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# PENGANTAR BIOINFORMATIKA

## IBT 431

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Pertemuan 6

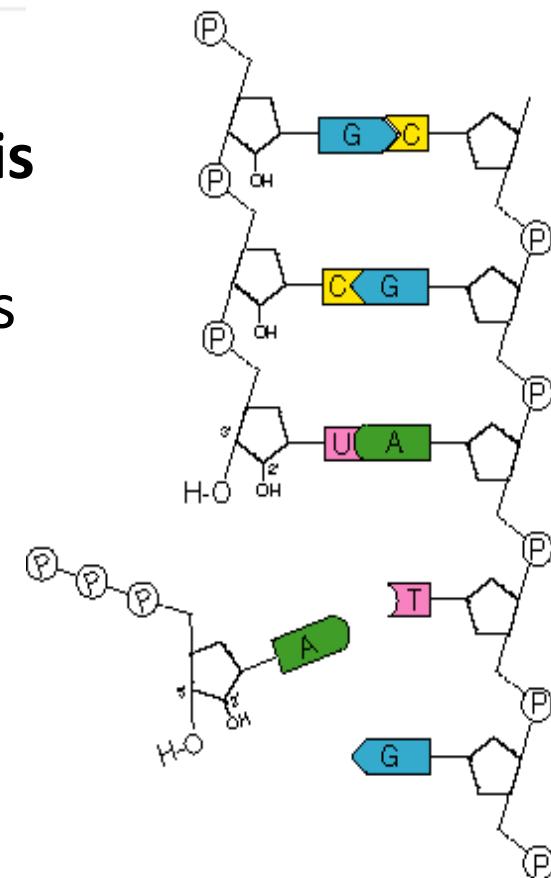
# DESAIN PRIMER

# Sasaran Perkuliahan

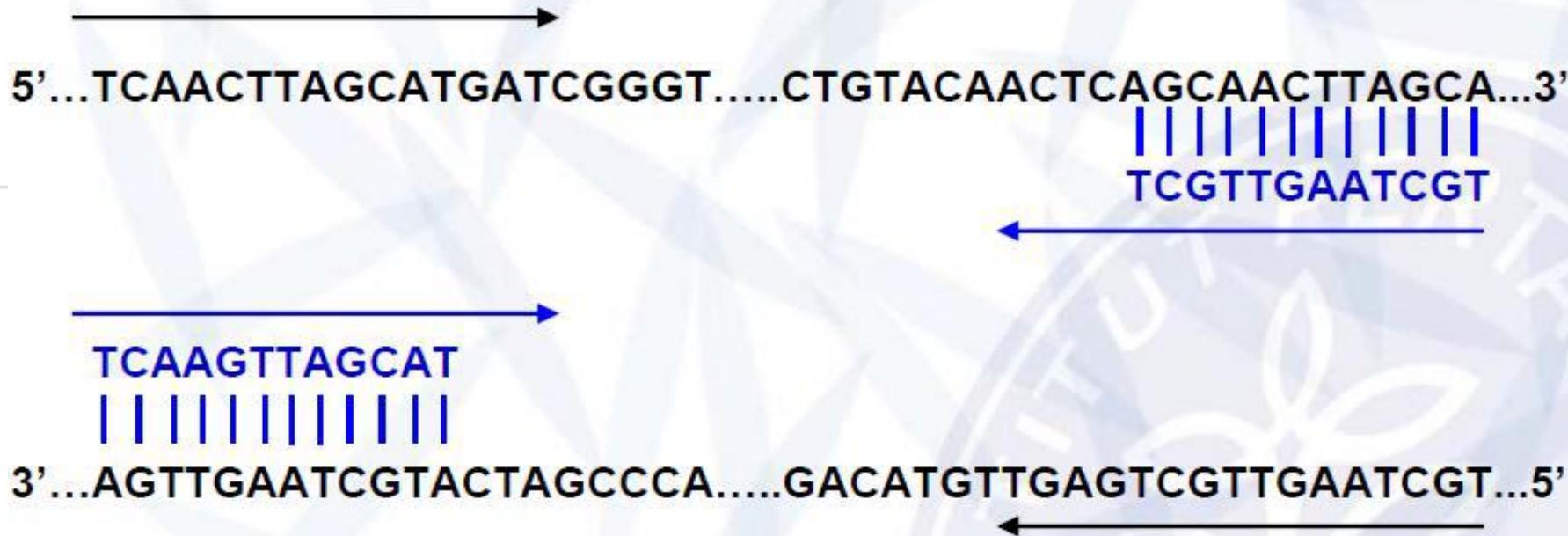
- Mahasiswa mampu menjelaskan tentang apa itu primer
- Mahasiswa Mampu menganalisis karakter primer yang baik
- Mahasiswa mampu mendesain urutan DNA primer sesuai dengan urutan gen yang diinginkan
- Mahasiswa mampu menghitung nilai Tm dan Ta primer

