



# DESAIN PRIMER UNTUK DNA BARKODING

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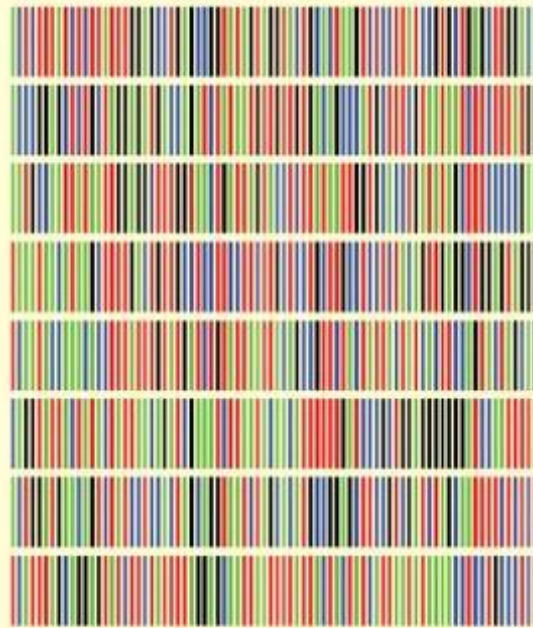
# DNA BARKODING

## Universal Product Code



- Ten unique states
- Twelve distinct positions

## DNA Barcode



- Four unique states
- Over 600 positions

**DNA-based  
identification  
system**

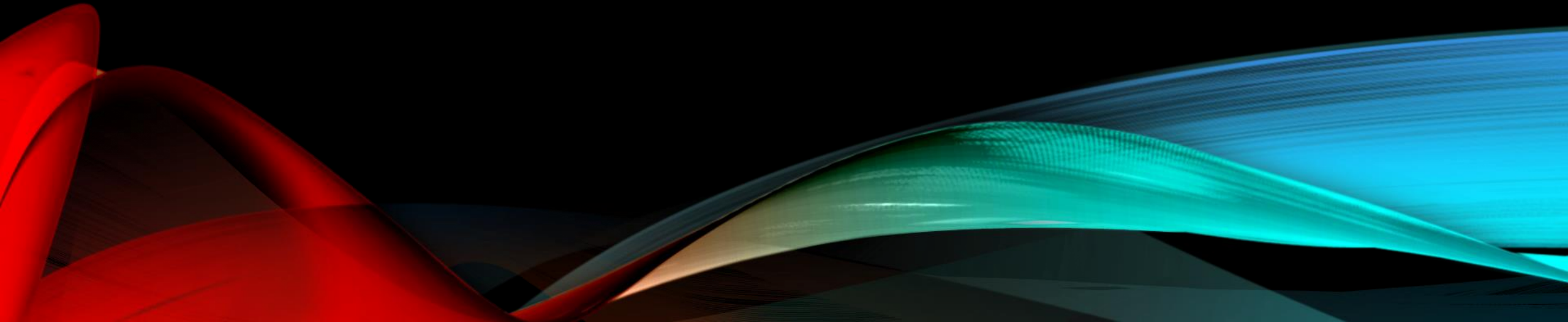
# DNA BARKODING

- Sample yang digunakan adalah DNA
  - Metode ekstraksi DNA.
- Gold standard locus:
  - COX1 (hewan),
  - rbcL dan matK (tumbuhan),
  - ITS (jamur),
  - 16s RNA (bakteri)
- Design primer
  - Tidak seluruh sekuen digunakan hanya sekuen “representatif”.
  - Disesuaikan dengan kebutuhan analisis/ identifikasi: level spesies, genus, famili, dst.





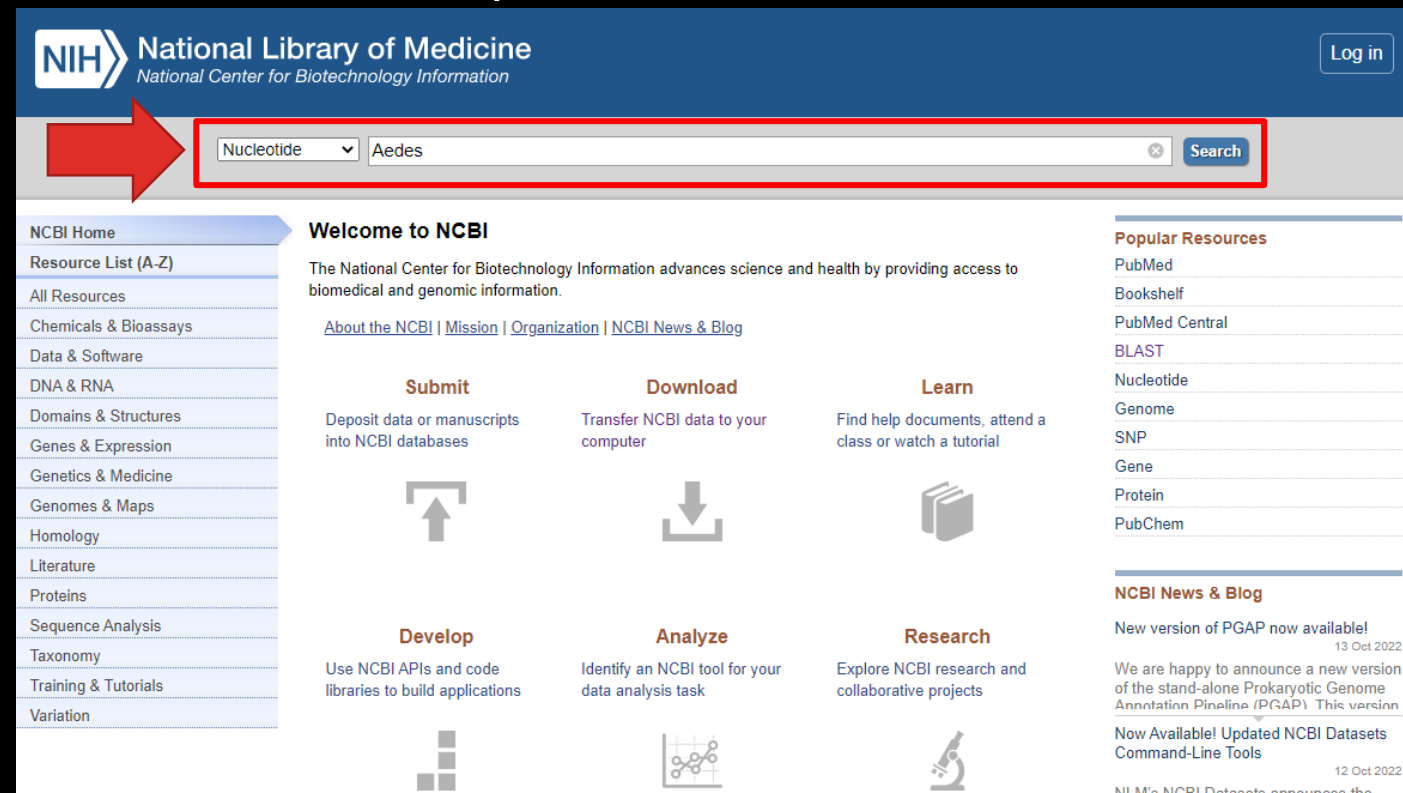
# 1. GEN TARGET



# PENGUMPULAN SEKUEN GEN TARGET

- Data sekuen gen target dapat dicari dan diunduh dari website NCBI (<https://www.ncbi.nlm.nih.gov/>).
  - Misal, anda diminta merancang primer gen cytochrome c oxidase 1 (COX1) dari beberapa spesies nyamuk, yaitu: *Aedes*, *Culex*, dan *Anopheles*.

- Lakukan pencarian *complete genome* pada NCBI
- Pilih “nucleotide” dari menu drop down
- Ketik genus organisme target anda.
- Lalu lakukan pencarian dengan klik “search”.



The screenshot shows the NCBI website interface. At the top, the NIH logo and 'National Library of Medicine' are visible. A search bar is prominently displayed, with a red arrow pointing to the dropdown menu set to 'Nucleotide' and the text 'Aedes' entered. The 'Search' button is highlighted with a red box. Below the search bar, the 'Welcome to NCBI' section provides information about the center's mission and offers links for 'Submit', 'Download', 'Learn', 'Develop', 'Analyze', and 'Research'. A sidebar on the left lists various resources, and a 'Popular Resources' section on the right includes links to PubMed, Bookshelf, and other tools.

# PENGUMPULAN SEKUEN GEN TARGET

Untuk merancang primer barkoding, gunakan *complete sequence* dari gen target.

Hindari *partial sequence* (panah)

The screenshot displays the NIH National Library of Medicine search interface. The search term 'aedes' is entered in the search bar. The results page shows a taxonomy summary for 'Aedes', stating it is a genus in the family Culicidae (mosquitos). Below this, a list of search results is shown, with the first result being 'Aedes cinereus isolate 4994997-1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial'. A red arrow points to this result, highlighting the word 'partial' in the title. The interface includes navigation options like 'Summary', '20 per page', and 'Sort by Default order'. There are also filters for species and molecule types on the left, and a 'Results by taxon' section on the right.

Nucleotide  Search

Species: Animals (793,495), Plants (87), Fungi (438), Protists (2,878), Bacteria (7,654), Archaea (1), Viruses (4,578), Customize ...

Molecule types: genomic DNA/RNA (335,089), mRNA (458,344), rRNA (481), Customize ...

Source databases: INSDC (GenBank) (720,396), RefSeq (88,670), Customize ...

Sequence Type: Nucleotide (385,981), EST (304,929), GSS (118,300)

Genetic compartments: Chloroplast (11), Mitochondrion (23,629), Plasmid (1,651), Plastid (11)

TAXONOMY

[Aedes](#)

*Aedes* is a genus in the family *Culicidae* (mosquitos).

Taxonomy ID: [7158](#)

[Taxonomy browser](#) [Genomes](#)

Was this helpful?

Items: 1 to 20 of 809210

1. [Aedes cinereus isolate 4994997-1 cytochrome oxidase subunit I \(COI\) gene, partial cds; mitochondrial](#)

658 bp linear DNA

Accession: [KM457571.1](#) GI: [730103364](#)

[Protein](#) [PubMed](#) [Taxonomy](#)

[GenBank](#) [FASTA](#) [Graphics](#)

2. [Aedes cinereus isolate 4606085 cytochrome oxidase subunit I \(COI\) gene, partial cds;](#)

Results by taxon

Top Organisms [\[Tree\]](#)

- [Aedes aegypti \(478415\)](#)
- [Aedes albopictus \(291821\)](#)
- [Ascogregarina taiwanensis \(2646\)](#)
- [Armigeres subalbatus \(2106\)](#)
- [Ochlerotatus sticticus \(1430\)](#)
- [All other taxa \(32792\)](#)

More...

Find related data

Database:

Search details

Recent activity

# PENGUMPULAN SEKUEN GEN TARGET

Lakukan penyaringan data dengan memilih menu yang ditunjuk oleh panah.

- RefSeq (dari menu source databases) dan
- Mitochondrion (dari menu genetic compartments)

Pada bagian kanan halaman hasil (kotak merah),

- Terdapat informasi mengenai spesies *Aedes* yang data *complete genome* dari gen COX1 sudah tersedia di NCBI.
- Unduh data complete sequence gen COX1 dari beberapa spesies *Aedes*, misal: *A. aegypti* dan *A. albopictus*

Nucleotide National Library of Medicine National Center for Biotechnology Information

Nucleotide Aedes Search

Species Animals (14) Customize ...

Molecule types genomic DNA/RNA (14) Customize ...

Source databases RefSeq (14) Customize ...

Sequence Type Nucleotide (14)

Genetic compartments Mitochondrion (14)

Sequence length Custom range...

Release date Custom range...

Revision date Custom range...

Clear all Show additional filters

TAXONOMY

**Aedes**

Aedes is a genus in the family Culicidae (mosquitos).

