Advanced Cell Biology & Biotechnology

Biotechnology Project Lab

Giovanna Gambarotta
& Isabella Tarulli

The lecture of November 15th 2021 is about to begin….

Summary of the previous lesson
Reaction ingredients
Sequence analysis

5′-CGTTAACTTGACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3′

5′-**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3′
3′-GCAATTGAACTGGTACACGTAGATCGAG**GTACCGTACG**-5′

Primer sense:	5′-**CGTTAACTTG**-3′
Primer antisense:	5′-**GCATGCCATG**-3′

5′-CGTTAACTTGACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3′

5′-**CGTTAACTTG**ACC**ATG**TGCATC**TAG**CTCCATGGCATGC-3′
3′-GCAATTGAACTGGTACACGTAGATCGAG**GTACCGTACG**-5′

Primer sense: 5′-**CGTTAACTTG**-3′
Primer antisense: 5′-**GCATGCCATG**-3′

5′-**AAGCTT**-3′
3′-**TTCGAA**-5′

5′-**CTGCAG**-3′
3′-**GACGTC**-5′

Primer sense: 5′-**AAGCTTCGTTAACTTG**-3′
Primer antisense: 5′-**CTGCAGGCATGCCATG**-3′

Primer sense: 5′-AAGCTTCGTTAACTTG-3′
Primer antisense: 5′-CTGCAGGCATGCCATG-3′

5′-AAGCTTCGTTAACTTG-3′ ->
3′-GCAATTGAACTGGTACACGTAGATCGAGGTACCGTACG-5′

5′-CGTTAACTTGACCATGTGCATCTAGCTCCATGGCATGC-3′
<- 3′-GTACCGTACGGACGTC-5′

5′-AAGCTTCGTTAACTTGACCATGTGCATCTAGCTCCATGGCATGC-3′
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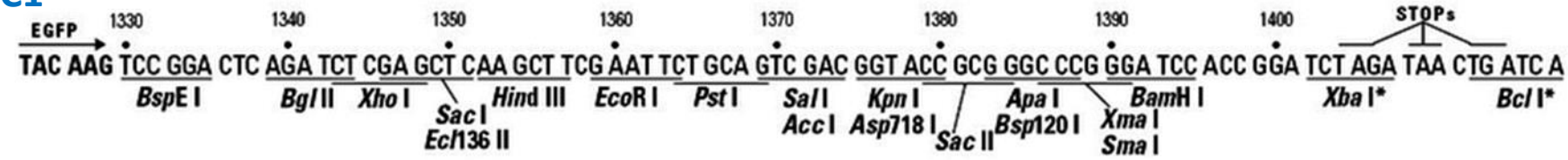
5′-AAGCTTCGTTAACTTGACCATGTGCATCTAGCTCCATGGCATGCCTGCAG-3′

3′-TTCGAAGCAATTGAACTGGTACACGTAGATCGAGGTACCGTACGGACGTC-5′

EGFP-C1



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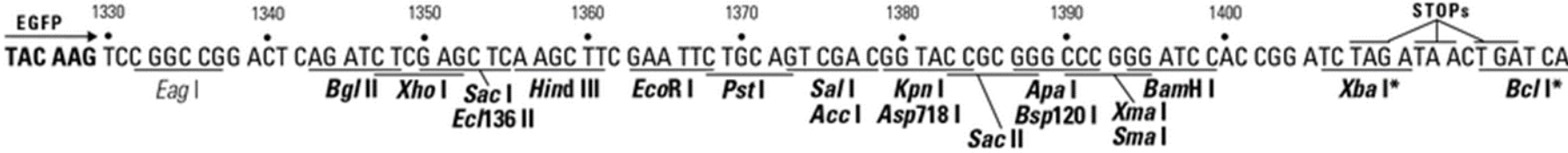

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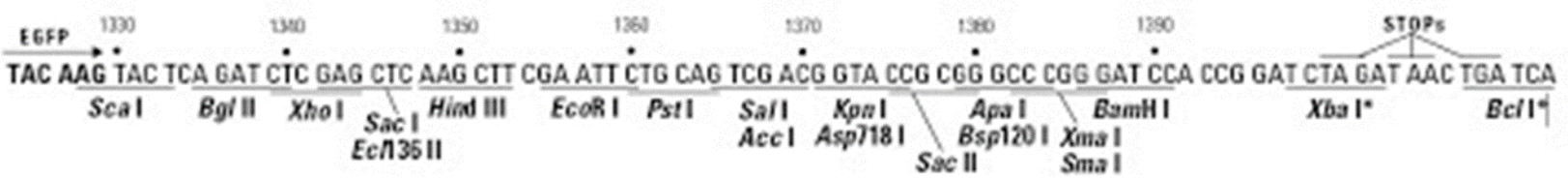

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EGFP

EGFP-C2

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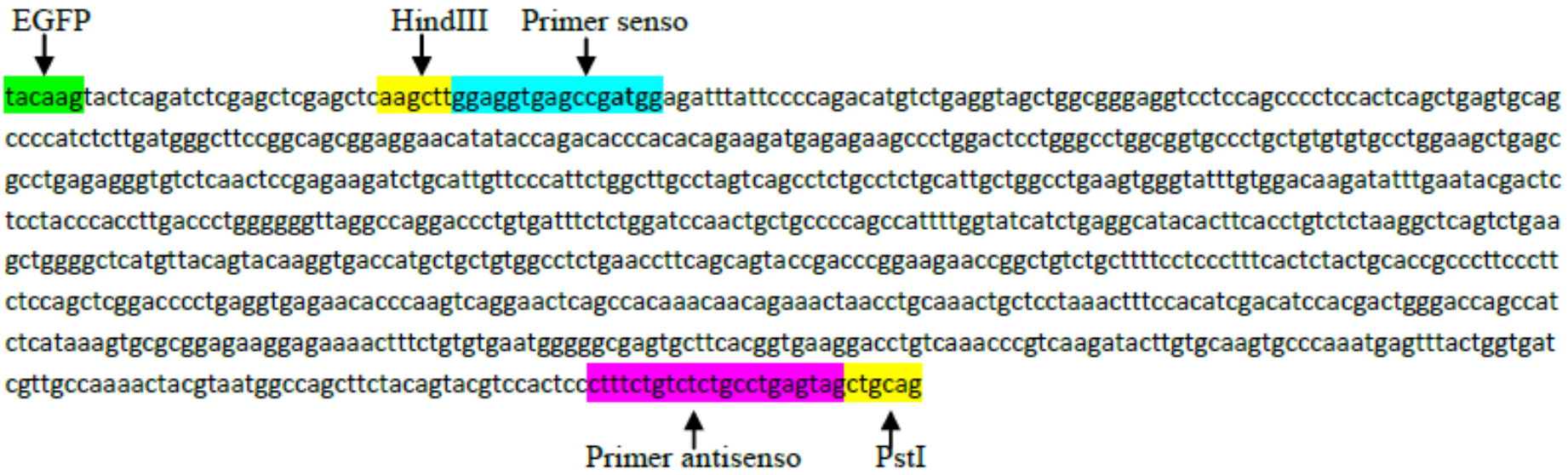
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PstI

pEGFP-C

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CTGCAG=PstI

pEGFP-C

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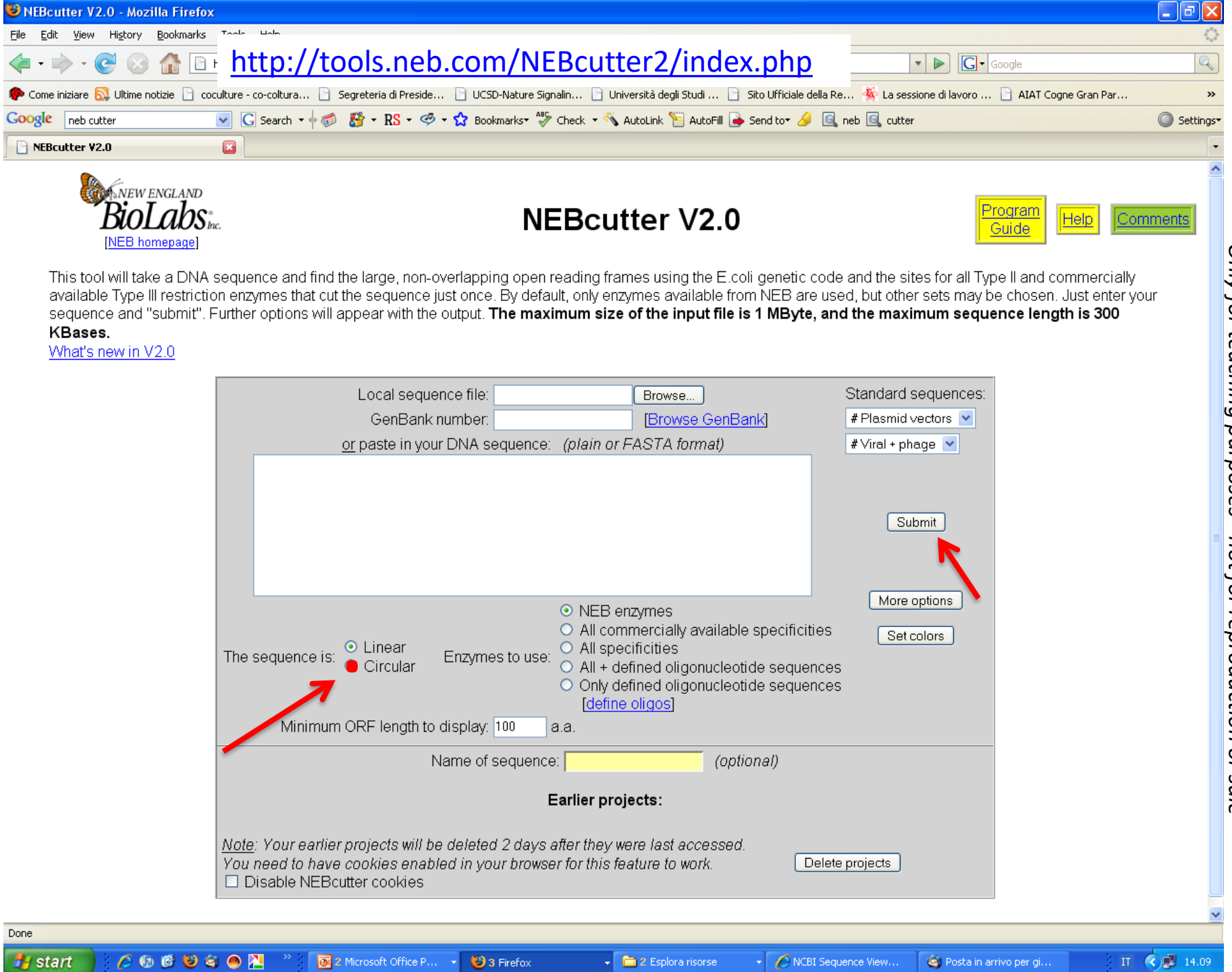
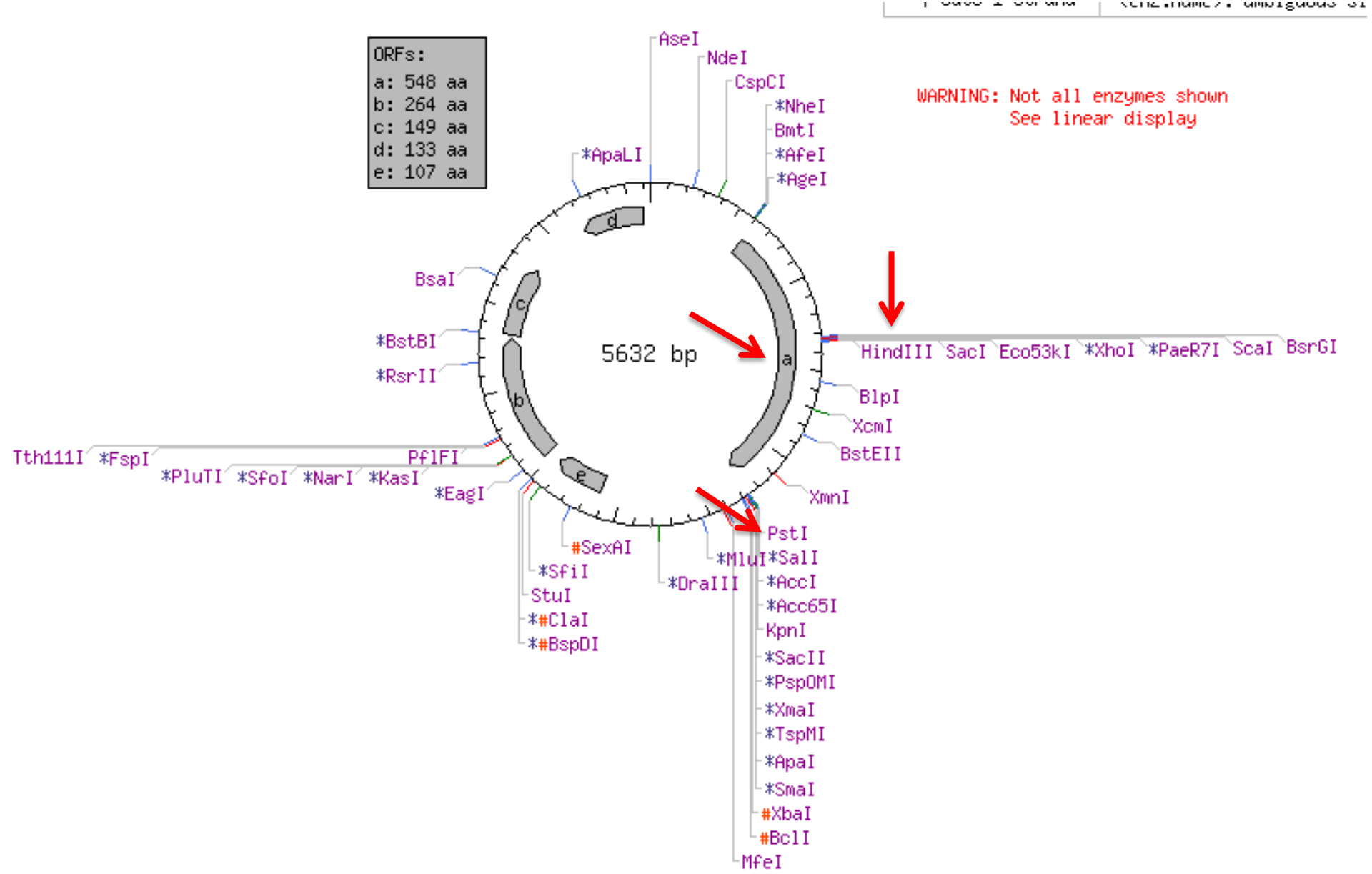
AAGCTT=HindIII CTGCAG=PstI