# What Is a Primer

- A short synthetic oligonucleotide
- Serves as a **starting point for DNA synthesis**
- Primer is required because the DNA polymerases can only add new nucleotides to the hydroxyl group at the 3'end of an existing strand of DNA.
- Used in many molecular techniques from PCR to DNA sequencing.
- The reverse complement of a region of template or target DNA to which we wish the primer to anneal.



# DNA REPLICATION & PRIMER



Common primer is an oligonucleotide, a short segment of single-stranded DNA, used to replicate DNA from a fragment.

# Good Primers Characteristics

- Unique
- Has an appropriate melting-(Tm) and annealing-temperature (Ta)
- Random base distribution, and average G+C content ; Avoid long A+T and G+C-rich region if possible
- Usually 15-30 nucleatides in length
- Absence of dimerizationcapability
- Absence of significant hairpin formation

## Uniqueness

- There shall be only one target site in the template DNA where the primer binds
- The longer → the more chance that it's unique
- The length  $\geq 15$  bases to ensure uniqueness

### Template DNA

5' .. TCAACCTTACGATGATCGGGTA .. GTAGCAGTTGACTGTACAACTCAGCAACTTAGCA .. 3'

TGCTAAGTTG

CAGTCAAATGCTAC

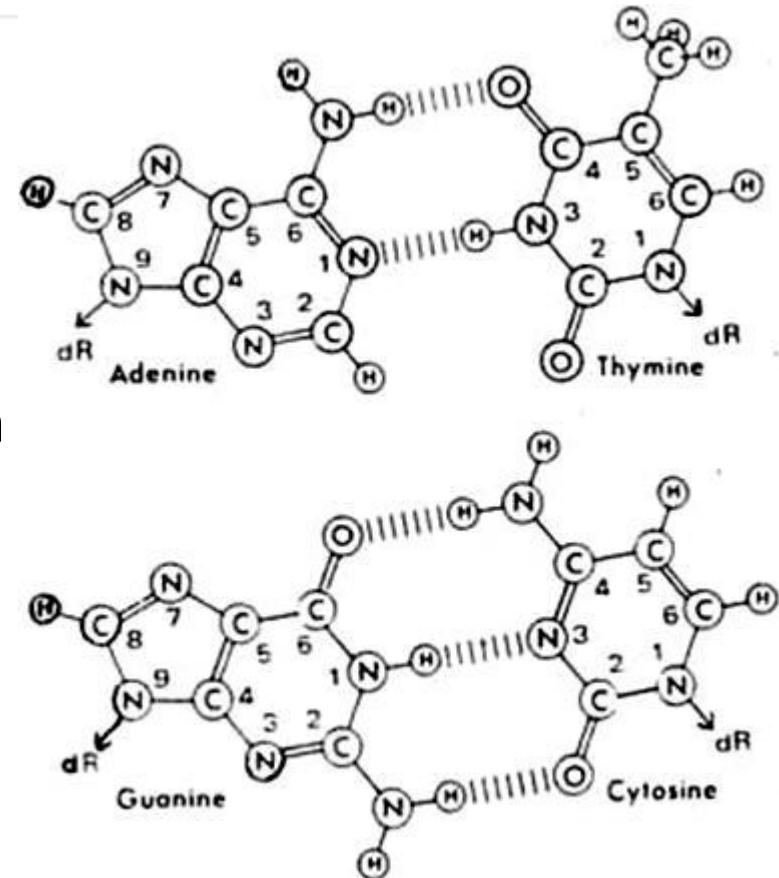
TGCTAAAGTTG

Primer candidate 1 5'-TGCTAAGTTG-3'      NOT UNIQUE!