Taxonomy ID: 7158

[Taxonomy browser](#) [Genomes](#)

Was this helpful? Like Dislike

Items: 14

Filters activated: RefSeq, Mitochondrion. Clear all

[Aedes aegypti strain LVP\\_AGWG mitochondrion, complete genome](#)

1. 16,790 bp circular DNA  
Accession: NC\_035159.1 GI: 1212890241  
[BioProject](#) [BioSample](#) [Protein](#) [Taxonomy](#)  
[GenBank](#) [FASTA](#) [Graphics](#)

[Aedes vexans mitochondrion, complete genome](#)

2. 15,857 bp circular DNA  
Accession: NC\_065121.1 GI: 2281539444  
[BioProject](#) [Protein](#) [Taxonomy](#)  
[GenBank](#) [FASTA](#) [Graphics](#)

[Aedes flavopictus mitochondrion, complete genome](#)

3. 16,060 bp circular DNA  
Accession: NC\_050044.1 GI: 1883997257  
[BioProject](#) [Protein](#) [Taxonomy](#)

Results by taxon

Top Organisms [Tree](#)

[Aedes koreicus \(1\)](#)  
[Aedes aegypti \(1\)](#)  
[Aedes albopictus \(1\)](#)  
[Aedes flavopictus \(1\)](#)  
[Aedes vexans \(1\)](#)  
All other taxa (9)  
[More...](#)

Analyze these sequences

Run BLAST

Find related data

Database: Select

Find items

Search details

("Aedes"[Organism] OR "Aedes"[Organism] OR Aedes[All Fields]) AND (refseq[filter] AND mitochondrion[filter])

Search See more...

Recent activity

Turn Off Clear

Aedes AND (refseq[filter] AND mitochondrion[filter]) (14)

# PENGUMPULAN SEKUEN GEN TARGET

- Pilih salah satu hasil pencarian setelah disaring, misal “Aedes aegypti” (panah merah)
- Kemudian gunakan shortcut find (**Ctrl+F**) untuk mencari “COX1” (panah kuning),
- kemudian klik tautan **geneID** untuk gen COX1 (panah hijau).

The screenshot displays the National Library of Medicine GenBank interface. On the left, the search results for "Aedes" are shown, with a red arrow (1) pointing to the first result: "Aedes aegypti strain LVP\_AGWG mitochondrion, complete genome". The main content area shows the gene details for "COX1", with a yellow arrow (2) pointing to the gene name and a green arrow (3) pointing to the "geneID" link. The COX1 gene sequence is displayed in the center, along with its translation and various annotations.

**Gene Details:**

- Gene: **COX1**
- Product: cytochrome c oxidase subunit I
- GeneID: 33307557
- Accession: NC\_035159.1

**Sequence:**

```
/locus_tag="CFI06_mgp12"
/product="tRNA-Tyr"
/db_xref="GeneID:33307556"
<1298..2834
/gene="COX1"
/locus_tag="CFI06_mgp12"
/db_xref="GeneID:33307557"
<1298..2834
/gene="COX1"
/locus_tag="CFI06_mgp12"
/note="start codon not determined; TAA stop codon is
completed by the addition of 3' A residues to the mRNA"
/codon_start=1
/transl_except=(pos:2834,aa:TERM)
/transl_table=5
/product="cytochrome c oxidase subunit I"
/protein_id="YP_009389261.1"
/db_xref="GeneID:33307557"
/translation="SRQWLFSTNHKDIGTLYFIFGVWSGMVGTSLILIRAELSHPGM
FIGNDQIYNVIIVTAHAFIMIFFMVMPIMIGGGFNWLVPLMLGAPDMFPRMNMFSWM
LPPSLTLLSSSMVENGAGTGWTVYPPPLSSGTAHAGASVDLAIFSLHLAGISSILGAV
NFITTVINMWSGGITLDRPLFVWVSVITAILLLLSLPVLAGAITHLLDRNLNLSFF
DPIGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKETFGTLGMIYAML
TIGLLGFIVWAHHMFTVGMVDVTRAYFATSATHIAVPTGIKIFSWLATLHGTLTYSF
ALLNLSLGFVFLFTVGGLTGVVLANSSIDIVLHDTYYVVAHFHYVLSMGAVFAIMAGFI
HWYPLLTGMVMPNSWLKAQFSMMFIVGNLTFPPQHFLGLAGMPRRYSDFPDSYLTWNI
ISSLGSITISLFAVIFFLFIWESMITQRTSPFPMQLSSSIEWYHTLPPAETHYSELPL
LSSN"
```



# PENGUMPULAN SEKUEN GEN TARGET

- Anda akan diarahkan ke halaman genome viewer yang menunjukkan lokasi COX1 pada genome nyamuk *Aedes aegypti*.
- Buka tautan FASTA pada tab baru (panah),
- Gambar kanan menunjukkan halaman FASTA seq COX1 organisme *Aedes aegypti*

**COX1 cytochrome c oxidase subunit I [ *Aedes aegypti* (yellow fever mosquito) ]**

Gene ID: 33307557, updated on 12-Aug-2022

**Summary**

Gene symbol: COX1  
Gene description: cytochrome c oxidase subunit I  
Locus tag: CF106\_mgp12  
Gene type: protein coding  
RefSeq status: REVIEWED  
Organism: *Aedes aegypti* (strain LVP\_AGWG)  
Lineage: Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Holometabola; Diptera; Nematocera; Culicoidea; Culicidae; Culicinae; Aedini; *Aedes*; *Stegomyia*

**Genomic context**

Annotation release	Status	Assembly	Chr	Location
.101	current	AaegL5.0 (GCF_002204515.2)	MT (non-nuclear)	NC_035159.1 (1298..2834)

**Genomic regions, transcripts, and products**

Genomic Sequence: NC\_035159.1 Chromosome MT Reference AaegL5.0 non-nuclear

**FASTA**

Nucleotide: Nucleotide

**Aedes aegypti strain LVP\_AGWG mitochondrion, complete genome**

NCBI Reference Sequence: NC\_035159.1

GenBank Graphics

```
>NC_035159.1:1298-2834 Aedes aegypti strain LVP_AGWG mitochondrion, complete genome
TCGCGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTATATTTTCATTTTTGGAGTATGAT
CTGGAATAGTCGGAACCTCTCTAAGAAATTTTAAATCGTGTGAACCTAGCCACCCCTGGTATATTTATGG
GAATGACCAAAATTTATAATGTAATTTAACAAGCTCATGCATTTATATAATTTTCTTATAGTAATGCCA
ATTATAATGGAGGATTTGGAAATGATAGTTCCTTTAATATTAGGAGCCCTGATATAGCTTCCCTC
GAATGAATAATATAAGTTTTGAATACTACCTCTTCAATGACTCTTCTATTATCAAGCTCAATAGTAGA
AAATGGGGCAGGAACCTGGGTGAACAGTTTATCCTCCTCTCTCAGGAAACAGCTCATGCTGGAGCTCT
GTTGATTTAGCTATTTTTCTCTCATTTAGCTGGAATTTCTCAATTTTAGGGCAGTAAATTTTATTA
CAACTGTGATTAATGATGATCGTCAGGGATTACTTTAGATCGACTACCCTTATTTGTTGATCGTAGT
TATTACAGCTACTTATTAATCTCTTCTCTCTGTTTATGCTGGAGCTATTACTATATTTATTAACAGAC
CGAAACTTAAATACATCTTCTTTGATCCAATCGAGGGGAGACCTATTTTATACCAACACTTATTTT
GATCTTTGGACCCCAAGAAGTTTATATTTAAATTTTACCCGGAATTTGGAATAATTTCTCATATATTAC
TCAAGAAAGCGGAAAAAAGAAACATTTGGAACCTTAGGAATAATTTATGCTAATTAACAATTTGGATTA
TTGGGATTTATGTTGAGCTCATCATATTTACAGTAGGTATAGACGTAGATACTCGACTTATTTTA
CTTCAGCAACTATAATTTGCTGCTCTACAGGAATTTAAATTTTAGTTGATAGCAACTTTACACGG
AACTCAATTAACATATAGTCCAGCCCTCTATGATCATTAGGATTTGATTTTATTTATTCAGTTGGAGT
TTAACAGGAGTAGTATTAGCTAATTTCTCAATGATATTTGTTCTTCATGATCTATTACAGTATGCCCC
ATTTCTATTACGTTTTTATCTATAGGAGCTGATTTGCTATTATAGCAGGATTTATTCATTTGATACCCCTT
ATTAACAGGAATAGTTATAAACCTTCATGATTAAGGCTCAATTTAGTATAATATTTATTTGGAGAAAT
CTACTTCTTTCTCAACATTTTTAGGGTTAGCTGGAATACCTCGACGATCACTAGATTTCTCTGATA
GCTACTAACTTGAATATTTCTCTCTTTAGGAAGAACATTTCACTATTTGCGGTTATTTCTTTTTT
ATTTATTATTGGAAAGTATAATTAACCAACGACCTTTCTTCCCTATACAAATATCTCATCTAAT
GAATGATATCATACACTCTCTCGAGAACACTATTATCAGAATACCACACTCTTCTCTAAT
```

**Change region shown**

Whole sequence  
 Selected region  
from: 1298 to: 2834

**Customize view**

**Analyze this sequence**

Run BLAST

Pick Primers

Highlight Sequence Features

**Related information**

Assembly

BioProject

BioSample

Protein

Taxonomy

# PENGUMPULAN SEKUEN GEN TARGET

- Lakukan langkah yang sama untuk spesies nyamuk lainnya dan simpan sekuen fasta gen COX1 pada “notepad”.

Berikut beberapa complete sequences COX1 gene:

- *Aedes aegypti*: NC\_035159 (location: 1298-2834)
- *Aedes albopictus*: NC\_006817 (location: 1436-2972)
- *Culex pipiens pallens*: NC\_015079 (location: 1446-2982)
- *Culex quinquefasciatus*: NC\_014574 (location: 1446-2982)
- *Anopheles cruzii*: NC\_04464 (location: 1445-2983)
- *Anopheles gambiae*: NC\_002084 (location: 1424-2960)

# PENGUMPULAN SEKUEN GEN TARGET

Tugas UAS (bagian 1):

- Cari dan tentukan kelompok organisme yang akan anda identifikasi menggunakan Teknik DNA Barkoding.
- Cari dan tentukan gen target yang sesuai untuk mengidentifikasi kelompok organisme tersebut.
- Kumpulkan sekuen gen target minimal dari 10 organisme yang ingin anda identifikasi.
- Simpan file dalam notepad.

# PENGUMPULAN SEKUEN GEN TARGET

***Gold standard daerah target*** untuk barcoding diantaranya:

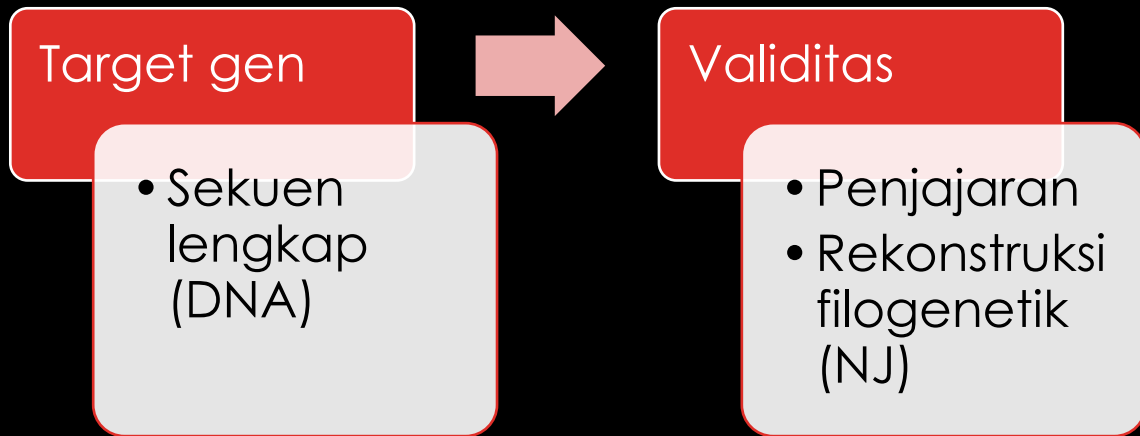
1. Hewan: *COX1* (cytochrome c oxidase 1)
2. Tumbuhan: *rbcL* (large subunit RuBisCo), *matK* (maturase K)

**Slow** evolving region: useful for distantly related taxa (genus, family, ordo)

**Fast** evolving region: needed when identifying more closely related species such as cryptic/sibling species groups.



# VALIDASI TARGET GEN



□ Target gen

Periksa terlebih dahulu apakah gen terpilih dapat dipergunakan sebagai DNA barcode.

Lakukan **penjajaran** dan rekonstruksi **pohon filogenetik**.