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NEW ENGLAND BioLabs Inc.
NEBcutter

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ORF Sequence

unnamed sequence

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ORFs:
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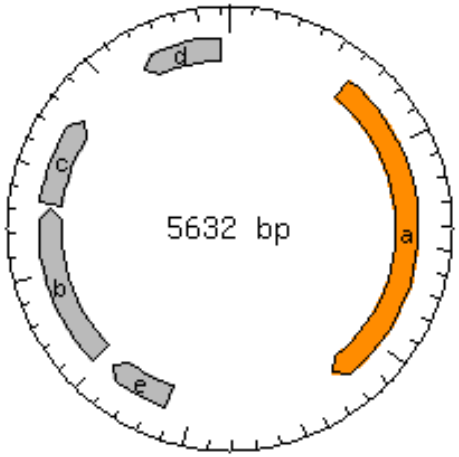
b: 264 aa
c: 149 aa
d: 133 aa
e: 107 aa



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EGFP

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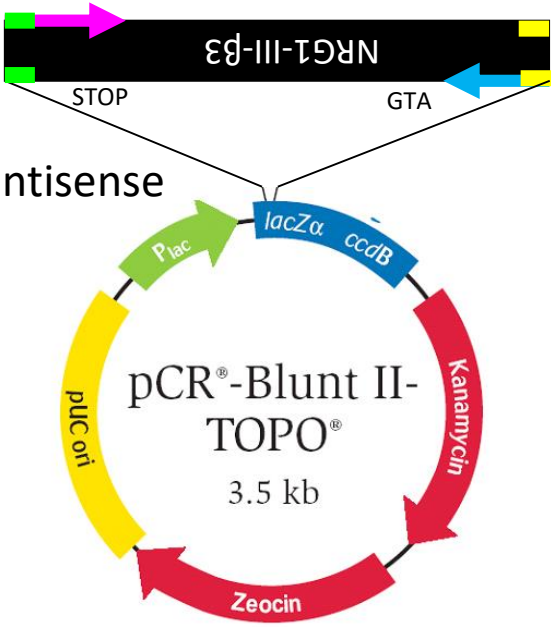
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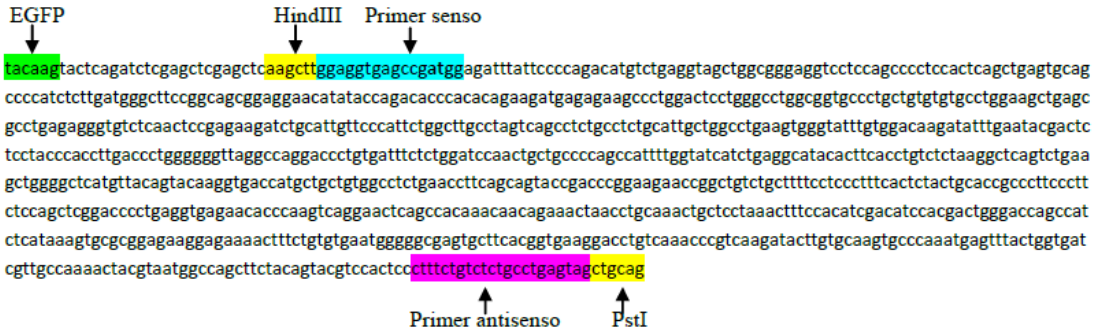
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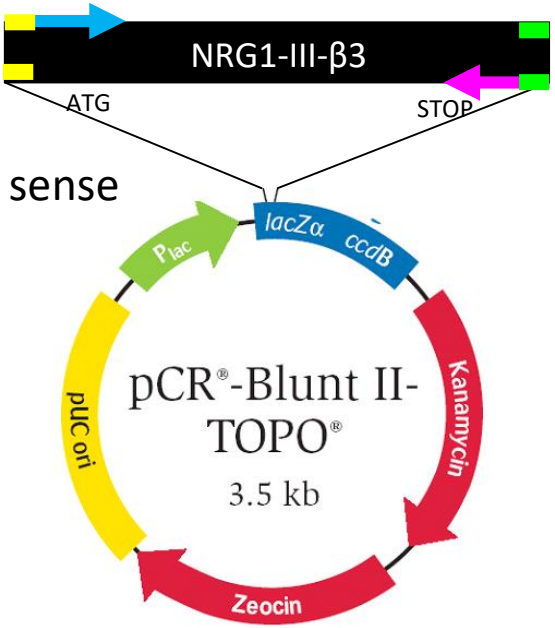
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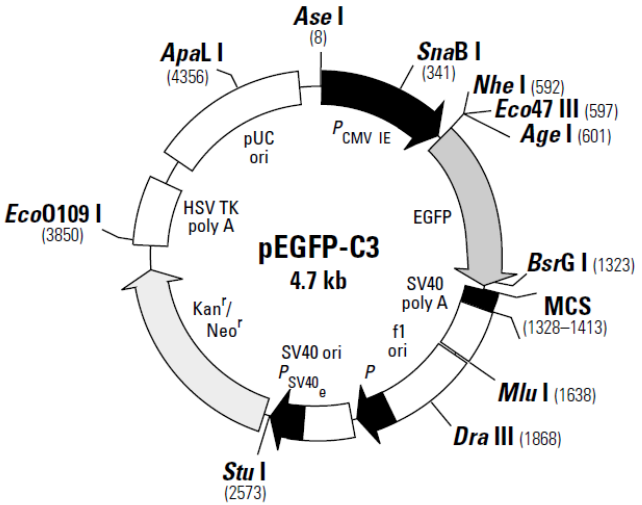
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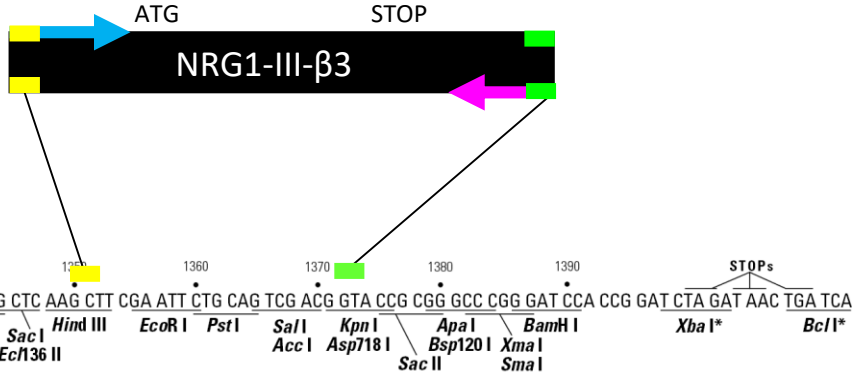
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BamH I
STOPs
Xba I*
Bcl I*

RNeasy® Mini

RNeasy Mini Kit

For purification of total RNA from animal cells, animal tissues, bacteria, and yeast, and for RNA cleanup

RNA extraction

by spectrofotometer

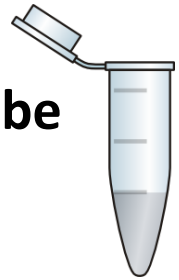
by Nanodrop

RNA quantification

• RNA extraction . After trizol extraction, RNA is resuspended in 20 µl H_2O

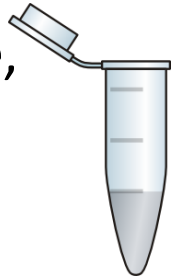
• Evaluation of RNA concentration at the spectrophotometer:
- you read **2 µl** in **1ml** H_2O (RNA diluted 1: ?) in a quartz cuvette
- reading at the spectrophotometer = **0.05 OD** (optical density)
- conversion factor: **1OD = 40µg RNA/ml**

Evaluate the concentration of RNA **in your original tube** by expressing it in **µg/µl**:



How many **µl** should I take to get **1 µg**?

How many **µg** do you have **in your original tube**, after concentration reading?



	stock	1 sample		final concentration
RNA	0.1 µg/ µl	µl		1 µg/reaction
Buffer	5x	µl		1x
BSA	1 µg/µl	µl		0.1 µg/ µl
triton	1%	µl		0.05%
dNTPs	10mM	µl		500 µM
random primers	50µM	µl		5 µM
RT	100u/µl	µl		200u/reaction
RNAsin	33u/µl	µl		33u/reaction
Water to 25µl		µl		
Tot		25 µl		

$C_sV_s=C_fV_f$

Concentration of the stock			Final concentration
NaCl	5M		0,5 M

Total volume 25 µl

C_s=stock concentration
V_s=stock volume
C_f=final concentration
Vf=final volume

$C_sV_s=C_fV_f$

$V_s=C_fV_f/C_s$

$V_s=0,5*\ 25/5=2,5$

DILUTION FACTOR

Concentration of the stock				Final concentration
NaCl	5M			0,5 M

Total volume 25 µl

How many fold do I have to dilute this ingredient?

DILUTION FACTOR

Concentration of the stock			Final concentration
NaCl	5M		0,5 M

Total volume 25 µl

How many fold do I have to dilute this ingredient?

5 : 0,5 = 10 fold = dilution factor

The final volume is 25 µl, how many µl do I have to use?

I have to dilute 10 fold this ingredient in the final volume of the solution
I will have to add 1/10 of the final volume

25 µl : 10 = 2,5 µl

DILUTION FACTOR

Concentration of the stock			Final concentration
NaCl	5M		0,5 M

Total volume 25 µl

How many fold do I have to dilute this ingredient?

C_s=stock concentration
V_s=stock volume
C_f=final concentration
Vf=final volume

Dilution factor = C_s/C_f = 5/0,5 = 10

V_s= V_f/dilution factor = 25/10 = 2,5

DILUTION FACTOR

Concentration of the stock			Final concentration
NaCl	5M		0,5 M

Total volume 25 µl

How many fold do I have to dilute this ingredient?

C_s=stock concentration
V_s=stock volume
C_f=final concentration
Vf=final volume

Dilution factor = C_s/C_f = 5/0,5 = 10

V_s= V_f/dilution factor = 25/10 = 2,5

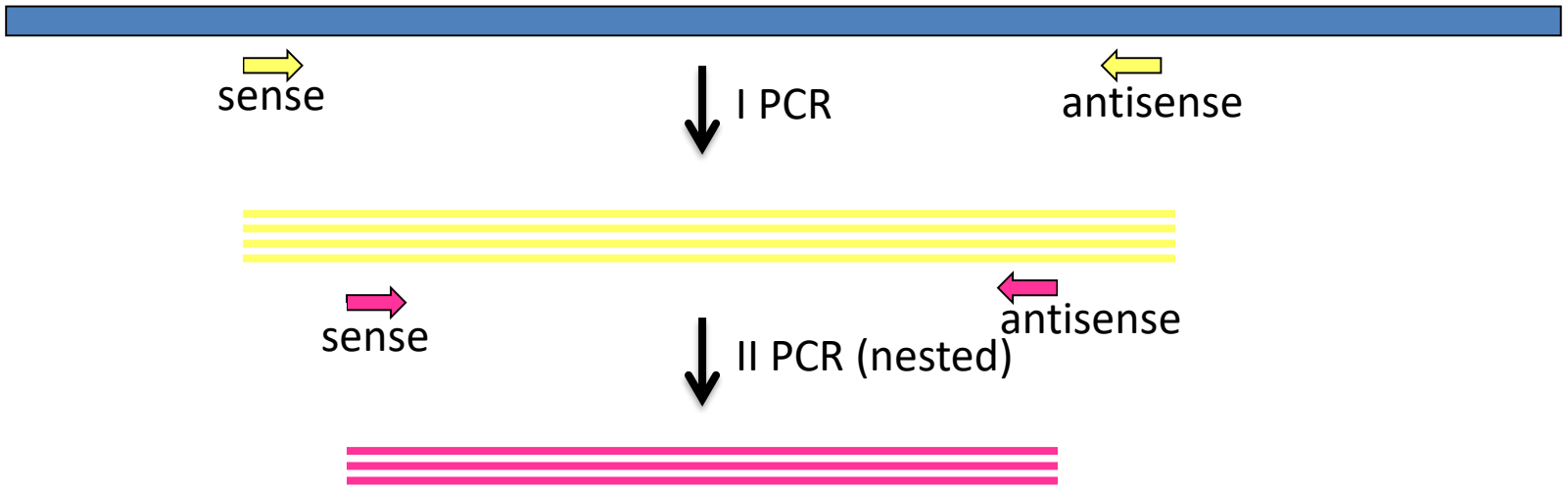
$V_s=V_f/(C_s/C_f)$

$V_s=V_fC_f/C_s$ = 25*0,5/5 = 2,5

	stock	1 sample		final concentration
RNA	0.1 µg/ µl	µl		1 µg/reaction
Buffer	5x	µl		1x
BSA	1 µg/µl	µl		0.1 µg/ µl
triton	1%	µl		0.05%
dNTPs	10mM	µl		500 µM
random primers	50µM	µl		5 µM
RT	100u/µl	µl		200u/reaction
RNAsin	33u/µl	µl		33u/reaction
Water to 25µl		µl		
Tot		25 µl		

