

IDENTIFIKASI BAKTERI ASAM LAKTAT BERBASIS MOLEKULER (DNA/RNA)

Pratama Nur Hasan

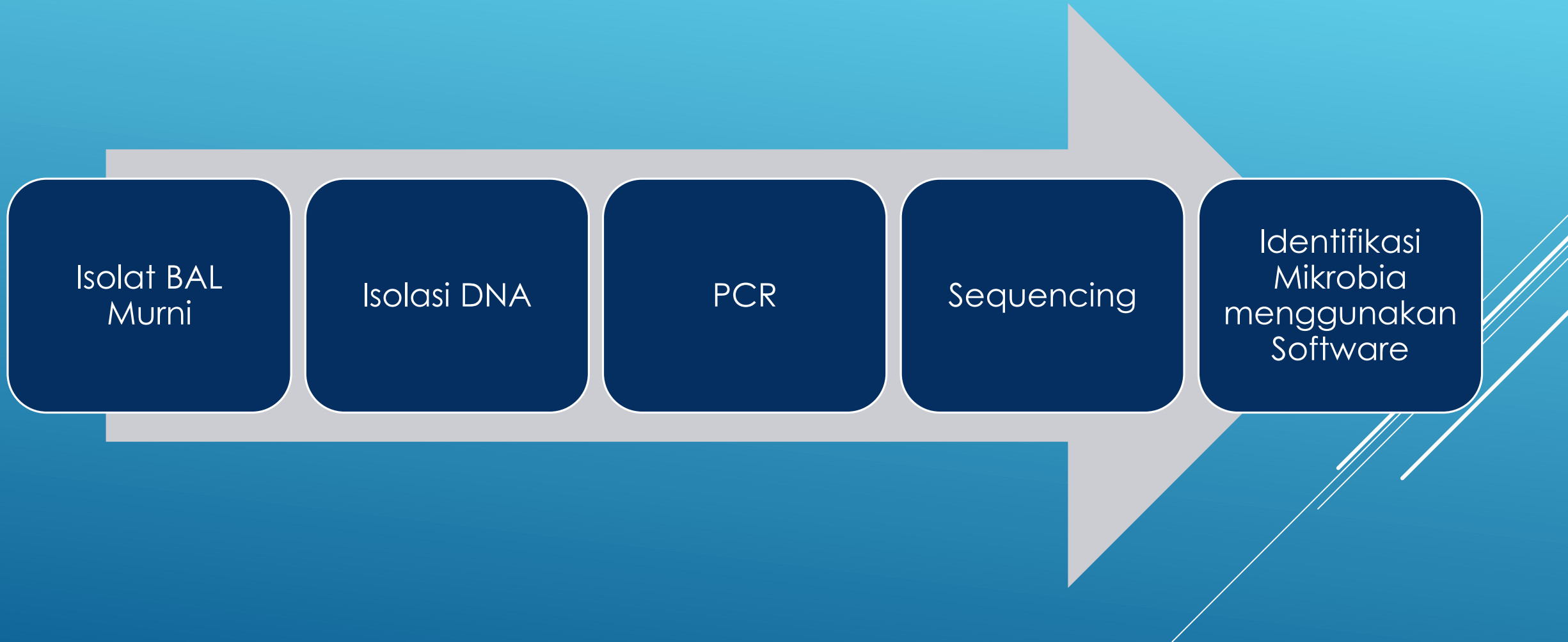
Endang S. Rahayu

Pusat Studi Pangan dan Gizi

Universitas Gadjah Mada

PNH & ESR - PSPG UGM

TAHAPAN IDENTIFIKASI BAL



ISOLASI BAL



Isolat Murni

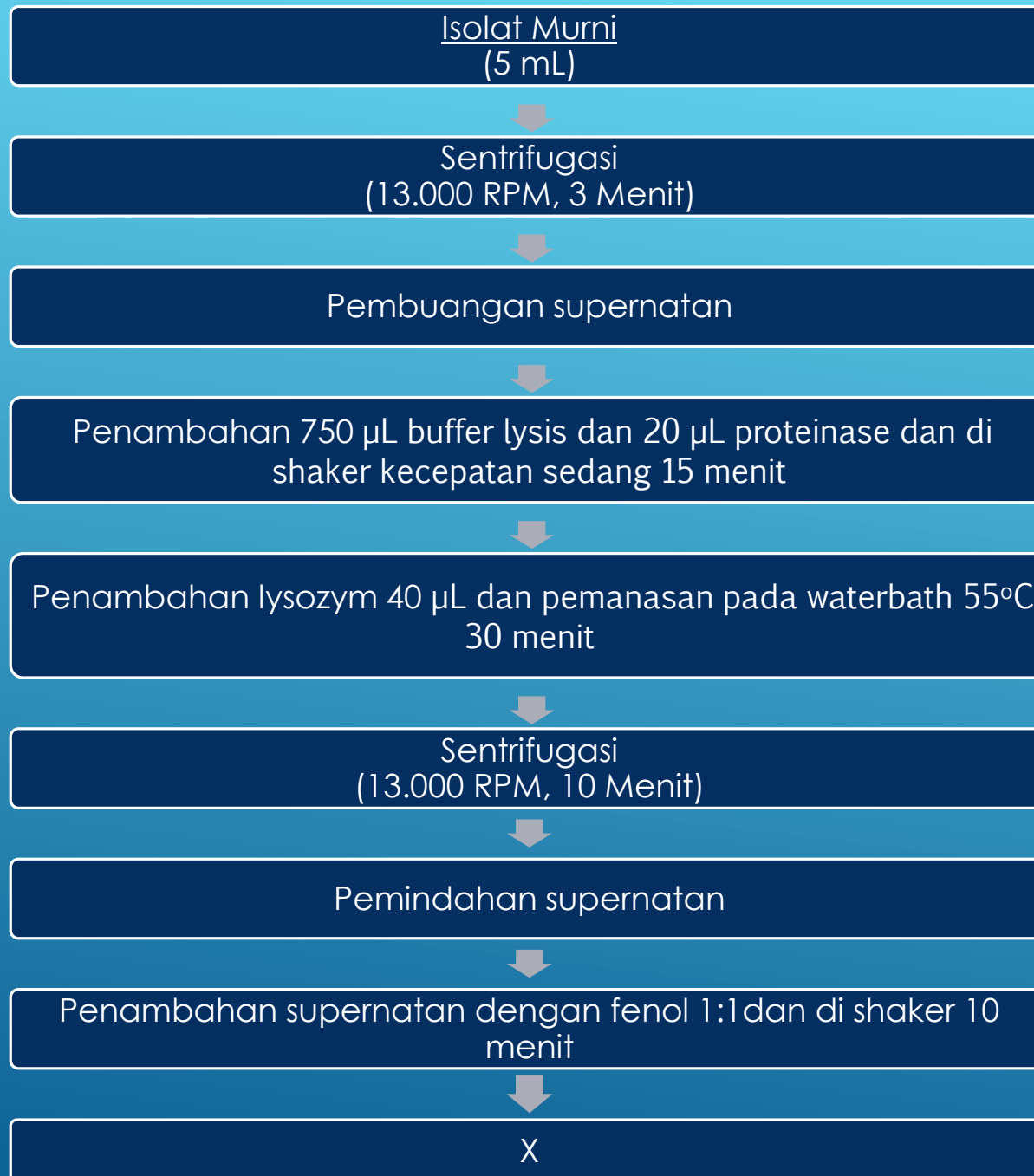


DNA Extraction

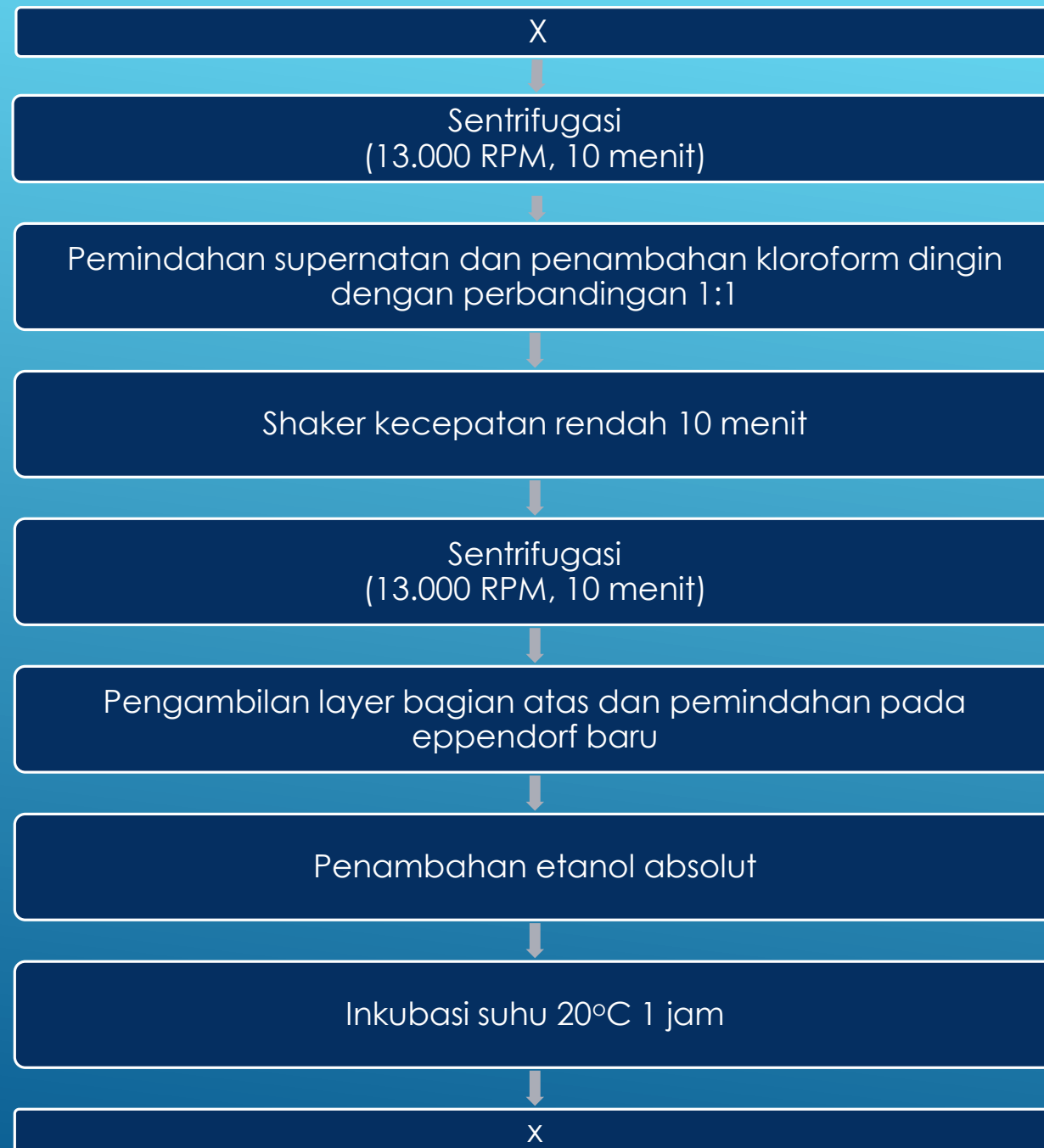


DNA isolat

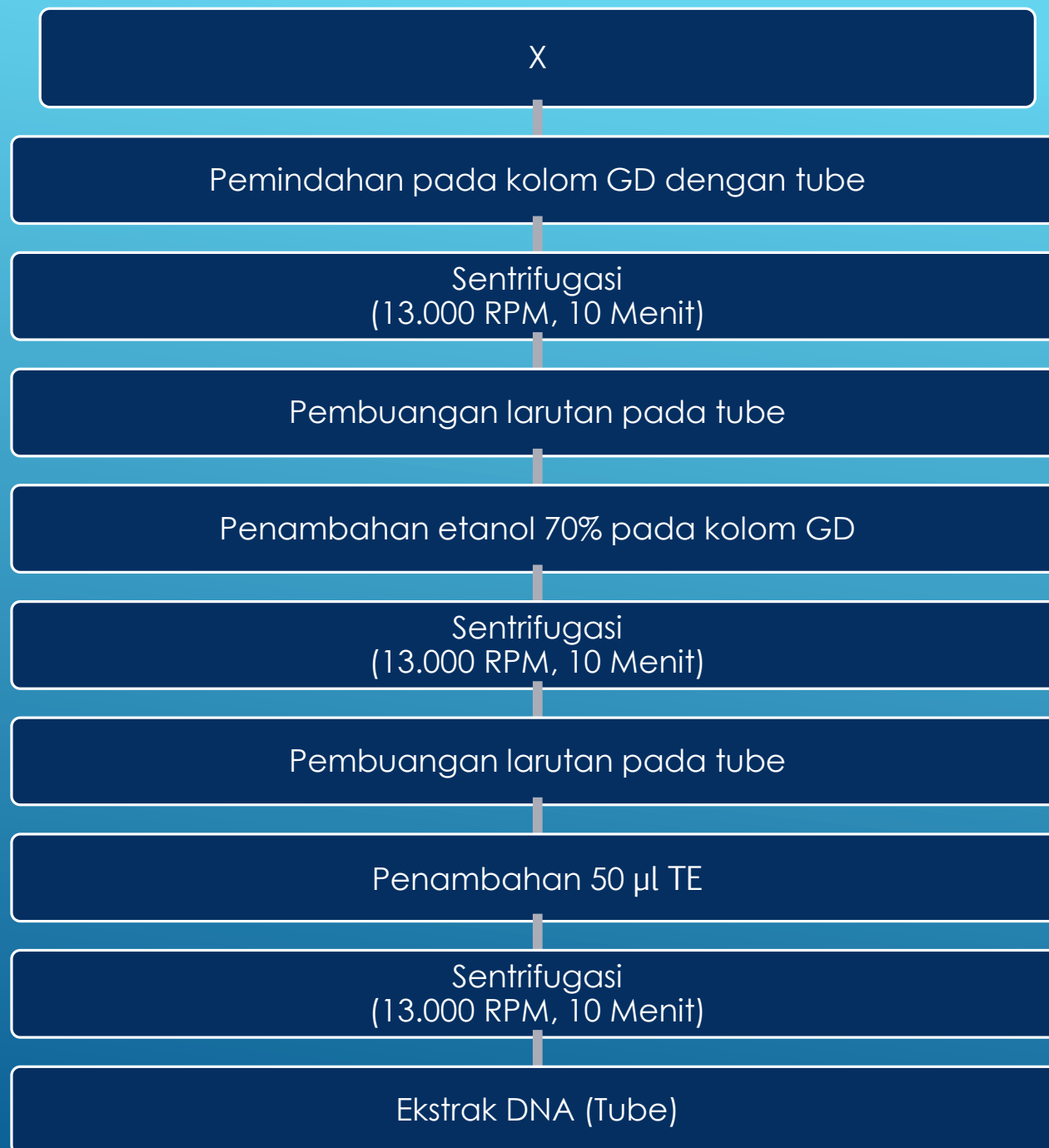
TAHAPAN ISOLASI DNA (MANUAL METHOD) 1



TAHAPAN ISOLASI DNA (MANUAL METHOD) 2



TAHAPAN ISOLASI DNA (MANUAL METHOD) 3

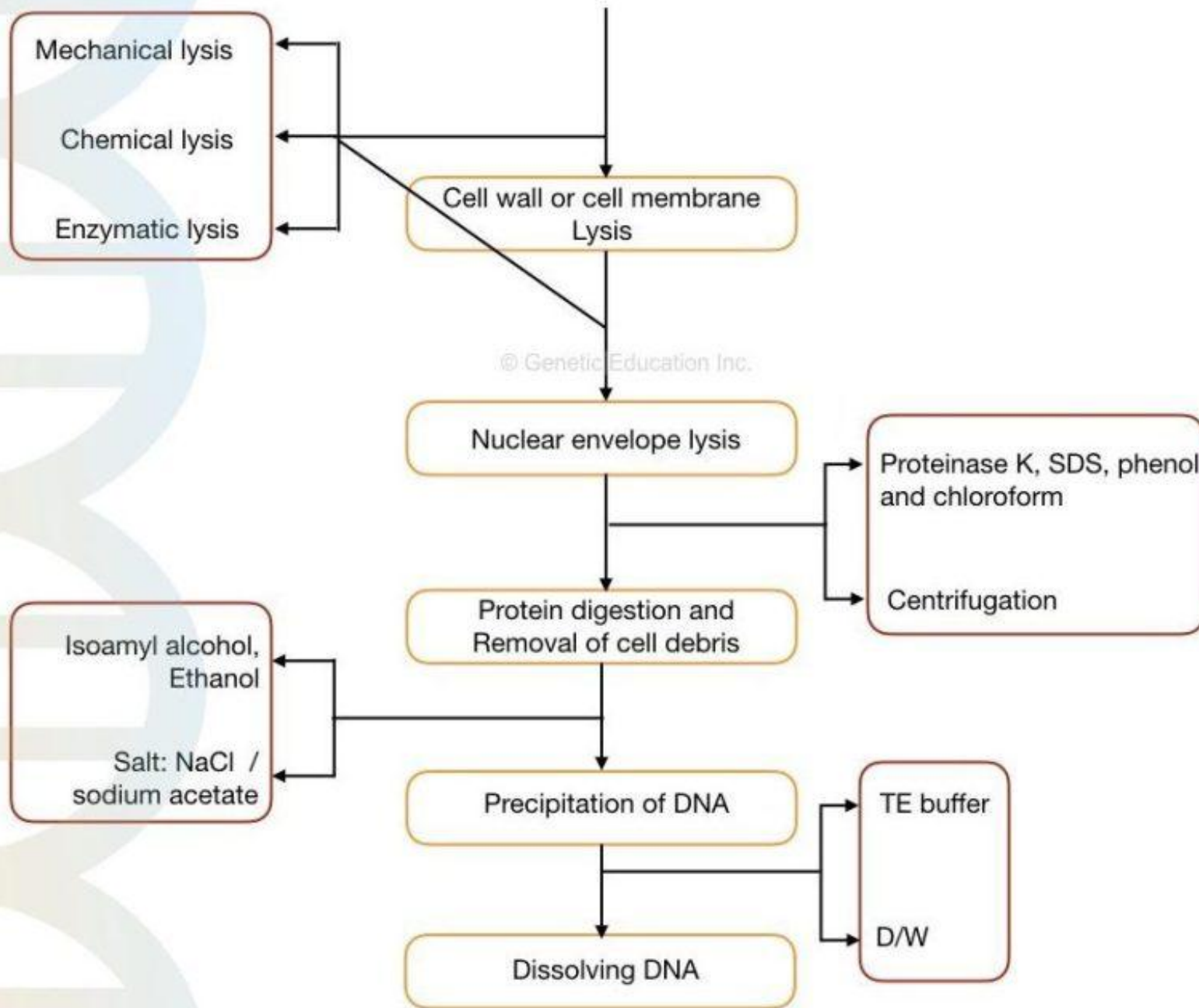


FUNGSI REAGENT

Reagent	Function
Buffer Lysis	Menjaga kondisi pH selama proses ekstraksi/isolasi DNA sehingga DNA tidak mengalami kerusakan akibat perubahan pH
Proteinase-K	Mendigest kontaminan protein serta mendegradasi enzim nuklease yang dimungkinkan ada selama proses isolasi
Lysozym	Enzim yang dapat memecah dinding sel dan aktif di suhu sekitar 50-55°C
Fenol	Membuka dari lipatan protein sehingga DNA mudah untuk terdenaturasi
Chloroform	Meningkatkan efisiensi dari fenol dalam denaturasi protein dan memberikan pemisahan yang baik antara fasa organik dan fasa cair dan DNA tetap tertahan dengan baik pada fasa cair