Primer candidate 2 5'-CAGTCAAATGCTAC-3'      UNIQUE!

## Melting Temperature (T<sub>M</sub>)

- the temperature at which 50% of the DNA duplex dissociates to become single stranded
- Mostly determined by primer length and base composition
- Longer primer length and higher G/C content increase T<sub>m</sub>
- The optimal G/C content: 45-55%

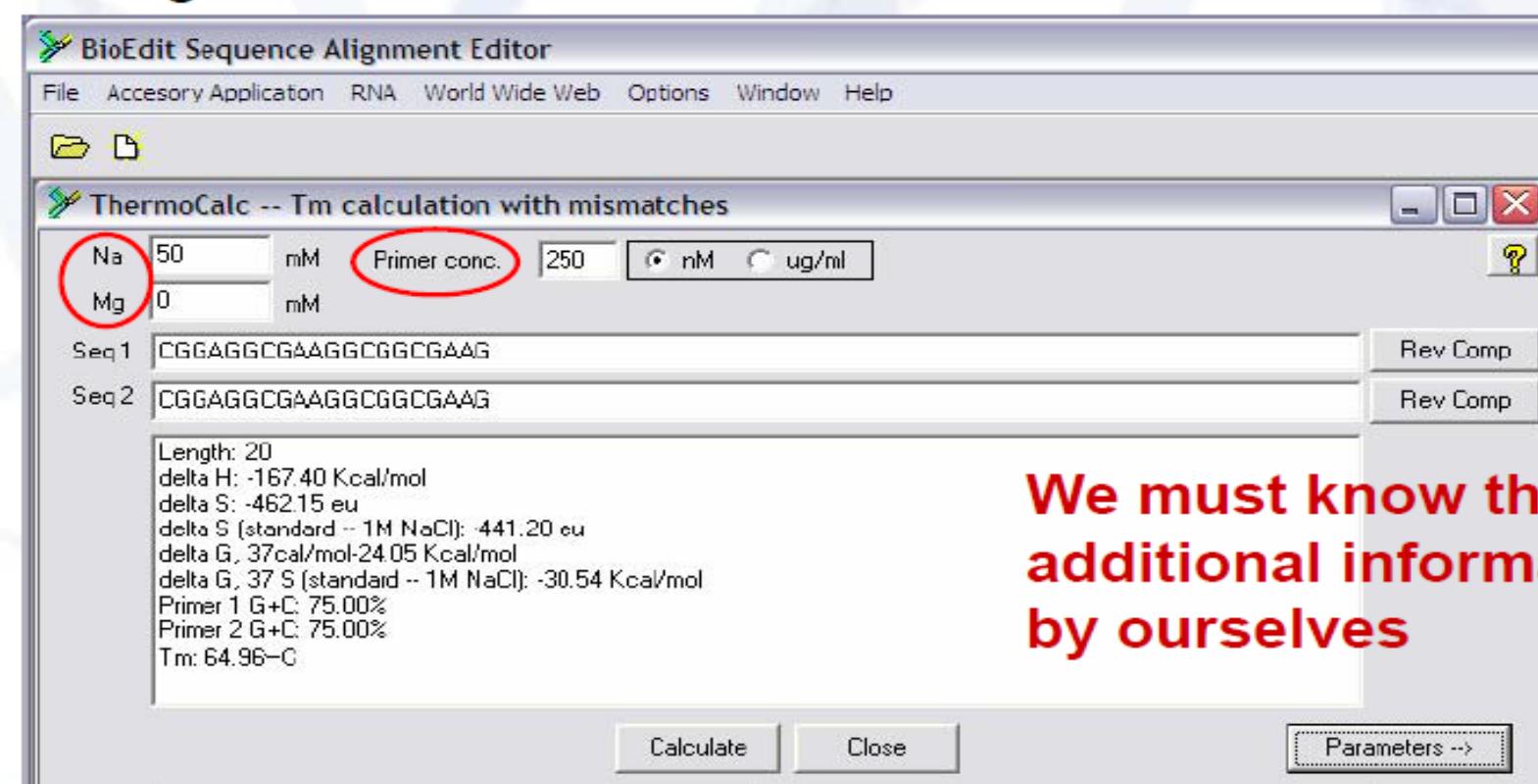


## Melting Temperature (Tm)

- Optimum Tm: 52–65 °C, but higher Tm is sometimes recommended for high GC content targets.
- If a pair of primer is used to amplify a fragment, the Tm difference between primer pair should be < 5 °C
- How to calculate Tm Using an equation:
- Shorter than :  $Tm = 2(A+T) + 4(G+C)$
- $Tm\ Gabungan = (Tm\ forward + Tm\ reverse)/2$   
Longer than :  $Tm = 64.9 + 41 * (G+C - 16.4) / (A+T+G+C)$

# Melting Temperature (Tm): how to calculate Tm

Using softwares:



We must know the additional information by ourselves

## 2. Untuk melihat GC content dan TM pada program online “ Oligo Calc”

The screenshot shows a web browser window with the title "OligoCalc: Oligonucleotide Properties Calculator". The URL in the address bar is [biotools.nubic.northwestern.edu/OligoCalc.html](http://biotools.nubic.northwestern.edu/OligoCalc.html). The main interface includes a sequence input field containing "TCA CGG CCA GCC TTG CTC CAC CTT", a reverse complement strand output field showing "AAG GTG GAG CAA GGC TGG CCG TGA", and various input fields for modifications, salt concentration, and absorbance. Below these are buttons for "Calculate", "Swap Strands", "BLAST", and "mfold". The interface is divided into sections for "Physical Constants" and "Melting Temperature (TM) Calculations", which include fields for length, molecular weight, GC content, and salt concentration. At the bottom, there are sections for "Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7." and "Deprecated Hairpin/self dimerization calculations". The browser toolbar at the bottom includes icons for Windows, Internet Explorer, File Explorer, Task View, Google Chrome, Edge, and Mozilla Firefox.