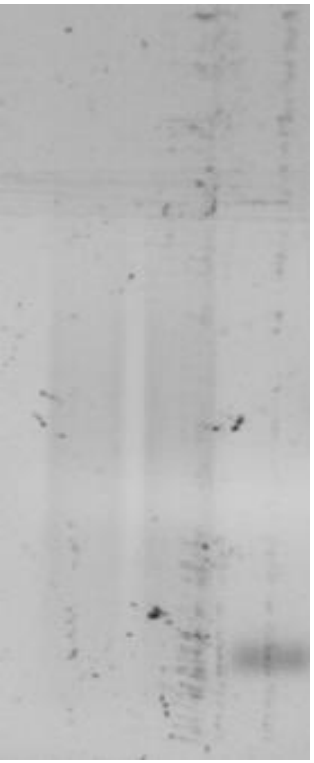
PCR reaction

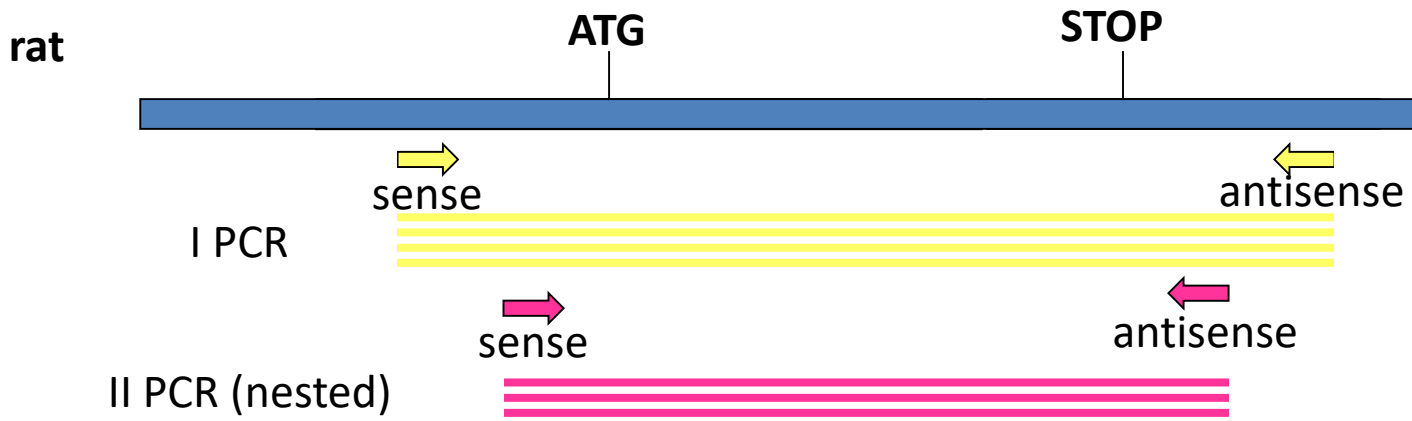
STOCK	RT+	RT -	final conc
cDNA	5 µl		
5x buffer Taq	µl		-> 1x
sense primer 10 µM	µl		-> 250 nM
antisense primer 10 µM	µl		-> 250 nM
100% glycerol	µl		-> 5%
10mM dNTPs	µl		-> 100 µM
Taq polimerase 1u/ul	µl		-> 1u
H_2O	µl		
total	50 µl		

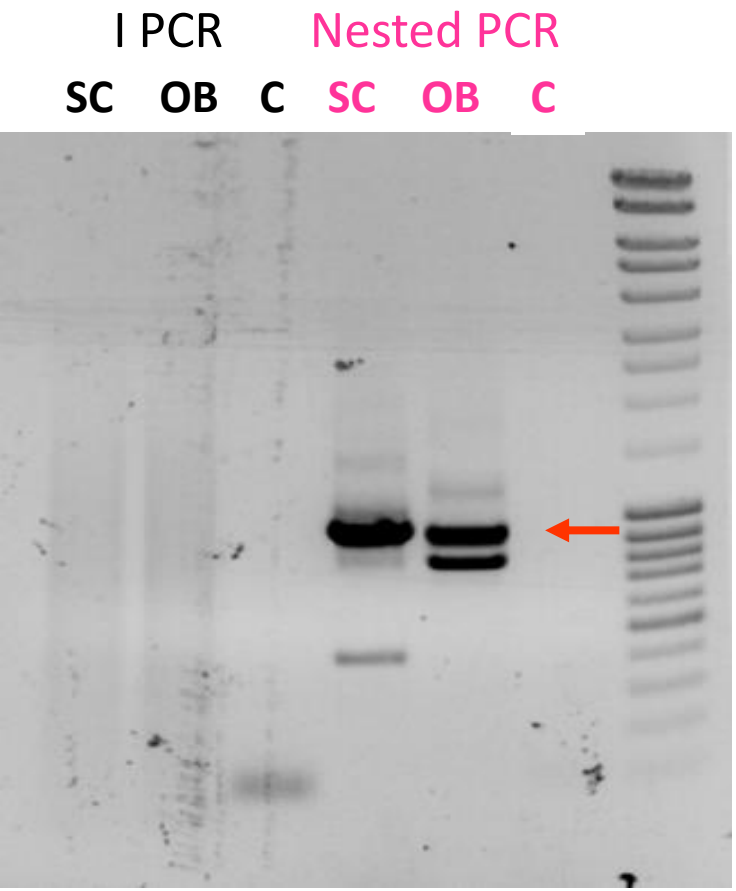


I PCR

SC OB C

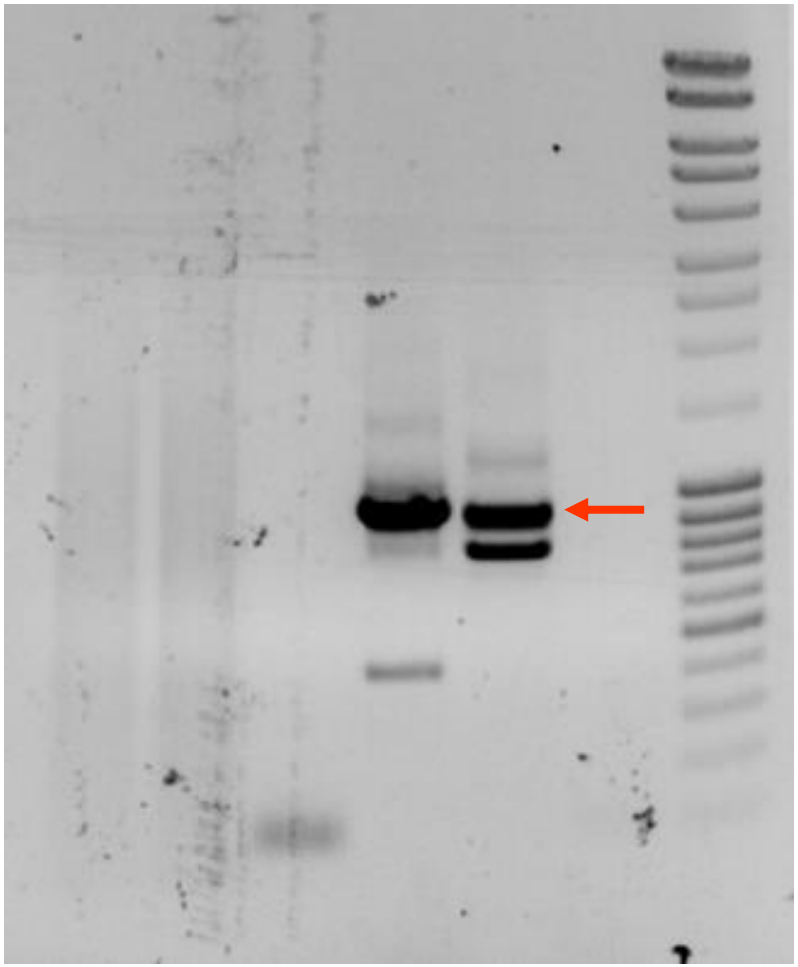






I PCR Nested PCR

SC OB C SC OB C



* cut with a blade the bands from the agarose gel

* elute DNA with a kit that allows to dissolve agarose

* use the obtained DNA for ligation

SC=Schwann cells
OB=olfactory bulb
C=control (PCR mix)

PASSAGES FROM THE LIGASE TO THE SEQUENCE

* ligase
* transformation of bacteria with ligase
* plating bacteria on petri dishes with antibiotic
* growth over-night at 37°C
* miniprep of plasmid DNA using a kit
* digestion to determine if the insert is present

* digestion to determine the orientation of the insert
    - which bands do you obtain if the orientation is "sense”?
    - which bands do you obtain if the orientation is "antisense"?

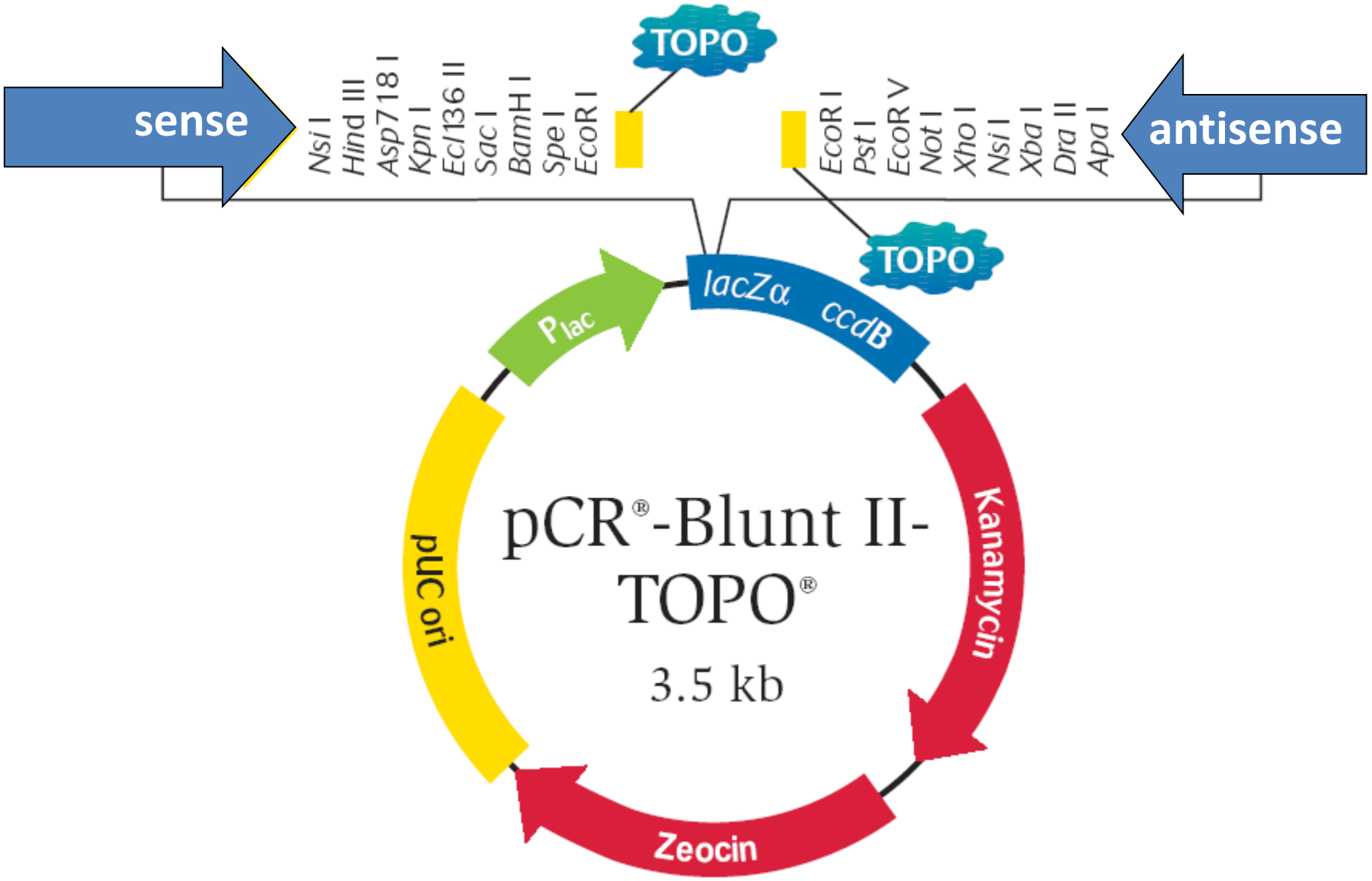
*sequencing using a primer upstream and a primer downstream of the insert

PASSAGES FROM THE LIGASE TO THE SEQUENCE

- ligase
- transformation of bacteria with ligase
- plating bacteria on petri dishes with antibiotic: which one?
- growth over-night at 37°C
- miniprep of plasmid DNA using a kit
- digestion to determine if the insert is present

- digestion to determine the orientation of the insert
  - which bands do you obtain if the orientation is "sense"?
  - which bands do you obtain if the orientation is "antisense"?

