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PENGANTAR BIOINFORMATIKA
IBT 431

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Pertemuan 6

DESAIN PRIMER

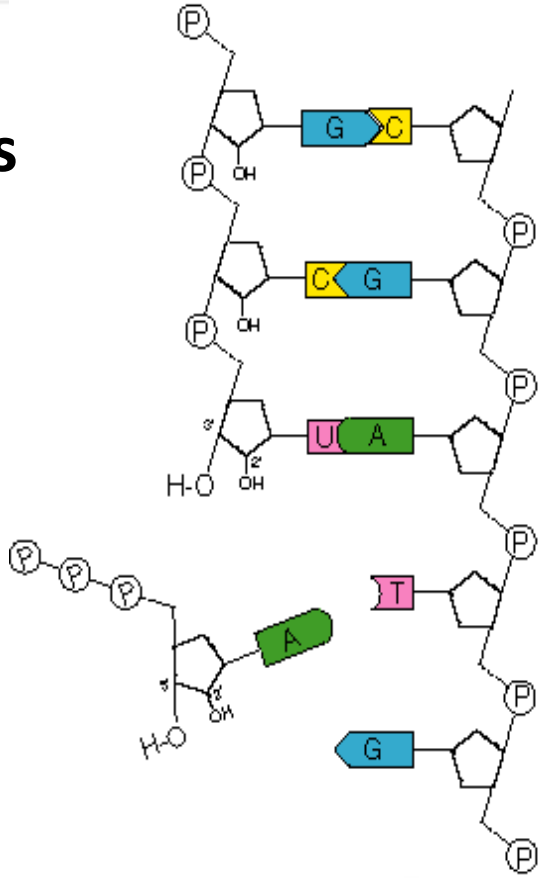
Universitas Esa Unggul

Sasaran Perkuliahan

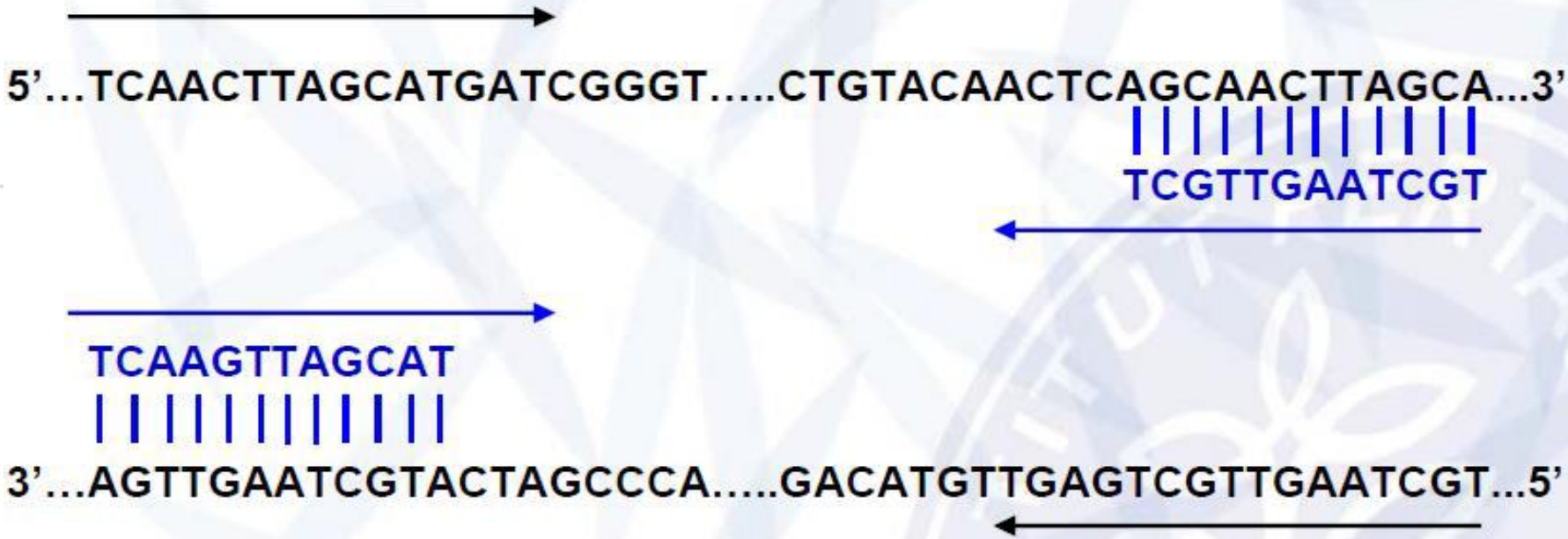
- Mahasiswa mampu menjelaskan tentang apa itu primer
- Mahasiswa Mampu menganalisis karakter primer yang baik
- Mahasiswa mampu mendesain urutan DNA primer sesuai dengan urutan gen yang diinginkan
- Mahasiswa mampu menghitung nilai Tm dan Ta primer

