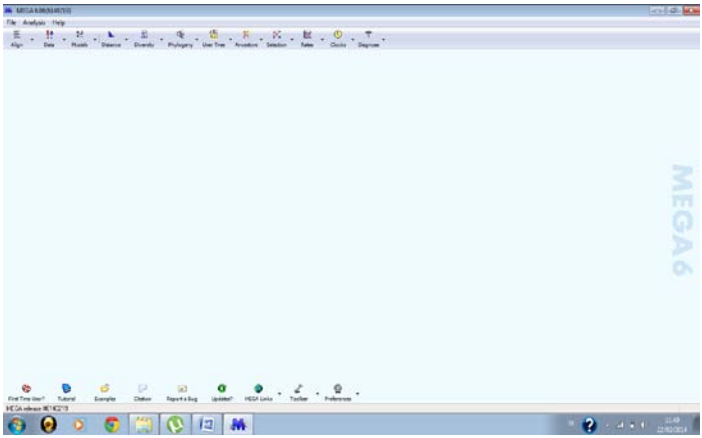
Hasil Analisis BLAST



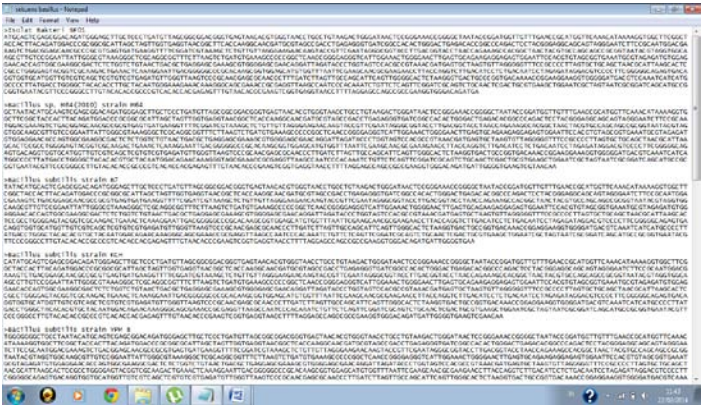
LAMPIRAN 5

PENENTUAN POHON FILOGENETIK ISOLAT BAKTERI SF01

Tampilan Program MEGA6



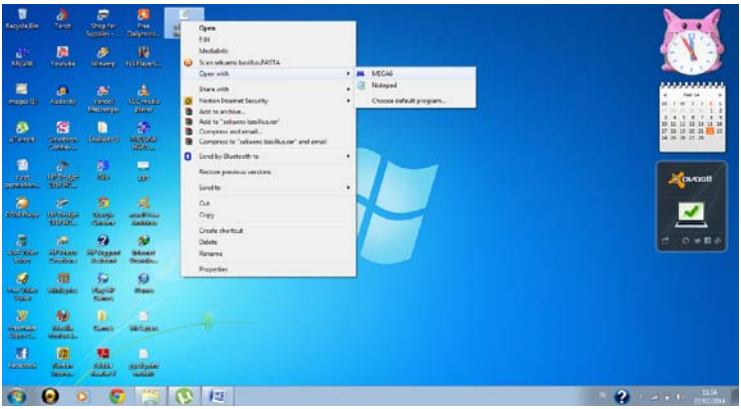
Tampilan Sekuens dari Isolat Bakteri SF01 dan Beberapa Bakteri Selulolitik Dalam Format FASTA