- sequensing using a primer upstream and a primer downstream of the insert

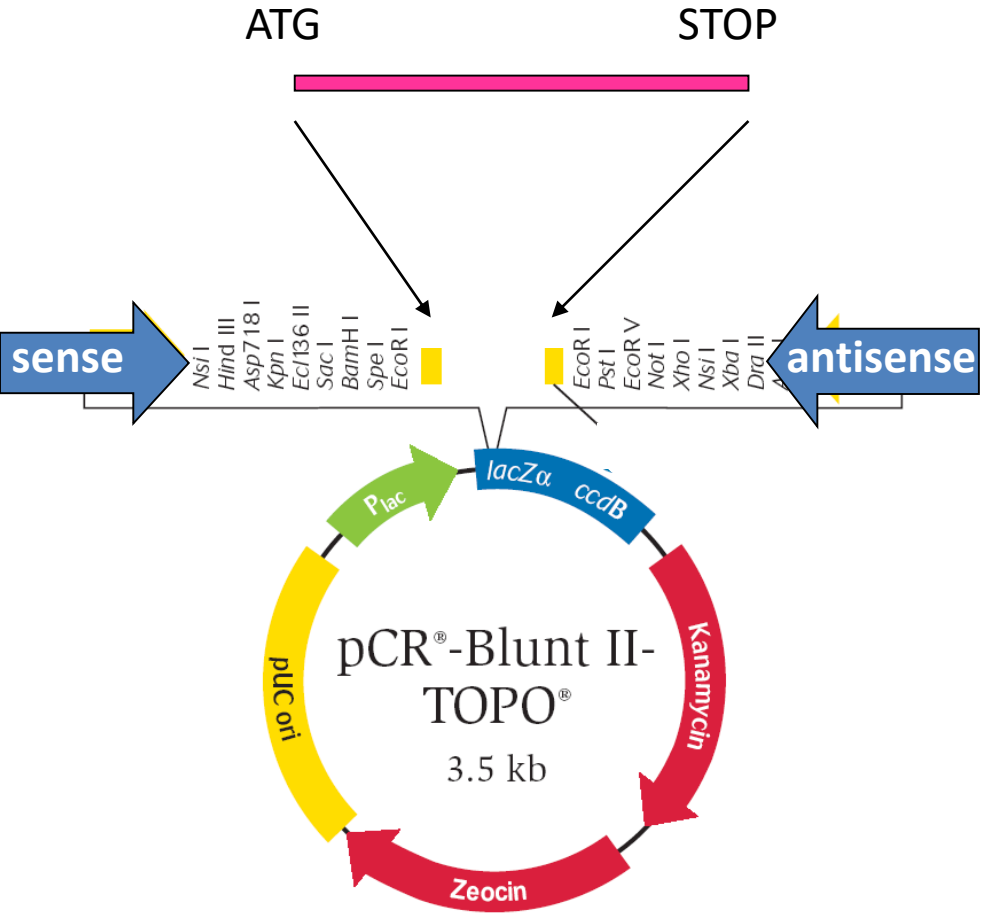


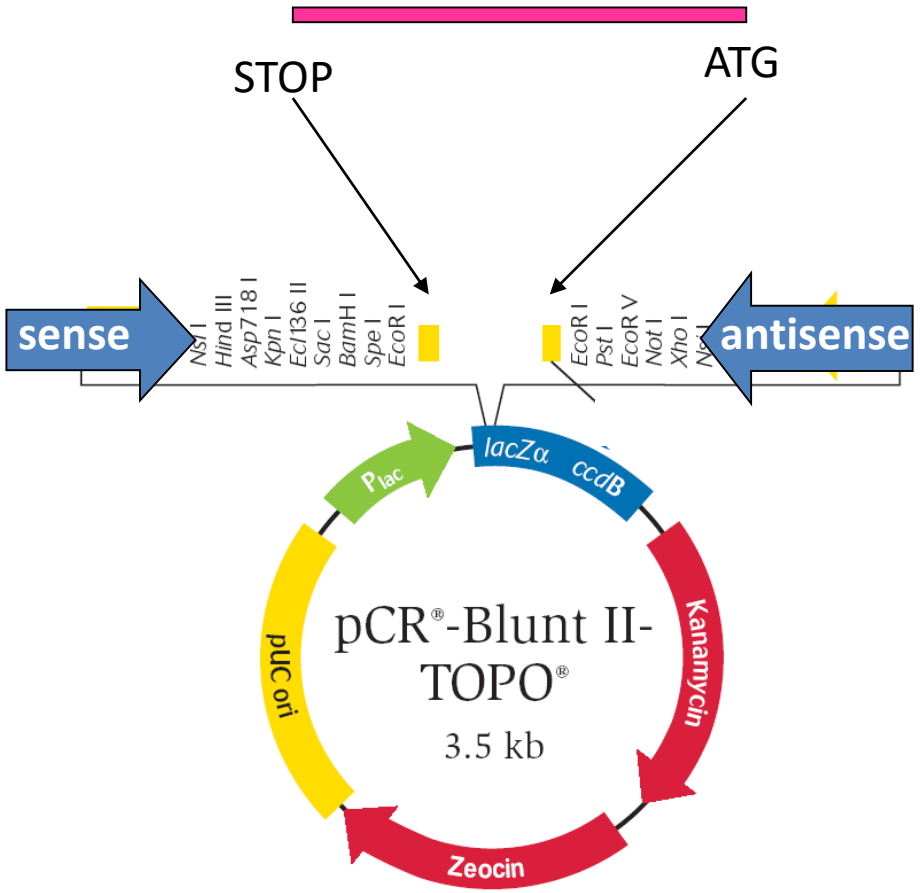
http://www.invitrogen.com/

Cloning vector for the RT-PCR amplification product

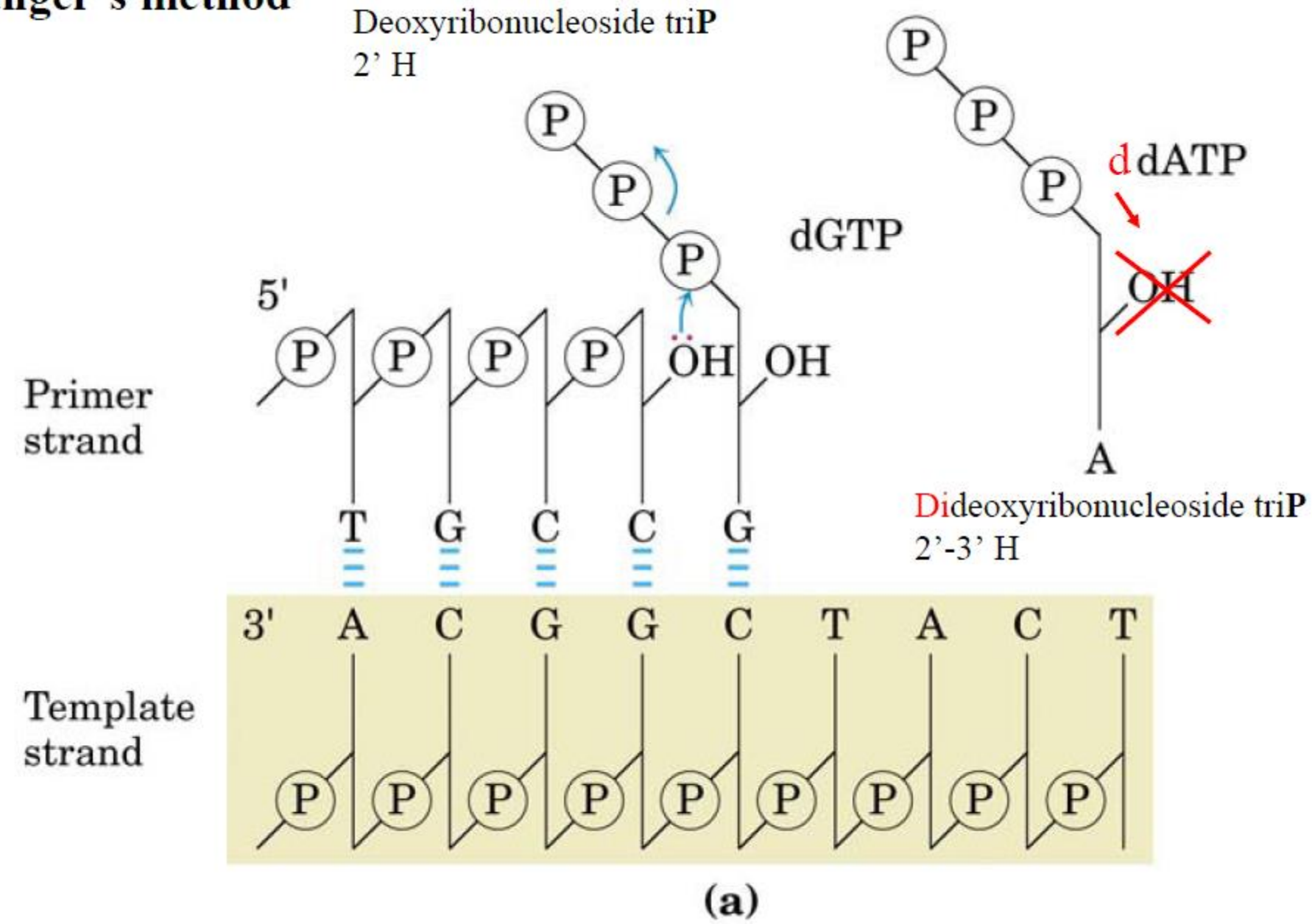
5′-**ATG** ——— TAG-3′