## **2. PENJAJARAN SEKUENS DAN REKONSTRUKSI POHON FILOGENETIK**



# LANGKAH MELAKUKAN PENJAJARAN MENGGUNAKAN SOFTWARE MEGA

The image displays the MEGA (Molecular Evolutionary Genetics Analysis) software interface, illustrating the steps for performing sequence alignment. The interface is divided into several panels, with numbered arrows indicating the sequence of actions:

- Step 1:** A red arrow points to the 'File' menu in the top-left corner.
- Step 2:** A red arrow points to the 'Edit/Build Alignment' option in the 'File' menu.
- Step 3:** An orange arrow points to the 'Do BLAST Search' option in the 'File' menu.
- Step 4:** A yellow arrow points to the 'DNA' button in the 'Data Type for Alignment' dialog box.
- Step 5:** A green arrow points to the 'Data' menu in the 'M11: Alignment Explorer' window.
- Step 6:** A teal arrow points to the '1. Sequence 1' entry in the 'DNA Sequences' list.
- Step 7:** A blue arrow points to the 'Align' button in the 'M11: Alignment Explorer' window.

The 'Alignment Editor' dialog box shows the following options:

- Select an Option
  - Create a new alignment
  - Open a saved alignment session
  - Retrieve a sequence from a file

The 'Data Type for Alignment' dialog box asks: "Are you building a DNA or protein sequence alignment?" with buttons for "DNA", "Protein", and "Cancel".

The 'M11: Alignment Explorer' window displays a list of DNA sequences and their corresponding protein sequences. The sequences are listed as follows:

Species/Abbrv	DNA Sequences	Translated Protein Sequences
1. NC_035159/Aedes aegypti cox1	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT
2. NC_050044/Aedes flavopictus cox1	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT
3. NC_046946/Aedes koreicus cox1	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT
4. NC_006817/Aedes albopictus cox1	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT	TTCGGGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGTATGAT
5. NC_044647/Anopheles cruzii cox1	TTCGGGACAATGATTATTTTCTACAAATCATAAAGATATTGGTACTTTTATATTTTTCATTTTGGGGCTTGAG	TTCGGGACAATGATTATTTTCTACAAATCATAAAGATATTGGTACTTTTATATTTTTCATTTTGGGGCTTGAG
6. NC_002084/Anopheles gambiae cox1	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG
7. NC_056265/Anopheles rivulorum cox1	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG
8. NC_064608/Anopheles moucheti cox1	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG
9. NC_014574/Culex quinquefasciatus cox1	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG
10. NC_015079/Culex pipiens pipiens cox1	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG
11. NC_038160/Culex gelidus cox1	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCAACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG
12. NC_028616/Culex tritaeniorhynchus cox1	TTCGGGACAATGATTATTTTCTACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCTACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG
13. Sequence 1	TTCGGGACAATGATTATTTTCTACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG	TTCGGGACAATGATTATTTTCTACAAATCATAAAGATATTGGAACCTTTATATTTTTCATTTTGGAGCTTGAG





# REKONSTRUKSI POHON FILOGENETIKA DENGAN MEGA SOFTWARE

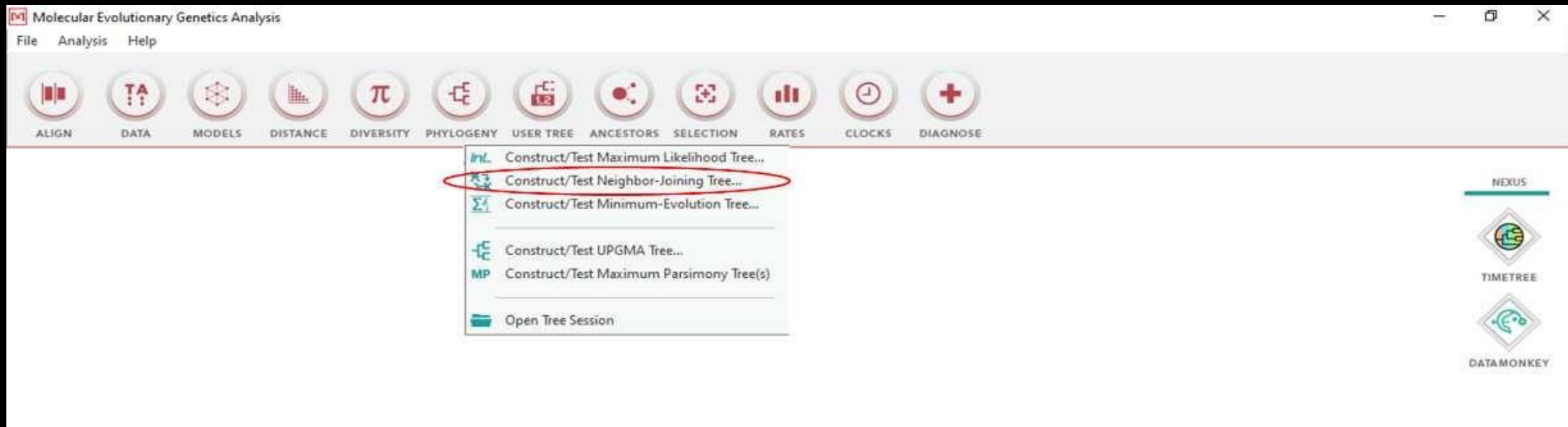
- Save/ export ke MEGA FORMAT



The screenshot displays the MEGA software interface. The main window is titled 'M11: Alignment Explorer (COX2.meg)'. The 'Data' menu is open, showing options like 'Create New', 'Open', 'Close', 'Phylogenetic Analysis', 'Save Session', 'Export Alignment', 'DNA Sequences', 'Protein Sequences', 'Translate/Untranslate', 'Genetic Code', 'Reverse Complement', and 'Quit'. The 'Export Alignment' option is highlighted, and a sub-menu is visible with 'MEGA Format' selected. A green arrow points to 'MEGA Format' in the sub-menu. The main window shows a sequence alignment with columns of nucleotides (A, T, C, G) and gaps. The status bar at the bottom indicates 'Site # 696' and 'Selected genetic code: Standard'. The bottom of the interface features a navigation bar with icons for 'HELP DOCS', 'EXAMPLES', 'CITATION', 'REPORT BUG', 'UPDATES', 'MEGA LINKS', 'TOOLBAR', and 'PREFERENCES'. There are also buttons for 'ANALYZE' and 'PROTOTYPE' and the MEGA logo.

# REKONSTRUKSI POHON FILOGENETIKA DENGAN MEGA SOFTWARE

Buka aplikasi MEGA X, Lalu pilih, menu **phylogeny**, kemudian pilih **construct/test Neighbor-Joining Tree**

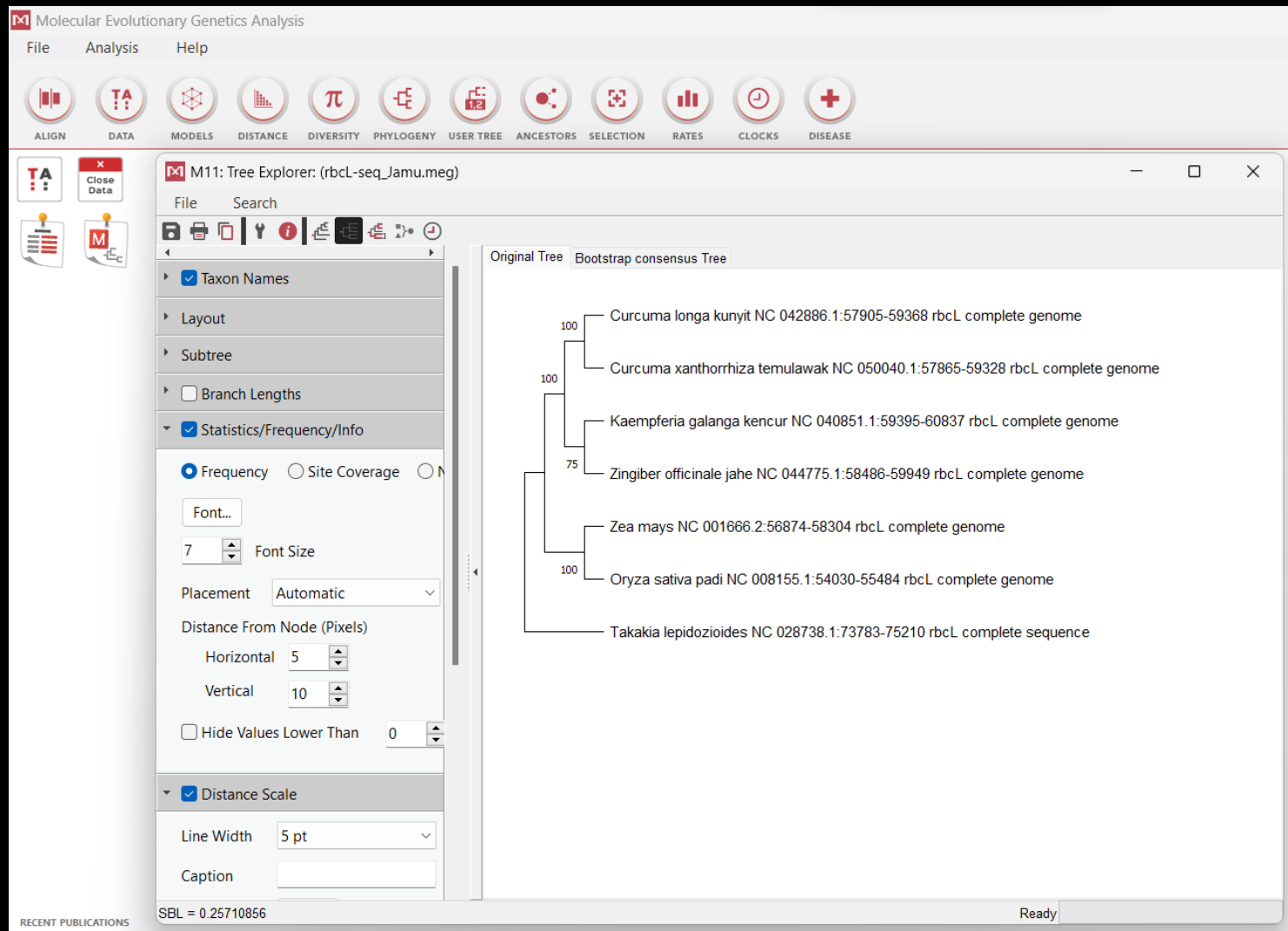


Option	Setting
<b>ANALYSIS</b>	
Scope →	All Selected Taxa
Statistical Method →	Neighbor-joining
<b>PHYLOGENY TEST</b>	
Test of Phylogeny →	Bootstrap method
No. of Bootstrap Replications →	1000
<b>SUBSTITUTION MODEL</b>	
Substitutions Type →	Nucleotide
Genetic Code Table →	Not Applicable
Model/Method →	p-distance
Fixed Transition/Transversion Ratio →	Not Applicable
Substitutions to Include →	d: Transitions + Transversions
<b>RATES AND PATTERNS</b>	
Rates among Sites →	Uniform Rates
Gamma Parameter →	Not Applicable
Pattern among Lineages →	Same (Homogeneous)
<b>DATA SUBSET TO USE</b>	
Gaps/Missing Data Treatment →	Complete 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"/> Nonc
<b>SYSTEM RESOURCE USAGE</b>	
Number of Threads →	1

# REKONSTRUKSI POHON FILOGENETIKA

- Pilih sekuen yang telah di jajarkan dalam bentuk format data MEGA.
- Kemudian pada menu **PHYLOGENY TEST** pilih **bootstrap method** dan **No. of bootstrap replications diisi 1000.**
- Pada menu **SUBSTITUTION MODEL** pilih model/method dengan **p-distance.** Biarkan parameter lain sesuai **default setting.**
- Pilih **OK**

# HASIL REKONSTRUKSI POHON FILOGENETIK



- Spesies yang merupakan bahan baku jamu, terpisah dengan spesies “kontaminan”
- rbcL dapat digunakan untuk melakukan pengujian kontaminasi tepung beras/ tepung jagung dalam sediaan jamu.



# SUMMARY



✓ Target gen

□ Target region

Analisis hasil **penjajaran** untuk menemukan daerah **polimorfik**.