What Is a Primer

- A short synthetic oligonucleotide
- Servesas 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 fromPCR 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

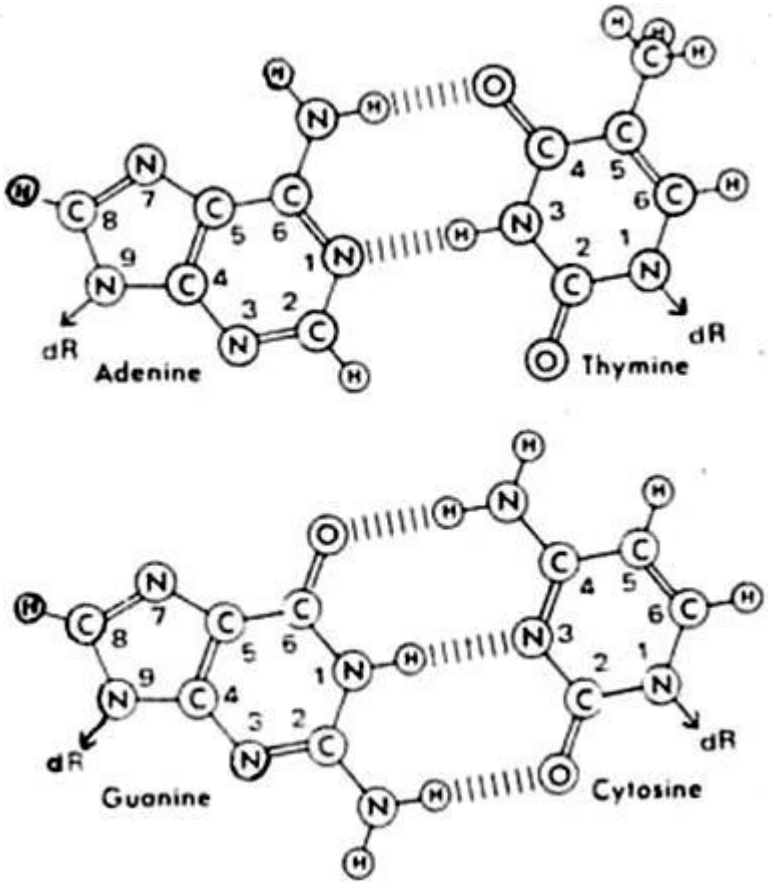
- Unique
- Has an appropriate melting-(Tm) and annealing-temperature (Ta)
- Random base distribution, and average G+C content ; Avoid long A+T and G+C-rich region if possible
- Usually 15-30 nucleatides in length
- Absence of dimerizationcapability
- Absence of significant hairpin formation

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 >= 15 bases to ensure uniqueness

Melting Temperature (TM)

- 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 Tm
- The optimal G/C content: 45-55%