3′-TAC ——— ATC-5′

5′-CTA ——— CAT-3′
3′-GAT ——— **GTA**-5′

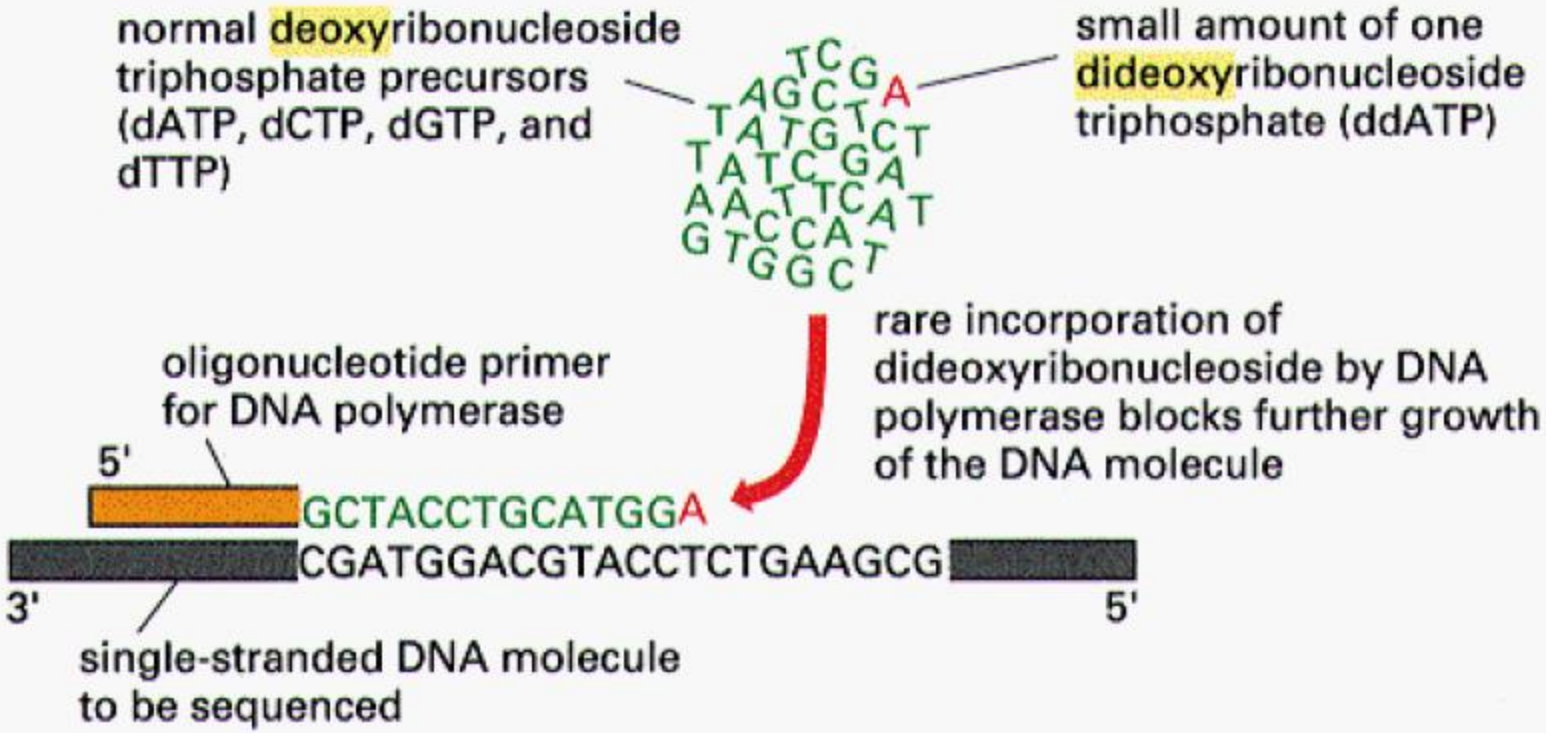


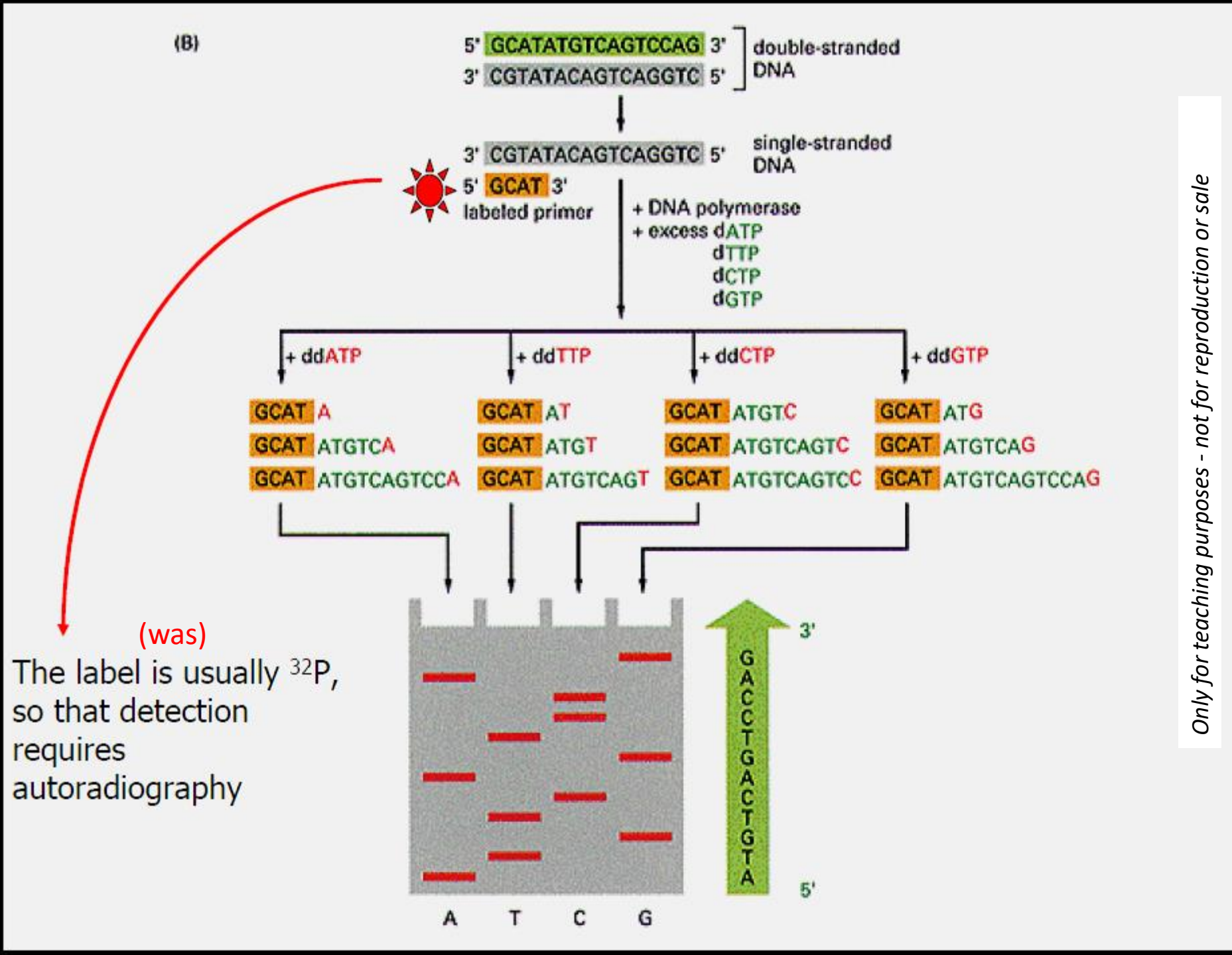


Sanger's method



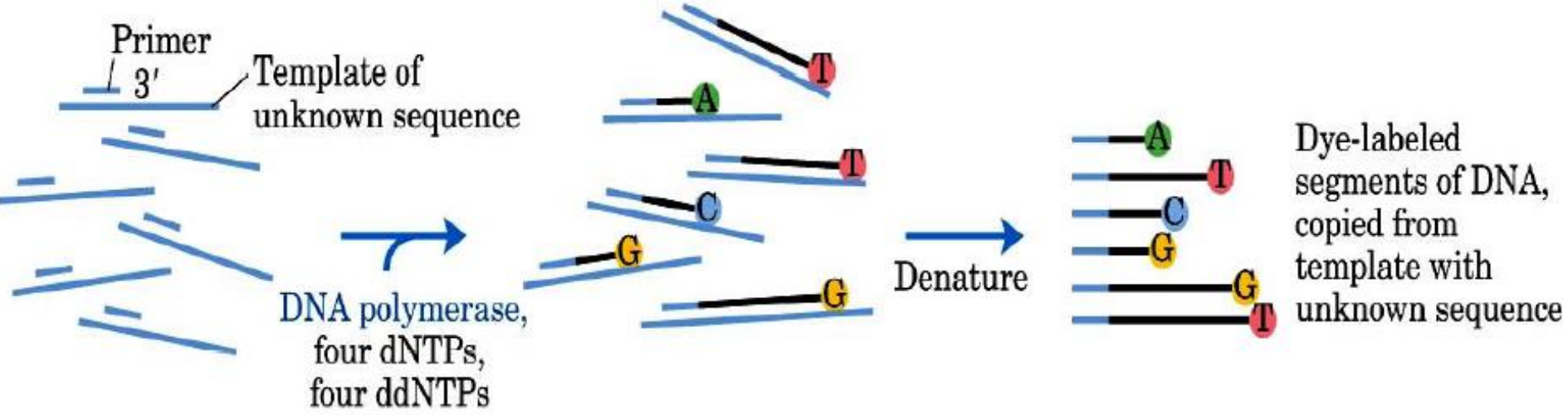
(A)

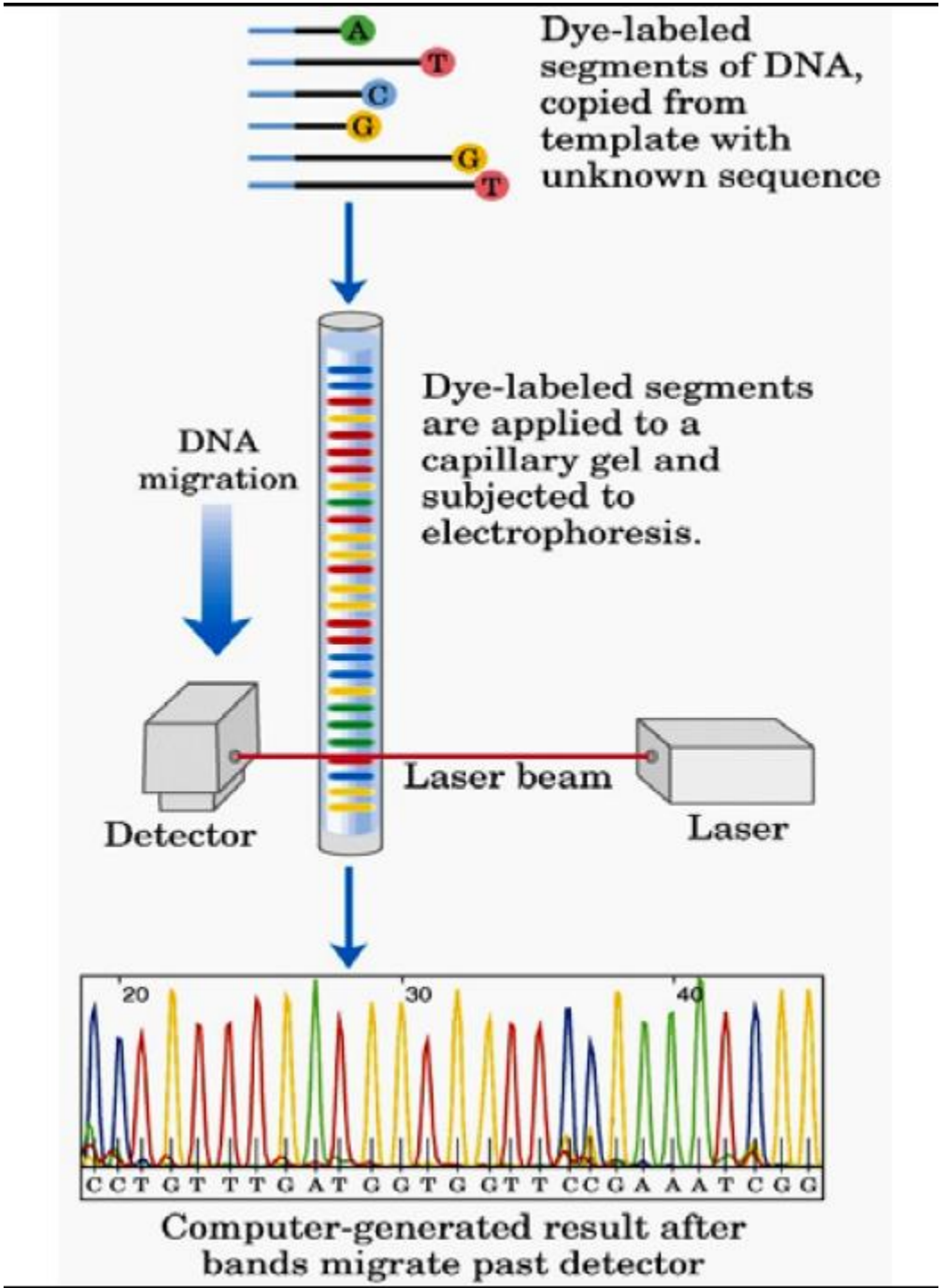


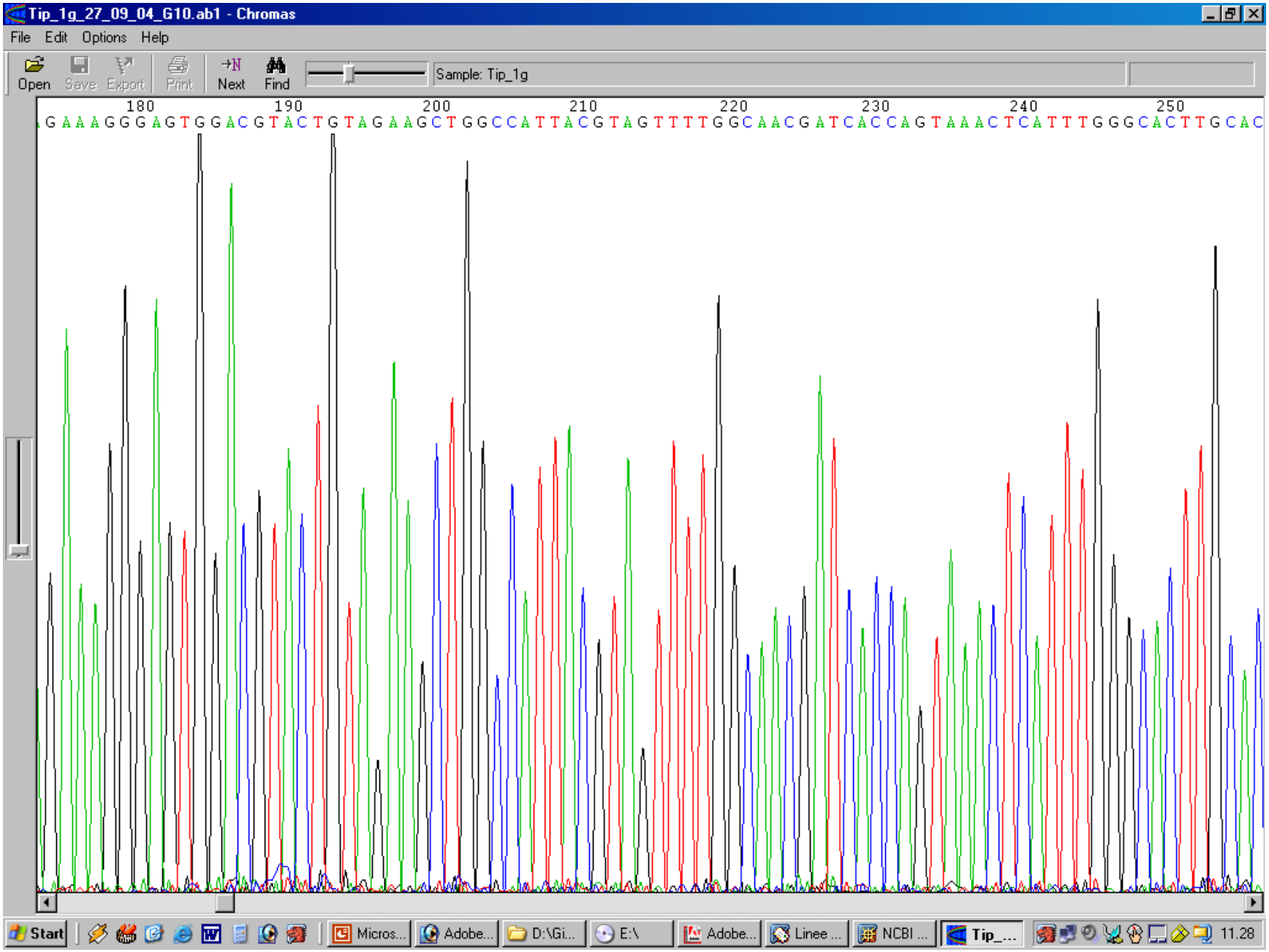


(was)

