Sequenze

The Nobel Prize in Chemistry 1980

Paul Berg
Prize share: 1/2

Walter Gilbert
Prize share: 1/4

Frederick Sanger
Prize share: 1/4

The Nobel Prize in Chemistry 1980 was divided, one half awarded to Paul Berg *"for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA"*, the other half jointly to Walter Gilbert and Frederick Sanger *"for their contributions concerning the determination of base sequences in nucleic acids"*.

Per sequenziare il DNA, si utilizzano 2 metodi:

Metodo chimico o di Maxam-Gilbert

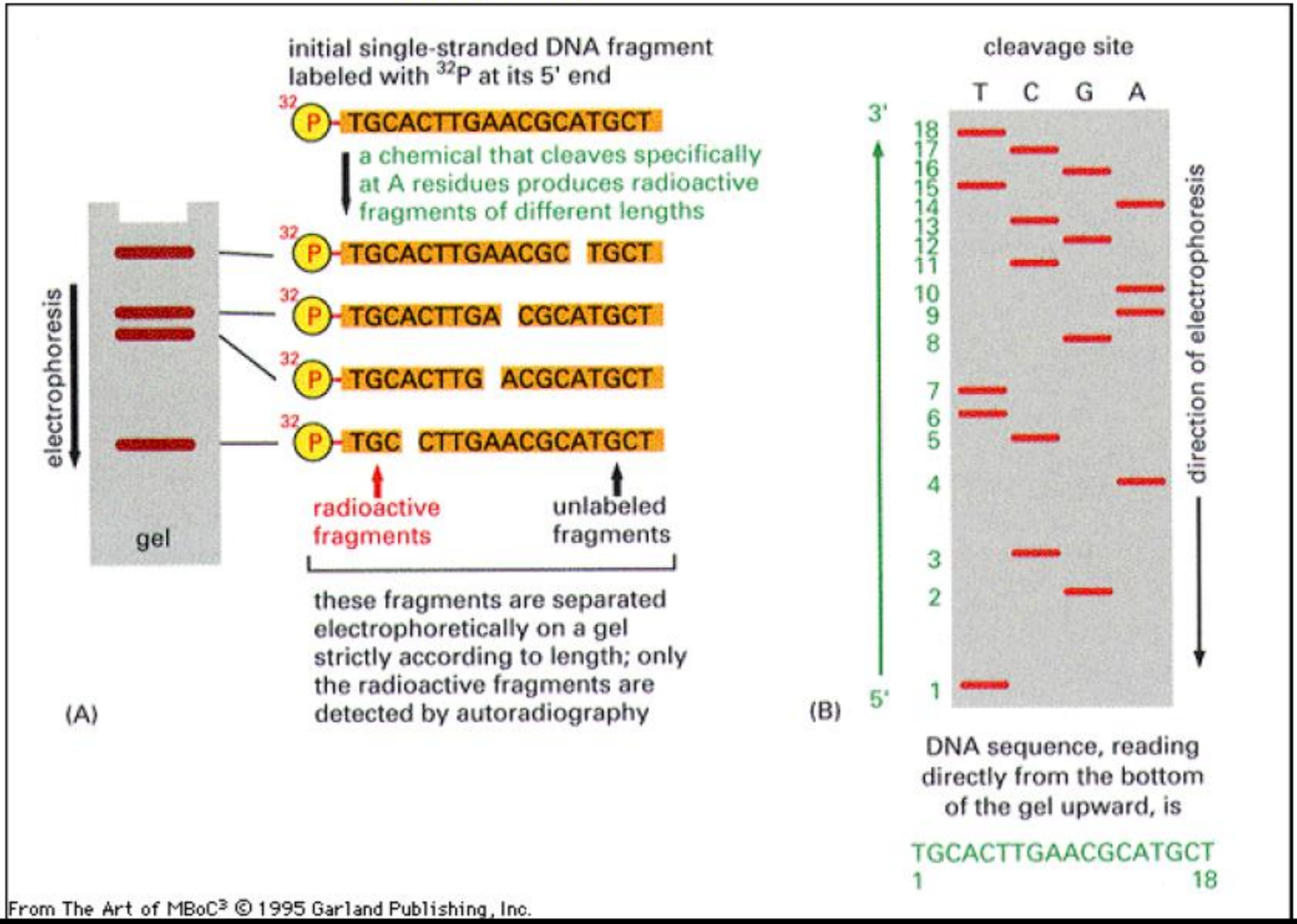
In questo caso, una molecola di DNA viene marcata con ^{32}P ad una estremità. Sulla molecola marcata, si eseguono poi quattro reazioni separate, che portano alla rottura del DNA in corrispondenza di una base specifica. Le reazioni vengono condotte in difetto del reagente, in modo che ogni molecola di DNA venga tagliata in media una volta sola. I frammenti prodotti dalle 4 reazioni vengono poi separati con elettroforesi ad alta risoluzione su gel di poliacrilamide, capace di separare (risolvere) due molecole di DNA che differiscono per lunghezza anche di un solo nucleotide.

Metodo enzimatico di Sanger

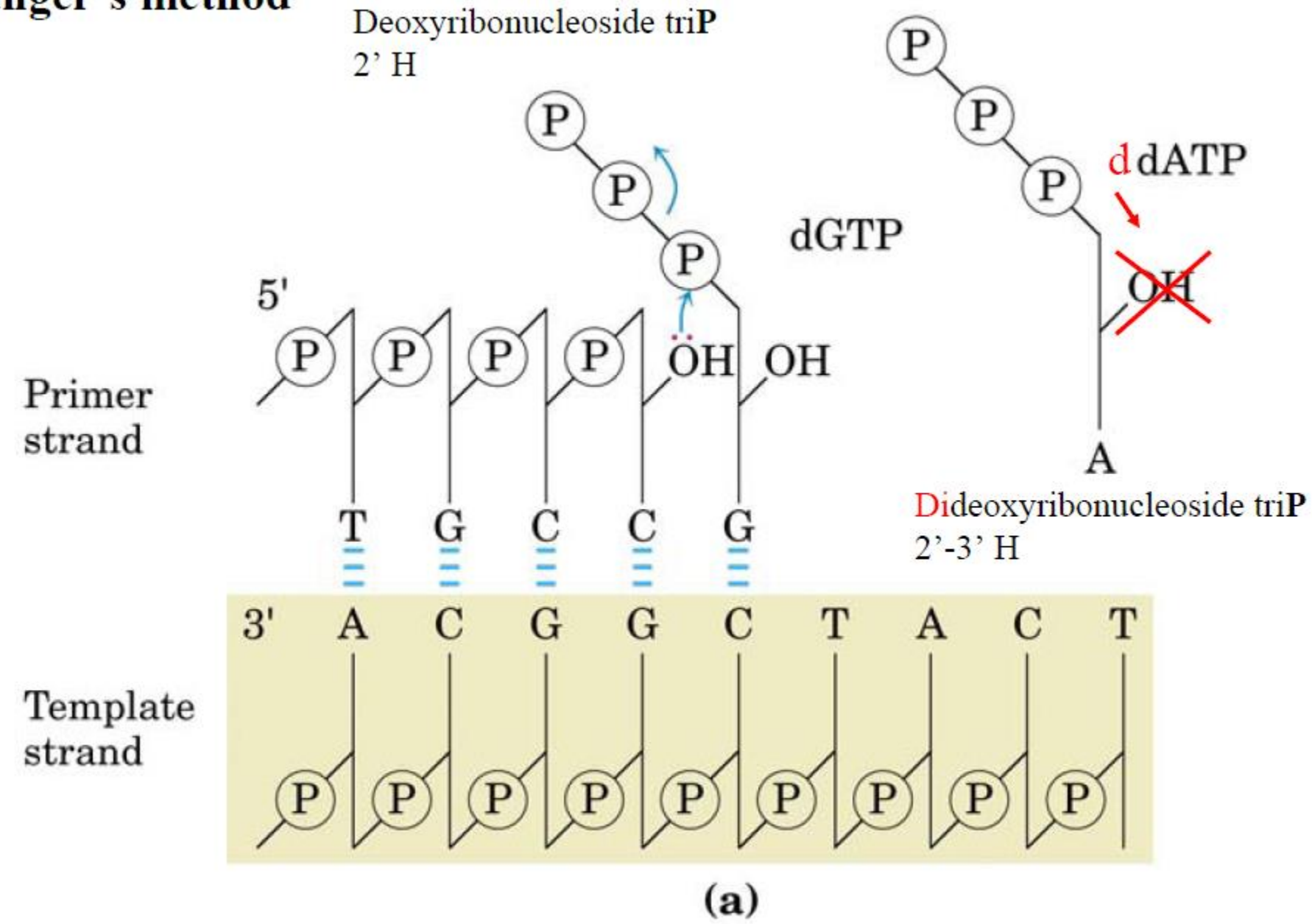
Questo metodo si basa sulla sintesi di nuove molecole di DNA, sullo stampo da sequenziare, troncate a livello di basi specifiche, e fa uso di speciali nucleotidi, preparati chimicamente, che mancano dell'ossidrile in posizione 3': i *2',3' dideossinucleotidi trifosfati*.