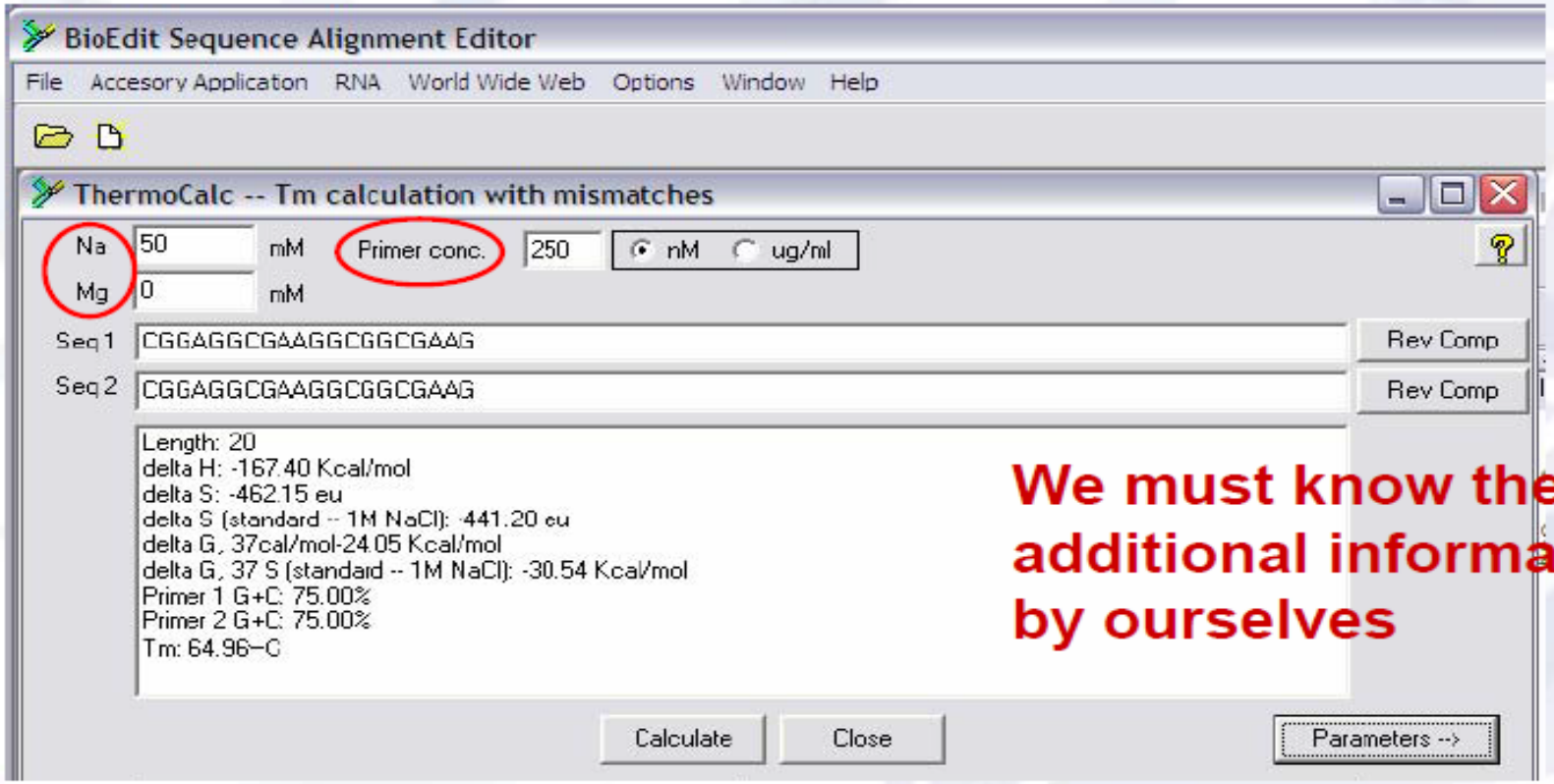


Melting Temperature (TM)

- Optimum Tm: 52–65 ^{0}C, but higher Tm is sometimes is 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 ^{0}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)*