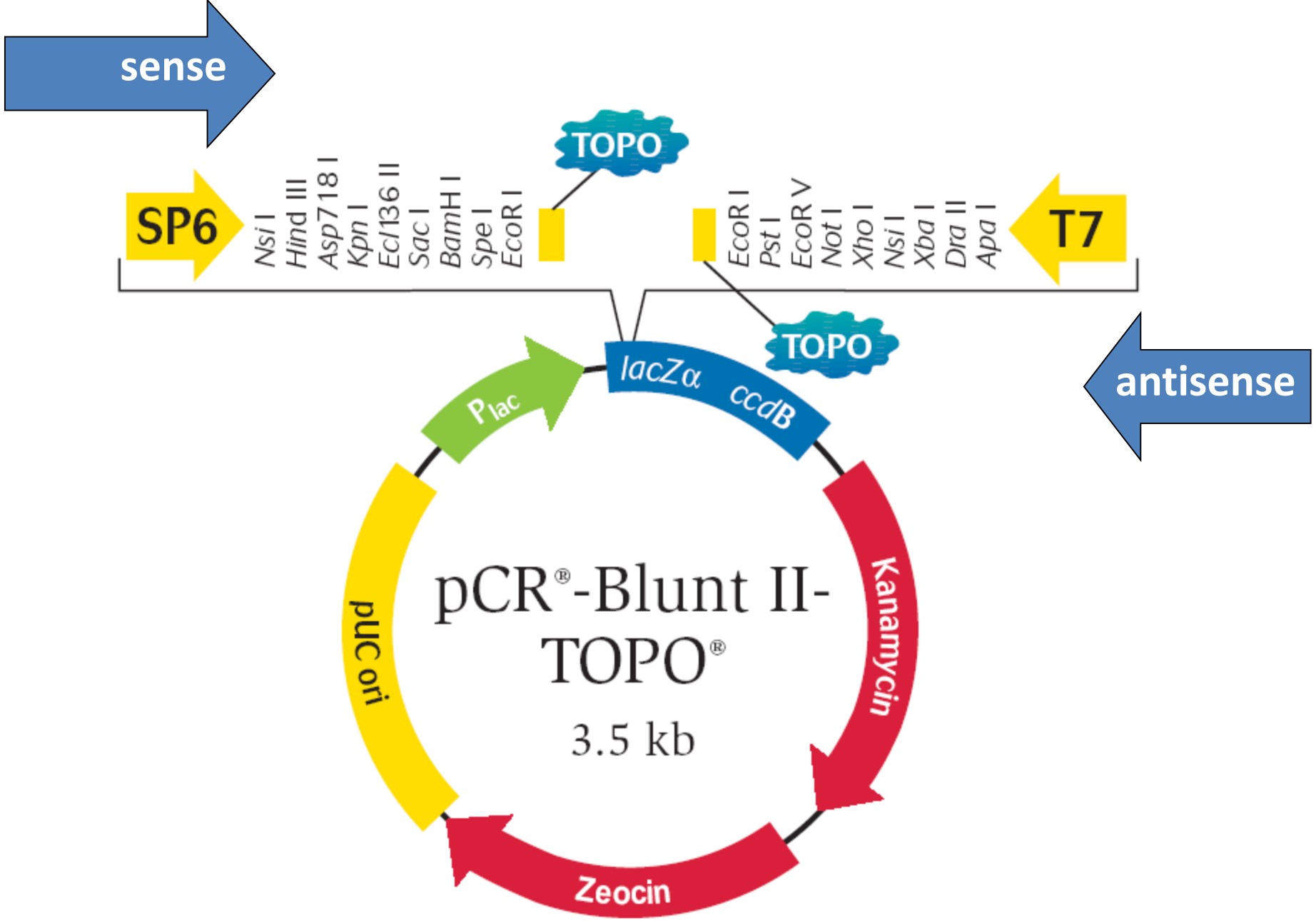
The label is usually ^{32}P, so that detection requires autoradiography