Maxam-Gilbert DNA chemical sequencing

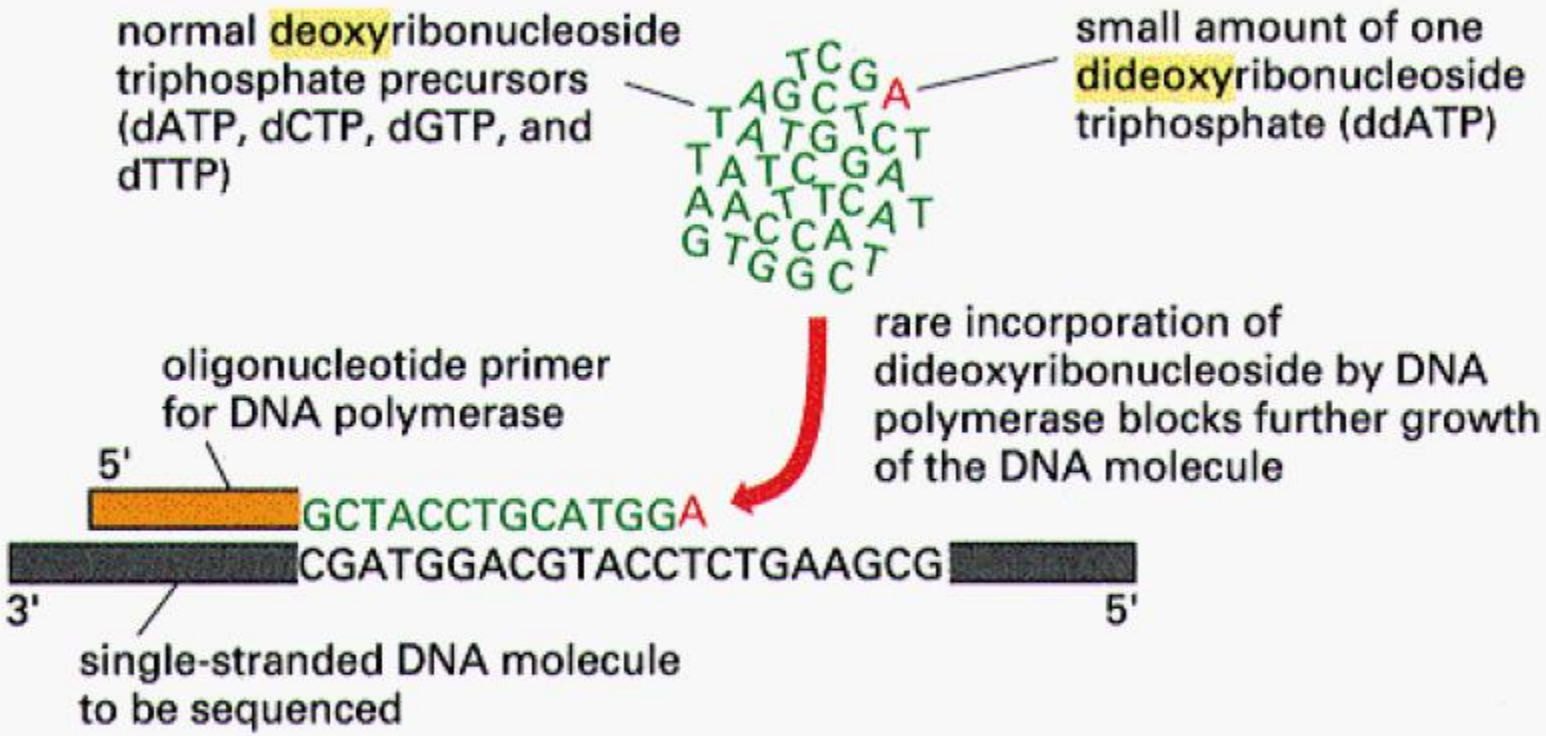
Example for A

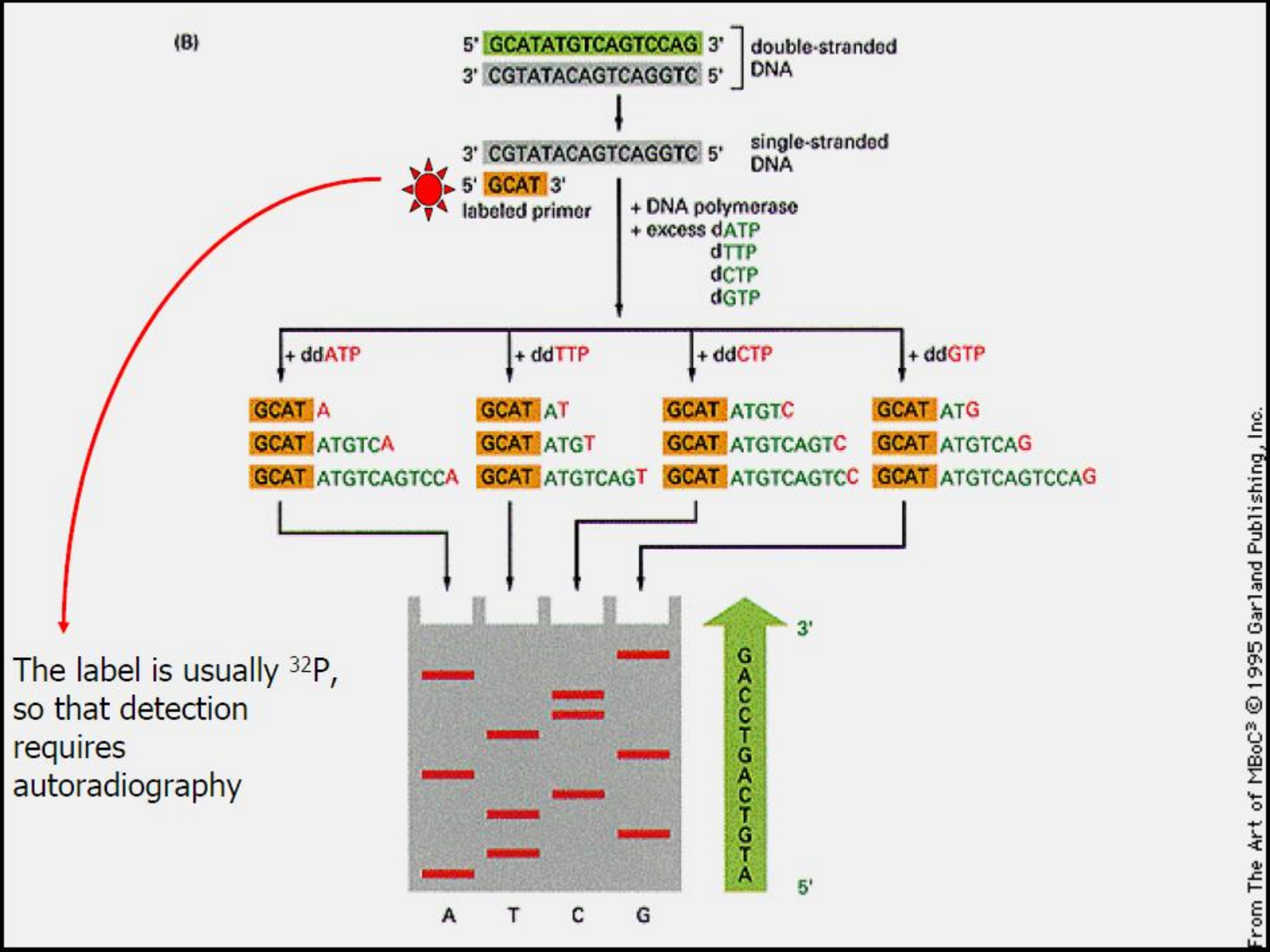


Sanger's method



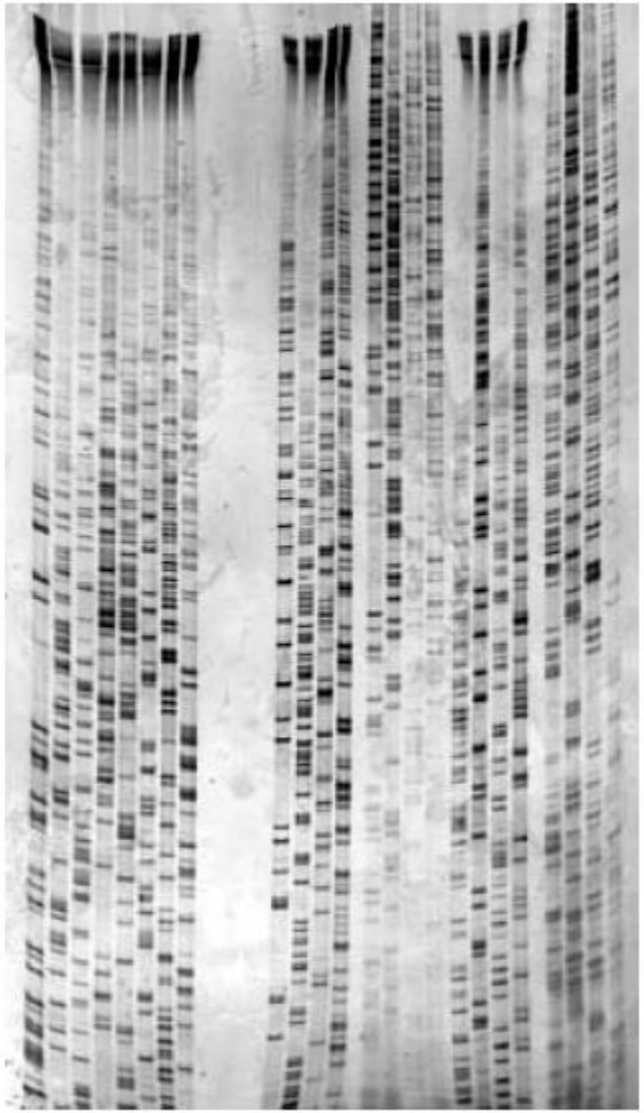
(A)