FUNGSI REAGENT

Reagent	Function
Ethanol Absolute	Mengendapkan DNA dalam bentuk pelet (presipitasi)
Ethanol 70%	Membersihkan DNA dari debris garam yang timbul selama proses ekstraksi dari buffer
TE Buffer	Melarutkan kembali DNA yang terpresipitasi



Reference :
<https://geneticeducation.co.in/phenol-chloroform-dna-extraction-basics-preparation-of-chemicals-and-protocol/>

PCR (POLYMERASE CHAIN REACTION)



- Proses penggandaan (copy) fragmen DNA spesifik.
- Terdiri atas beberapa tahap :
 - Predenaturasi
 - Denaturasi
 - Annealing
 - Extension
 - Last extension

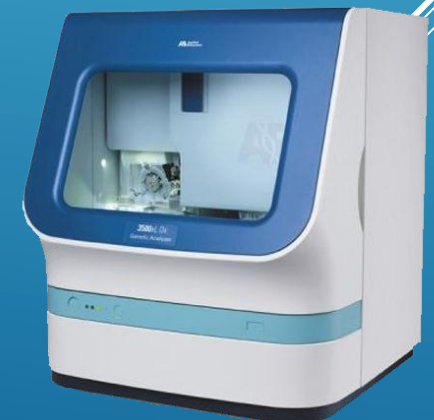
Reagent	Jumlah
Nuclease Free Water	9,5 uL
Mix PCR (Biolane HS)	12,5 uL
Primer 27F	1 uL
Primer 1492R	1 uL
DNA Template	1 uL
Total	25 uL

Tahapan	Suhu	Waktu
Predenaturasi	96°C	4 Menit
Denaturasi	94°C	1 Menit
Annealing	51,5°C	1 Menit 30 detik
Extension	68°C	8 Menit
Last extention	68°C	10 Menit

} 30x

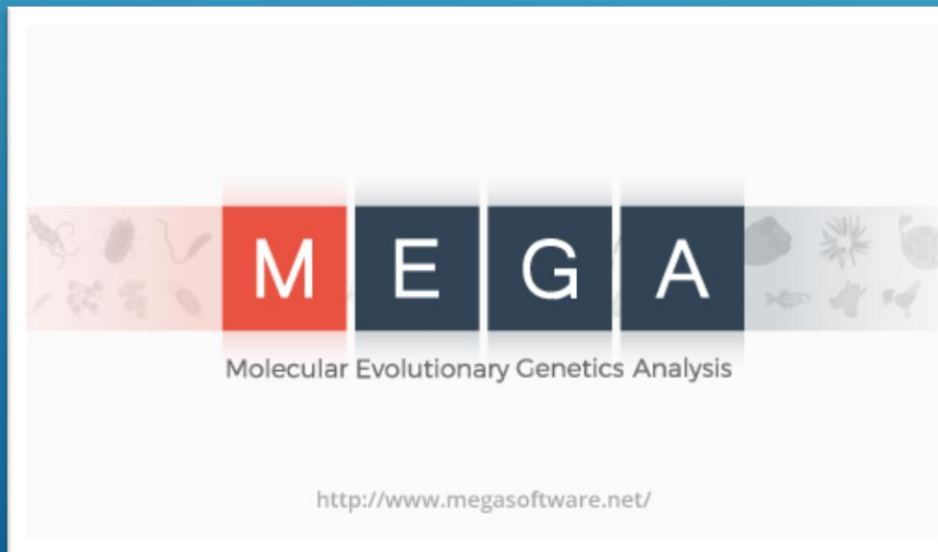
DNA SEQUENCING

- Setelah DNA si duplikasi menggunakan PCR DNA akan di sequencing pada alat DNA Sequencer.
- Pada tahap ini sampel diserahkan kepada teknisi di LPPT-UGM



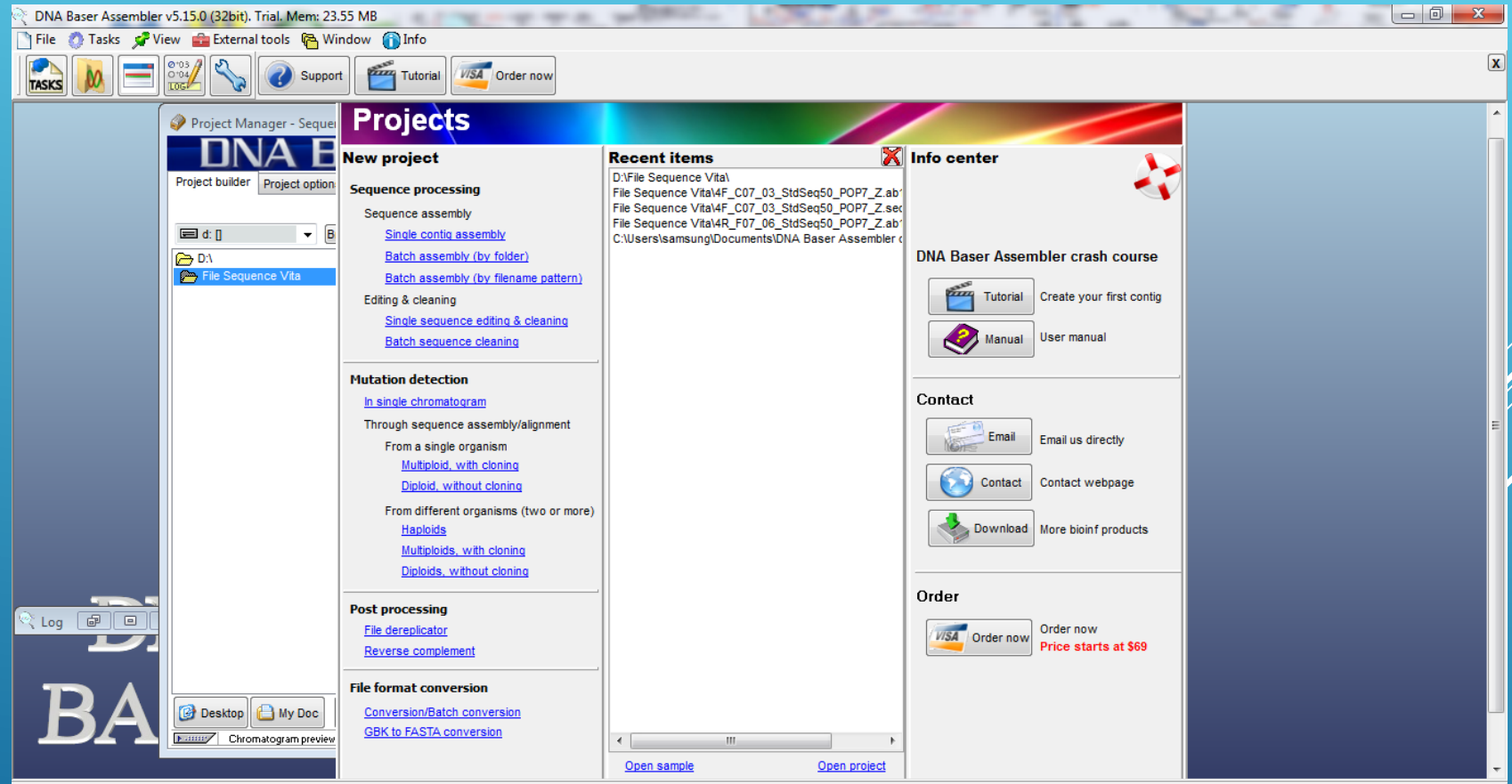
IDENTIFIKASI BAL

- ▶ Untuk melakukan identifikasi molekuler mikrobial dapat menggunakan beberapa software. Pada penjelasan ini menggunakan program DNA Baser Assembler dan MEGA X. Untuk database menggunakan web NCBI-BLAST.