# **3. IDENTIFIKASI DAERAH POLIMORFIK**



# PENJAJARAN (*ALIGNMENT*)

- Penjajaran sekuen (*alignment*) dilakukan untuk mengidentifikasi daerah lestari (*conserved region*) dan daerah polimorfik (*polymorphic region*).
  - Daerah lestari gen: memiliki sekuen DNA yang sama pada tingkatan taksonomi yang berbeda
  - Daerah polimorfik gen: daerah yang memiliki variasi sekuen DNA pada tingkatan taksonomi yang berbeda.
  - Primer di desain pada daerah yang lestari sehingga sepasang primer dapat dipergunakan untuk **mengamplifikasi DNA** dari beberapa organisme.
  - Urutan daerah polimorfik digunakan untuk **mengidentifikasi organisme**.

# PENJAJARAN (ALIGNMENT)

Lakukan alignment/penjajaran pada sekuen gen COX1 yang sudah dikumpulkan, menggunakan tools **clustalW** pada software **MEGA**, BioEdit, atau **MUSCLE** (web-based tools).

The image displays the MEGA (Molecular Evolutionary Genetics Analysis) software interface, illustrating the steps for performing a sequence alignment. The interface is divided into several panels, and the workflow is indicated by numbered arrows:

- 1**: A red arrow points to the 'File' menu in the top-left corner.
- 2**: A red arrow points to the 'Edit/Build Alignment' option in the 'File' menu.
- 3**: An orange arrow points to the 'Open Saved Alignment Session...' option in the 'File' menu.
- 4**: A yellow arrow points to the 'DNA' button in the 'Data Type for Alignment' dialog box, which asks 'Are you building a DNA or protein sequence alignment?'. The 'Protein' and 'Cancel' buttons are also visible.
- 5**: A green arrow points to the 'M11: Alignment Explorer' window, which is the main workspace for viewing and editing alignments.
- 6**: A teal arrow points to the 'M11: Alignment Explorer' window, specifically to the 'DNA Sequences' tab, which lists the sequences being aligned.
- 7**: A blue arrow points to the 'M11: Alignment Explorer' window, specifically to the 'Translated Protein Sequences' tab, which displays the aligned protein sequences.

The 'M11: Alignment Explorer' window shows a list of sequences under the 'DNA Sequences' tab, including:

- 1. NC\_035159/Aedes aegypti cox1
- 2. NC\_050044/Aedes flavopictus cox1
- 3. NC\_046946/Aedes koreicus cox1
- 4. NC\_006817/Aedes albopictus cox1
- 5. NC\_044647/Anopheles cruzii cox1
- 6. NC\_002084/Anopheles gambiae cox1
- 7. NC\_056265/Anopheles rivulorum cox1
- 8. NC\_064608/Anopheles moucheti cox1
- 9. NC\_014574/Culex quinquefasciatus cox1
- 10. NC\_015079/Culex pipiens pipiens cox1
- 11. NC\_038160/Culex gelidus cox1
- 12. NC\_028616/Culex tritaeniorhynchus cox1
- 13. Sequence 1

The 'Translated Protein Sequences' tab shows the corresponding amino acid sequences for each of these DNA sequences, aligned together.

# PENJAJARAN (ALIGNMENT)

Lakukan alignment/penjajaran pada sekuen gen COX1 yang sudah dikumpulkan, menggunakan tools **clustalW** pada software MEGA, **BioEdit**, atau **MUSCLE** (web-based tools).

The screenshot shows the BioEdit Sequence Alignment Editor interface. The 'Open File ...' dialog box is open, displaying a list of files in the 'Seq.Praktikum' directory. The file 'Mosquito\_cox1\_seq(v2)' is selected. The 'Accessory Application' menu is open, and 'ClustalW Multiple alignment' is highlighted. The main window shows a sequence alignment view with a scale bar at the bottom.

1. Open File ...

2. Mosquito\_cox1\_seq(v2)

3. ClustalW Multiple alignment

4. Accessory Application

5. ClustalW Multiple alignment

Accession	Species	Sequence
NC_035159	Ae	TTCGGACAAATGGT
NC_050044	Ae	TTCGGACAAATGGT
NC_046946	Ae	TTCGGACAAATGGT
NC_006817	Ae	TTCGGACAAATGGT
NC_044647	An	TTCGGACAAATGAT
NC_002084	An	TTCGGACAAATGAT
NC_056265	An	TTCGGACAAATGAT
NC_064608	An	TTCGGACAAATGAT
NC_014574	Cu	TTCGGACAAATGAC
NC_015079	Cu	TTCGGACAAATGAC
NC_038160	Cu	TTCGGACAAATGAC
NC_028616	Cu	TTCGGACAAATGAC



# PENJAJARAN (ALIGNMENT)

Lakukan alignment/penjajaran pada sekuen gen COX1 yang sudah dikumpulkan, menggunakan tools **clustalW** pada software MEGA, BioEdit, atau **MUSCLE** (web-based tools).

The screenshot shows the MUSCLE web interface. At the top, there is a teal header with the word "MUSCLE" in white. Below the header, there are navigation tabs: "Input form", "Web services", "Help & Documentation", and "Bioinformatics Tools FAQ". A "Feedback" link is also present. The main content area has a breadcrumb trail: "Tools > Multiple Sequence Alignment > MUSCLE". The title "Multiple Sequence Alignment" is displayed in teal. Below the title, there is a paragraph explaining that MUSCLE stands for Multiple Sequence Comparison by Log-Expectation and is claimed to be more accurate and faster than ClustalW2 or T-Coffee. An important note states that the tool can align up to 500 sequences or a maximum file size of 1 MB. The interface is divided into two steps. Step 1, "Enter your input sequences", contains a large text area for pasting sequences and a file upload section with a "Choose File" button. Step 2, "Set your Parameters", features a dropdown menu for "OUTPUT FORMAT" which is currently set to "ClustalW".

**MUSCLE**

Input form | Web services | Help & Documentation | Bioinformatics Tools FAQ | Feedback

Tools > Multiple Sequence Alignment > MUSCLE

## Multiple Sequence Alignment

MUSCLE stands for **M**ultiple **S**equence **C**omparison by **L**og- **E**xpectation. MUSCLE is claimed to achieve both better average accuracy and better speed than [ClustalW2](#) or [T-Coffee](#), depending on the chosen options.

**Important note:** This tool can align up to 500 sequences or a maximum file size of 1 MB.

**STEP 1 - Enter your input sequences**

Enter or paste a set of sequences in any supported format:

Or upload a file:  No file chosen [Use a example sequence](#) | [Clear sequence](#) | [See more example inputs](#)

**STEP 2 - Set your Parameters**

OUTPUT FORMAT:  
ClustalW

Masukan  
sekuen disini

Pilih ClustalW

# PENJAJARAN (ALIGNMENT)

Identifikasi daerah lestari (*conserved*) yang mengapit daerah polimorfisme.

M11: Alignment Explorer (rbcL-seq\_Jamu.meg)

Data Edit Search Alignment Web Sequencer Display Help

DNA Sequences Translated Protein Sequences

Species/Abbrv

Species/Abbrv	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	C	A	A	A	T	T	G	A	A	T	T	A	T	T	A	T	A	C	T	C	C	T	G	A	C	T	A	C	G	A	A	G	T	C	A	A	A	G	A	T	A	C	T	G	A	T	A	T	C	T	T	G	G	C	A	G	C	A	T
1. Curcuma longa kunyit NC_042886.1:57905-59368 rbcL complete genome	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	C	A	A	A	T	T	G	A	A	T	T	A	T	T	A	T	A	C	T	C	C	T	G	A	C	T	A	C	G	A	A	G	T	C	A	A	A	G	A	T	A	C	T	G	A	T	A	T	C	T	T	G	G	C	A	G	C	A	T
2. Curcuma xanthorrhiza temulawak NC_050040.1:57865-59328 rbcL complete genome	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	C	A	A	A	T	T	G	A	A	T	T	A	T	T	A	T	A	C	T	C	C	T	G	A	C	T	A	C	G	A	A	G	T	C	A	A	A	G	A	T	A	C	T	G	A	T	A	T	C	T	T	G	G	C	A	G	C	A	T
3. Kaempferia galanga kencur NC_040851.1:59395-60837 rbcL complete genome	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	C	A	A	A	T	T	G	A	C	T	T	A	T	T	A	T	A	C	T	C	C	T	G	A	C	T	A	C	G	A	A	G	T	C	A	A	A	G	A	T	A	C	T	G	A	T	A	T	C	T	T	G	G	C	A	G	C	A	T
4. Zingiber officinale jahe NC_044775.1:58486-59949 rbcL complete genome	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	C	A	A	A	T	T	G	A	C	T	T	A	T	T	A	T	A	C	T	C	C	T	G	A	C	T	A	C	G	A	A	G	T	C	A	A	A	G	A	T	A	C	T	G	A	T	A	T	C	T	T	G	G	C	A	G	C	A	T
5. Zea mays NC_001666.2:56874-58304 rbcL complete genome	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	T	A	A	A	T	T	G	A	C	T	T	A	C	T	A	C	A	C	C	C	C	G	G	A	G	T	A	C	G	A	A	A	C	C	A	A	G	G	A	T	A	C	T	G	A	T	A	T	C	T	T	G	G	C	A	G	C	A	T
6. Oryza sativa padi NC_008155.1:54030-55484 rbcL complete genome	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	T	A	A	A	T	T	G	A	C	T	T	A	C	T	A	C	A	C	C	C	C	G	G	A	G	T	A	C	G	A	A	A	C	C	A	A	G	G	A	C	A	C	T	G	A	T	A	T	C	T	T	G	G	C	A	G	C	A	T
7. Takakia lepidozoides NC_028738.1:73783-75210 rbcL complete sequence	A	A	A	G	C	T	G	G	T	G	T	T	A	A	A	G	A	T	T	A	C	A	G	T	T	A	A	C	T	C	A	T	T	A	C	A	C	T	C	C	G	A	A	T	A	T	G	A	G	A	C	C	A	A	G	A	T	A	C	C	G	A	T	A	T	T	C	T	T	G	G	C	A	G	C	A	T		

Conserved region

Polymorphic region

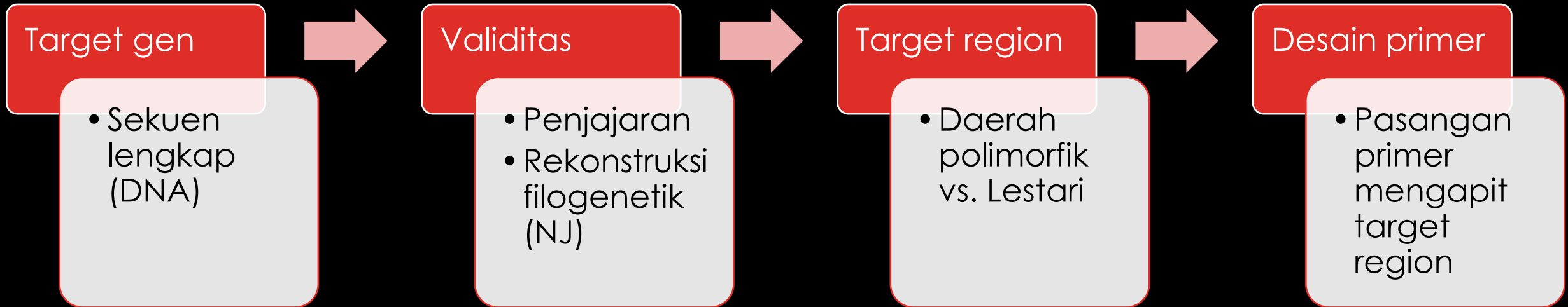
# PENJAJARAN SEKUEN

Tugas UAS (bagian 2):

Lakukan penjajaran sekuen gen target pilihan anda menggunakan:

1. Offline tools (installation needed): Mega, BioEdit
2. Web based tools provided by ebi.ac uk
  - MUSCLE: <https://www.ebi.ac.uk/Tools/msa/muscle/>
  - Clustal omega: <https://www.ebi.ac.uk/Tools/msa/clustalo/>