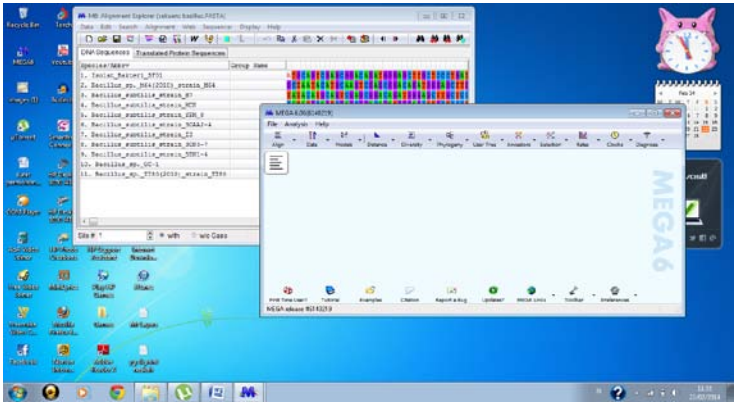


LAMPIRAN 6

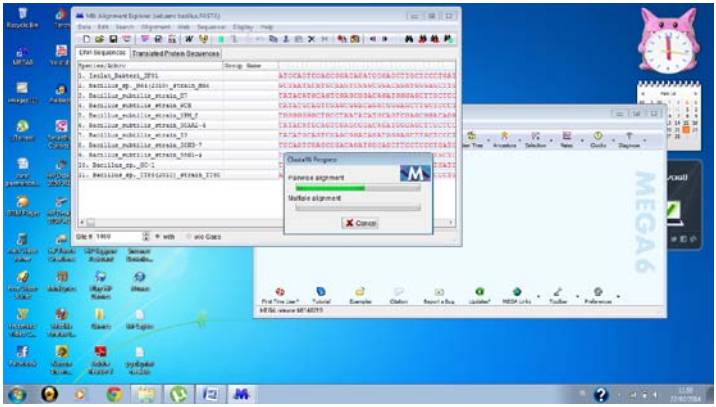
CARA PENGGUNAAN PROGRAM MEGA6

Buka file sekuens dalam format FASTA dengan program MEGA6

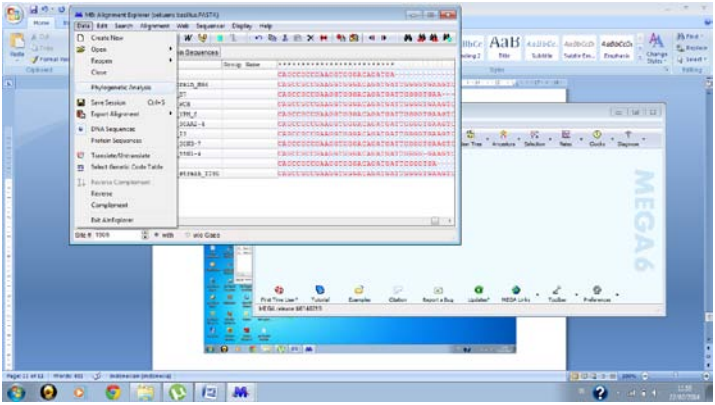




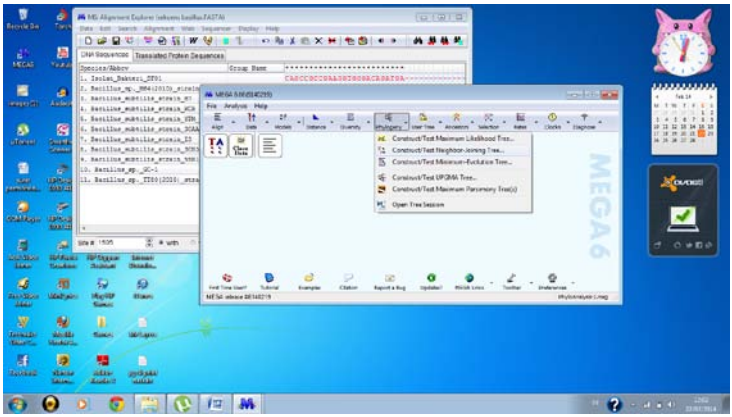
Pilih semua data sekuens yang diinginkan kemudian pilih *alignment → align by ClustalW→ok*



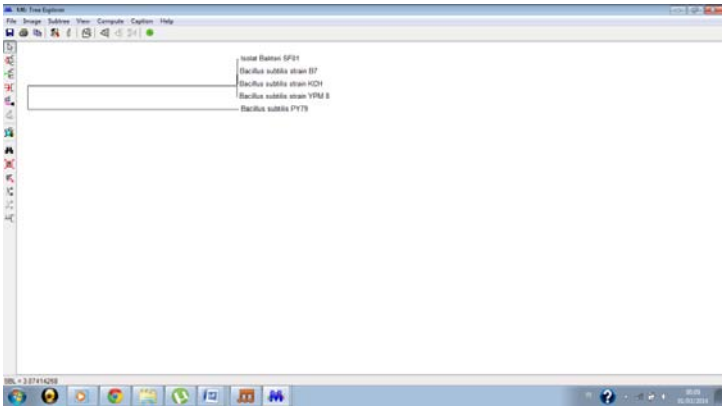
Pilih data → *Phylogenetic Analysis*→ yes