Mendapatkan 2 TIKET untuk ke ... X OligoCalc: Oligonucleotide Pro X + biotools.nubic.northwestern.edu/OligoCalc.html Cari Cari Sering Mampir Perkenalan Suggested Sites Web Slice Gallery General Manager Biot... Fwd: DNA Sequencing... Pesanan Saya Sejarah Ilmu Anatomi ... Nucleotide BLAST: Sea...

Oligo Calc: Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below  
OD calculations are for single-stranded DNA or RNA

Nucleotide base codes

TCA CGG CCA GCC TTG CTC CAC CTT

Reverse Complement Strand(5' to 3') is:

AAG GTG GAG CAA GGC TGG CCG TGA

5' modification (if any) 3' modification (if any) Select molecule  
nM Primer mM Salt (Na<sup>+</sup>) Measured Absorbance at 260 nanometers

Calculate Swap Strands BLAST mfold

Physical Constants Melting Temperature (TM) Calculations

Length: 24 Molecular Weight: 7200.74 GC content: 63 % 1 62.5 °C (Basic)  
1 ml of a sol'n with an Absorbance of at 260 nm 2 NaN °C (Salt Adjusted)  
is 0 microMolar 5 and contains micrograms. 3 NaN °C (Nearest Neighbor)

Thermodynamic Constants Conditions: 1 M NaCl at 25°C at pH 7.

RlnK NaN cal/(°K\*mol) deltaH 209.4 Kcal/mol  
deltaG 36.4 Kcal/mol deltaS 541.6 cal/(°K\*mol)

Deprecated Hairpin/self dimerization calculations

# Annealing Temperature (Ta)

- Ta is the temperature at which primers anneal to the template DNA.
- Ta can be calculated from Tm.
- General rule: Ta is 5°C lower than Tm ( $Ta = Tm - 5^{\circ}C$ )  
 $Suhu\ Annealing = Tm\ Gabungan - 5^{\circ}C$
- Higher Ta enhances specific amplification but may lower yields

# Primer Dimer

- Primer molecules that have attached (hybridized) to each other because of strings of complementary bases in the primers.
- Primer dimer could decrease the PCR efficiency dramatically.