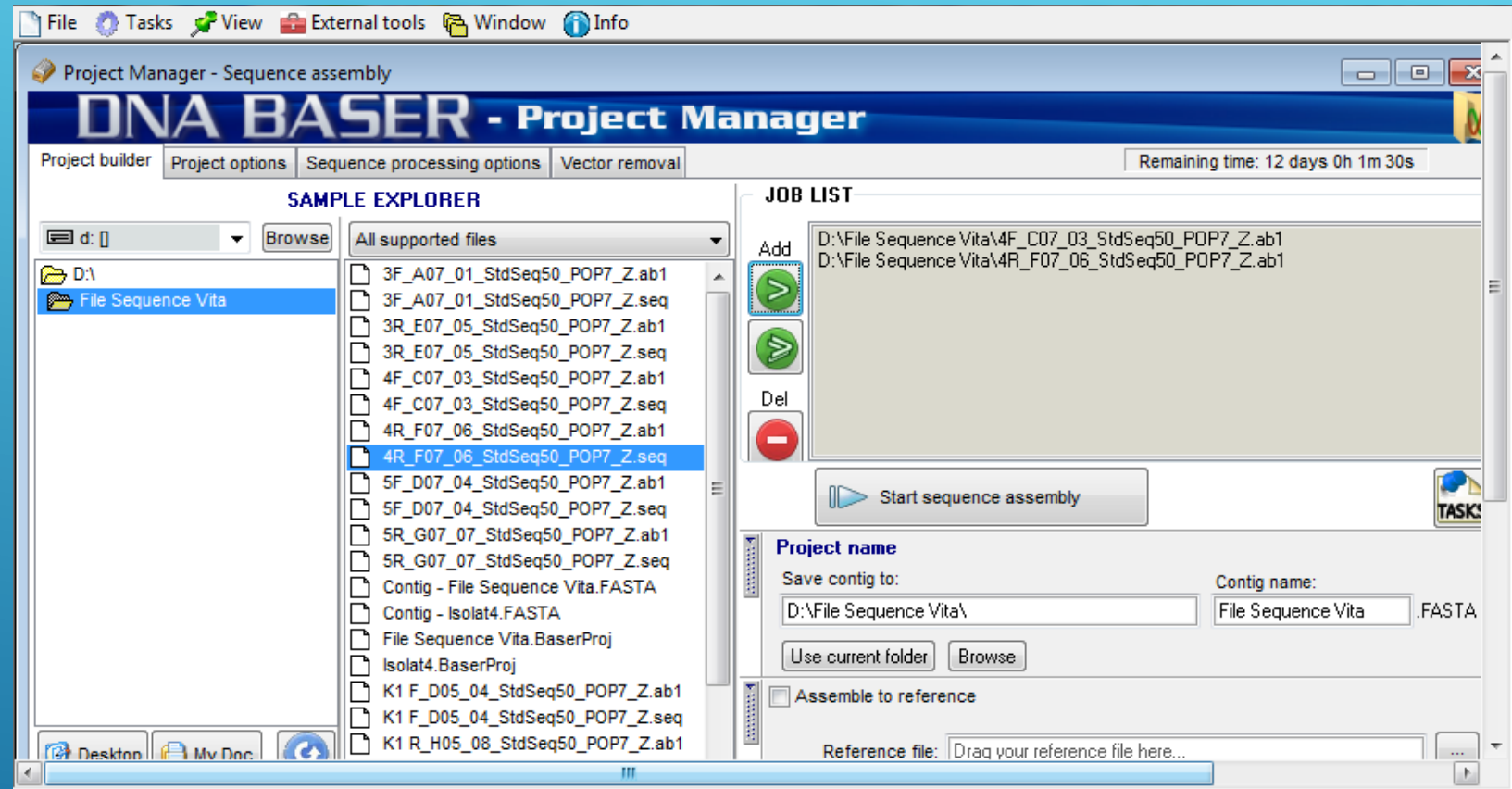
IDENTIFIKASI BAL

- ▶ Setelah proses sequencing akan didapatkan data urutan genom dari isolat terpilih dalam bentuk file. Untuk mengolah file tersebut menggunakan program DNA Baser Assembler



DNA BASER

- ▶ Program tersebut untuk melakukan contig yaitu mengedit dengan cara memotong urutan genom File hasil sequencing forward dan reverse dilakukan proses assembly.
- ▶ Contoh pada file isolat 4 (4F dan 4R)



DNA BASER

- ▶ Contoh hasil contig menggunakan DNA Baser Assembler
- ▶ File yang disimpan berupa fasta file (.fasta)



BLAST (BASIC LOCAL ALIGNMENT SEARCH TOOLS)

- ▶ Merupakan web based database yang menyimpan database mikrobial di seluruh dunia.

The screenshot shows the NCBI BLAST website. At the top, there are logos for NIH (U.S. National Library of Medicine) and NCBI (National Center for Biotechnology Information), along with a "Sign in to NCBI" link. The main header includes "BLAST" and navigation links for "Home", "Recent Results", "Saved Strategies", and "Help". A prominent red banner contains a COVID-19 alert: "COVID-19 is an emerging, rapidly evolving situation. Get the latest public health information from CDC: <https://www.coronavirus.gov>. Get the latest research from NIH: <https://www.nih.gov/coronavirus>." Below this, the "Basic Local Alignment Search Tool" is described as a program that finds regions of similarity between biological sequences. A "NEWS" box highlights a new "Search Betacoronavirus Database" for SARS-CoV-2 sequences, dated Mon, 03 Feb 2020 10:00:00 EST. The "Web BLAST" section features three main options: "Nucleotide BLAST" (nucleotide to nucleotide), "blastx" (translated nucleotide to protein), and "Protein BLAST" (protein to protein). The "tblastn" option (protein to translated nucleotide) is also visible but partially obscured.

- Untuk proses BLAST digunakan option Nucleotide BLAST
- Digunakan File fasta dari hasil Contig untuk memasukkan sequence yang akan di BLAST



Standard Nucleotide BLAST

[blastn](#) [blastp](#) [blastx](#) [tblastn](#) [tblastx](#)

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#) [Query subrange](#) [?](#)

From
To

```
>Isolat4
GKGAACGSTTCTTTTCMCCGGGAGCTTGCTCCACCGGAAAAAGAGGAGTGKYKAACGGGTGAGTAACAC
GTGGGTAACCTGCCCATCAGAAGGGGAT AACACTTGGAAACAGGTGCTAATACCGTATAACAATCRAAAC
CGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTT
GGTGAGGTAACGGCTACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACT
```

Or, upload file No file chosen [?](#)

Job Title
Enter a descriptive title for your BLAST search [?](#)

[Align two or more sequences](#) [?](#)

Choose Search Set

Database Standard databases (nr etc.): rRNA/ITS databases Genomic + transcript databases Betacoronavirus

[?](#)

Organism Optional exclude [+](#)
 exclude
 Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude Optional Models (XM/XP) Uncultured/environmental sample sequences

Limit to Optional Sequences from type material

Entrez Query Optional
Enter an Entrez query to limit search [?](#) [YouTube](#) [Create custom database](#)

▶ Contoh hasil Blast pada isolat no 4

BLAST[®] » blastn suite » results for RID-9GTNOV1J014 Home Recent Results Saved Strategies Help

[← Edit Search](#) [Save Search](#) [Search Summary ▾](#) [? How to read this report?](#) [BLAST Help Videos](#) [↶ Back to Traditional Results Page](#)

i Your search is limited to records that exclude: uncultured/environmental sample sequences

Job Title Isolat4

RID [9GTNOV1J014](#) Search expires on 04-17 22:07 pm [Download All ▾](#)

Program BLASTN [? Citation ▾](#)

Database nt [See details ▾](#)

Query ID lcl|Query_25633

Description Isolat4

Molecule type dna

Query Length 1412

Other reports [Distance tree of results](#) [MSA viewer](#) [?](#)

Filter Results

Organism only top 20 will appear exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to **E value** to **Query Coverage** to

[Filter](#) [Reset](#)

Descriptions [Graphic Summary](#) [Alignments](#) [Taxonomy](#)

Sequences producing significant alignments [Download ▾](#) [Manage Columns ▾](#) Show [?](#)

select all 100 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Enterococcus faecium strain CAU3862 16S ribosomal RNA gene, partial sequence	2507	2507	99%	0.0	98.23%	MF429683.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain 7-10 16S ribosomal RNA gene, partial sequence	2507	2507	99%	0.0	98.23%	KJ702545.1
<input checked="" type="checkbox"/>	Enterococcus durans strain CAU6810 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.23%	MF583035.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain CAU9113 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.22%	MF424050.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain D1 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.22%	KJ702551.1

- Cari isolat yang memiliki query cover 100% atau mendekati 100%
- Klik bagian Accession number untuk membuka informasi mengenai isolat tersebut

Sequences producing significant alignments Download ▾ Manage Columns ▾ Show 100 ▾ ?

select all 100 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Enterococcus faecium strain CAU3862 16S ribosomal RNA gene, partial sequence	2507	2507	99%	0.0	98.23%	MF429683.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain 7-10 16S ribosomal RNA gene, partial sequence	2507	2507	99%	0.0	98.23%	KJ702545.1
<input checked="" type="checkbox"/>	Enterococcus durans strain CAU6810 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.23%	MF583035.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain CAU9113 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.22%	MF424050.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain D1 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.22%	KJ702551.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain TW5-3 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.16%	KJ026572.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain IGM5-9 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	MT197265.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain IGM4-6 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	MT197247.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain VVEswe-R chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP041261.2
<input checked="" type="checkbox"/>	Enterococcus faecium strain VVEswe-S chromosome, complete genome	2503	15014	99%	0.0	98.15%	CP041270.2
<input checked="" type="checkbox"/>	Enterococcus faecium strain SRR24 chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP038996.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain ZY2 chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP039729.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain ZY11 chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP038995.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain ME3 chromosome	2503	10013	99%	0.0	98.15%	CP043865.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain 4 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	MH236305.2
<input checked="" type="checkbox"/>	Enterococcus faecium strain AHC4 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	MN749966.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain VRE chromosome, complete genome	2503	15014	99%	0.0	98.15%	CP046077.1
<input checked="" type="checkbox"/>	Enterococcus durans strain APED4 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.22%	MN595072.1
<input checked="" type="checkbox"/>	Enterococcus faecium strain LAC7.2 chromosome, complete genome	2503	15001	99%	0.0	98.15%	CP045012.1

- Pada bagian “send to” klik dan pilih file lalu pilih format fasta lalu download/send file
- File tersebut digunakan dalam proses elanjutnya menggunakan program MEGA X
- Pilih setidaknya 5-6 database yang mirip (query mendekati 100%)

GenBank

Enterococcus faecium strain CAU3862 16S ribosomal RNA gene,
GenBank: MF429683.1
[FASTA](#) [Graphics](#) [PopSet](#)

Go to:

LOCUS MF429683 1423 bp DNA linear BCT 10-AUG-2017
DEFINITION Enterococcus faecium strain CAU3862 16S ribosomal RNA gene, partial sequence.
ACCESSION MF429683
VERSION MF429683.1
KEYWORDS .
SOURCE Enterococcus faecium
ORGANISM [Enterococcus faecium](#)
Bacteria; Firmicutes; Bacilli; Lactobacillales; Enterococcaceae; Enterococcus.
REFERENCE 1 (bases 1 to 1423)
AUTHORS Ren,F. and Zhao,L.
TITLE Direct Submission
JOURNAL Submitted (04-JUL-2017) College of Food Science & Nutritional Engineering, China Agricultural University, No. 17 Qinghua East Road, Beijing 100083, China
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..1423
/organism="Enterococcus faecium"

Send to:

Choose Destination
 File Clipboard
 Collections Analysis Tool

Download 1 item.