# SUMMARY

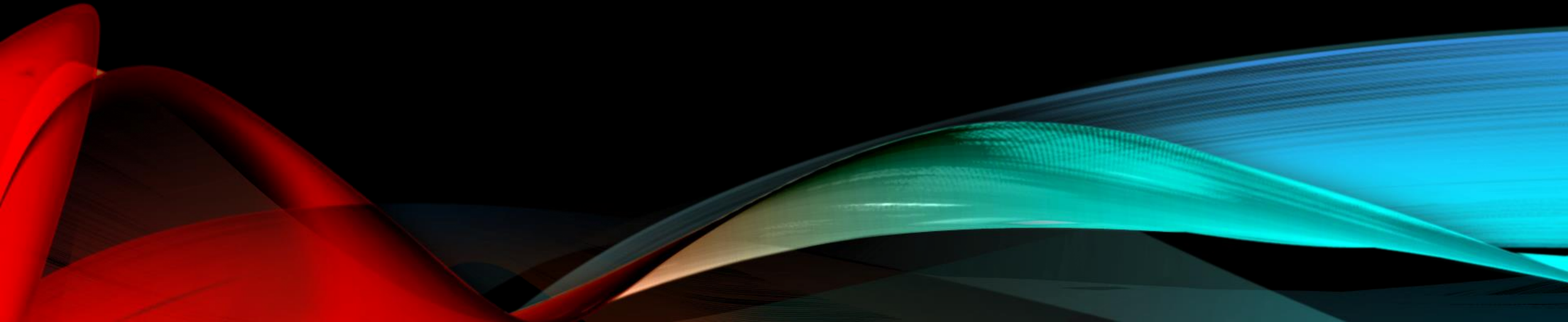


✓ Target gen

✓ Target region

□ Desain primer untuk amplifikasi target region

# DESAIN PRIMER





**Primer** adalah urutan sekuen nukleotida pendek (18-30 basa) yang biasanya digunakan untuk amplifikasi sekuen DNA spesifik secara *in vitro* pada proses *polymerase chain reaction* (PCR)

**Desain Primer** dapat dilakukan dengan menggunakan software bioinformatik. Bisa menggunakan software berbasis web atau pun perangkat lunak yang di install pada komputer

# TAHAP DESAIN PRIMER

1. Menentukan dan mencari sekuen DNA target: gen, intergenic, ekson, intron, UTR
2. Penjajaran (*alignment*) sekuen DNA
3. Menentukan daerah DNA yang akan diamplikasi: *polymorphic region*
4. Uji kualitas primer yang telah didesain
5. Uji spesifisitas primer yang telah di desain ( *in silico* PCR)

# What makes a good primer?

## Primer length: 18–22bp

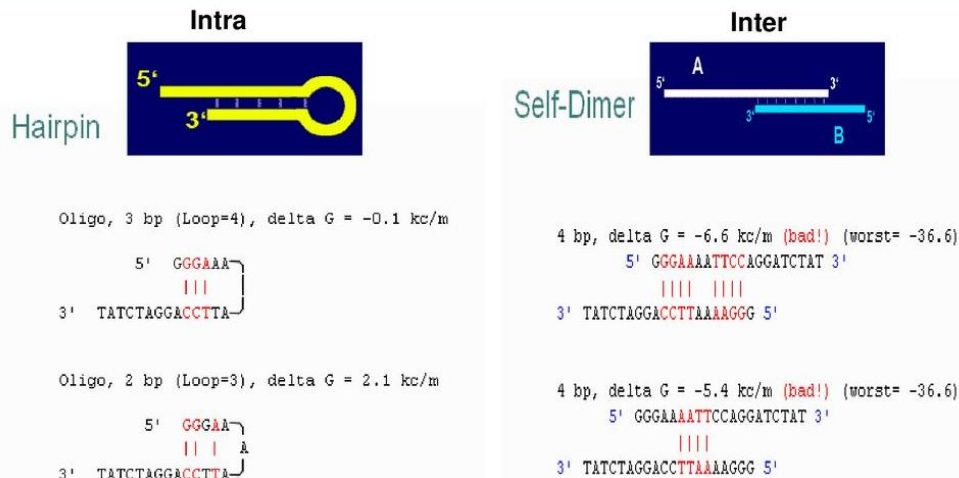
Too small a qPCR primer (<18bp) can increase the likelihood that it will bind to elsewhere in the genome, ie it is not specific enough to the target of interest. Otherwise, too large a qPCR primer (>22bp) can raise the primer melting temperature ( $T_m$ ) which will impact on the annealing temperature and primer binding properties of the reaction.



$T_m$ : 59–65°C

GC content: 50–60%

## Examples of Primer-Dimer Formation



The primer pair is specific to the target of interest

## Primers contain a GC clamp

Another feature which I like to include in both my primers is a **GC clamp**. Simply, a GC clamp is the presence of either a guanine (G) or cytosine (C) base in the last 5 bases of the primer.

The reasoning behind using a GC clamp in primers is the fact that G and C bases contain stronger hydrogen bonds, compare with adenine (A) and thymine (T) bases. Therefore, by including at least one G or C base towards the end of the primer will ensure it binds completely to the template sequence.



Other examples of a GC clamp (in red) in PCR primers include are listed below.

- 5'-CTCTGTAGGGTCGCGACTAC-3'
- 5'-CGCTACCACCATCGATTGAT-3'
- 5'-GGATCTGGCTGCATGCTATG-3'

Notice that it does not matter where in the last 5 bases the G or C base is in order for them to be referred to as a GC clamp.

## Avoid nucleotide repeats



# SOFTWARE DESAIN PRIMER

Software **berbasis web** yang dapat digunakan untuk desain primer

- **Primer-Blast (NCBI)**
- **Primer3**
- GenScript
- PerlPrimer
- RExPrimer

Software pada **personal komputer** yang digunakan untuk desain primer

- PrimerSelect
- PrimerPremier6
- Fast PCR
- PrimerDesign
- Oligo 6
- Geneious

# WEBPAGE DESAIN PRIMER

- Pencarian data sekuen gen target:
  - NCBI: <https://www.ncbi.nlm.nih.gov/>
- Desain primer:
  - NCBI primer BLAST: [https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK\\_LOC=BlastHome](https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome)
  - Primer3 Plus: <https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>
- Quality check kandidat primer:
  - OligoAnalyzer: <https://sg.idtdna.com/>
  - Beacon designer: <http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1>
  - NCBI primer BLAST



# WHAT DO YOU NEED HELP WITH?

## Techniques

Helpful advice on lab techniques

VIEW

## Lab Recipes

Learn how to create solutions

VIEW

## Stats & Maths

Guides on all things maths

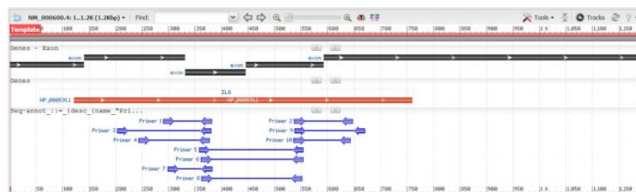
VIEW

### 13 Free PCR Primer Design Programs




Gone are the days where you have to design primers by hand. Instead, we have a plethora of excellent resources available that utilises complex algorithms to determine the optimal primers for your PCR reaction. These give the best theoretical chance of your PCR reaction working, thus saving time and money. Best of all, the majority of them are free to use or download. Here is a list of 13 free primer design programs to use when designing primers:

#### 1 Primer-BLAST



# Desain primer dengan primer-BLAST

 U.S. National Library of Medicine  
National Center for Biotechnology Information

[Log in](#)

## Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

**Primers for target on one template** | Primers common for a group of sequences

[Retrieve recent results](#) | [Publication](#) | [Tips for finding specific primers](#) | [Save search parameters](#) | [Reset page](#)

### PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) [?](#) [Clear](#)

```
>NC_029243.1A. sinensis_rpoC1
TTGATCGGAATGAATGAATCAGAATTTTCTTCTATGATCGACCGATATAAACATCAACAACCTC
AAATAG
GATCGGTTTCGCCTCAACAAATAAGTGCTTGGGCTAAGAAAATCCTACCCAATGGAGAGATA
GTTGGAGA
CGATCGA...
```

Or, upload FASTA file  No file chosen

Range [?](#) [Clear](#)

Forward primer  From  To

Reverse primer

### Primer Parameters

Use my own forward primer (5'->3' on plus strand)  [?](#) [Clear](#)

Use my own reverse primer (5'->3' on minus strand)  [?](#) [Clear](#)

PCR product size  Min  Max

# of primers to return

Primer melting temperatures (T<sub>m</sub>)  Min  Opt  Max  Max T<sub>m</sub> difference [?](#)

### Primer Pair Specificity Checking Parameters

Specificity check  Enable search for primer pairs specific to the intended PCR template [?](#)

Search mode  [?](#)

Database  [?](#)

Exclusion  Exclude predicted Refseq transcripts (accession with XM, XR prefix)  Exclude uncultured/environmental sample sequences [?](#)

Organism  [Add organism](#)

Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type. [?](#)

Entrez query (optional)  [?](#)

Primer specificity stringency  
Primer must have at least  total mismatches to unintended targets, including  
at least  mismatches within the last  bps at the 3' end. [?](#)  
Ignore targets that have  or more mismatches to the primer. [?](#)

Max target amplicon size  [?](#)


Allow splice variants  Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input) [?](#)

[Get Primers](#) |  Show results in a new window  Use new graphic view [?](#)

Note: Parameter values that differ from the default are highlighted in yellow

[+ Advanced parameters](#)

# Desain primer dengan primer-BLAST


**U.S. National Library of Medicine**  
 National Center for Biotechnology Information

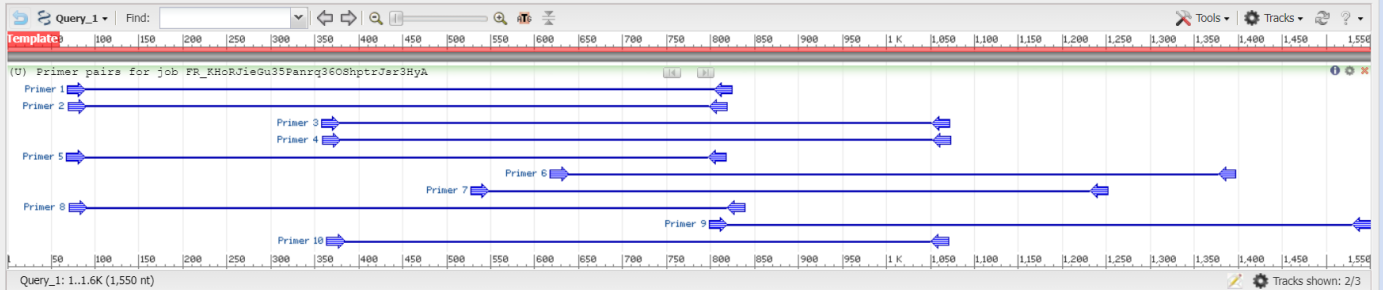
[Log in](#)

**Primer-BLAST** > JOB ID:FR\_KHoRJeGu35Panrq360ShptrJsr3HyA

**Primer-BLAST Results**

Input PCR template NC\_029243|A.sinensis\_rpoC1  
 Range 1 - 1550  
 Specificity of primers Primers may **not** be specific to the input PCR template as targets were found in selected database:Nucleotide collection (nt) (Organism limited to Aquilaria)...[help on specific primers](#)  
 Other reports [Search Summary](#)

**Graphical view of primer pairs**



**Detailed primer reports**

You can re-search for specific primers by accepting some of the unintended targets, check the box(es) next to the ones you accept and try again to re-search for specific primers [Submit](#)

**Primer pair 1**

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGATCGGTTTCGCCTCAAC	Plus	20	69	88	60.39	55.00	4.00	3.00
Reverse primer	ACCTCGACGGTTATACCCCA	Minus	20	824	805	60.03	55.00	6.00	2.00
Product length	756								

**Products on intended targets**  
 >[MN720647.1](#) Aquilaria sinensis chloroplast, complete genome

product length = 756  
 Forward primer 1 AGGATCGGTTTCGCCTCAAC 20  
 Template 25085 ..... 25066  
 Reverse primer 1 ACCTCGACGGTTATACCCCA 20  
 Template 24330 ..... 24349

**Products on potentially unintended templates**  
 >[NC\\_052859.1](#) Aquilaria subintegra chloroplast, complete genome

product length = 761  
 Forward primer 1 AGGATCGGTTTCGCCTCAAC 20  
 Template 25070 ..... 25051  
 Reverse primer 1 ACCTCGACGGTTATACCCCA 20  
 Template 24310 ..... 24329

# TAHAP DESAIN PRIMER: PRIMER3PLUS

- Buka website Primer3Plus
- Copy-paste salah satu sekuen gen COX1 ke kotak sekuen yang disediakan.