## Self-Dimer

8 bp

3' GGGAAAATTCCAGGATCTAT 5'  
      |||||   |||||

5' TATCTAGGA**CCTTAA**AAGGG 3'

4 bp

3' GGGAAAATTCCAGGATCTAT 5'  
      |||||

5' TATCTAGGAC**CTTAA**AAGGG 3'

## Dimer

forward primer

5' TATCTAGG**ACCTTAAA**AGGG 3'

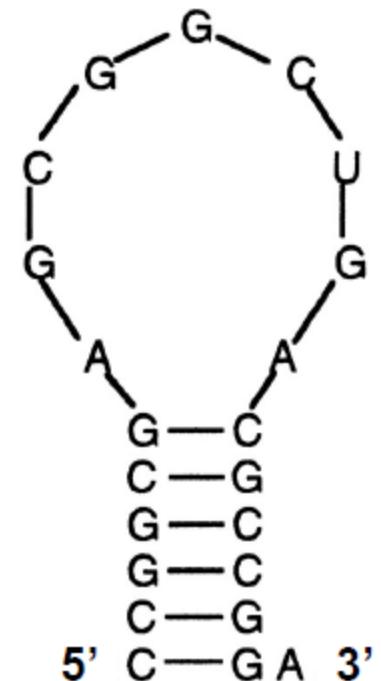
|||||

3' **CATGGAAACGTAGGAGAC** 5'

reverse primer

# Hairpin Formation

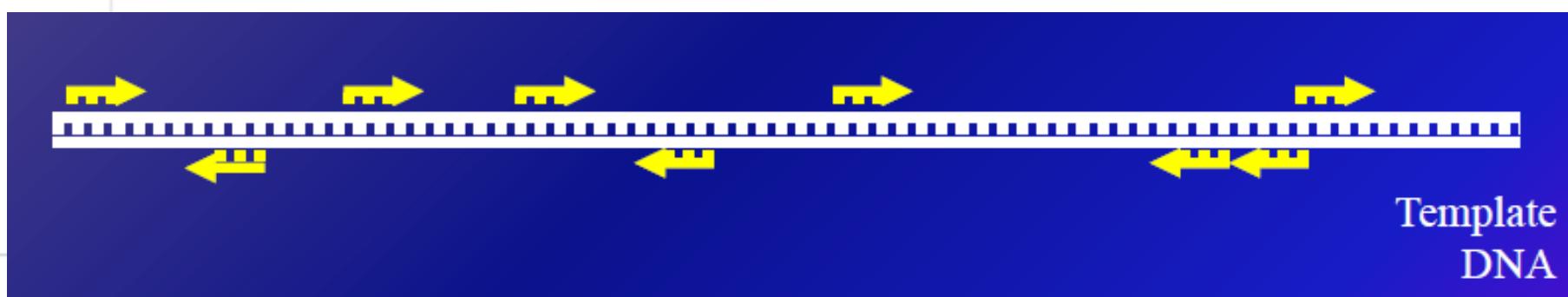
- Occurs when two regions of the same strand of the primer base-pair resulting lollipop-shaped structure
- Hairpin formation will lead unfunctional primer and could decrease the PCR efficiency dramatically.
- Sometimes primer dimer and hairpins are harmless when the annealing temperature does not allow them to take form. (i.e. some dimers or hairpins form at 30 °C while during PCR cycle, the lowest temperature only drops to 60 °C).



# Application of Primers

- Molecular Marker (RAPD, SSR, ISSR, etc.)
- Gene cloning *gene specific primer, degenerate primer, adapter primer*
- PCR-based gene expression studies *PCR, Real-time PCR*
- Constructing probes *Northern-, Southern-blot, Micro-array*
- Sequencing

# RAPD Primers



- Only 1 primer per reaction
- Uses 10 base primers for PCR
- Primer binds to many locations on the template DNA
- Only when primer binding sites are close and oriented in opposite directions so the primers point toward each other will amplification take place