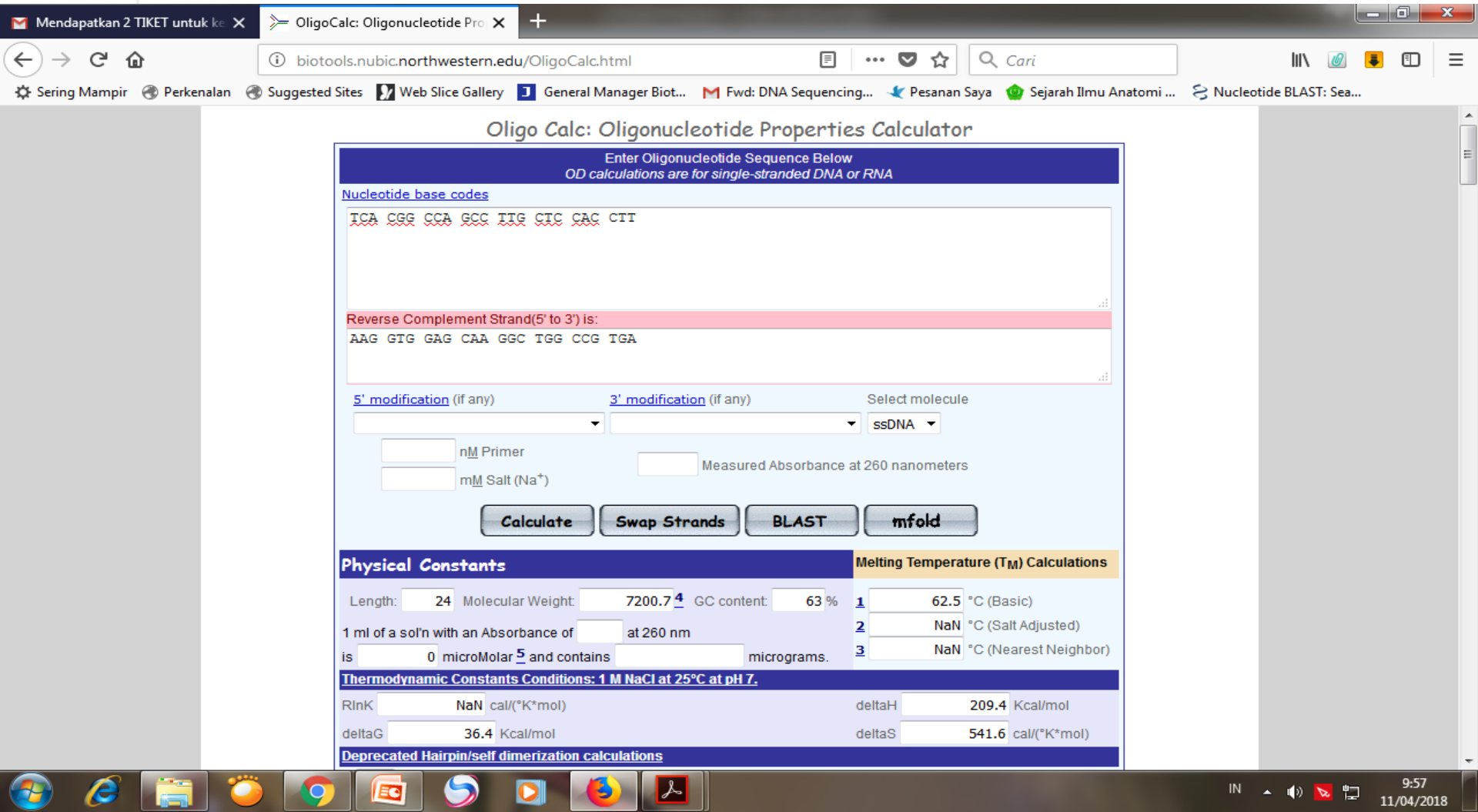
Melting Temperature (Tm): how to calculate Tm

Using softwares:



We must know the additional information by ourselves

2. Untuk melihat GC content dan TM pada program online " Oligo Calc"



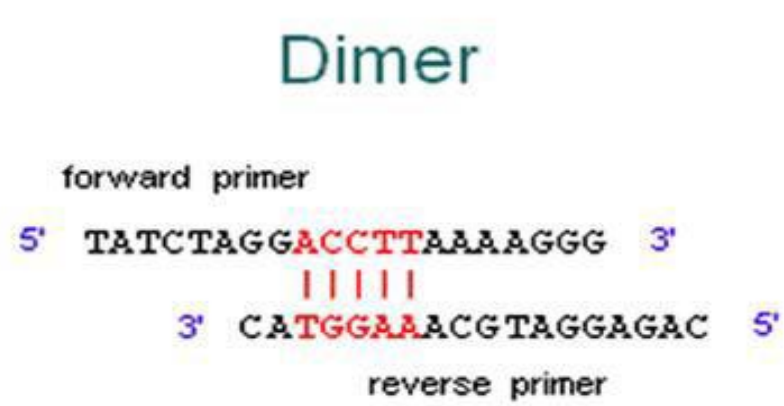
Annealing Temperature (Ta)