The label is usually ^{32}P, so that detection requires autoradiography

SEQUENZIAMENTO DEL DNA



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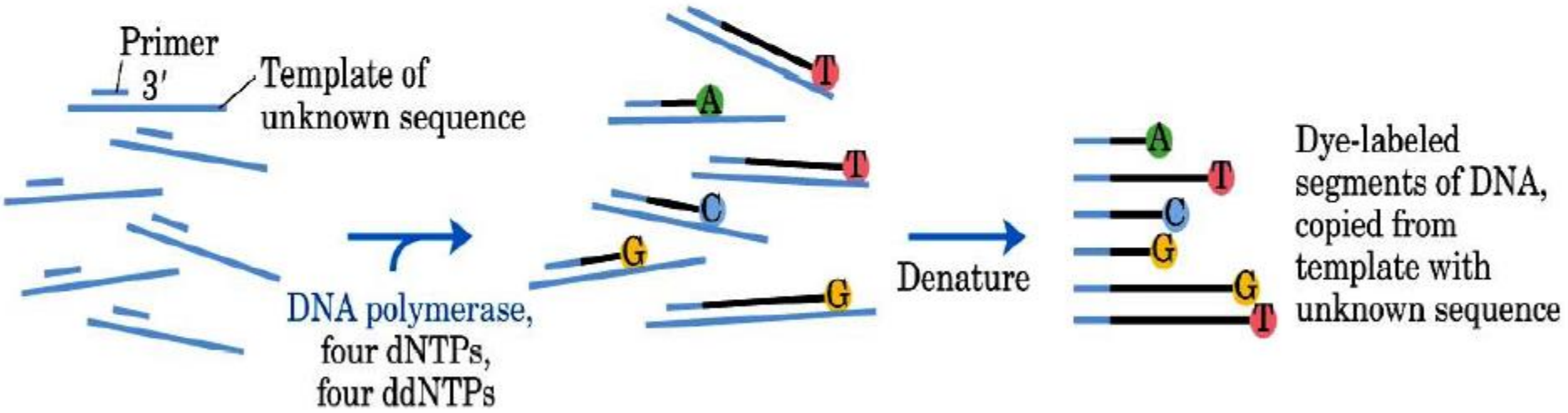
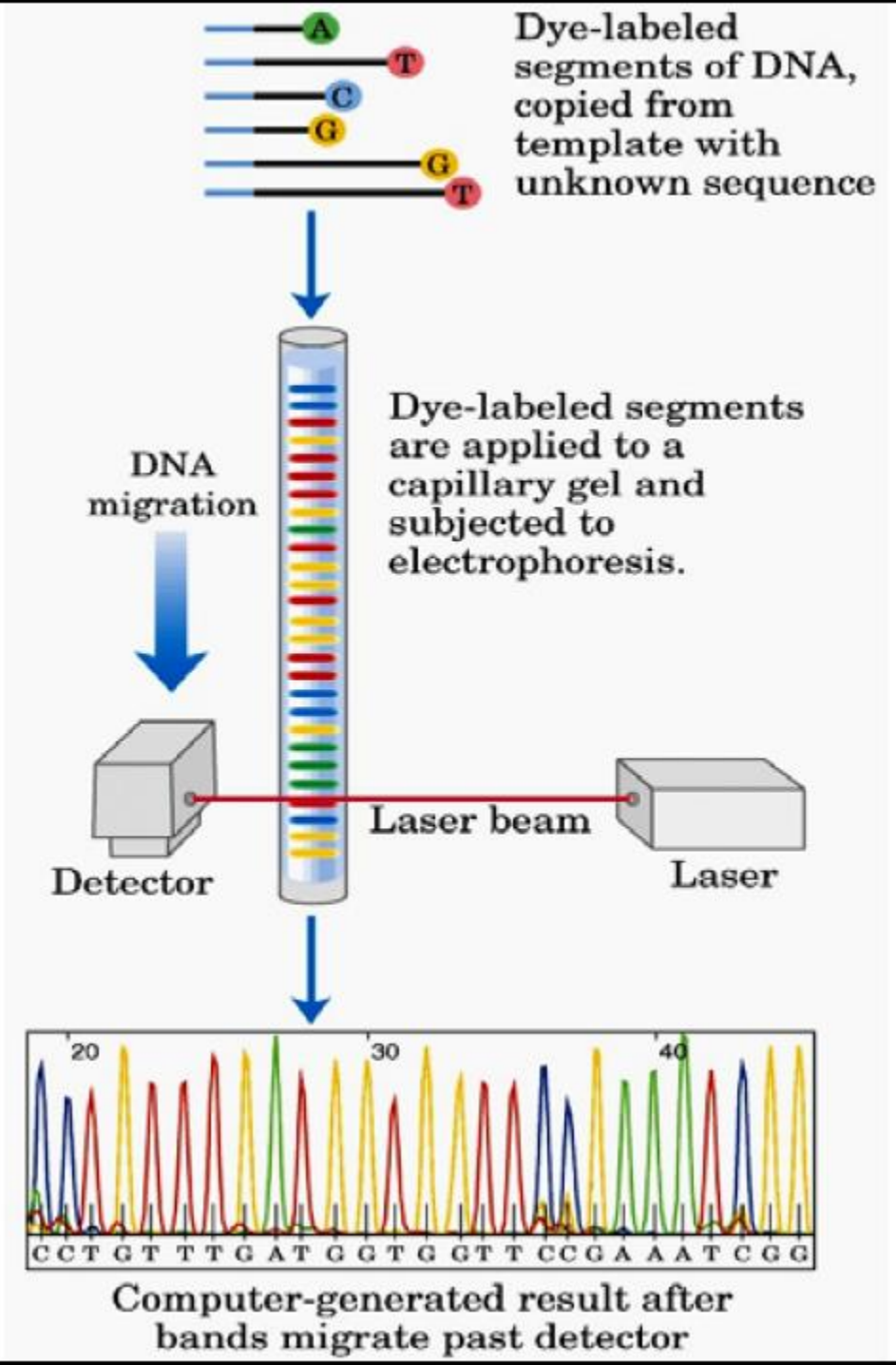
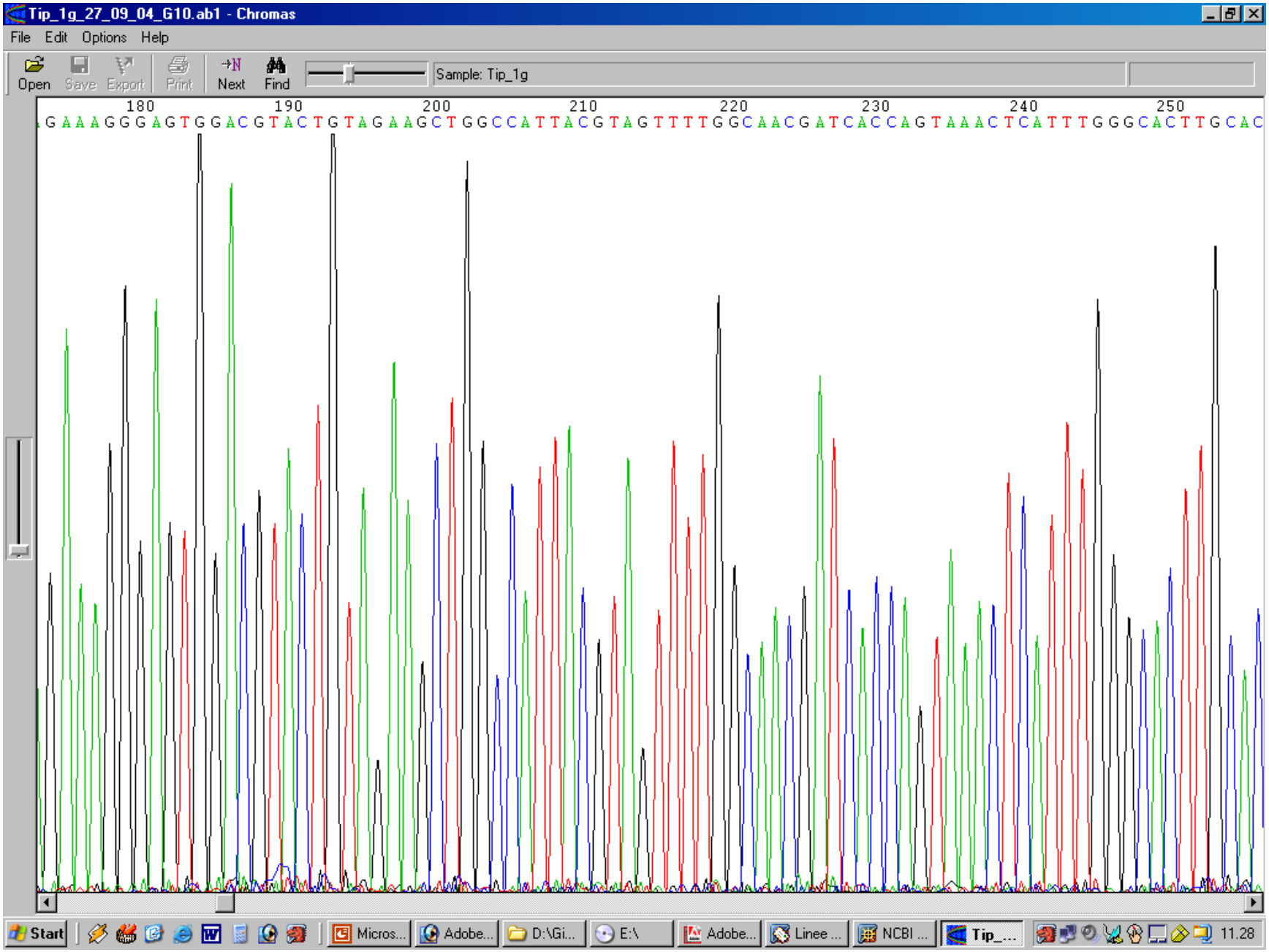
```

SEQUENZIATORE AUTOMATICO

Una successiva evoluzione del metodo ha permesso l'automazione del sequenziamento del DNA. In questo caso, i nucleotidi dideossi-terminatori sono marcati mediante l'addizione di un gruppo chimico fluorogeno, diverso per ogni base.




A
T
C
G
G
T
Dye-labeled segments of DNA, copied from template with unknown sequence
DNA migration
Dye-labeled segments are applied to a capillary gel and subjected to electrophoresis.
Detector
Laser beam
Laser
20
30
40
C C T G T T T G A T G G T G G T T C C G A A A T C G G
Computer-generated result after bands migrate past detector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11.28


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File Edit Options Help
Open Save Export Print Next Find
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Disegno Forme
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PCR.rtf - Mic...