**Primer3Plus** [Primer3Manager](#) [Help](#)  
pick primers from a DNA sequence [About](#) [Source Code](#)

**Task:** Detection   Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.

**Main** **General Settings** **Advanced Settings** **Internal Oligo** **Penalty Weights** **Sequence Quality**

**Sequence Id:** NC\_029243|A.sinensis\_rpo

[Paste source sequence below](#) Or upload sequence file:  No file chosen

```
TTGATCGGAATGAATGAATCAGAATTTTCTTCTATGATCGACCGATATAAACATCAACAACCTCCAAATAGGATCGGGTTTC
GCCTCAACAAATAAGTGCTTGGGCTAAGAAAATCCTACCCAATGGAGAGATAGTTGGAGAGGTGACAAAACCCCTATACTT
TTCATTACRAAAACCAATAAACCGGAAAAGATGGATTATTTTGTGARAAGATTTTGGGCTACAAAAGTGGAAATTTGC
GCTTGTGGAATATCGAATAATGGAAATGAAAAGAGGATCCTAAATTTTGTGAACAATCGCGAGTTGAATTTGTTGA
TTCTCGGATACGAAGATATCAATGGGATACATAAACTGGCATGCCAGTAACTCATGTGTGGTATTTGAAACGCTTTC
CTAGTTATATTGCGAATCTTTAGATAAACCTCTTAAAGAATTAGAGAGTCTAGTATACTGCGATGTGTGATTTGATCGA
AATTATGATTTAACAGATTAGGAATGAGAACTGTCATCCCATTCATTGAATGGGATGCCCGAGATCGACATGCTC
TTGGGAGGAGTAAGATGAAGCTCAGAATTATGGGTGTATTCAAGACCACCAATAAAAAGGGGAATTGGTCTATGGTCGAG
TTAGTAAAAAAGAAATAGGAATTTGAAAGTTGTACCTCGTGAATAAAAGACTTCTTCTTTTCCCATTCCCATTCTT
TTAGAAAAGAAATGATGTTCAAGTAAGCAAATATGTCATGGTTACAGGAGTCTATACATCGCATATAGGCTTTAAGGAAG
GCATTTGGGGTATAACCGTCGAGGTAAGGCGGACCTAATAGATCGAGCGGAACAGTACATAGACAAGTAAATCCCTTATG
AATGAATCCAAGACATTCCTTTAATTTAATATAAAAAGGGATTCATGATTTGAAGGAAAGTAGACTACTCAAGAATTT
```

Mark selected region:

**Excluded Regions:** <  >  
**Targets:** [  ]  
**Included Region:** {  }

<input checked="" type="checkbox"/> Pick left primer or use left primer below. <input type="text"/>	<input type="checkbox"/> Pick hybridization probe (internal oligo) or use oligo below. <input type="text"/>	<input checked="" type="checkbox"/> Pick right primer or use right primer below (5'->3' on opposite strand). <input type="text"/>
---	---	---

# TAHAP DESAIN PRIMER: PRIMER3PLUS

- Pada primer3plus, daerah target dapat ditandai dengan mengapit sekuen dengan tanda “[ ]”

- < > : excluded region
- [ ] : target region
- { } : included region

- *Note: gunakan shortcut Ctrl+F untuk mencari sekuen target anda*

**Primer3Plus**  
pick primers from a DNA sequence

[Primer3Manager](#) [Help](#)  
[About](#) [Source Code](#)

**Task:** Detection

Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.

**Main** | General Settings | Advanced Settings | Internal Oligo | Penalty Weights | Sequence Quality

Sequence Id:

Paste source sequence below  No file chosen

```
>NC_035159|Aedes aegypti cox1
TCGCGACAATGGTTATTTTCAACAAATCATAAAGATATTGGAACTTTATATTTTCATTTTTGGAGTATGAT
CTGGAATAGTCGGAACCTCTCTAAGAATTTTAATTCGTGCTGAACCTAGCCACCCTGGTATATTTATTGG
GAATGACCAAATTTATAAT [GTAATTGTACAGCTCATGCATTTATTATAATTTTCTTTATAGTAATGCCA
ATTATAATTGGAGGATTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCTGATATAGCTTTCCTC
GAATGAATAATATAAGTTTTTGAATACTACCTCCTCATTGACTTCTATTATCAAGCTCAATAGTAGA
AAATGGGGCAGGAACCTGGGTGAACAGTTTATCCTCCTCTCTCTCAGGAACAGCTCATGCTGGAGCTTCT
GTTGATTAGCTATTTTTCTTTCATTTAGCTGGAATTTCTCAATTTTAGGGCAGTAAATTTATTA
CAACTGTGATTAATATGTGATCGTCAGGGATTACTTTAGATCGACTACCCTTATTGTTGATCTGTAGT
TATTACAGCTATCTTACTTCTTCTCTCCTGTTTTAGCTGGAGCTATTACTATATTTAACAGAC
CGAACTTAAATACATCTTTCTTTGATCCAATCGGAGGGGAGCCCTATTTTATACCAAC}CTTATTTT
GATTCTTTGGACACCCAGAAGTTTATATTTAATTTTACCCTGGATTTGGAATAATTTCTCATATTATTAC
```

Mark selected region:

**Excluded Regions:**  >

**Targets:** [  ]

**Included Region:** {  }

Pick left primer or use left primer below.

Pick hybridization probe (internal oligo) or use oligo below.

Pick right primer or use right primer below (5'->3' on opposite strand).



# TAHAP DESAIN PRIMER: PRIMER3PLUS

- Pilih general, tentukan:
  - ✓ Ukuran produk PCR
  - ✓ Panjang primer,
  - ✓ Tm,
  - ✓ GC% dan max. Tm Difference
- Klik [*pick primers*]

**Primer3Plus** [Primer3Manager](#) [Help](#)  
pick primers from a DNA sequence [About](#) [Source Code](#)

**Task:** Detection

Select primer pairs to detect the given template sequence. Optional included/excluded regions can be specified.

**Main** **General Settings** **Advanced Settings** **Internal Oligo** **Penalty Weights** **Sequence Quality**

**Product Size Ranges** 600-700

<b>Primer Size</b>	Min: <input type="text" value="18"/>	Opt: <input type="text" value="23"/>	Max: <input type="text" value="27"/>
<b>Primer Tm</b>	Min: <input type="text" value="57.0"/>	Opt: <input type="text" value="60.0"/>	Max: <input type="text" value="63.0"/>
<b>Primer GC%</b>	Min: <input type="text" value="20.0"/>	Opt: <input type="text"/>	Max: <input type="text" value="80.0"/>

**Max Tm Difference:**

**Fix the**  **prime end of the primer**

**Concentration of monovalent cations:**  **Annealing Oligo Concentration:**

**Concentration of divalent cations:**  **Concentration of dNTPs:**

**Mispriming/Repeat Library:**

**Load and Save**  
**Please select special settings here:**  (use Activate Settings button to load the selected settings)  
To upload or save a settings file from your local computer, choose here:  
 No file chosen

# TAHAP DESAIN PRIMER: PRIMER3PLUS

**Primer3Plus**  
pick primers from a DNA sequence

[Primer3Manager](#) [Help](#)  
[About](#) [Source Code](#)

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Pair 1:

Left Primer 1: NC\_035159|Aedes aegypti cox1\_F  
Sequence: TTTAATTCGTGCTGAACCTAGCC  
Start: 99 Length: 23 bp Tm: 59.8 °C GC: 39.1 % ANY: 5.0 SELF: 3.0

Right Primer 1: NC\_035159|Aedes aegypti cox1\_R  
Sequence: AGAAATTATCCAAATCCGGGTA  
Start: 759 Length: 23 bp Tm: 59.9 °C GC: 34.8 % ANY: 5.0 SELF: 2.0

Product Size: 661 bp Pair Any: 5.0 Pair End: 3.0

[Send to Primer3Manager](#) [Reset Form](#)

1	TCGCGACAAT	GGTTATTTTC	AACAAATCAT	AAAGATATG	GAACCTTATA
51	TTTCATTTT	GGAGTATGAT	CTGGAATAGT	CGGAACCTCT	CTAAGAATTT
101	TAATTCGTGC	TGAACCTAGC	CACCCCTGGTA	TATTTATTGG	GAATGACCAA
151	ATTTATAATG	TAATTGTAAC	AGCTCATGCA	TTTATTATAA	TTTTCTTTAT
201	AGTAATGCCA	ATTATAATTG	GAGGATTTGG	AAATTGATTA	GTTCCCTTAA
251	TATTAGGAGC	CCCTGATATA	GCTTTCCCTC	GAATGAATAA	TATAAGTTTT
301	TGAATACTAC	CTCCTTCAT	GACTCCTCTA	TTATCAAGCT	CAATAGTAGA
351	AAATGGGGCA	GGAACCTGGT	GAACAGTTTA	TCCTCCTCTC	TCCTCAGGAA
401	CAGCTCATGC	TGGAGCTTCT	GTTGATTTAG	CTATTTTTTC	TCTTCATTTA
451	GCTGGAATTT	CCTCAATTTT	AGGGGCGAGTA	AATTTTATTA	CAACTGTGAT
501	TAATATGTGA	TCGTACAGGA	TACTTTAGA	TCGACTACCC	TTATTTGTTT
551	GATCTGTAGT	TATTACAGCT	ATCTTATTAC	TTCTTTCTCT	TCCTGTTTTA
601	GCTGGAGCTA	TACTATATTT	ATTAACAGAC	CGAAACTTAA	ATACATCTTT
651	CCTTGATCCA	ATCGGAGGGG	GAGACCCTAT	TTTATACCAA	CACCTATTTT
701	GATTCTTTGG	ACACCCAGAA	GTTTATATTT	TAATTTTACC	CGGATTTGGA
751	ATAATTTCTC	ATATTATTAC	TCAAGAAAGC	GGAAAAAGG	AAACATTTGG
801	AACTTTAGGA	ATAAATTTATG	CTATATTAAC	AATTGGATTA	TTGGGATTTA
851	TTGTTTGAGC	TCATCATATA	TTTACAGTAG	GTATAGACGT	AGATACTCGA
901	GCTTATTTTA	CTTCAGCAAC	TATAATTATT	GCTGTTCCCTA	CAGGAATTTAA
951	AATTTTTAGT	TGATTAGCAA	CTTTACACGG	AACTCAATTA	ACATATAGTC
1001	CAGCCCTTCT	ATGATCATT	GGATTTGTAT	TTTTATTTAC	AGTTGGAGGT
1051	TTAACAGGAG	TAGTATTAGC	TAATTCCTCA	ATTGATATTG	TTCTTCATGA
1101	TACTTATTAC	GTAGTTGCC	ATTTTCATTA	CGTTTTATCT	ATAGGAGGTG
1151	TATTTGCTAT	TATAGCAGGA	TTTATTCATT	GATACCCTTT	ATTAACAGGA
1201	ATAGTTATAA	ACCCTTCATG	ATTAAGGCT	CAATTTAGTA	TAATATTTAT

Primer3 akan memberikan beberapa kandidat primer (biasanya 5 pasang primer). Seleksi lebih lanjut dilakukan dengan mencari primer yang memenuhi kriteria dan **target-specific**

Pair 2:

Left Primer 2: NC\_035159|Aedes aegypti cox1\_1\_F  
Sequence: TTTAATTCGTGCTGAACCTAGCC  
Start: 99 Length: 23 bp Tm: 59.8 °C GC: 39.1 % ANY: 5.0 SELF: 3.0

Right Primer 2: NC\_035159|Aedes aegypti cox1\_1\_R  
Sequence: AATTATCCAAATCCGGGTA  
Start: 756 Length: 23 bp Tm: 59.8 °C GC: 30.4 % ANY: 5.0 SELF: 0.0

Product Size: 658 bp Pair Any: 5.0 Pair End: 2.0

[Send to Primer3Manager](#) [Reset Form](#)

Pair 3:

Left Primer 3: NC\_035159|Aedes aegypti cox1\_2\_F  
Sequence: TTTAATTCGTGCTGAACCTAGCC  
Start: 99 Length: 23 bp Tm: 59.8 °C GC: 39.1 % ANY: 5.0 SELF: 3.0

Right Primer 3: NC\_035159|Aedes aegypti cox1\_2\_R  
Sequence: AAATTTCCAAATCCGGGTA  
Start: 757 Length: 23 bp Tm: 59.8 °C GC: 30.4 % ANY: 5.0 SELF: 0.0

Product Size: 659 bp Pair Any: 5.0 Pair End: 3.0

[Send to Primer3Manager](#) [Reset Form](#)

Pair 4:

Left Primer 4: NC\_035159|Aedes aegypti cox1\_3\_F  
Sequence: AGCCACCCTGGTATATTATTGG  
Start: 118 Length: 23 bp Tm: 60.3 °C GC: 43.5 % ANY: 4.0 SELF: 0.0

Right Primer 4: NC\_035159|Aedes aegypti cox1\_3\_R  
Sequence: AGAAATTATCCAAATCCGGGTA  
Start: 759 Length: 23 bp Tm: 59.9 °C GC: 34.8 % ANY: 5.0 SELF: 2.0

Product Size: 642 bp Pair Any: 6.0 Pair End: 0.0

[Send to Primer3Manager](#) [Reset Form](#)

# SESUAIKAN PRIMER SETTINGS HINGGA DIPEROLEH PASANGAN PRIMER YANG MEMENUHI KRITERIA

## Primer3Plus

pick primers from a DNA sequence

[Primer3Manager](#) [Help](#)  
[About](#) [Source Code](#)

**Task:**  *Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.*

**Main** | **General Settings** | **Advanced Settings** | **Internal Oligo** | **Penalty Weights** | **Sequence Quality**

[Product Size Ranges](#)

**Primer Size** Min:  Opt:  Max:   
**Primer Tm** Min:  Opt:  Max:  [Max Tm Difference:](#)   
**Primer GC%** Min:  Opt:  Max:  [Fix the](#)  [prime end of the primer](#)

[Concentration of monovalent cations:](#)  [Annealing Oligo Concentration:](#)   
[Concentration of divalent cations:](#)  [Concentration of dNTPs:](#)

[Mispriming/Repeat Library:](#)



**Load and Save**  
[Please select special settings here:](#)  (use Activate Settings button to load the selected settings)  
To upload or save a settings file from your local computer, choose here:  
 No file chosen




## Primer3Plus

pick primers from a DNA sequence

[Primer3Manager](#) [Help](#)  
[About](#) [Source Code](#)

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Pair 1:  
 **Left Primer 1:**    
Sequence:    
Start: 118 Length: 23 bp Tm: 60.3 °C GC: 43.5 % ANY: 4.0 SELF: 0.0

**Right Primer 1:**    
Sequence:    
Start: 902 Length: 27 bp Tm: 57.2 °C GC: 44.4 % ANY: 8.0 SELF: 3.0  
Product Size:   Pair Any: 6.0 Pair End: 1.0

```
1 TCGCGACAAT GGTATTTTC AACAAATCAT AAAGATATTG GAACCTTTATA
51 TTTCATTTTT GGAGTATGAT CTGGAATAGT CGGAACTTCT CTAAGAATTT
101 TAATTCGTGC TGAACCTAGC CACCCTGGTA TATTTATTGG GAATGACCAA
151 ATTTATAATG TAATTGTAAC AGCTCATGCA TTTATTATAA TTTTCTTTAT
201 AGTAATGCCA ATTATAATTG GAGGATTGG AAATGATTA GTTCCTTTAA
251 TATTAGGAGC CCCTGATATA GCTTCCCTC GAATGAATA TATAAGTTTT
301 TGAATACTAC CTCCTTCATT GACTCTTCTA TTATCAAGCT CAATAGTAGA
351 AAATGGGCA GGAACCTGGT GAACAGTTA TCCTCCTCTC TCCTCAGGAR
401 CAGCTCATGC TGGAGCTTCT GTTGAATAG CTATTTTTTC TCCTCATTTA
451 GCTGGAATTT CCTCAATTT AGGGGCGATA AATTTTATA CAACCTGTAT
501 TAATATGTGA TCGTCAGGGA TTACTTTAGA TCGACTACC TTATTTGTTT
551 GATCTGTAGT TATTACAGCT ATCTTATTAC TTCTTTCTCT TCCTGTTTTA
601 GCTGGAGCTA TTAATATATT ATTAACAGAC CGAACTTAA ATACATCTTT
651 CTTTGATCCA ATCGGAGGGG GAGACCCTAT TTTATACCAA CACTTATTTT
701 GATTCCTTGG ACACCCAGAA GTTTATATTT TAATTTTACC CGGATTTGGA
751 ATAATTTCTC ATATTATTAC TCAAGAAAGC GGAAAAAAGG AAACATTTGG
801 AACCTTAGGA ATAATTTATG CTATATTAAC AATTTGATTA TTGGGATTTA
851 TTGTTTGGC TCATCATATA TTTACAGTAG GTATAGACGT AGATAGCTCGA
901 GCTTATTTTA CTTACGCAAC TATAATTATT GCTGTTCTTA CAGGAATTTA
951 AATTTTATG TGATTAGCAA CTTTACACGG AACTCAATTA ACATATAGTC
1001 CAGCCCTTCT ATGATCATTG GGATTTGTAT TTTTATTTAC AGTTGGAGGT
1051 TTAACAGGAG TAGTATTAGC TAATCTTCA ATTGATATTG TTCTTCATGA
1101 TACTTATTAC GTAGTTGCC ATTTTCATTA CGTTTTATCT ATPAGGAGCTG
1151 TATTTGCTAT TATAGCAGGA TTTATTCATT GATACCCTTT ATTAACAGGA
```

# ANALISIS KUALITAS PRIMER MENGGUNAKAN OLYGOANALYZER

1. Akses website  
(<https://sg.idtdna.com/>).

Pada bagian paling bawah website  
klik **OligoAnalyzer Tool**

The screenshot displays the IDT (Integrated DNA Technologies) website homepage. At the top, there is a navigation bar with the IDT logo, a search bar, and links for 'GET HELP', 'EN', and 'SIGN IN'. Below the navigation bar, a main banner features the text 'Change is coming' and a 'READ THE FAQS' button. The page is organized into several product and service categories, each with a brief description and a list of sub-services:

- COVID-19 solutions:** SARS-CoV-2 probes and other COVID-19 research reagents.
- DNA & RNA:** Save time and resources with industry-leading oligos manufactured to your specifications.
- CRISPR genome editing:** Achieve higher efficiency genome editing and avoid toxicity or innate immune responses with...
- Functional genomics:** Achieve increased potency and better specificity in loss-of-function studies using short oligos. Sub-services include RNA interference, Antisense oligos, and miRNA inhibitors.
- Reagents & kits:** High-quality genomics reagents to complete experimental workflows. Sub-services include Mutation detection, Nuclease detection & control, and Buffers & solutions.
- GMP, OEM & integrations:** Customized products and services to meet rigorous quality requirements for clinical and molecular diagnostic applications. Sub-services include GMP services, GMP products, OEM services, and Integrations.
- Optional services:** Customized services to enhance the purity, QC, formulation, or other specifications of select standard product offerings. Sub-services include PAGE & HPLC purification, Formulation & packaging, and Custom quality control.

At the bottom of the page, there is a large orange arrow pointing down to a row of tool icons: OligoAnalyzer™ Tool, PrimerQuest™ Tool, More tools, and How to order.



# ANALISIS KUALITAS PRIMER MENGUNAKAN OLYGOANALYZER

2. Lakukanlah *sign in* jika sudah memiliki akun, namun jika belum lakukan *register* terlebih dahulu kemudian *sign in*
3. Pilihlah salah satu primer forward dari kandidat primer hasil pick primer di Primer3, kemudian *paste* sekuen primer tersebut di OlygoAnalyzer. Lalu pilih *analyze*. Lakukan hal yang sama untuk mengetahui kualitas primer reverse

The screenshot displays the IDT Olygo Analyzer web interface. The main content area is titled "OlygoAnalyzer" and features a "Sequence" input field containing the text "CACTAGCACAAATGGATGCCC". A red arrow points to this field, accompanied by a text box stating "Masukkan urutan sekuen primer yang akan dianalisis". To the right of the sequence input is a "Parameter sets" section with various dropdown menus and input fields for "Target type" (set to DNA), "Oligo Conc" (0.25 µM), "Na<sup>+</sup> Conc" (50 mM), "Mg<sup>++</sup> Conc" (0 mM), and "dNTPs Conc" (0 mM). On the right side of the interface, a vertical menu of analysis options is visible, with the "ANALYZE" button highlighted in a red oval. Other options include "HAIRPIN", "SELF-DIMER", "HETERO-DIMER", "NCBI BLAST", and "TM MISMATCH". At the bottom of this menu is an "ADD TO ORDER" button. The top of the page shows the IDT logo and navigation menus.



# ANALISIS KUALITAS PRIMER MENGUNAKAN OLYGOANALYZER

4. Setelah pilih *analyze*, OlygoAnalyzer akan menampilkan rincian dari primer yang dianalisis, seperti: panjang sekuen primer, suhu *melting*, suhu *annealing* dan sebagainya. Untuk mengetahui struktur sekunder internal primer, klik bagian self-dimer dan hairpin

The screenshot displays the OlygoAnalyzer interface with the following elements:

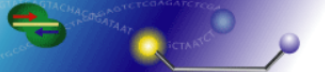
- Analysis Results:** Five panels showing secondary structure predictions for a primer sequence. Each panel includes the  $\Delta G$  value, the number of base pairs, and the sequence alignment with vertical bars indicating base pairing.
- Navigation Menu:** A vertical list of buttons on the right side of the interface, including "ANALYZE", "HAIRPIN", "SELF-DIMER", "HETERO-DIMER", "NCBI BLAST", "TM MISMATCH", and "ADD TO ORDER".

**Analysis Results Summary:**

Delta G (kcal/mole)	Base Pairs
-5.09	3
-4.16	4
-3.14	2
-3.14	2
-3.07	2

# ANALISIS KUALITAS PRIMER MENGGUNAKAN BEACON DESIGNER

- Buka website Beacon Designer. Isikan identitas primer.
- Pada drop-down [Type], pilih “Primer pair”. Salin urutan primer forward dan reverse pada kotak tersedia. Klik [Analyze]

**Beacon Designer Free Edition**  **PREMIER Biosoft International**

[Beacon Designer Full Version](#)

**Oligo Analysis** | **Sequence Analysis**

**Assay Type:**

TaqMan®  SYBR®Green

Name:  [ Optional]

Description:  [ Optional]

Type:  ▼

Sense Primer: 5'  3'  bp

Anti-sense Primer: 5'  3'  bp

Probe: 5'  3'  bp

**Reaction Conditions:**

Nucleic Acid Concentration:  nM

Mono Ion Concentration:  mM

Free Mg<sup>++</sup> Concentration:  mM

Total Na<sup>+</sup> Concentration:  mM

# ANALISIS KUALITAS PRIMER MENGUNAKAN BEACON DESIGNER

Name: NC\_029243|A.sinensis\_rpoC1 November 10, 2021

Description: Assay Type: TaqMan®

Reaction Conditions:

Nucleic Acid Concentration (nM)	0.25	Monovalent Concentration (mM)	50
Free Mg <sup>++</sup> Concentration (mM)	5	Total Na <sup>+</sup> Concentration (mM)	332.84

Sense Primer: GTGTGGTATTTGAAACGTCTTCCT

Length (bp)	T <sub>m</sub> (°C)	GC%	GC Clamp	Cross Dimer (ΔG)	Self Dimer (ΔG)	Hairpin (ΔG)
24	58.43	41.67	2	-2.0	<u>-3.3</u>	-0.6

Anti-sense Primer: CTCTCATCCGGCTCAAGTAGTTA

Length (bp)	T <sub>m</sub> (°C)	GC%	GC Clamp	Cross Dimer (ΔG)	Self Dimer (ΔG)	Hairpin (ΔG)
23	58.1	47.83	2	-2.0	<u>-4.3</u>	0.0

As a rule of thumb:

- Never accept primers where the 3' end has 3 bp matches, as these will tend to form primer dimers preferentially over hybridizing with the sequence.
- If self-dimers or cross dimers cannot be avoided, chose primers with the highest  $-\Delta G$  (meaning the least negative number—the one closest to zero). Discard primers with  $\Delta G$ s more negative than  $-3.5$  kcal/mol.