Format
 GenBank
 Summary
 GenBank
 GenBank (full)
FASTA
 ASN.1
 XML
 INSDSeq XML
 TinySeq XML
 Feature Table
 Accession List
 GI List
 GFF3

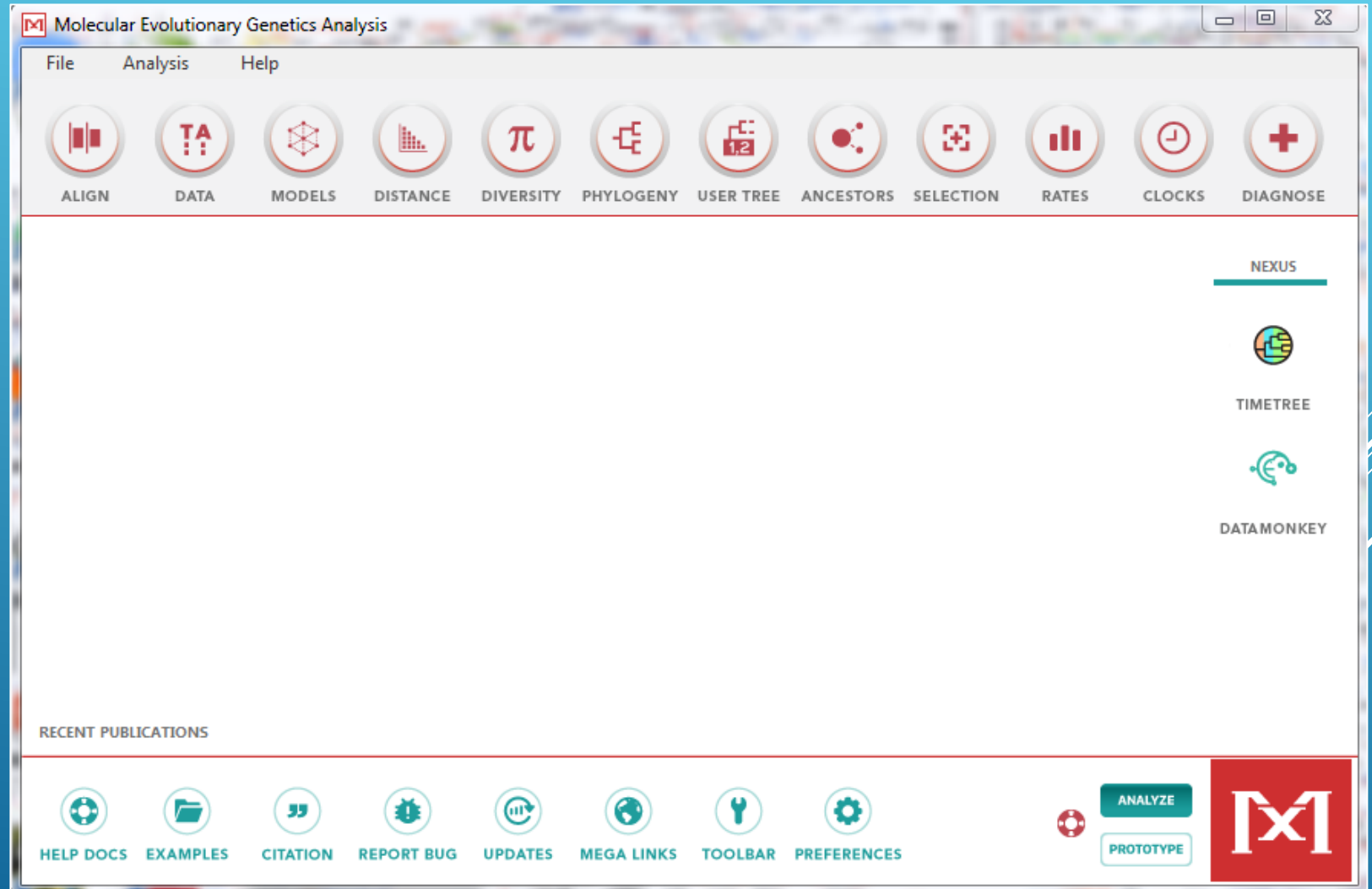
Related information
 Taxonomy
 PopSet

LinkOut to external resources
 SILVA SSU Database [SILVA]

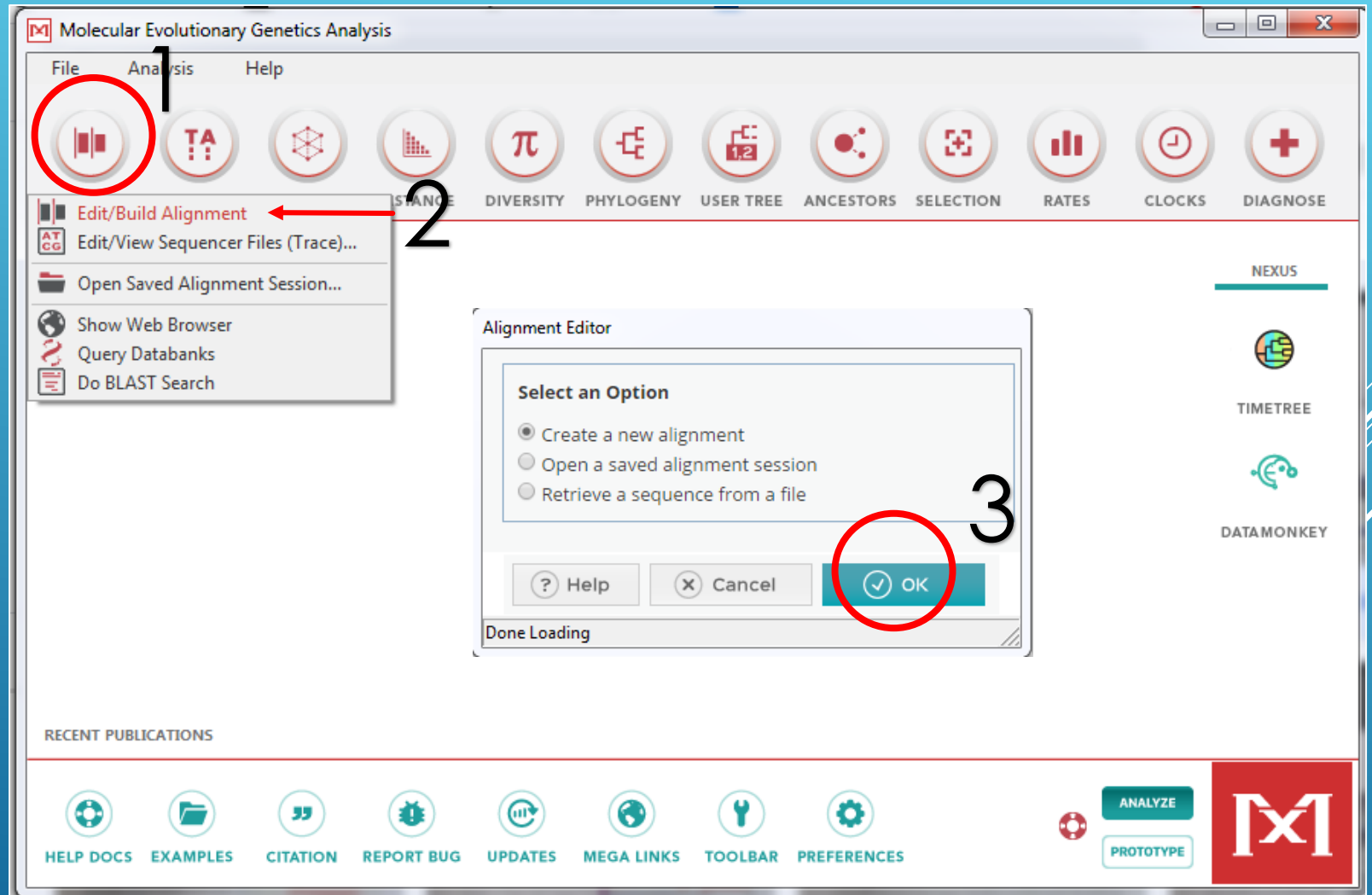
Recent activity

MEGA (MOLECULAR EVOLUTIONARY GENETIC ANALYSIS)