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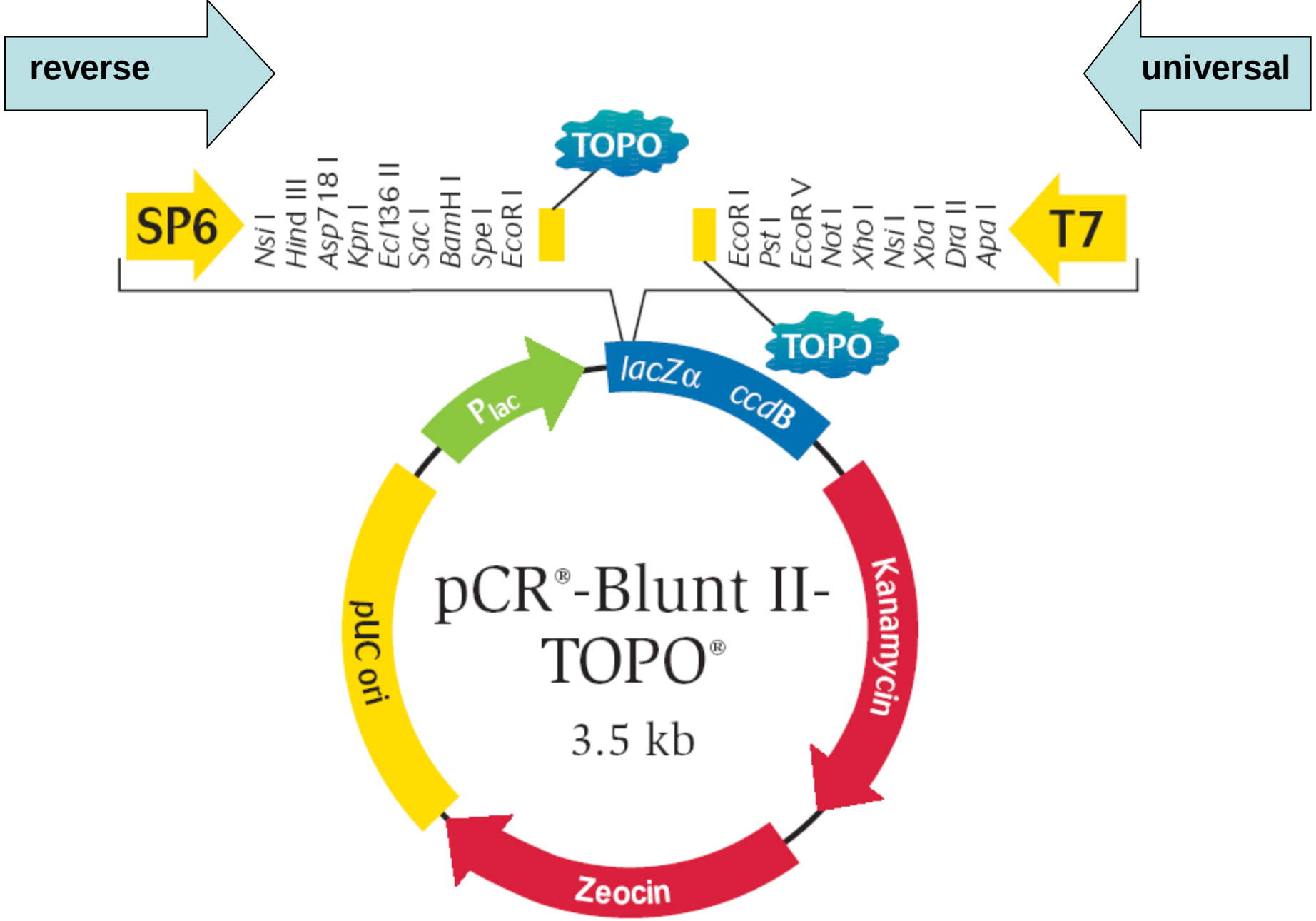
reverse
universal
TOPO
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Hind III
Asp718 I
Kpn I
Ecl136 II
Sac I
BamH I
Spe I
EcoR I
EcoR I
Pst I
EcoR V
Not I
Xho I
Nsi I
Xba I
Dra II
Apa I
T7
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ccdB
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Zeocin
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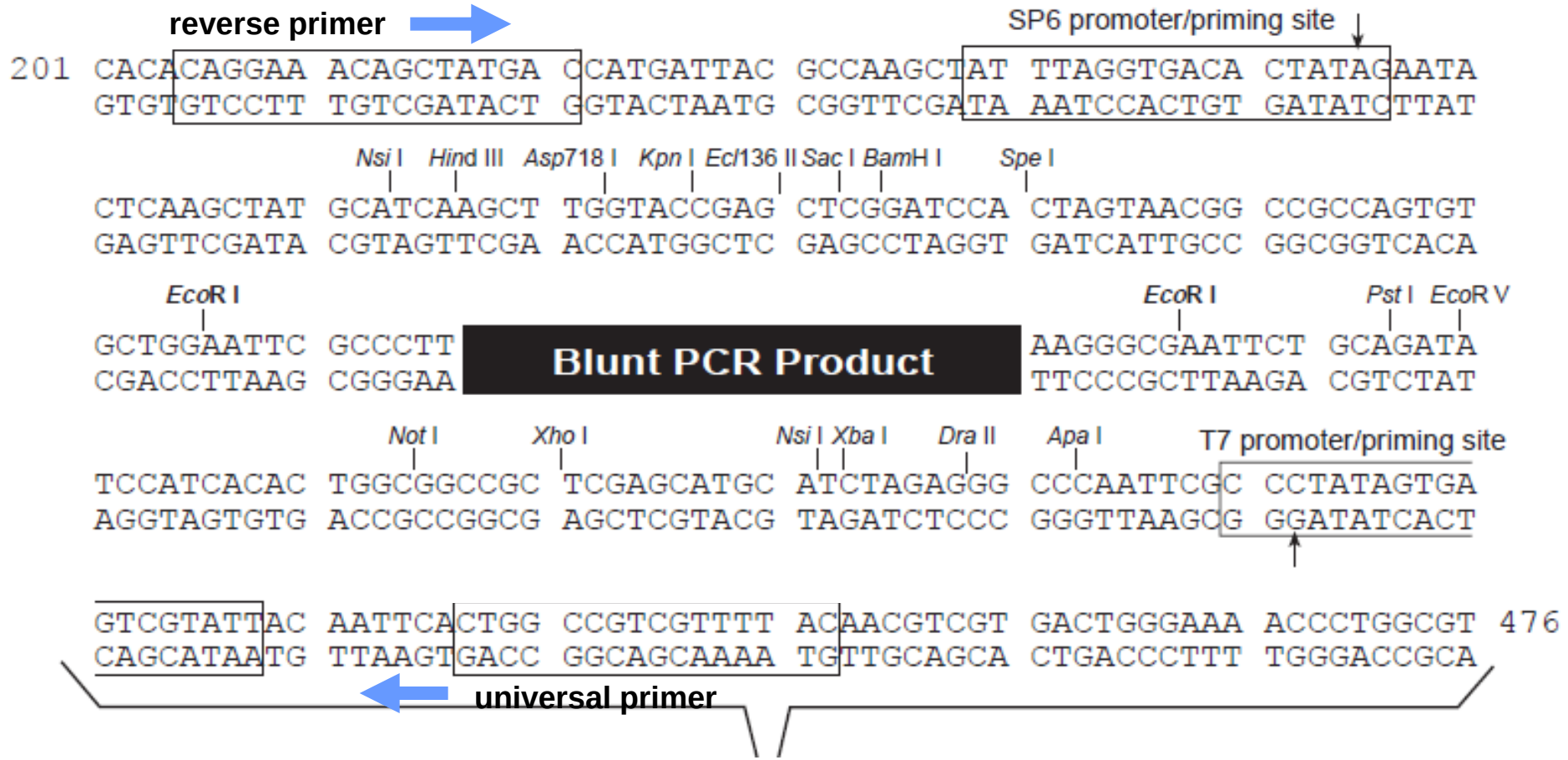
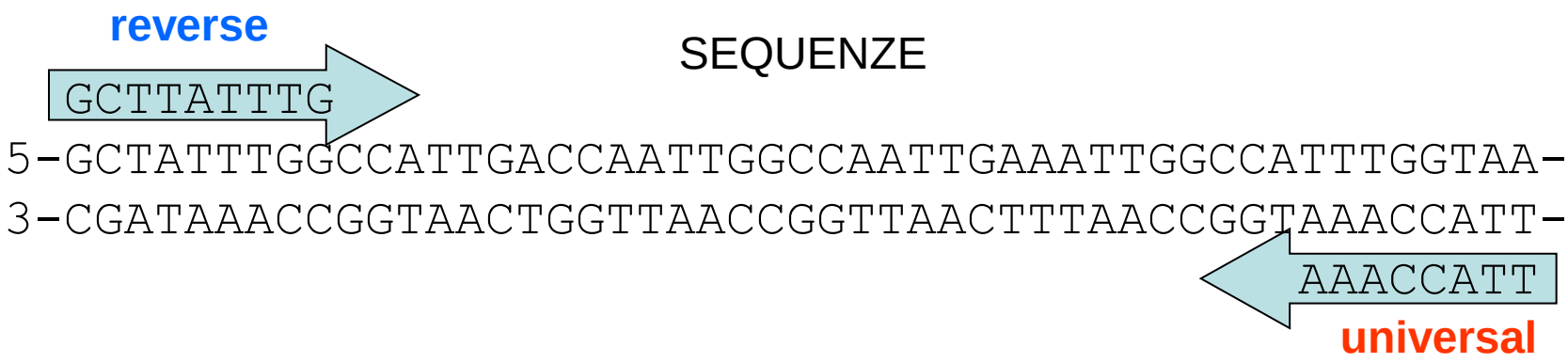


reverse primer:
5-CAGGAAACAGCTATGAC-3′

universal primer:
5′-GTAAAACGACGGCCAG-3′

SEQUENZE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3-CGATAAACCGGTAACTGGTTAACCGGTTAACTTTAACCGGTAAACCATT-5′
AAACCATT
universal


reverse
GCTTATTTG
CGATAAACCGGTAACTGGTTAACCGGTTAACTTTAACCGGTAAACCATT
Sequenza con reverse:
5′ - CCATTGACCAATTGGCCAATTGAAATTGGCCATTTGGTAA – 3′

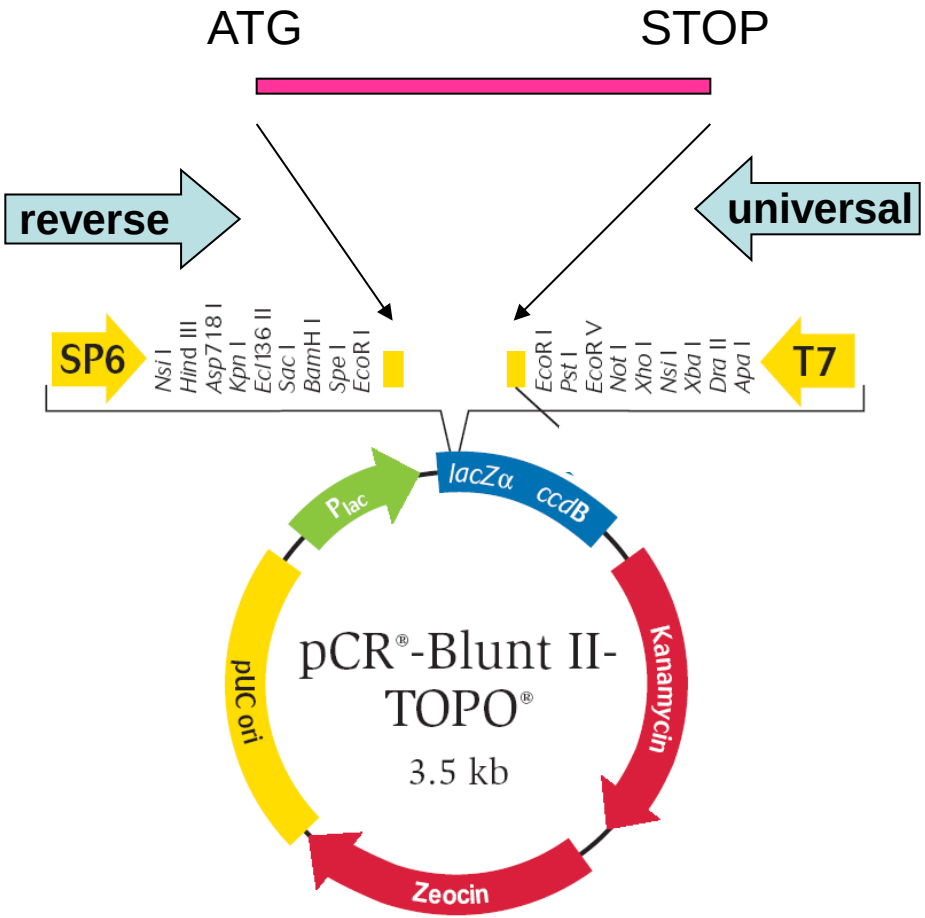

GCTATTTGGCCATTGACCAATTGGCCAATTGAAATTGGCCATTTGGTAA
AAACCATT
universal
Sequenza con universal:
5′- TGGCCAATTTCAATTGGCCAATTGGTCAATGGCCAAATAGC – 3′

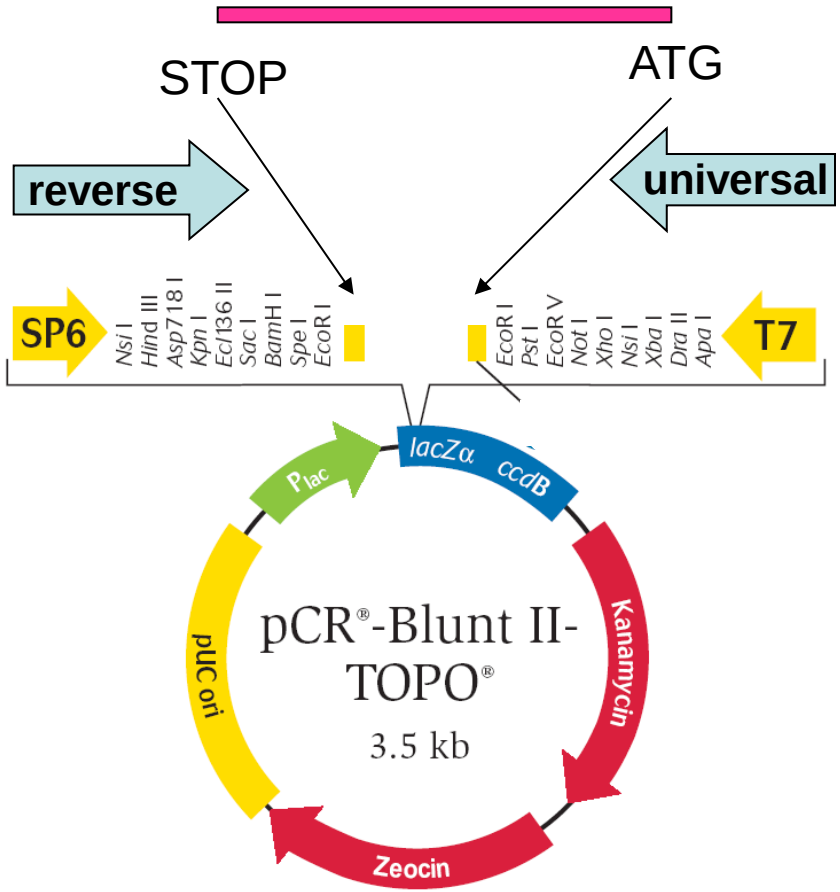