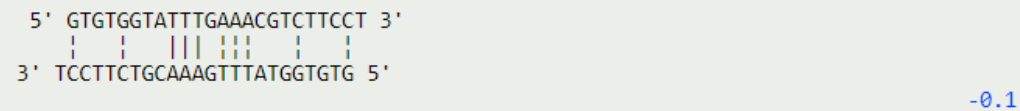
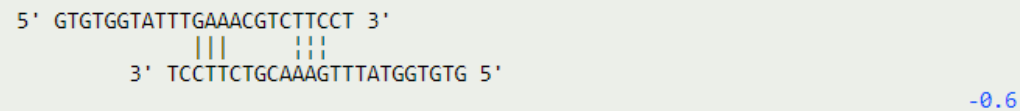
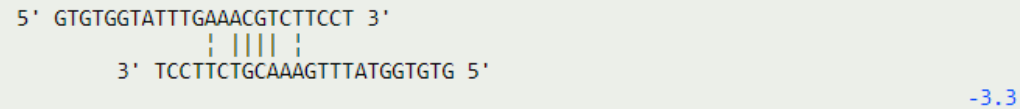
Delta G Base Pairs	-6.14 kcal/mole 3	Delta G Base Pairs	-0.96 kcal/mole 2
<pre> 5' GGGCTAACTTCAATGTCATCCC        : : : : : : : : : 3' CCCTACTGTAACCTCAATCGGG                     </pre>		<pre> 5' GGGCTAACTTCAATGTCATCCC    :    : 3' CCCTACTGTAACCTCAATCGGG                     </pre>	
Avoid 3' bp matches >2bp		Example of acceptable self-dimer	

Reference: Thornton and Basu, 2011 (<https://doi.org/10.1002/bmb.20461>)

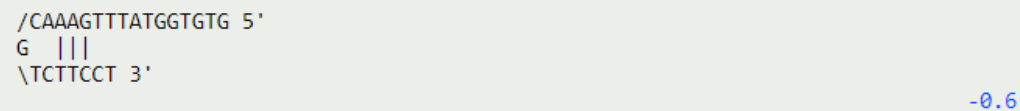
# ANALISIS KUALITAS PRIMER MENGGUNAKAN BEACON DESIGNER SECONDARY STRUCTURE OF PRIMERS

## Secondary Structures for Sense Primer

### Dimer:-

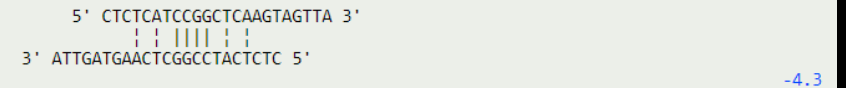


### Hairpin:-



## Secondary Structures for Anti-sense Primer

### Dimer:-

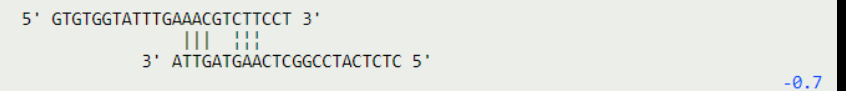
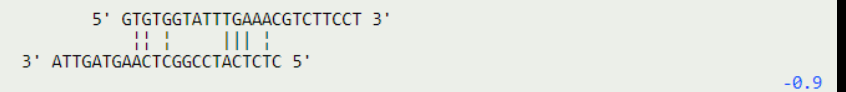
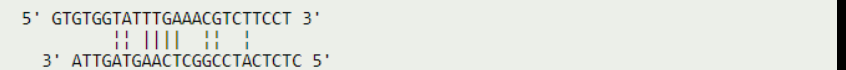


### Hairpin:-

Not Found

### Cross Dimer

### Cross Dimer between Sense Primer and Anti-sense Primer:-



# UJI SPESIFISITAS PRIMER (IN SILICO) MENGGUNAKAN BLAST PRIMER NCBI

## 1. Akses Website NCBI, pilih Blast, Kemudian Pilih Blast Primer

NCBI Resources How To

NCBI National Center for Biotechnology Information

All Databases

**COVID-19 Information**  
[Public health information \(CDC\)](#) | [Research information \(NIH\)](#) | [SARS-CoV-2 data \(NCBI\)](#) | [Prevention and treatment inform.](#)

**UNITE**  
 A new NIH initiative to end structural racism and achieve racial equity in the biomedical re  
[LEARN MORE](#)

**NCBI Home**

**Resource List (A-Z)**

- All Resources
- Chemicals & Bioassays
- Data & Software
- DNA & RNA
- Domains & Structures
- Genes & Expression
- Genetics & Medicine
- Genomes & Maps
- Homology
- Literature

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BLAST® Home Recent Results Saved Strategies Help

**Basic Local Alignment Search Tool**

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

**NEWS**  
 A new feature was added to the NCBI IgBLAST webpage  
 IgBLAST is now able to determine Ig isotypes  
 Mon, 01 Nov 2021 12:00:00 EST [More BLAST news...](#)

**Web BLAST**

**Nucleotide BLAST**  
 nucleotide ► nucleotide

**blastx**  
 translated nucleotide ► protein

**tblastn**  
 protein ► translated nucleotide

**Protein BLAST**  
 protein ► protein



# UJI SPESIFISITAS PRIMER (IN SILICO) MENGGUNAKAN BLAST PRIMER NCBI

2. Copy dan paste primer forward dan primer reverse yang didapat dari hasil [pick primer] pada Primer3 ke Primer-BLAST
3. Gunakan *default* setting pada menu “Primer parameter” dan “Exon/intron selection”.
4. Pada bagian “Primer pair specificity checking parameters”, pilih ‘nr’ pada menu ‘Database’ dan kosongkan menu ‘Organism’.

Klik [Get primer]

U.S. National Library of Medicine  
National Center for Biotechnology Information

Primer-BLAST  
A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

Primer Parameters

Use my own forward primer (5'->3' on plus strand): GTGTGGTATTTGAAACGCTTCCT

Use my own reverse primer (5'->3' on minus strand): CTCTCATCCGGCTCAAGTAGTTA

PCR product size: Min 70, Max 1000

# of primers to return: 10

Primer melting temperatures (T<sub>m</sub>): Min 57.0, Opt 60.0, Max 63.0, Max T<sub>m</sub> difference 3

Primer Pair Specificity Checking Parameters

Search mode: Automatic

Database: nr

Exclusion:  Exclude predicted Refseq transcripts (accession with XM, XR prefix)  Exclude uncultured/environmental sample sequences

Organism:  Add organism

Primer specificity stringency: Primer must have at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps at the 3' end. Ignore targets that have 6 or more mismatches to the primer.

Max target length: 4000

Allow primer to amplify mRNA splice variants:  Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)

Get Primers

Show results in a new window Use new graphic view

# UJI SPESIFISITAS PRIMER (IN SILICO) MENGGUNAKAN BLAST PRIMER NCBI

4. Perhatikan hasil blast primer, pastikan spesies yang terdapat pada hasil blast merupakan spesies yang sama atau satu genus dengan spesies yang digunakan pada saat desain primer. Apabila sudah sama, maka pasangan primer tersebut dapat digunakan untuk amplifikasi sekuen DNA target dengan metode PCR

Primer-BLAST Results ?

Input PCR template none

Specificity of primers Target templates were found in selected database: Nucleotide collection (nt)

Other reports > Search Summary

### — Detailed primer reports

**Primer pair 1**

	Sequence	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTGTGGTATTTGAAACGCTTCCT	24	59.49	41.67	6.00	2.00
Reverse primer	CTTCATCCGGCTCAAGTAGTA	23	59.37	47.83	4.00	2.00

**Products on target templates**

>NC\_059004.1 *Lanea coromandelica* chloroplast, complete genome

product length = 809

Forward primer 1 GTGTGGTATTTGAAACGCTTCCT 24

Template 25713 ..... 25690

Reverse primer 1 CTTCATCCGGCTCAAGTAGTA 23

Template 24905 ..... 24927

>NC\_059000.1 *Spondias dulcis* chloroplast, complete genome

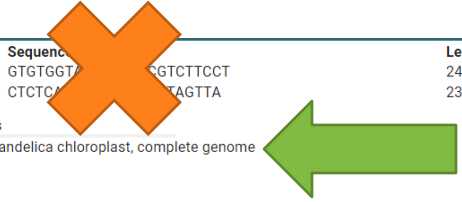
product length = 808

Forward primer 1 GTGTGGTATTTGAAACGCTTCCT 24

Template 25288 ..... 25265

Reverse primer 1 CTTCATCCGGCTCAAGTAGTA 23

Template 24481 ..... 24503



# HASIL PRIMER-BLAST APABILA MENU 'ORGANISM' DIISI DENGAN SPESIES TARGET

Primer-BLAST Results ?

Input PCR template none

Specificity of primers Target templates were found in selected database: Nucleotide collection (nt) (Organism limited to Aquilaria)

Other reports [▶ Search Summary](#)

## — Detailed primer reports

### Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTGTGGTATTTGAAACGTCTTCCT	24	59.49	41.67	6.00	2.00
Reverse primer	CTCTCATCCGGCTCAAGTAGTTA	23	59.37	47.83	4.00	2.00

#### Products on target templates

>[NC\\_052859.1](#) Aquilaria subintegra chloroplast, complete genome

product length = 803

Forward primer 1 GTGTGGTATTTGAAACGTCTTCCT 24  
Template 24760 ..... 24737

Reverse primer 1 CTCTCATCCGGCTCAAGTAGTTA 23  
Template 23958 ..... 23980

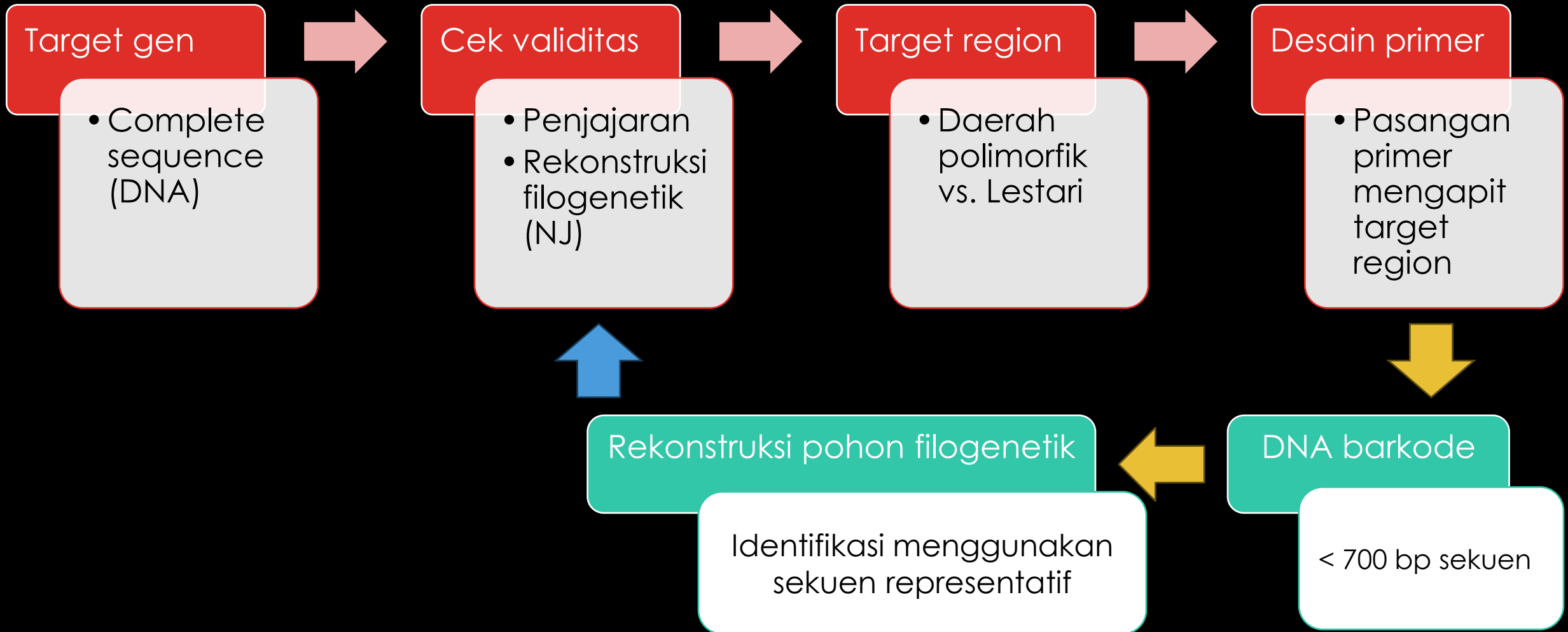
>[MN147870.1](#) Aquilaria sinensis chloroplast, complete genome

product length = 798

Forward primer 1 GTGTGGTATTTGAAACGTCTTCCT 24  
Template 24774 ..... 24751

Reverse primer 1 CTCTCATCCGGCTCAAGTAGTTA 23  
Template 23977 ..... 23999

# SUMMARY



TERIMA KASIH