Pilih Phylogeny → *construct/test Neighbor-Joining Test → Compute*

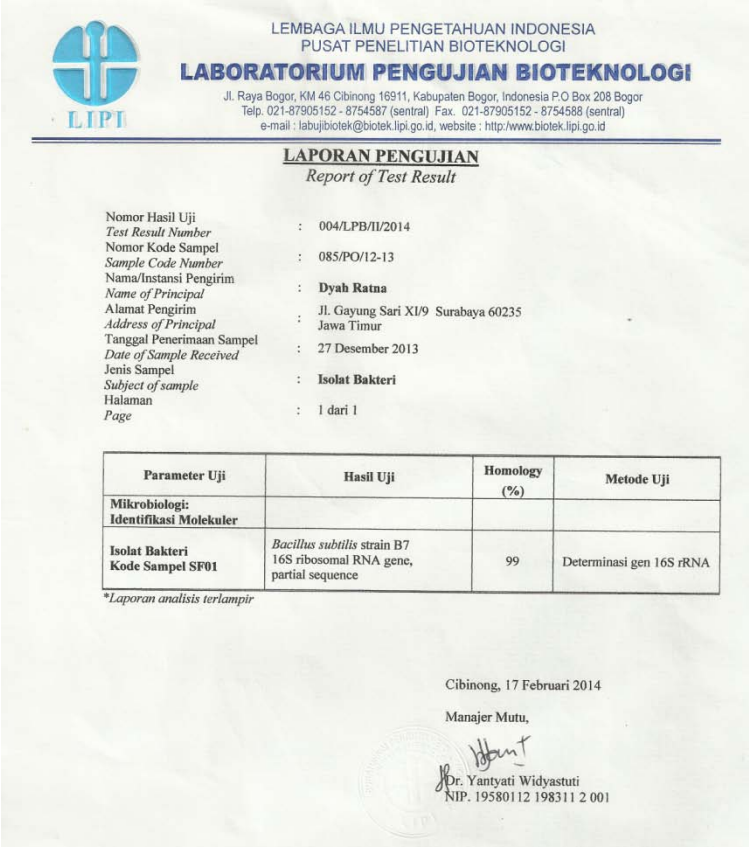


Hasil desain Pohon Filogenetik



LAMPIRAN 7

SERTIFIKAT HASIL ANALISIS AMPLIFIKASI GEN PENYANDI 16S rRNA DARI LIPI BOGOR



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e-mail : labujibiotek@biotek.lipi.go.id, website : http:/www.biotek.lipi.go.id

LAPORAN PENGUJIAN
Report of Test Result

Nomor Hasil Uji *Test Result Number*	:	004/LPB/II/2014
Nomor Kode Sampel *Sample Code Number*	:	085/PO/12-13
Nama/Instansi Pengirim *Name of Principal*	:	**Dyah Ratna**
Alamat Pengirim *Address of Principal*	:	Jl. Gayung Sari XI/9 Surabaya 60235 Jawa Timur
Tanggal Penerimaan Sampel *Date of Sample Received*	:	27 Desember 2013
Jenis Sampel *Subject of sample*	:	**Isolat Bakteri**
Halaman *Page*	:	1 dari 1

Parameter Uji	Hasil Uji	Homology (%)	Metode Uji
Mikrobiologi: Identifikasi Molekuler			
Isolat Bakteri Kode Sampel SF01	*Bacillus subtilis* strain B7 16S ribosomal RNA gene, partial sequence	99	Determinasi gen 16S rRNA

**Laporan analisis terlampir*

Cibinong, 17 Februari 2014

Manajer Mutu,

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