SENSO

5′-**ATG**CGTACCTTTAACTCG**TAG**-3′
3′-TACGCATGGAAATTGAGCATC-5′

ANTISENSO

5′-CTACGAGTTAAAGGTACGCAT-3′
3′-**GAT**GCTGAATTTCCATGC**GTA**-5′





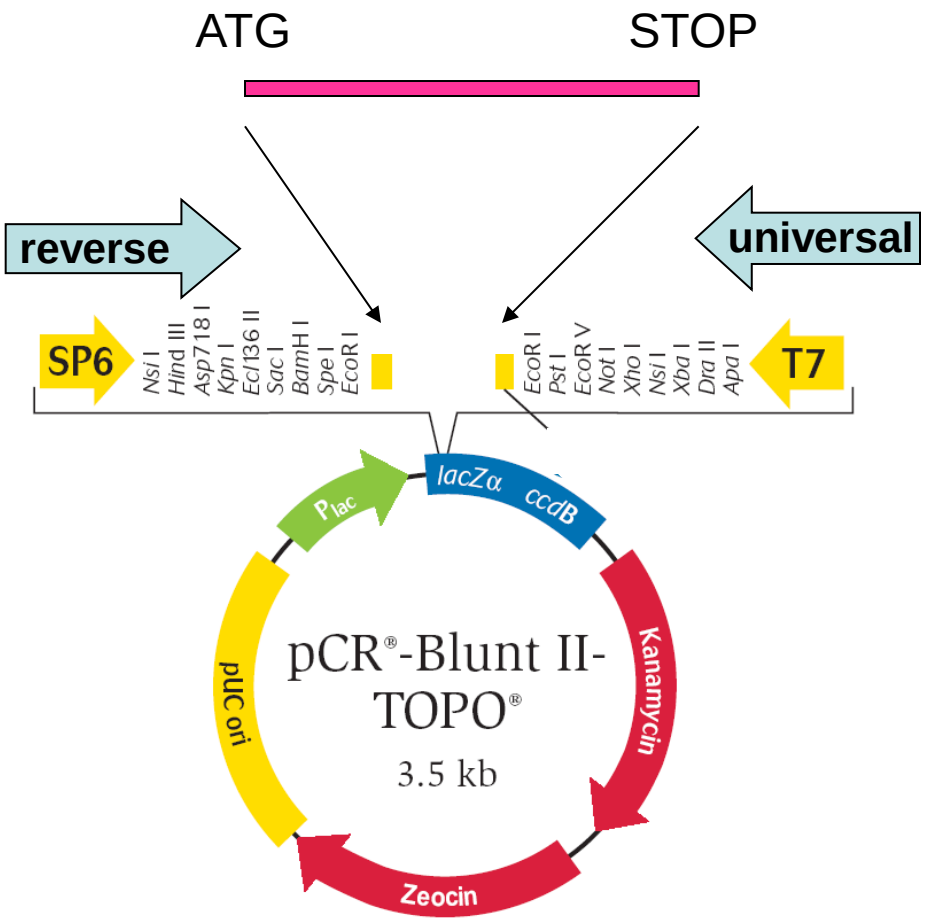
SENSO

reverse

5′–**ATG**CGTACCTTTAACTCG**TAG**–3′

3′–TACGCATGGAAATTGAGCATC–5′

universal



SENSO

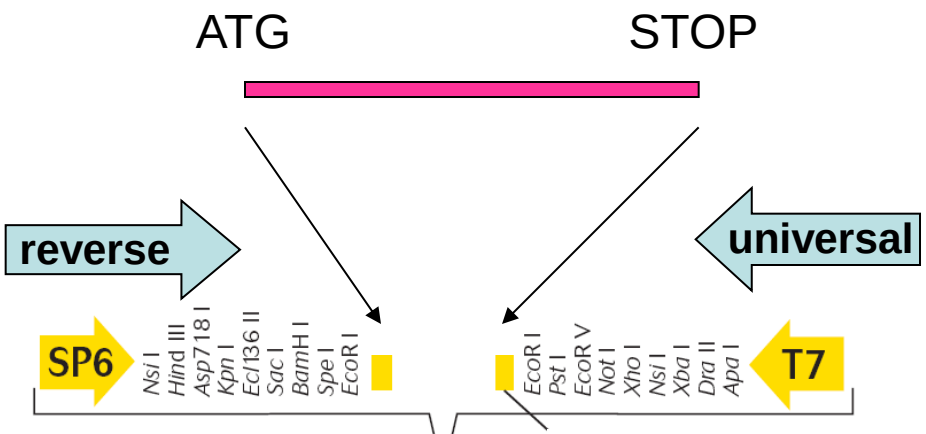
reverse

5′-**ATG**CGTACCTTTAACTCG**TAG**-3′

3′-TACGCATGGAAATTGAGCATC-5′

universal

REVERSE: NNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN



SENSO

reverse

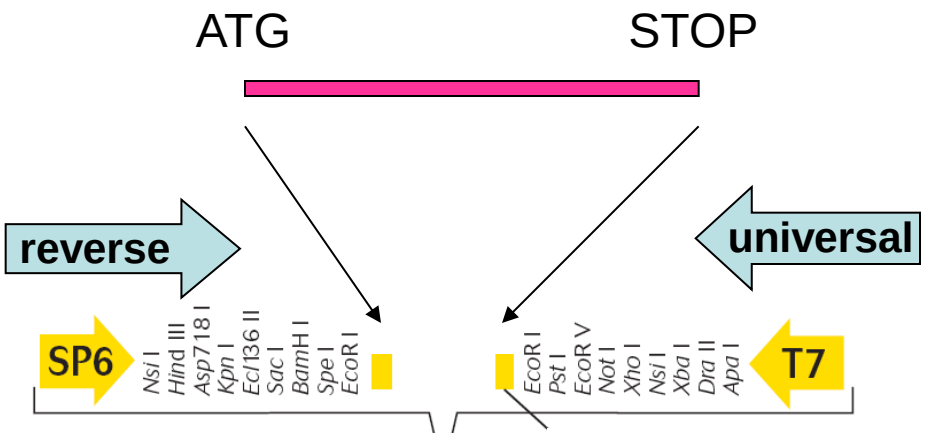
5′-**ATG**CGTACCTTTAACTCG**TAG**-3′

3′-TACGCATGGAAATTGAGCATC-5′

universal

REVERSE: NNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN

UNIVERSAL: NNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN



SENSO

200bp 100bp

reverse

5′-**ATG**CGTACCTTTAACTCG**TAG**-3′

3′-TACGCATGGAAATTGAGCATC-5′

universal

REVERSE: NNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNN

UNIVERSAL: NNNNNNCTACGAGTTAAAGGTACGCATNNNNNN

BLAST:

Query: 1 **ATG**CGTACCTTTAACTCG**TAG** 21

||||||||||||||||||||

Subjct Reverse: 201 **ATG**CGTACCTTTAACTCG**TAG** 221