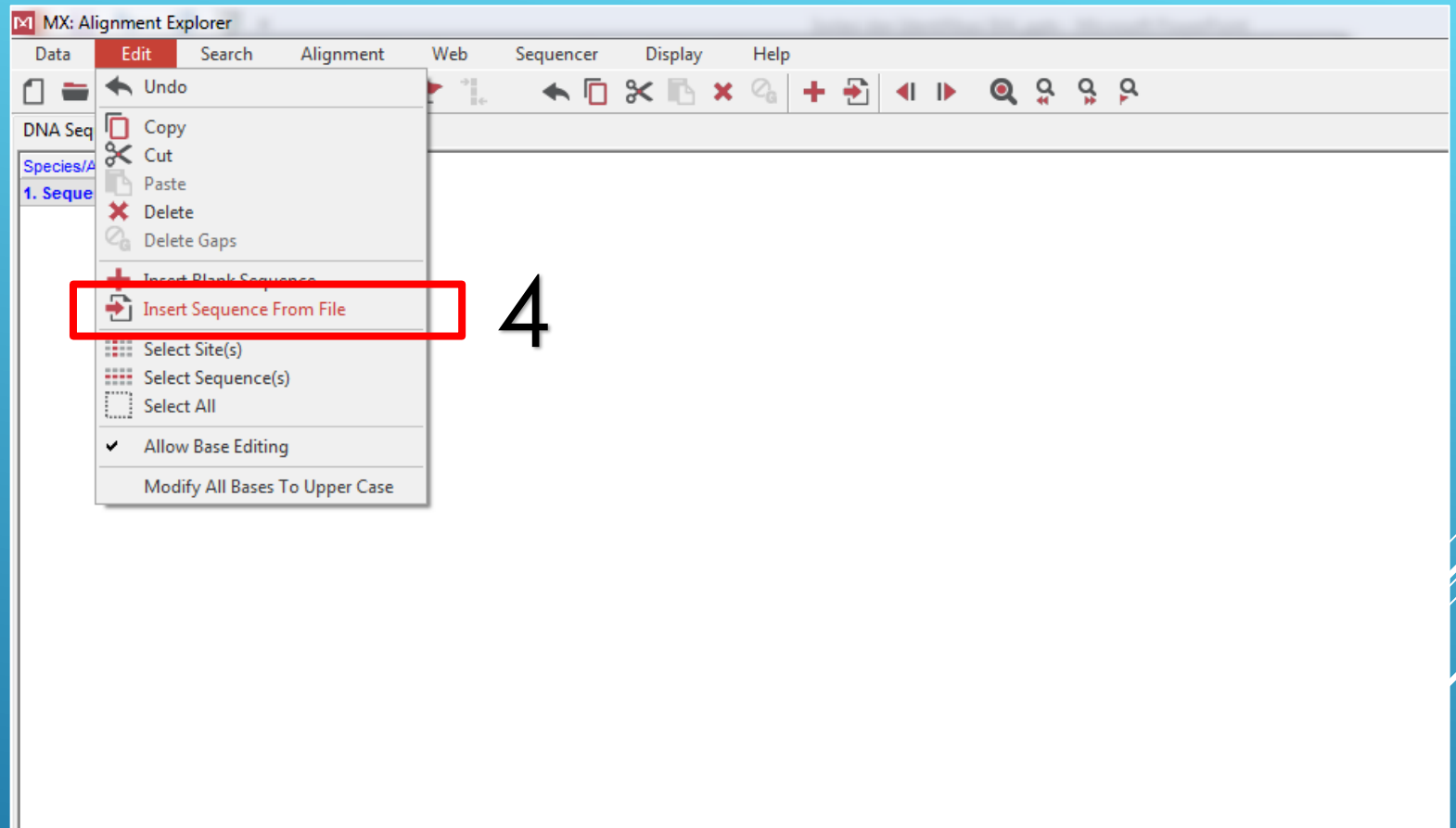
- Tahapan selanjutnya menggunakan software MEGA (Molecular Evolutionary Genetic Analysis). Di sini digunakan MEGA versi 10 (MEGA X)



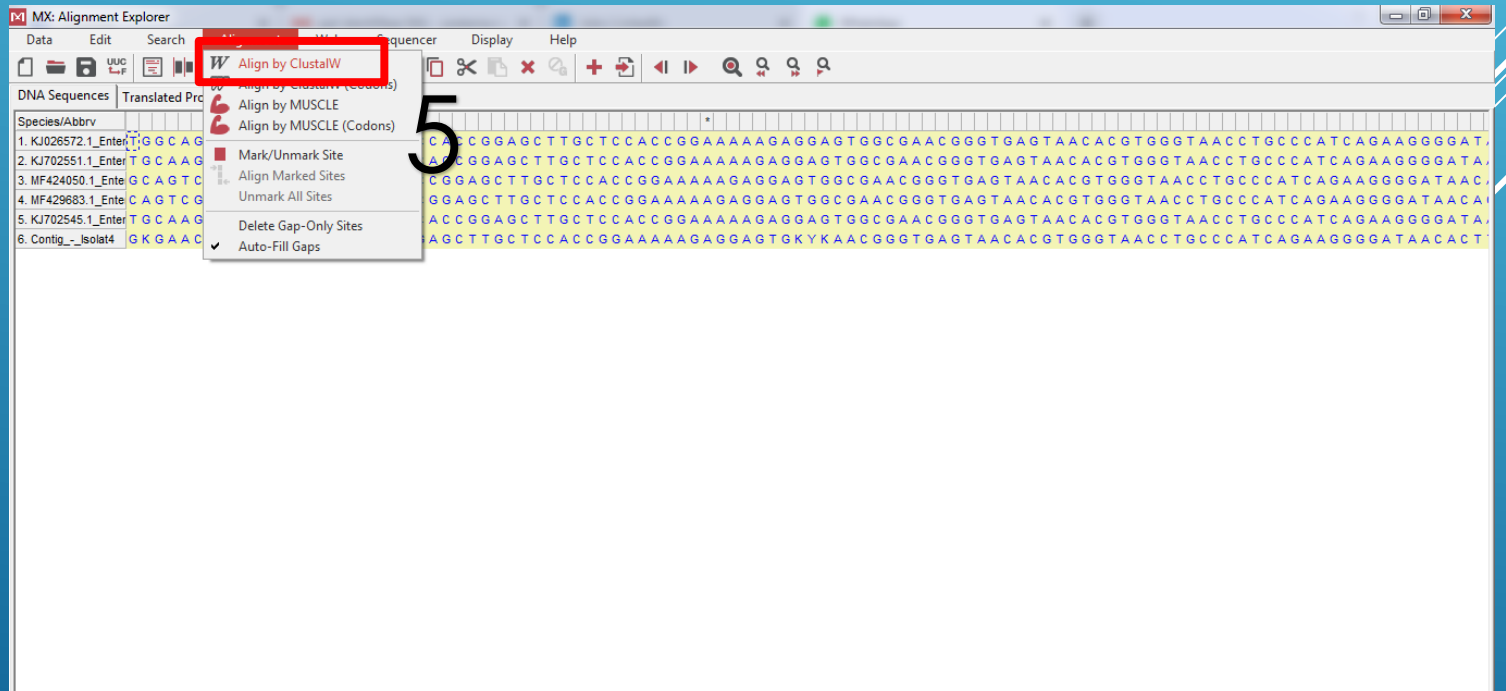
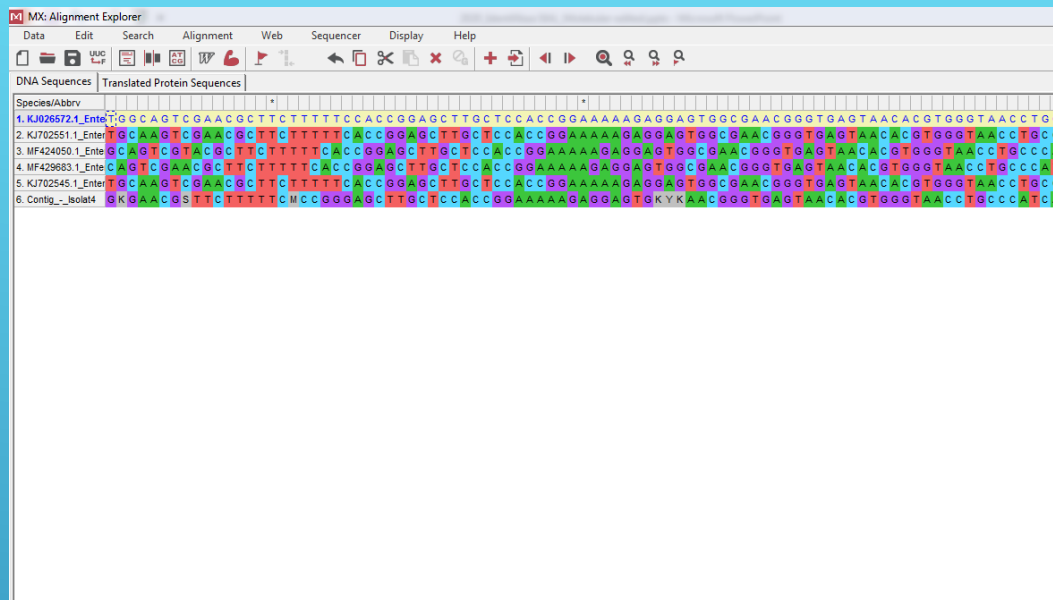
- Untuk membuat pohon filogeni dilakukan pengaturan sequences agar sequence menjadi teratur
- Tahapan pertaman dapat dilihat dari slide