- 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 ^{o}C)

  Suhu *Annealing = Tm Gabungan – 5°C*
- Higher Ta enhances specific amplification but may lower yields

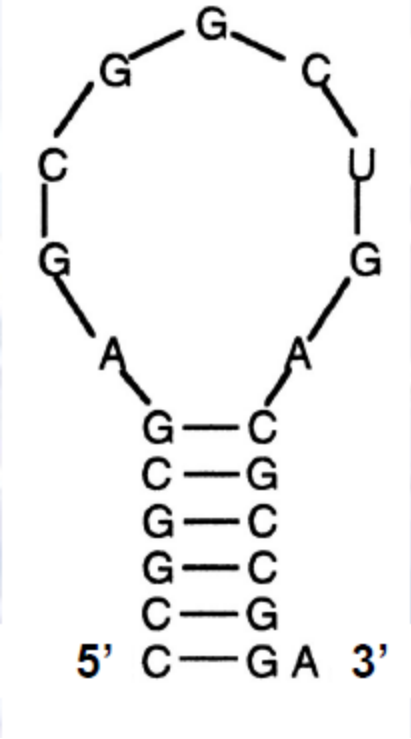
Primer Dimer

- 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.



Hairpin Formation

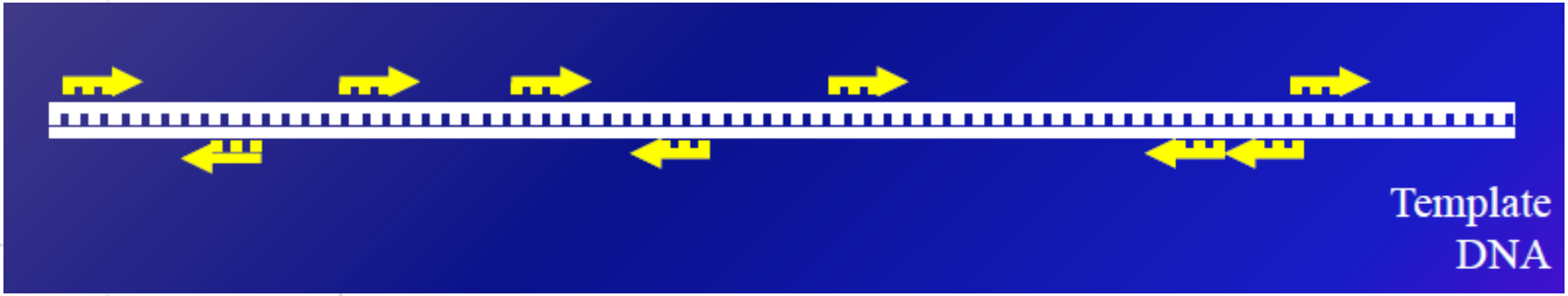
- Occurs when two regions of the same strand of the primer base-pair resulting lollipop-shaped structure
- Hairpin formation will lead unfunctionalprimer and could decrease the PCR efficiency dramatically.
- Sometimes primer dimerand hairpinsareharmless when the annealing temperature does not allow them to take form. (i.e. some dimersor 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 areclose and oriented in opposite directionso the primers point toward each other will amplification take place