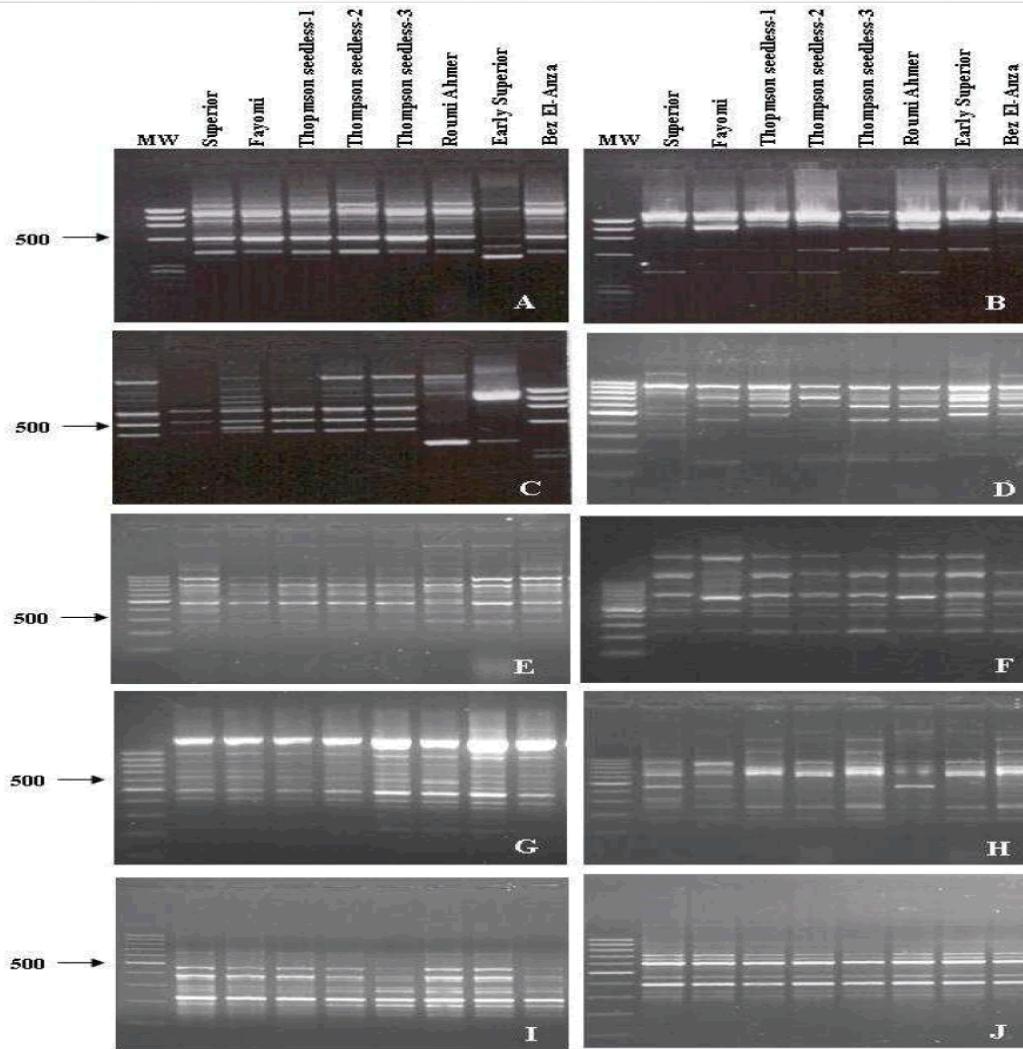


Figure 2: RAPD fingerprinting of 8 local Egyptian grape cultivars (*Vitis vinifera* L.). (A)Primer OP-A03; (B)Primer OP-A07; (C) Primer OP-A11; (D)Primer OP-A15; (E) Primer OP-B02; (F)Primer OP-B07; (G)Primer OP-B16 ; (H)Primer OP-B19; (I)Primer OP-G19 and (J)Primer OP-G20

# Primers Design in Gene Cloning

- Primers work in pairs –forward primer and reverse primer.
- location of the primer on the template DNA should consider the start-and stop-codon.