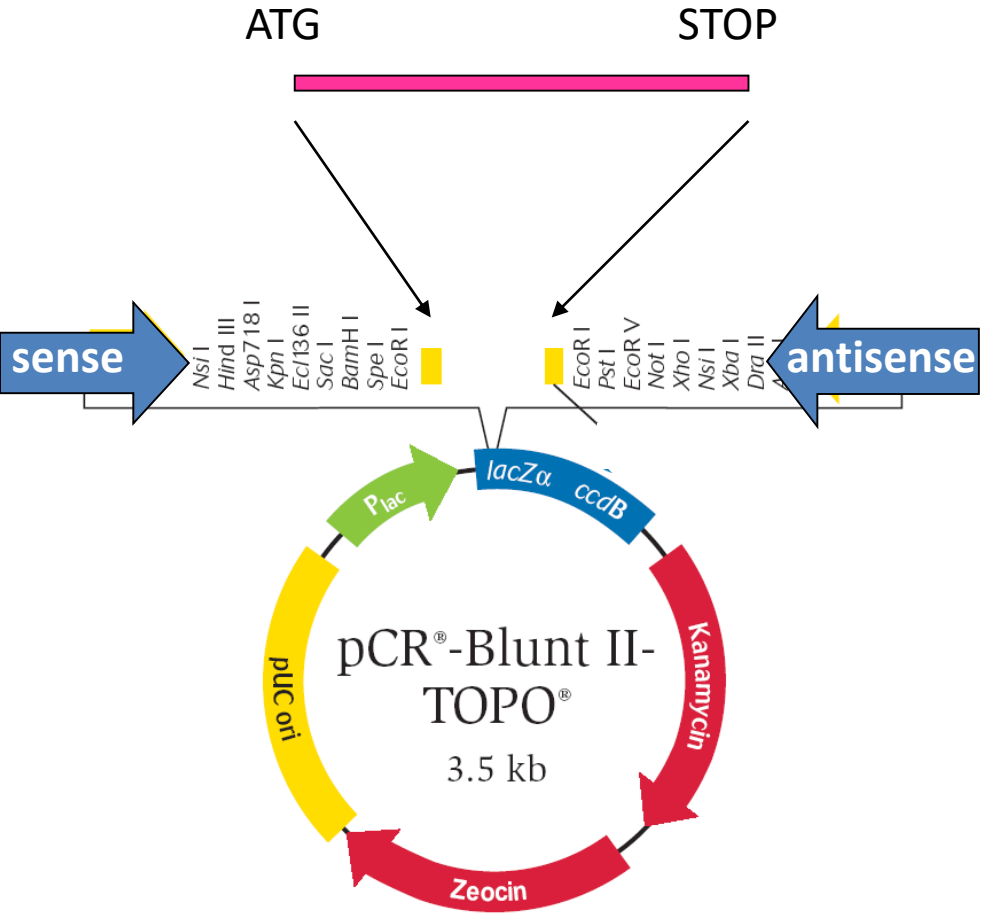


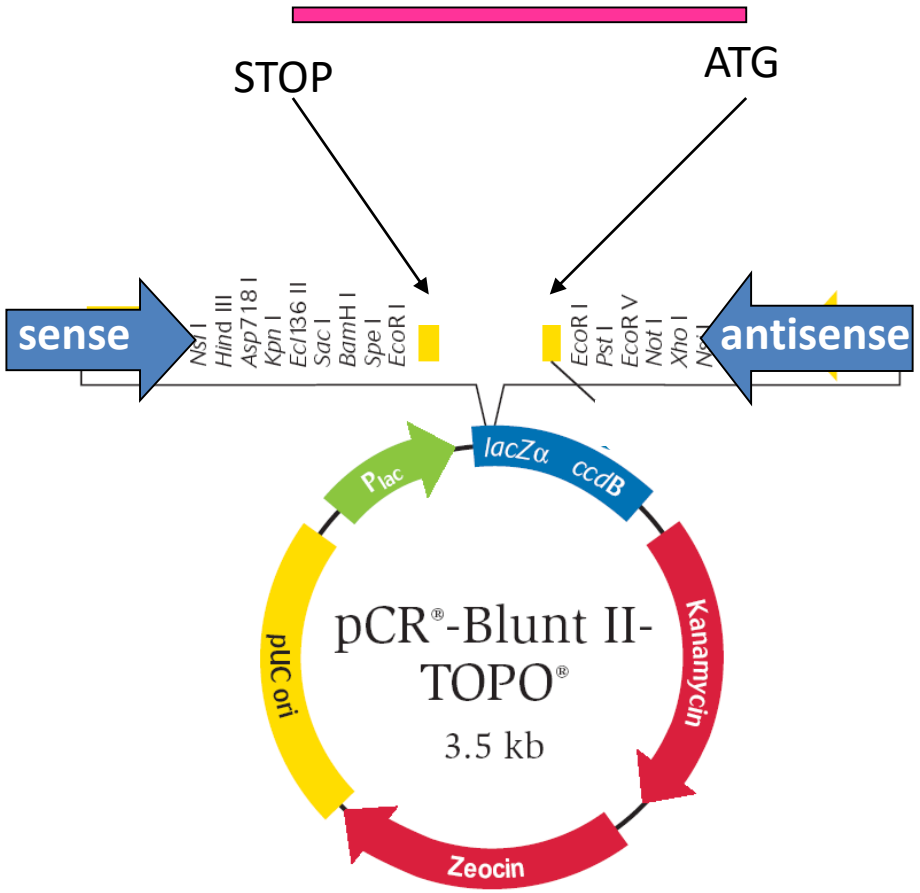
Sequencing of rat-NRG1-typeIIIβ3

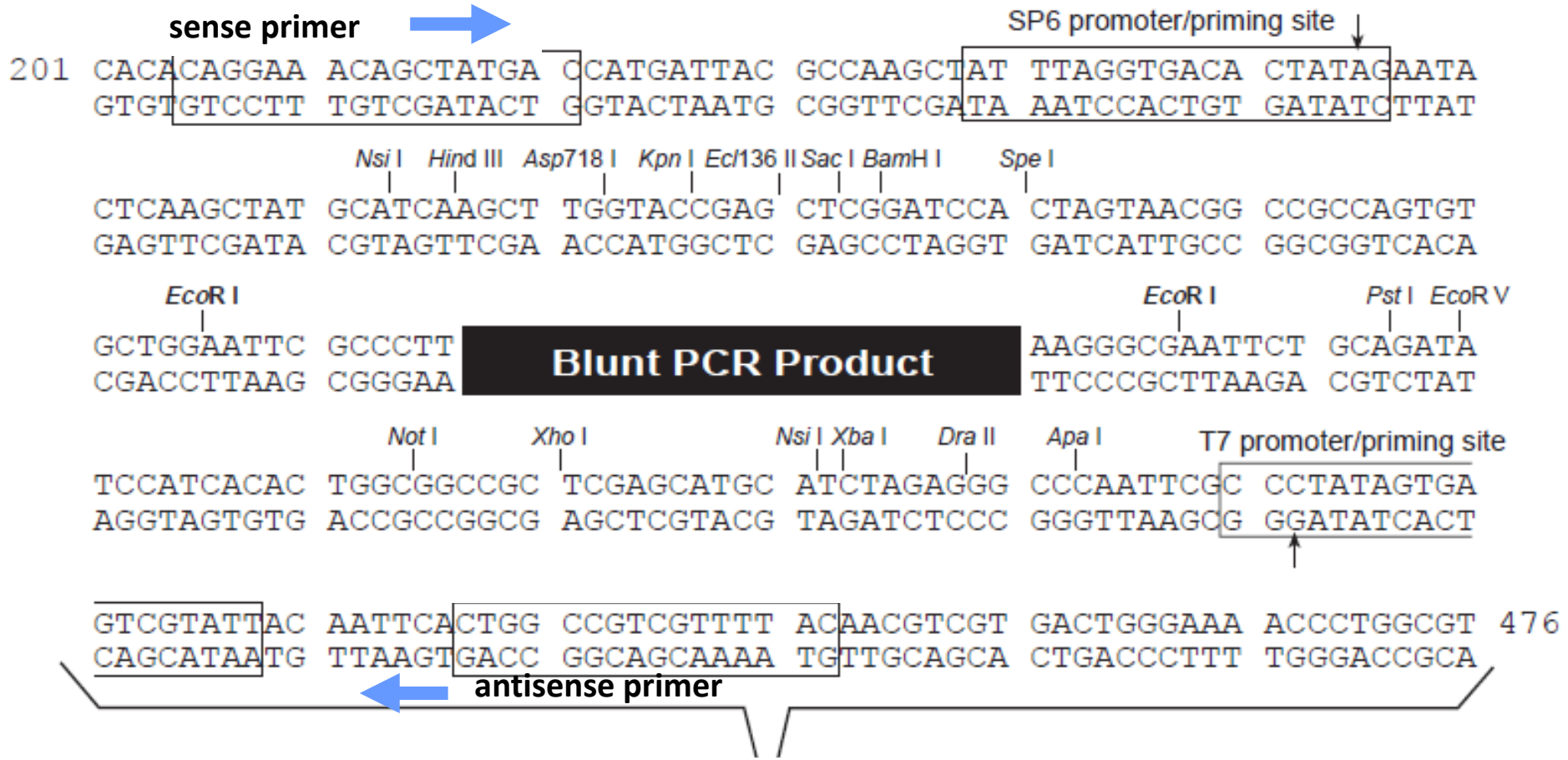


5′-**ATG** ——— TAG-3′
3′-TAC ——— ATC-5′

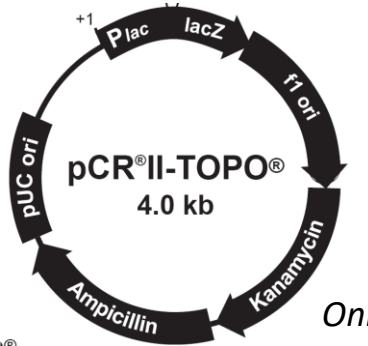
5′-CTA ——— CAT-3′
3′-GAT ——— **GTA**-5′



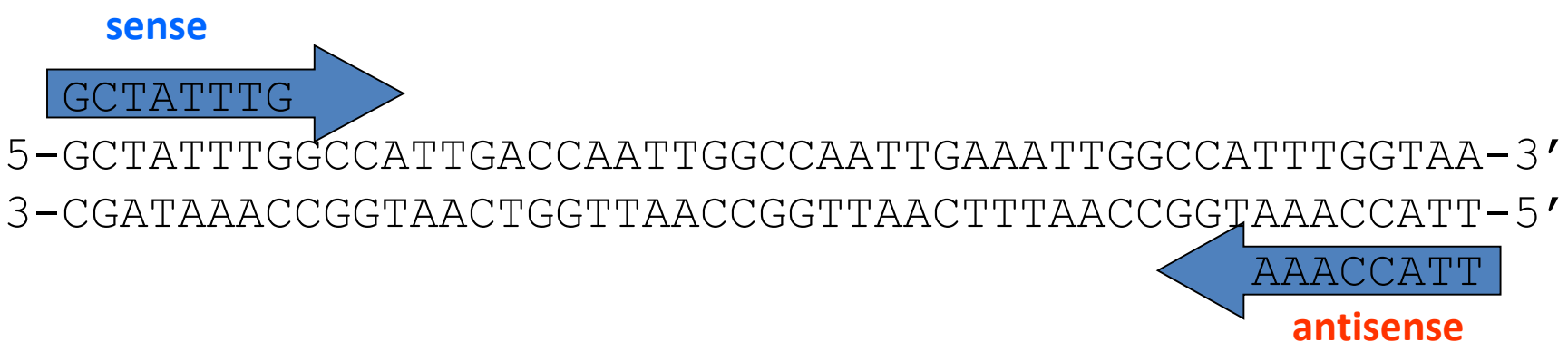


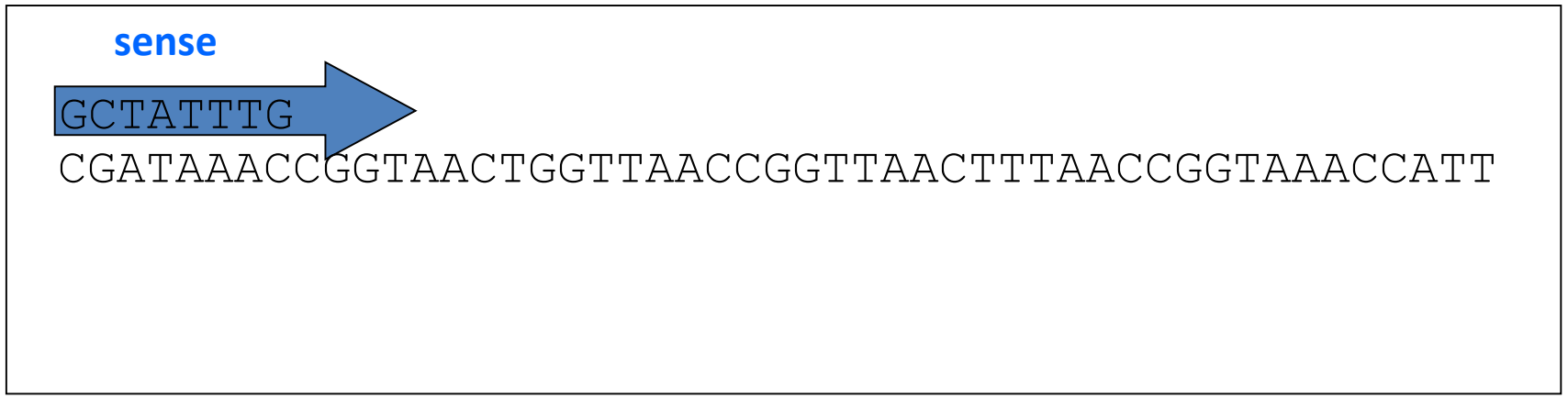


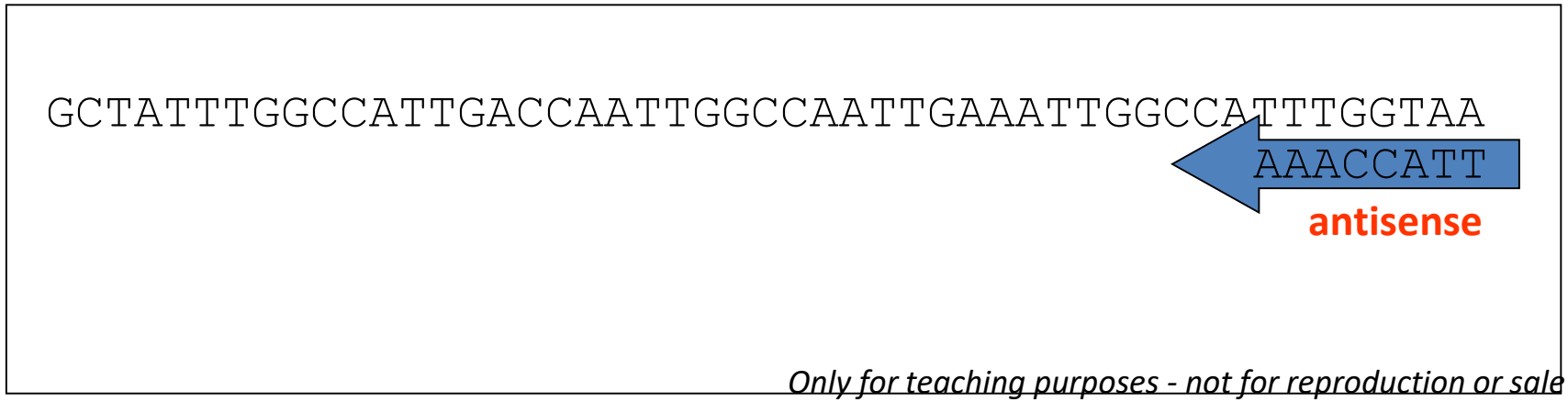
sense primer:
5-CAGGAAACAGCTATGAC-3′

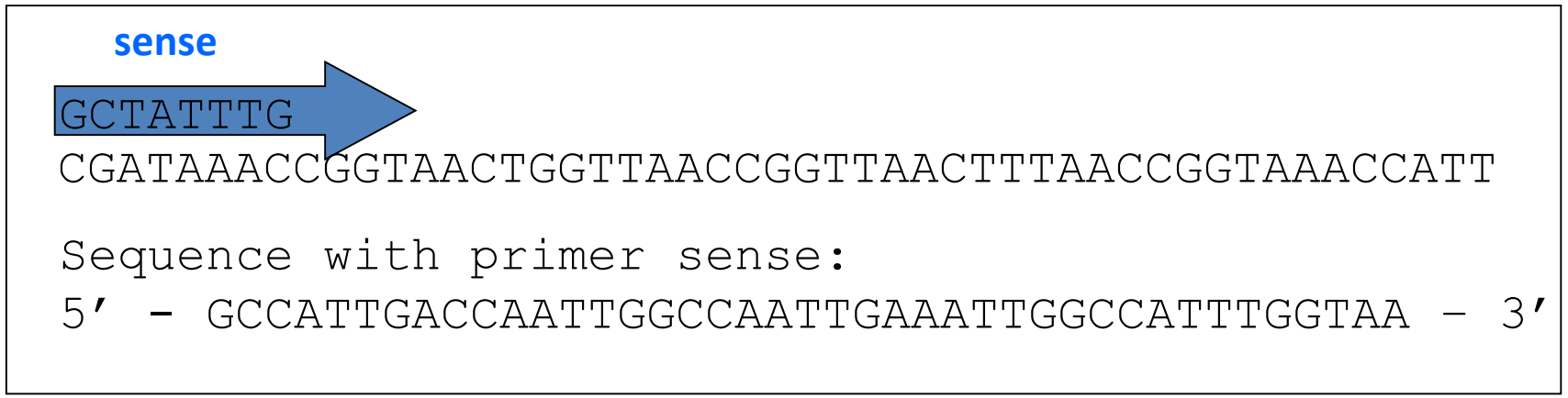


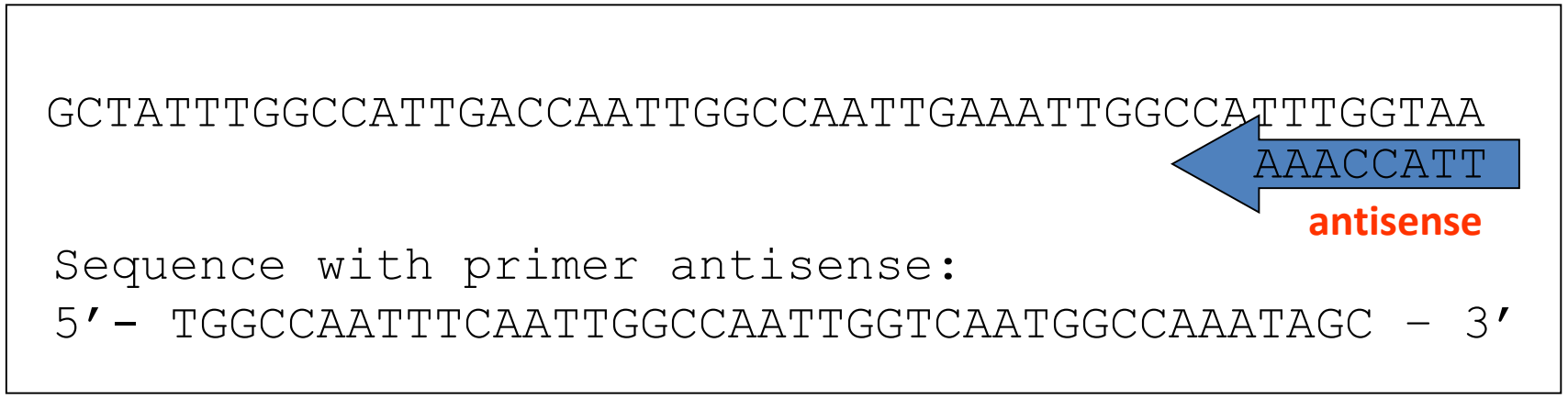
antisense primer:
5′-GTAAAACGACGGCCAG-3′







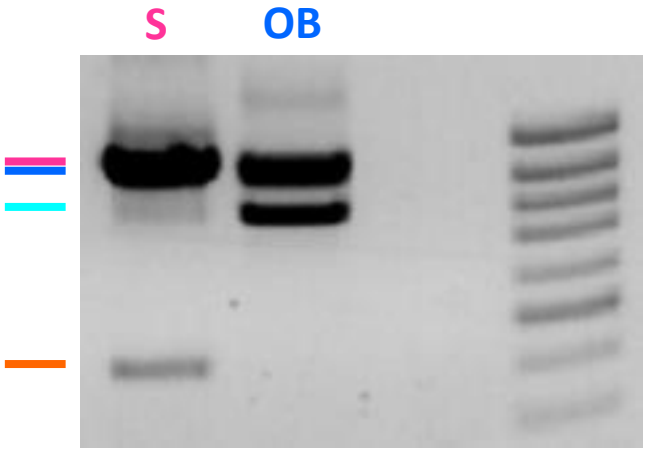




sequence **electroferograms**

 # primer

1a sense	olfactory bulb low molecular weight	1a
1b antisense		1b
1c sense	olfactory bulb high molecular weight	1c
1d antisense		1d
1e sense	Schwann cells low molecular weight	1e
1f antisense		1f
1g sense	Schwann cells high molecular weight	1g
1h antisense		1h



* please, analyze the sequences to verify the correctness of the cloning and to understand the orientation of the insert