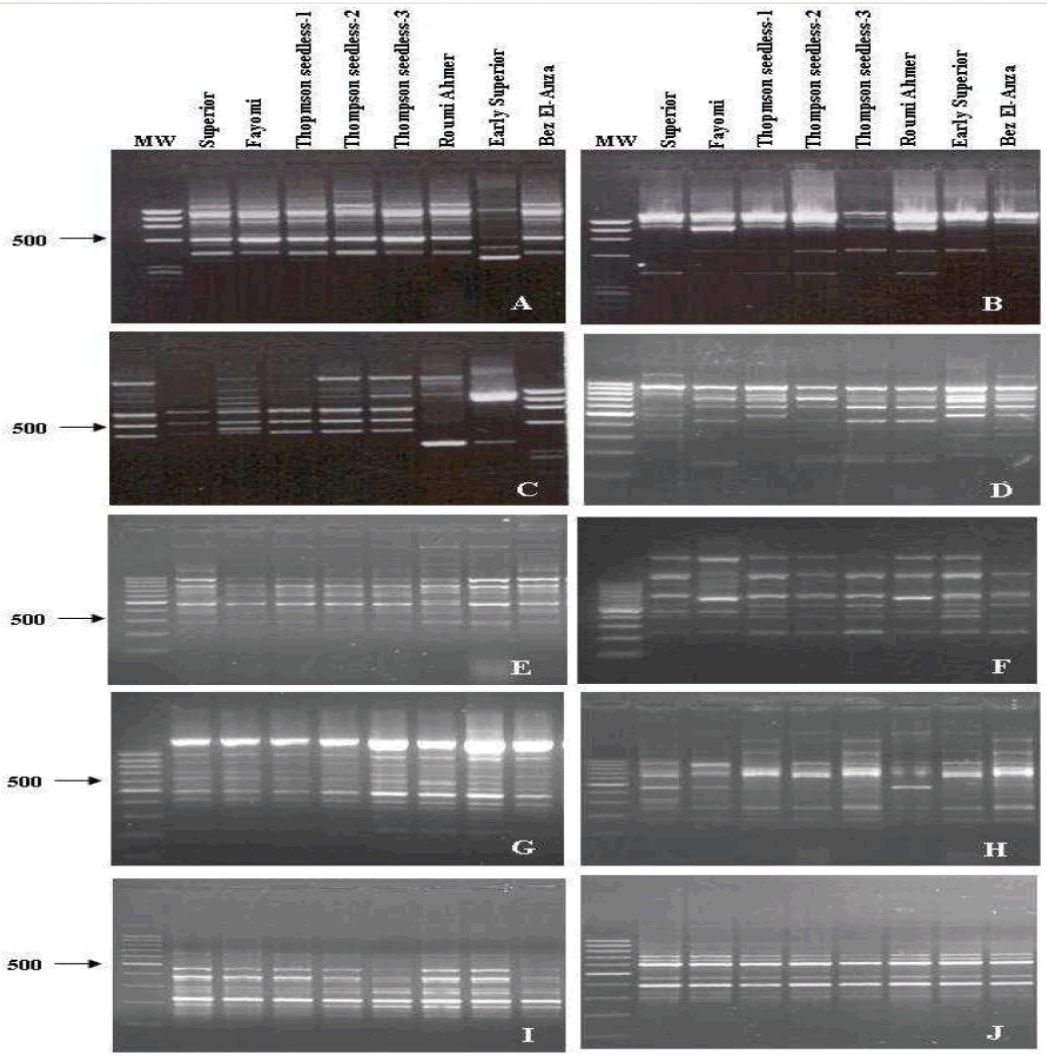
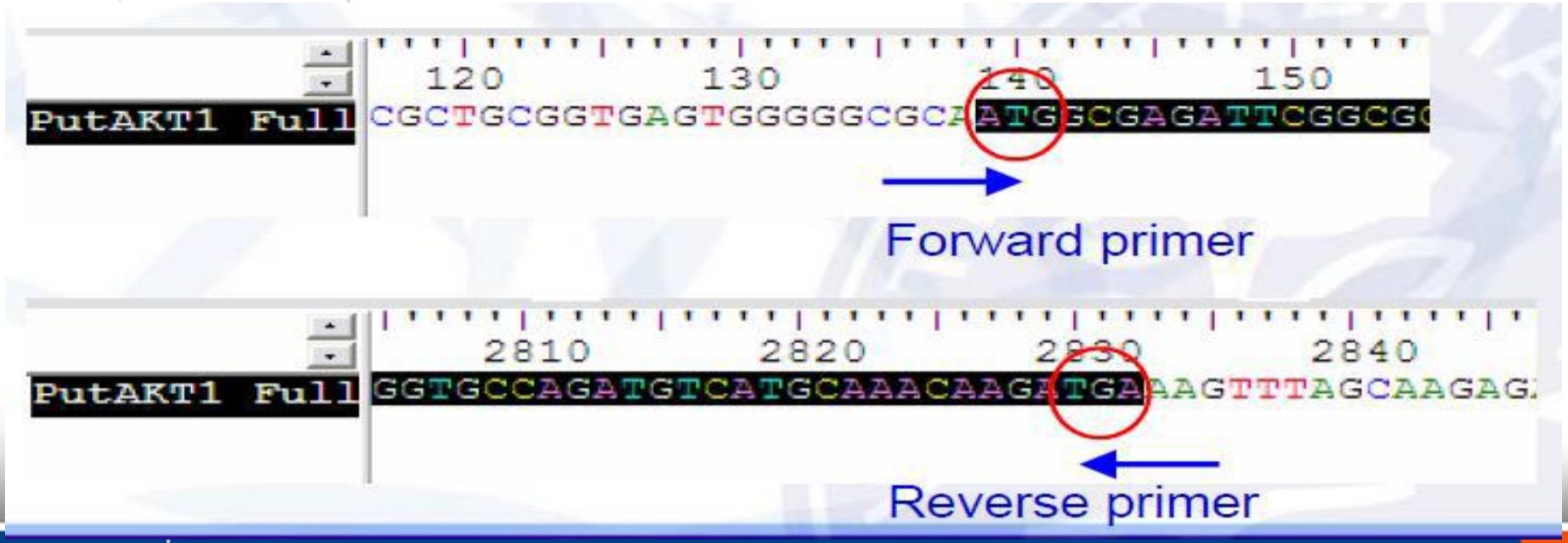