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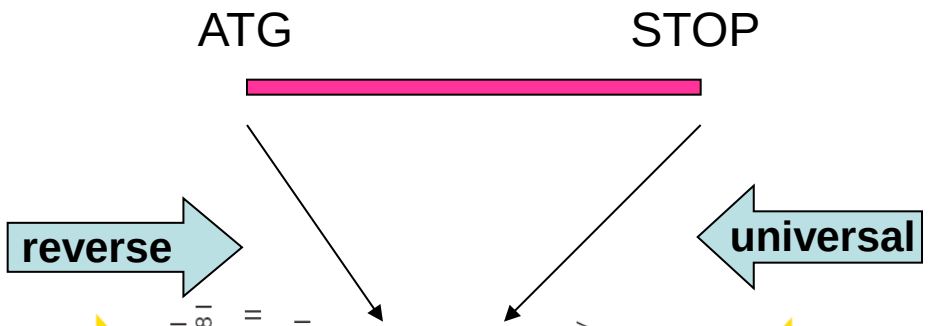


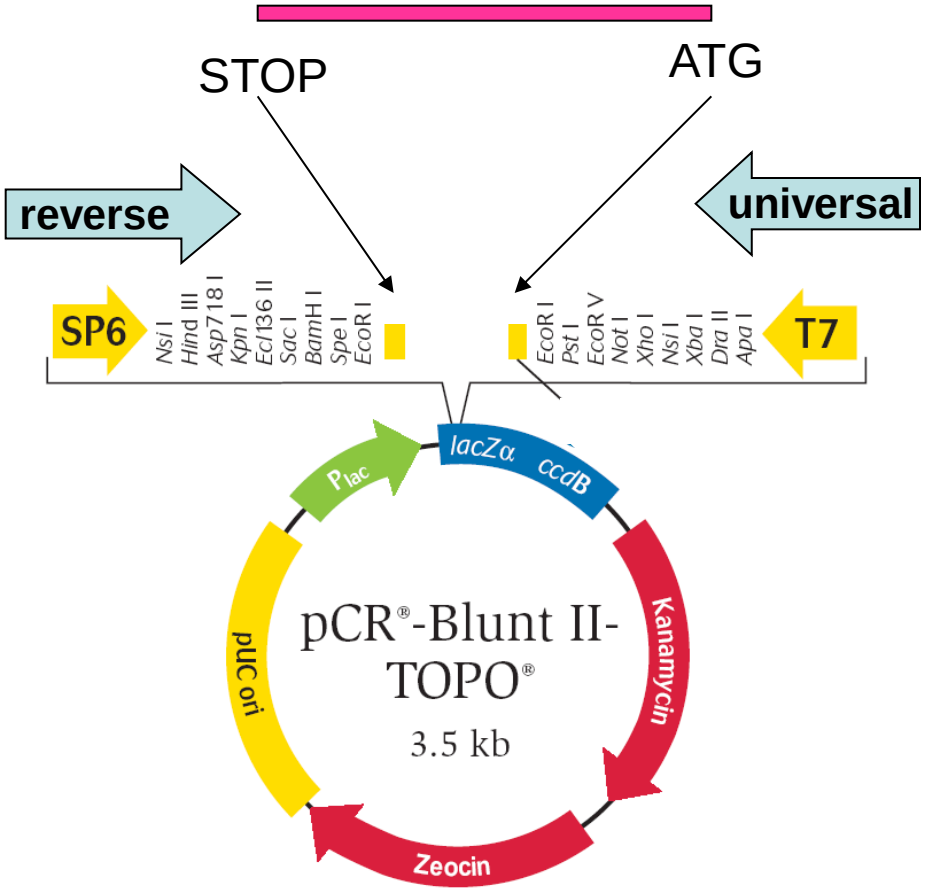
ANTISENSO

reverse

5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

universal



ANTISENSO

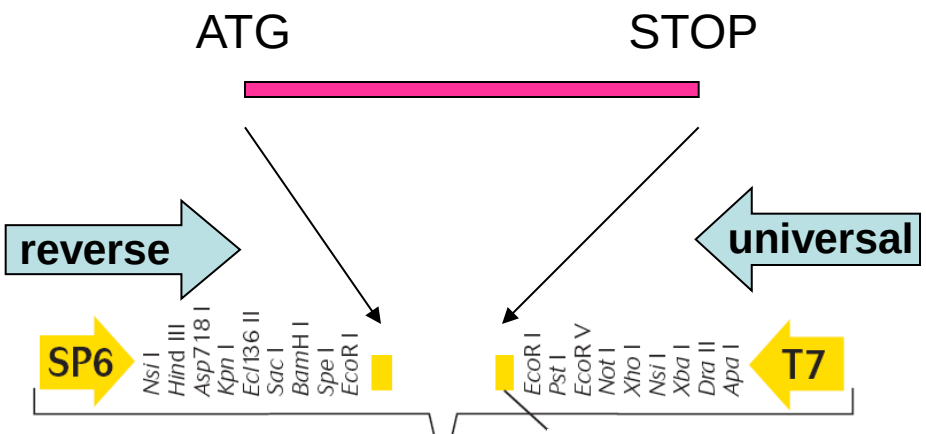
reverse

5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

universal

REVERSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN



ANTISENSO

reverse

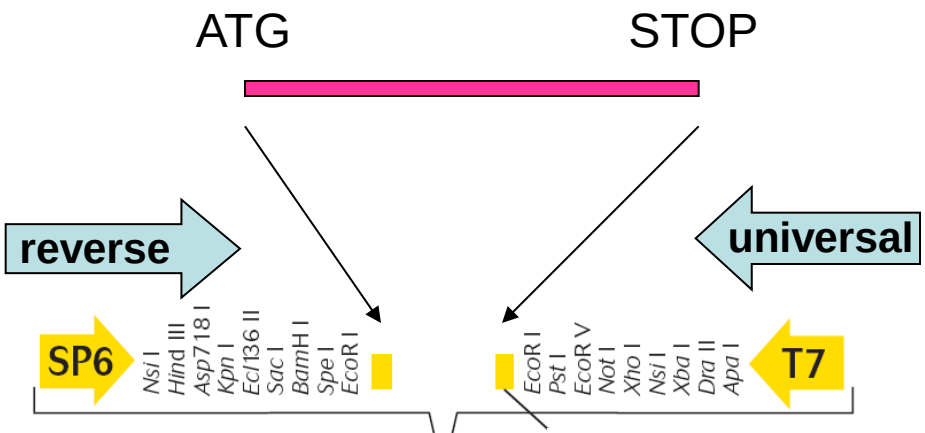
5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

universal

REVERSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN

UNIVERSAL: NNNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN



ANTISENSO

200bp 100bp

reverse 5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′ universal

REVERSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN

UNIVERSAL: NNNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN

BLAST:

Query: 1 **ATG**CGTACCTTTAACTCG**TAG** 21

||||||||||||||||||||

Subjct Reverse: 221 **ATG**CGTACCTTTAACTCG**TAG** 201

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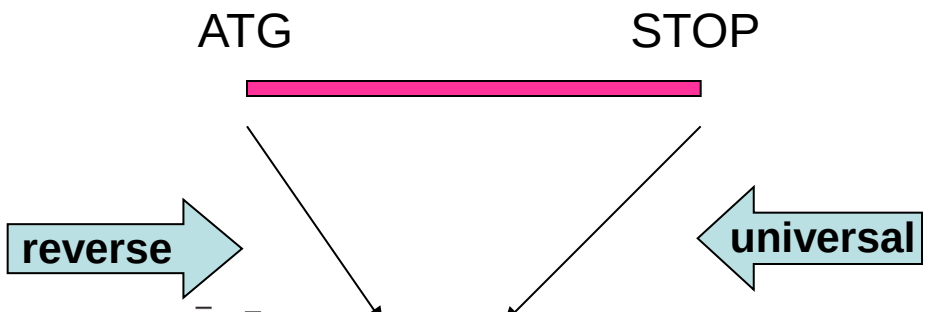


C -> T

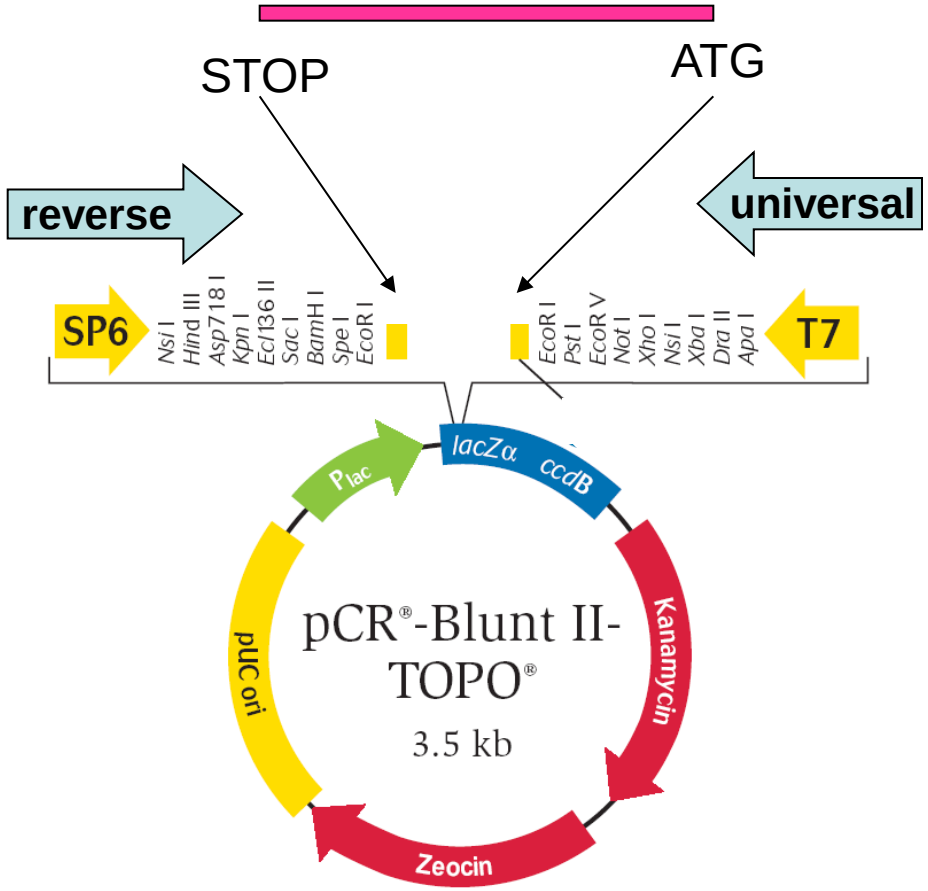