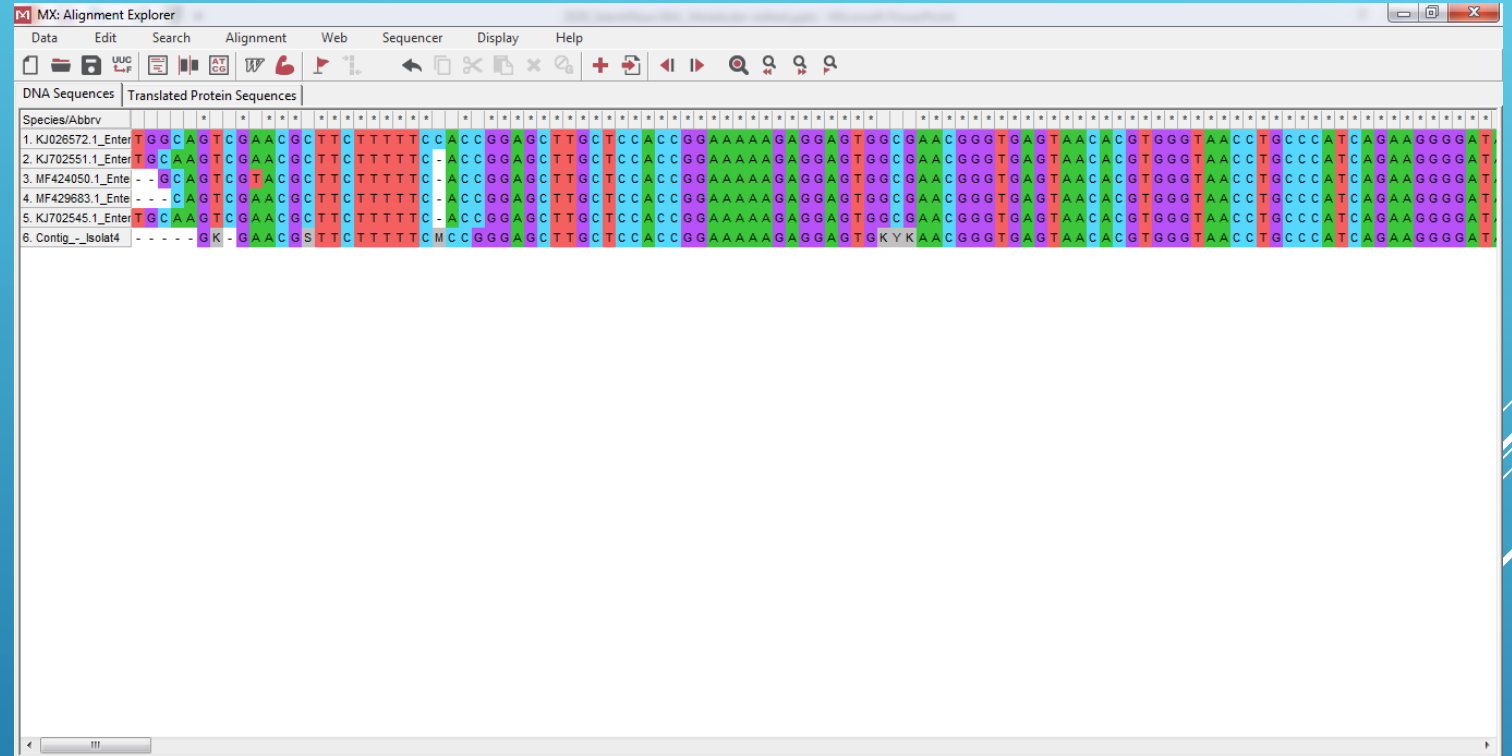
- ▶ Masukkan semua sequence yang telah di unduh dari BLAST dan sequence isolat 4 (Fasta File)



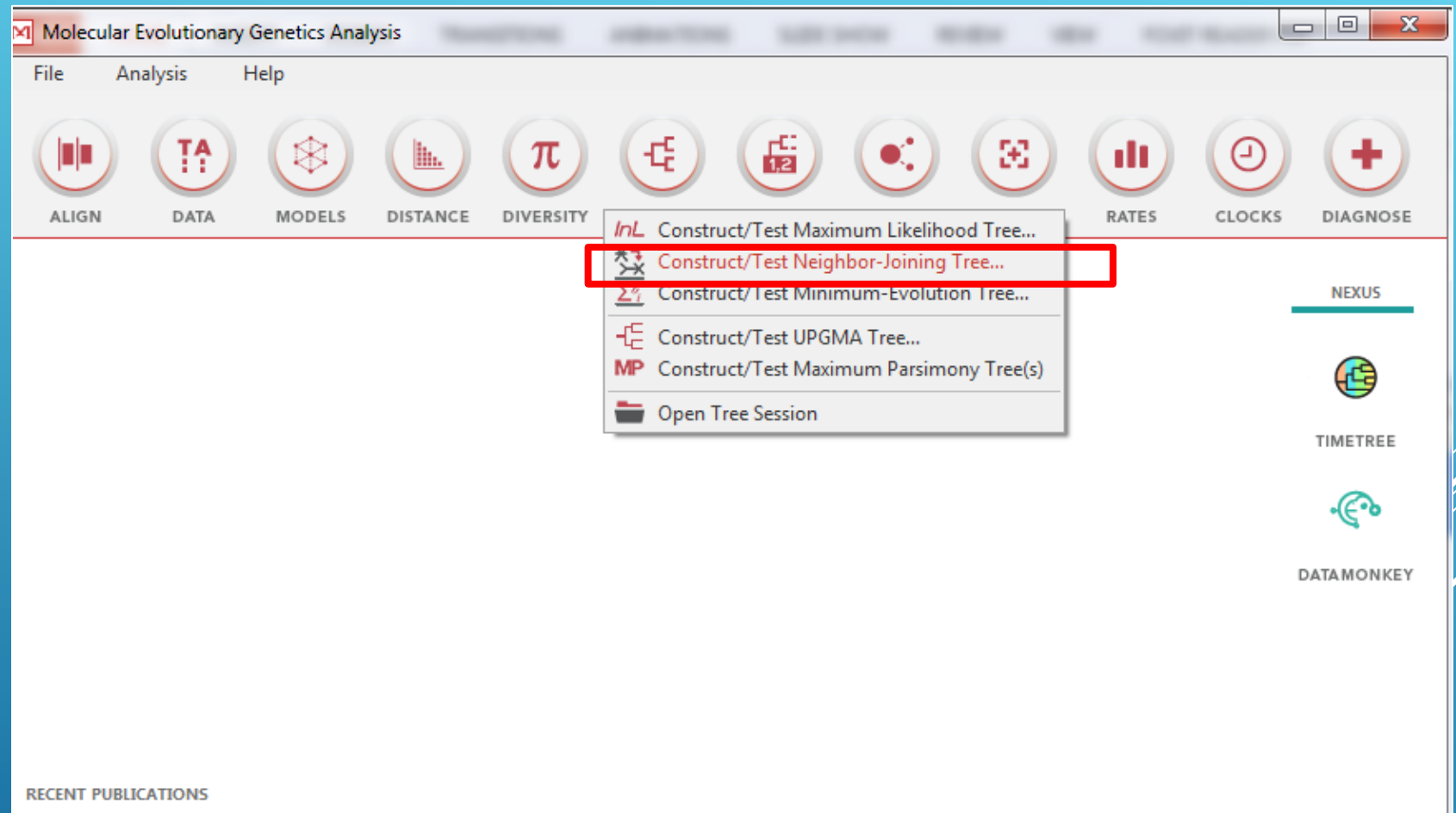
- Masukkan semua sequence yang telah di unduh dari BLAST dan sequence isolat 4 (Fasta File)
- Highlight semua sequence lalu pilih alignment – align by ClustalW



- Sequence yang sudah di align di simpan dalam format mega untuk pembuatan pohon filogeni
- File disimpan dalam file berekstensi .meg



- ▶ Untuk pembuatan pohon filogeni menggunakan menu phylogeny – construct/test neighbor joining tree
- ▶ Lalu pilih file mega yang sudah disimpan tadi



- Untuk preferences mengikuti option seperti gambar di samping

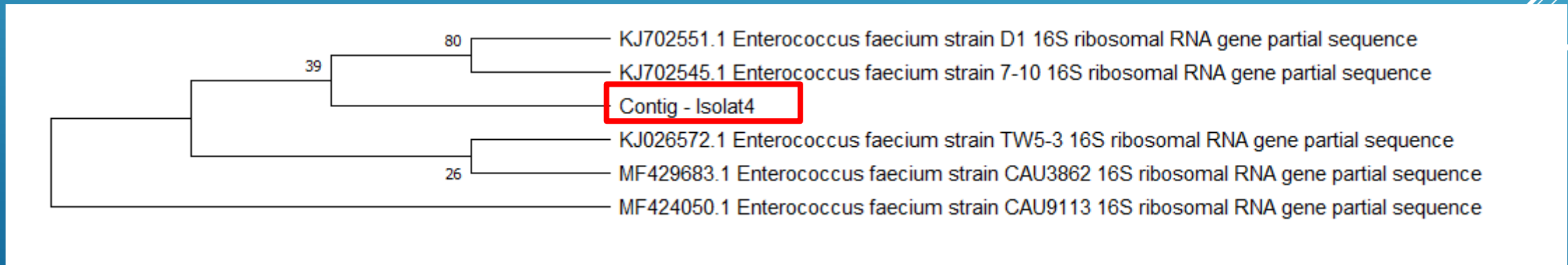
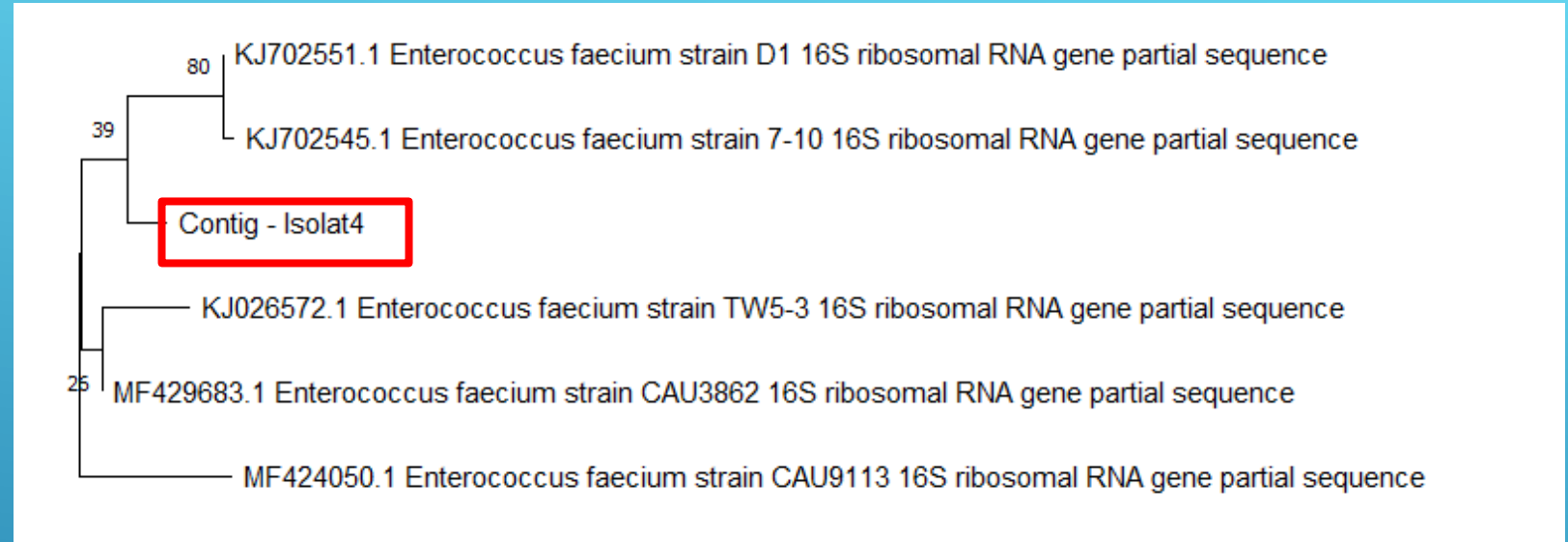
MX: Analysis Preferences