Figure 2: RAPD fingerprinting of 8 local Egyptian grape cultivars (*Vitis vinifera* L.). (A)Primer OP-A03; (B)Primer OP-A07; (C) Primer OP-A11; (D)Primer OP-A15; (E) Primer OP-B02; (F)Primer OP-B07; (G)Primer OP-B16 ; (H)Primer OP-B19; (I)Primer OP-G19 and (J)Primer OP-G20

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.

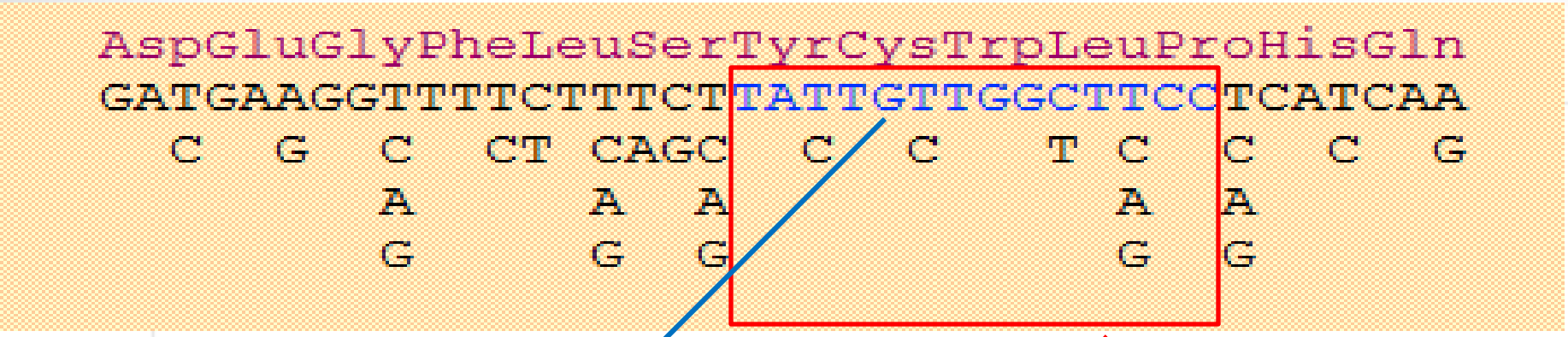


Degenerate Primers

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

Degenerate Primers

- Digunakan secraa 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



A total of 32 unique oligonucleotides would be generated
TATTGTTGGCTTCC
TACTGTTGGTTCCC
TATTGCTGGCTTCC
TACTGCTGGCTTCC
etc.

TAYTGYTGGYTNCC

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

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

-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 **Tm** masing – masing primer dan nilai **Ta** nya
2. bandingkan hasil keduanya dan analisis kelebihan dan kekurangannya


THANK
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