ANTISENSO
MUTATO

reverse

5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

universal



C -> T

ANTISENSO
MUTATO

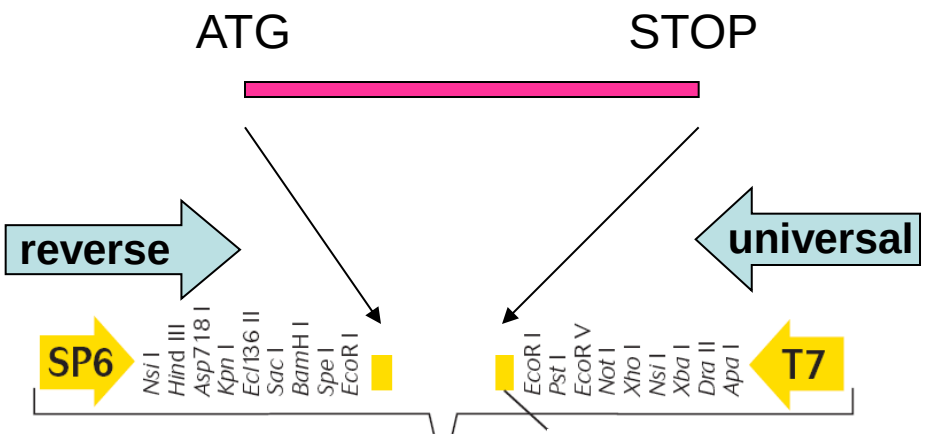
reverse

5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

universal

REVERSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN



ANTISENSO
MUTATO

C -> T

reverse

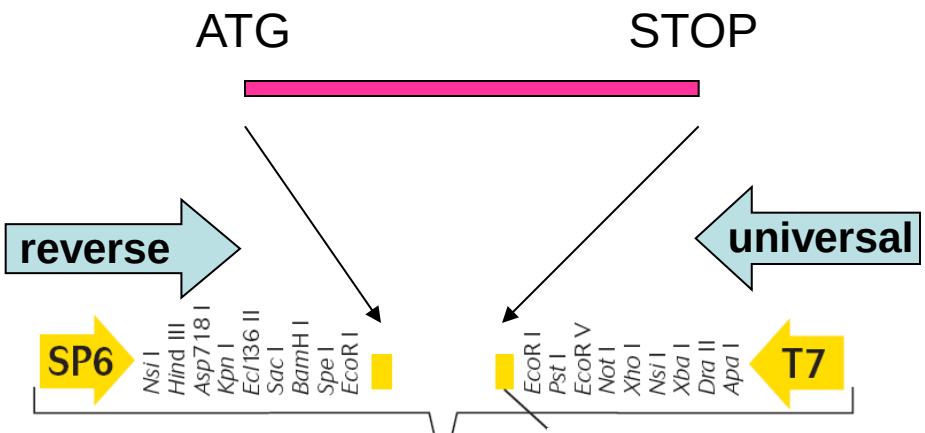
5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

universal

REVERSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN

UNIVERSAL: NNNNNNN**ATG**CGTACTTTTAACTCG**TAG**NNNNNNN



ANTISENSO MUTATO

200bp C -> T 100bp

reverse

5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

universal

REVERSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN

UNIVERSAL: NNNNNNN**ATG**CGTACTTTTAACTCG**TAG**NNNNNNN

BLAST:

Query: 1 **ATG**CGTACCTTTAACTCG**TAG** 21

||||||| ||||||||||||

Subjct Reverse: 221 **ATG**CGTACTTTTAACTCG**TAG** 201

Query: 1 **ATG**CGTACCTTTAACTCG**TAG** 21

||||||| ||||||||||||

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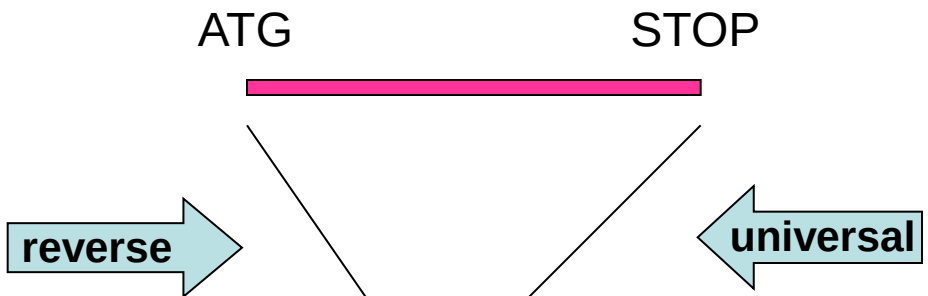

ANTISENSO MUTATO

200bp C -> T 100bp

REVERSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN
UNIVERSAL: NNNNNNN**ATG**CGTACTTTTAACTCG**TAG**NNNNNNN

BLAST:

Query: 1 **ATG**CGTACCTTTAACTCG**TAG** 21
||||||| ||||||||||
Subjct Reverse: 221 **ATG**CGTACTTTTAACTCG**TAG** 201

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Identifico la posizione della mutazione sulle sequenze Query, Reverse ed Universal:

Query	Reverse	Universal
9	213	109


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Tip_1g_reverse.ab1 - Chromas
File Edit Options Help
Open Save Export Print Next Find
Sample: Tip_1g
780 790 800 810 820 830 840 850
A T G C A G A G G C A G A G G C T G A C T A G G C A A G C C A G A A T G G G A A C A A T G C A G A T C T T C T C G G A G T T G A G A C A C C C T C T C A G G C G C T C A
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5 Adobe R...
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ANTISENSO
MUTATO

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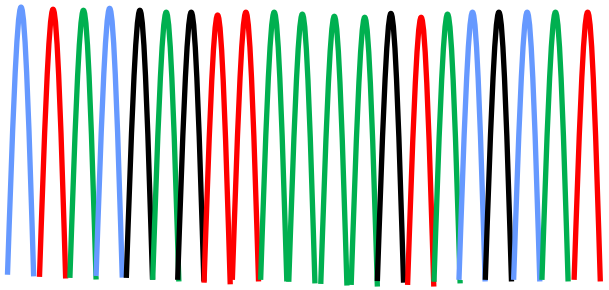
REVERSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN



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UNIVERSAL: NNNNNNNATGCGTACTTTTAACTCGTAGNNNNNNN