Phylogeny Reconstruction

Option	Setting
ANALYSIS	
Scope →	All Selected Taxa
Statistical Method →	Neighbor-joining
PHYLOGENY TEST	
Test of Phylogeny →	Bootstrap method
No. of Bootstrap Replications →	100
SUBSTITUTION MODEL	
Substitutions Type →	Nucleotide
Genetic Code Table →	Not Applicable
Model/Method →	Kimura 2-parameter model
Fixed Transition/Transversion Ratio →	Not Applicable
Substitutions to Include →	d: Transitions + Transversions
RATES AND PATTERNS	
Rates among Sites →	Uniform Rates
Gamma Parameter →	Not Applicable
Pattern among Lineages →	Same (Homogeneous)
DATA SUBSET TO USE	
Gaps/Missing Data Treatment →	Pairwise deletion
Site Coverage Cutoff (%) →	Not Applicable
Select Codon Positions →	<input checked="" type="checkbox"/> 1st <input checked="" type="checkbox"/> 2nd <input checked="" type="checkbox"/> 3rd <input checked="" type="checkbox"/> Noncoding Sites
SYSTEM RESOURCE USAGE	
Number of Threads →	3

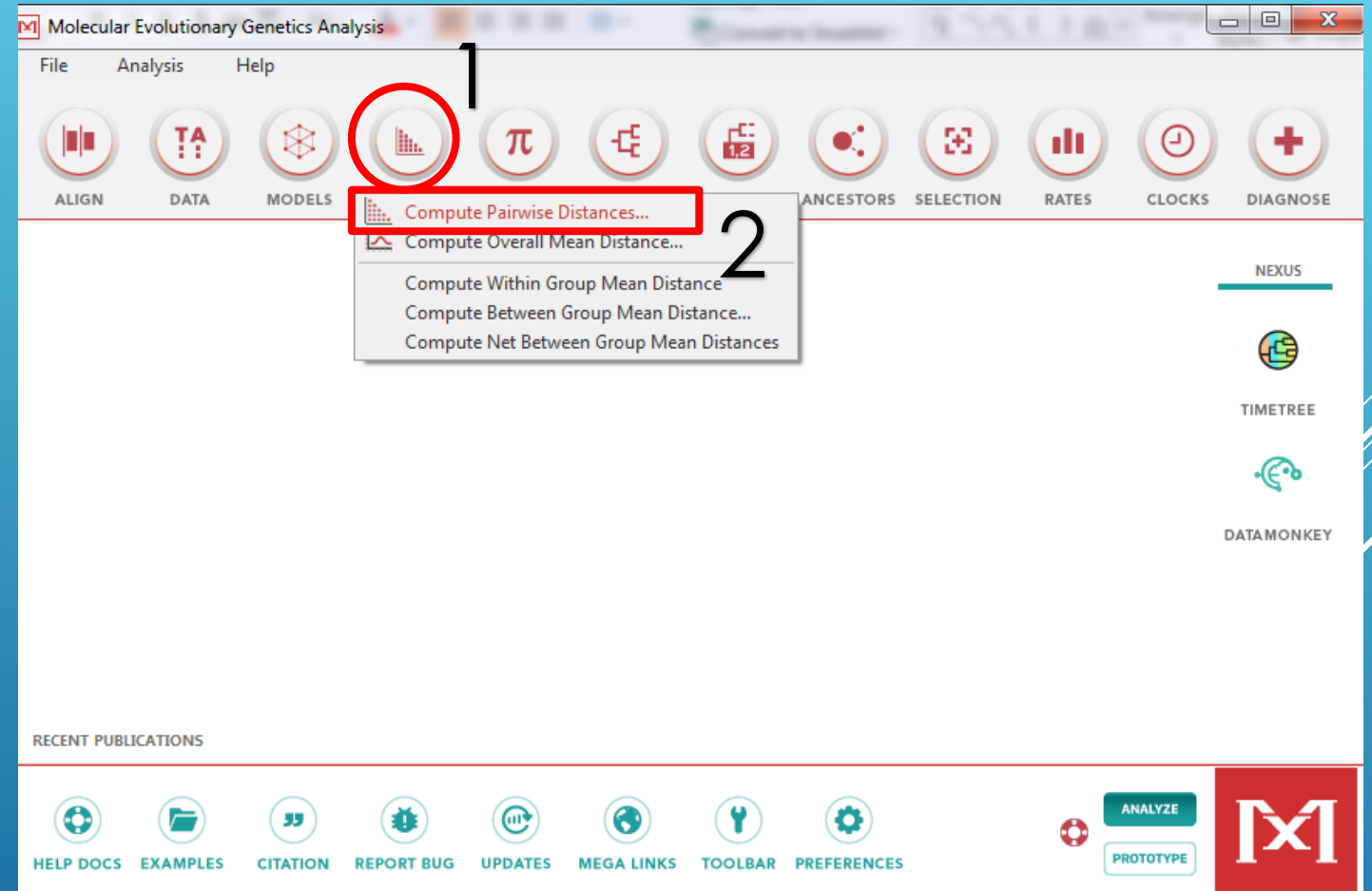
? Help X Cancel ✓ OK

► Contoh hasil pembuatan pohon filogeni



SEQUENCE SIMILARITY

- ▶ Untuk mengetahui tingkat kemiripan antara strain isolat dengan isolat lain dapat menggunakan distances menu
- ▶ Dari menu distances pilih compute pairwise distance
- ▶ Gunakan file hasil align (.meg)



SEQUENCE SIMILARITY

- ▶ Preferensi dalam model penghitungan tingkat similarity seperti ditunjukkan dalam gambar

MX: Analysis Preferences

Distance Estimation

Option	Setting
ANALYSIS	
Scope →	<i>Pairs of taxa</i>
ESTIMATE VARIANCE	
Variance Estimation Method →	<i>None</i>
No. of Bootstrap Replications →	<i>Not Applicable</i>
SUBSTITUTION MODEL	
Substitutions Type →	<i>Nucleotide</i>
Genetic Code Table →	<i>Not Applicable</i>
Model/Method →	<i>Kimura 2-parameter model</i>
Fixed Transition/Transversion Ratio →	<i>Not Applicable</i>
Substitutions to Include →	<i>d: Transitions + Transversions</i>
RATES AND PATTERNS	
Rates among Sites →	<i>Uniform Rates</i>
Gamma Parameter →	<i>Not Applicable</i>
Pattern among Lineages →	<i>Same (Homogeneous)</i>
DATA SUBSET TO USE	
Gaps/Missing Data Treatment →	<i>Pairwise deletion</i>
Site Coverage Cutoff (%) →	<i>Not Applicable</i>
Select Codon Positions →	<input checked="" type="checkbox"/> 1st <input checked="" type="checkbox"/> 2nd <input checked="" type="checkbox"/> 3rd <input checked="" type="checkbox"/> Noncoding Sites

? Help X Cancel ✓ OK

SEQUENCE SIMILARITY

- ▶ Hasil kalkulasi akan ditunjukkan seperti tabel di bawah

	1	2	3	4	5	6
1. Contig - Isolat4						
2. KJ026572.1 Enterococcus faecium strain TW5-3 16S ribosomal RNA gene partial sequence	0.0014445656					
3. KJ702551.1 Enterococcus faecium strain D1 16S ribosomal RNA gene partial sequence	0.0007229352	0.0021235206				
4. MF424050.1 Enterococcus faecium strain CAU9113 16S ribosomal RNA gene partial sequence	0.0014456098	0.0021126775	0.0028362544			
5. MF429683.1 Enterococcus faecium strain CAU3862 16S ribosomal RNA gene partial sequence	0.0014444349	0.0007036061	0.0007085918	0.0014088232		
6. KJ702545.1 Enterococcus faecium strain 7-10 16S ribosomal RNA gene partial sequence	0.0014444349	0.0021100782	0.0000000000	0.0028182694	0.0007036061	

Angka ini menunjukkan similaritas sebesar 99,86%

► Hasil identifikasi isolat 4 adalah sebagai berikut :

Strain	Related Species Name	Acc. No	Identity	Identification by 16S rRNA Gene
Isolat 4	<i>Enterococcus faecium</i> TW5-3	KJ026572	99,86%	<i>Enterococcus faecium</i> group
	<i>Enterococcus faecium</i> D1	KJ702551	99,93%	
	<i>Enterococcus faecium</i> CAU9113	MF424050	99,86%	
	<i>Enterococcus faecium</i> CAU 3862	MF 429683	99,86%	
	<i>Enterococcus faecium</i> 7-10	KJ702545	99,86%	