# Degenerate Primers

- Untuk identifikasi anggota baru / gen dari gen yang sama pada individu yang berbeda
- Berasal dari percampuran/ sequen yang sama
- Mengandung perbedaan urutan nekleetida pada lokasi tertentu
-

# Degenerate Primers

- Digunakan secara bersamaan dengan harapan salah satu urutan DNA akan melengkapai gen target
- Urutan asam amino dari protein yang dikenal
- Primer yang dibentuk untuk mendapatkan sekuen DNA yang belum di ketahui di ambil dari urutan asam amino protein yang dikenal

Asp Glu Gly Phe Leu Ser Tyr Cys Trp Leu Pro His Gln

GAT GAA AGG TTT CT TCT TATT GTT GGCT TCC TCATCAA

C	G	C	CT	CAGC	C	C	T	C	C	C	G
A		A	A				A	A			
G		G	G				G	G			

A total of 32 unique oligonucleotides  
would be generated

TATT GTT GGCT TCC

TACT GTT GGTT CCC

TATT GCT GGCT TCC

TACT GCT GGCT TCC

etc.

TAY TGY TGG YT NCC

# Standard MixBase Definitions

R	A, G
Y	C, T
M	A, C
K	G, T
S	C, G
W	A, T
H	A, C, T
B	C, G, T
V	A, C, G
D	A, G, T
N	A, C, G, T

Amino Acid	3-Letter Code	1-Letter Code
Alanine	Ala	A
Cysteine	Cys	C
Aspartic acid or aspartate	Asp	D
Glutamic acid or glutamate	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asn	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T
Valine	Val	V
Tryptophan	Trp	W
Tyrosine	Tyr	Y

## **Universal Genetic Code Chart**

### **Messenger RNA Codons and Amino Acids for Which They Code**

Second base					
	U	C	A	G	
U	UUU } PHE UUC } UUA } LEU UUG }	UCU } UCC } SER UCA } UCG }	UAU } TYR UAC } UAA } STOP UAG }	UGU } CYS UGC } UGA } STOP UGG } TRP	U C A G
	CUU } CUC } LEU CUA } CUG }	CCU } CCC } PRO CCA } CCG }	CAU } HIS CAC } CAA } GLN CAG }	CGU } CGC } CGA } ARG CGG }	U C A G
	AUU } ILE AUC }	ACU } ACC } ACA } THR ACG }	AAU } ASN AAC } AAA } LYS AAG }	AGU } SER AGC } AGA } ARG AGG }	U C A G
	AUA } MET or AUG } START				
G	GUU } VAL GUC }	GCU } GCC } ALA GCA } GCG }	GAU } ASP GAC } GAA } GLU GAG }	GGU } GGC } GGA } GLY GGG }	U C A G

# UNiversal Primers

Primers can be designed to amplify only one product.

Primers can also be designed to amplify multiple products (“universal primers”).

Strategy:

1. Align groups of sequences you want to amplify.
2. Find the most conservative regions at 5' end and at 3' end.
3. Design forward primer at the 5' conservative region.
4. Design reverse primer at the 3' conservative regions.
5. Matching forward and reverse primers to find the best pair.
6. Ensure uniqueness in all template sequences.
7. Ensure uniqueness in possible contaminant sources

# Computer – aided primer design

Some primer design programs we use:

-**Oligo:Life Science Software, standalone application**

-GCG: Accelrys, ICBR maintains the server.

-**Primer3:MIT, standalone / web application**

[http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi)

-BioTools:BioTools, Inc. ICBR distributes the license.

-**Others:GeneFisher, Primer!, Web Primer, NBI oligo program, etc.**

Melting temperature calculation software:

-**BioMath: <http://www.promega.com/biomath/calc11.htm>**

## TUGAS PRAKTIKUM ---- KUMPULKAN MINGGU DEPAN

1. Buatlah Desain primer dari suatu gen tertentu atau dari spesies tertentu. lakukan analisis primer tersebut secara online dan secara manual, Nilai **T<sub>m</sub>** masing – masing primer dan nilai **T<sub>a</sub>** nya
2. bandingkan hasil keduanya dan analisis kelebihan dan kekurangannya

THANK  
YOU

GOOGLEADWORDS.COM

Novlani's blog