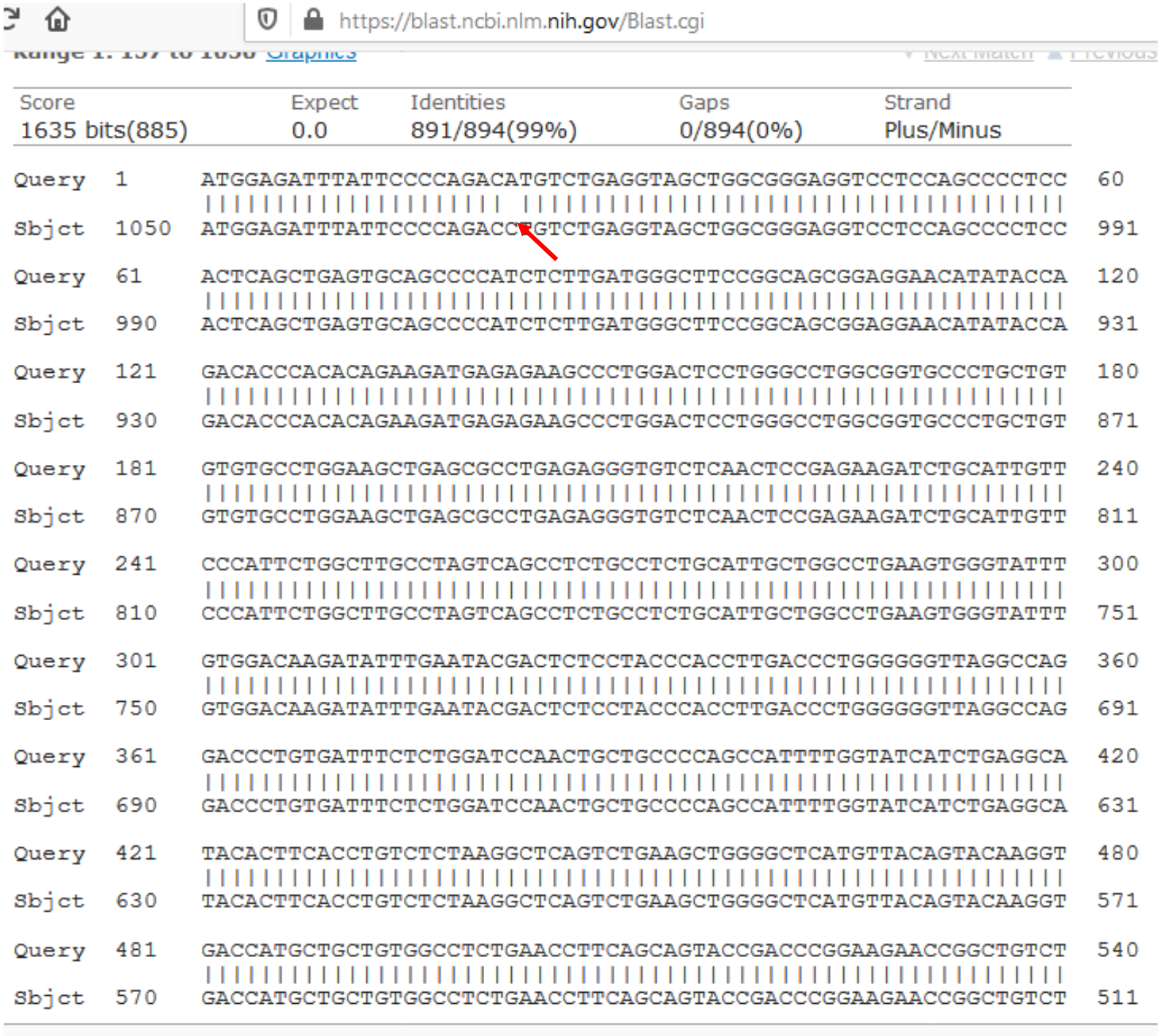
* open sequences.seq with the program notepad, copy them and compare them to the rat NRG1-typeIII-beta3 sequence
(use the BLAST program “align two sequences”)

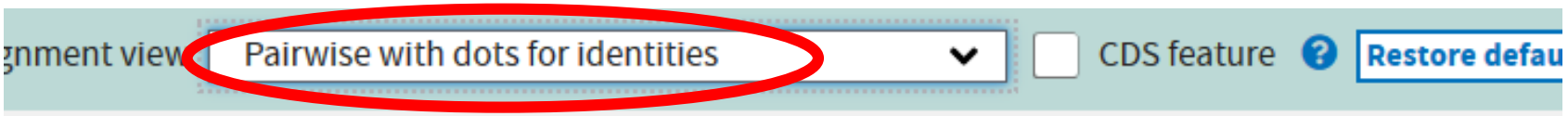
* as **Query** you can use the rat **NRG1** sequence **from ATG to STOP**

* as **Sbjct** use **sequences.seq** that you want to analyze (**c** or **d**)

*write down the coordinates of the mutations on the expected theoretical sequence (Query) and on the Sbjct sequence to be able to compare - for each mutation – sense and antisense sequences

* don’t worry about possible gaps





equences selected

Download Graphics

Sequence ID: Query_1525 Length: 1282 Number of Matches: 1

Range 1: 157 to 1050 Graphics ▼ Next Match ▲ Previous

Score	Expect	Identities	Gaps	Strand
1635 bits(885)	0.0	891/894(99%)	0/894(0%)	Plus/Minus

```
Query  1     ATGGAGATTTATTCCCCAGACATGTCTGAGGTAGCTGGCGGGAGGTCCTCCAGCCCCTCC  60
Sbjct  1050  ....................C.....................................  991

Query  61    ACTCAGCTGAGTGCAGCCCCATCTCTTGATGGGCTTCCGGCAGCGGAGGAACATATACCA  120
Sbjct  990   ............................................................  931

Query  121   GACACCCACACAGAAGATGAGAGAAGCCCTGGACTCCTGGGCCTGGCGGTGCCCTGCTGT  180
Sbjct  930   ............................................................  871

Query  181   GTGTGCCTGGAAGCTGAGCGCCTGAGAGGGTGTCTCAACTCCGAGAAGATCTGCATTGTT  240
Sbjct  870   ............................................................  811

Query  241   CCCATTCTGGCTTGCCTAGTCAGCCTCTGCCTCTGCATTGCTGGCCTGAAGTGGGTATTT  300
Sbjct  810   ............................................................  751

Query  301   GTGGACAAGATATTTGAATACGACTCTCCTACCCACCTTGACCCTGGGGGGTTAGGCCAG  360
Sbjct  750   ............................................................  691

Query  361   GACCCTGTGATTTCTCTGGATCCAACTGCTGCCCCAGCCATTTTGGTATCATCTGAGGCA  420
Sbjct  690   ............................................................  631

Query  421   TACACTTCACCTGTCTCTAAGGCTCAGTCTGAAGCTGGGGCTCATGTTACAGTACAAGGT  480
```

https://blast.ncbi.nlm.nih.gov/Blast.cgi

Range 1: 157 to 1050 Graphics

Score	Expect	Identities	Gaps	Strand
1635 bits(885)	0.0	891/894(99%)	0/894(0%)	Plus/Minus

```
Query  1     ATGGAGATTTATTCCCCAGACATGTCTGAGGTAGCTGGCGGGAGGTCCTCCAGCCCCTCC  60
Sbjct  1050  ......................C.......................................  991

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Sbjct  990   ............................................................  931

Query  121   GACACCCACACAGAAGATGAGAGAAGCCCTGGACTCCTGGGCCTGGCGGTGCCCTGCTGT  180
Sbjct  930   ............................................................  871

Query  181   GTGTGCCTGGAAGCTGAGCGCCTGAGAGGGTGTCTCAACTCCGAGAAGATCTGCATTGTT  240
Sbjct  870   ............................................................  811

Query  241   CCCATTCTGGCTTGCCTAGTCAGCCTCTGCCTCTGCATTGCTGGCCTGAAGTGGGTATTT  300
Sbjct  810   ............................................................  751

Query  301   GTGGACAAGATATTTGAATACGACTCTCCTACCCACCTTGACCCTGGGGGGTTAGGCCAG  360
Sbjct  750   ............................................................  691

Query  361   GACCCTGTGATTTCTCTGGATCCAACTGCTGCCCCAGCCATTTTGGTATCATCTGAGGCA  420
Sbjct  690   ............................................................  631

Query  421   TACACTTCACCTGTCTCTAAGGCTCAGTCTGAAGCTGGGGCTCATGTTACAGTACAAGGT  480
Sbjct  630   ............................................................  571

Query  481   GACCATGCTGCTGTGGCCTCTGAACCTTCAGCAGTACCGACCCGGAAGAACCGGCTGTCT  540
Sbjct  570   ............................................................  511

Query  541   GCTTTTCCTCCCTTTCACTCTACTGCACCGCCCTTCCCTTCTCCAGCTCGGACCCCTGAG  600
Sbjct  510   ............................................................  451

Query  601   GTGAGAACACCCAAGTCAGGAACTCAGCCACAAACAACAGAAACTAACCTGCAAACTGCT  660
Sbjct  450   ............................................................  391

Query  661   CCTAAACTTTCCACATCAACATCCACGACTGGGACCAGCCATCTCATAAAGTGTGCGGAG  720
Sbjct  390   ................G...........................................C......  331

Query  721   AAGGAGAAAACTTTCTGTGTGAATGGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAAC  780
Sbjct  330   ............................................................  271

Query  781   CCGTCAAGATACTTGTGCAAGTGCCCAAATGAGTTTACTGGTGATCGTTGCCAAAACTAC  840
Sbjct  270   ............................................................  211

Query  841   GTAATGGCCAGCTTCTACAGTACGTCCACTCCCTTTCTGTCTCTGCCTGAGTAG  894
Sbjct  210   ..................................................  157
```

Position of the mutation on the rat NRG1 sequence (numbered from ATG)	Position of mutation on c	Position of mutation on d
Position of the mutation on the rat NRG1 sequence (numbered from ATG)	Position of mutation on g	Position of mutation on h

EXERCISE #1

* compare your theoretical NRG1-III-beta3 sequence from ATG to STOP to sequences c & d and g & h.
c/d belong to the same clone, g/h to the same clone.

* prepare a table containing the list of mutations that you find in the sense and antisense sequences, marking - for each mutated nucleotide - the coordinates in the theoretical sequence, the coordinates in the obtained sequence (c or d, gor h), the type of mutation (ie: A -> G)

* compare the mutations found in the sequence obtained with the primer sense with the nucleotides found in the sequence obtained with the primer antisense

The sequences are much more correct, as closer to the primer;
if possible mutations are in regions with a high number (> 800), you can resolve the ambiguity by analyzing the complementary sequence obtained with the other primer

⁕ open sequences.seq with the program notepad, copy them and compare them to the rat NRG1-typeIII-beta3 sequence
(use the BLAST program “align two sequences”)

⁕ as **Query** you can use the rat **NRG1** sequence **from ATG to STOP**

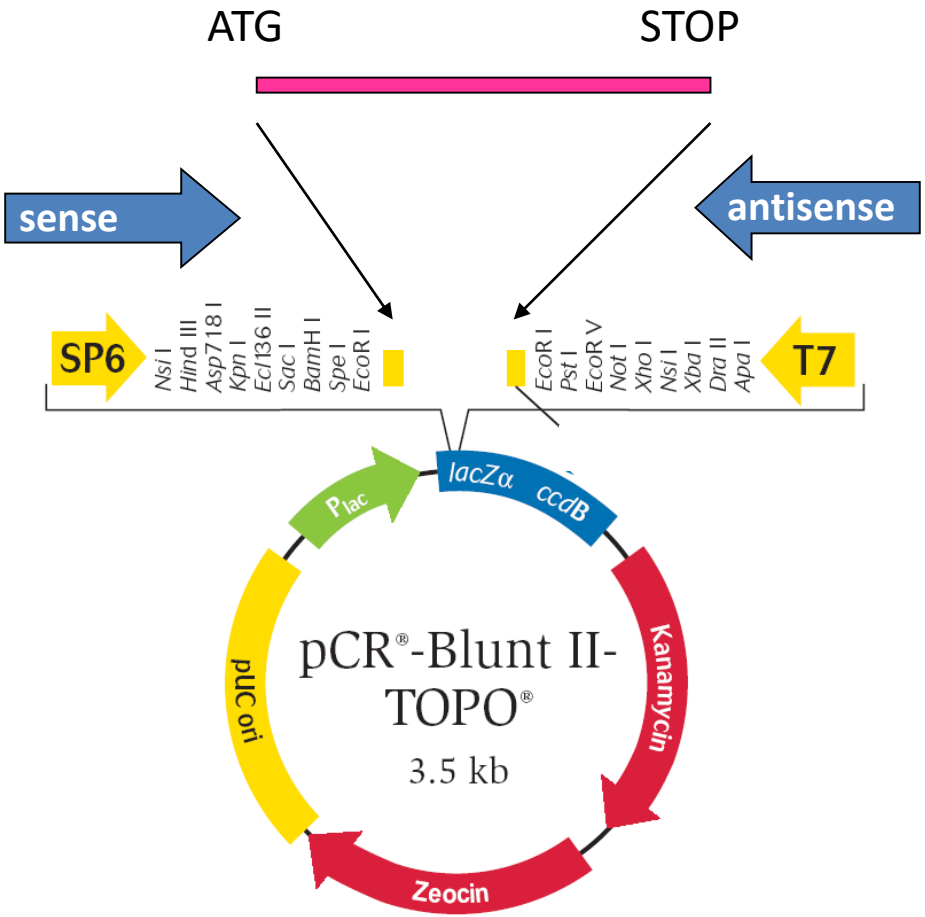
⁕ as **Sbjct** use **sequences.seq** that you want to analyze (c/d or g/h)

⁕write down the coordinates of the mutations on the expected theoretical sequence (Query) and on the Sbjct sequence to be able to compare - for each mutation – sense and antisense sequences

Sequence analysis didactic examples

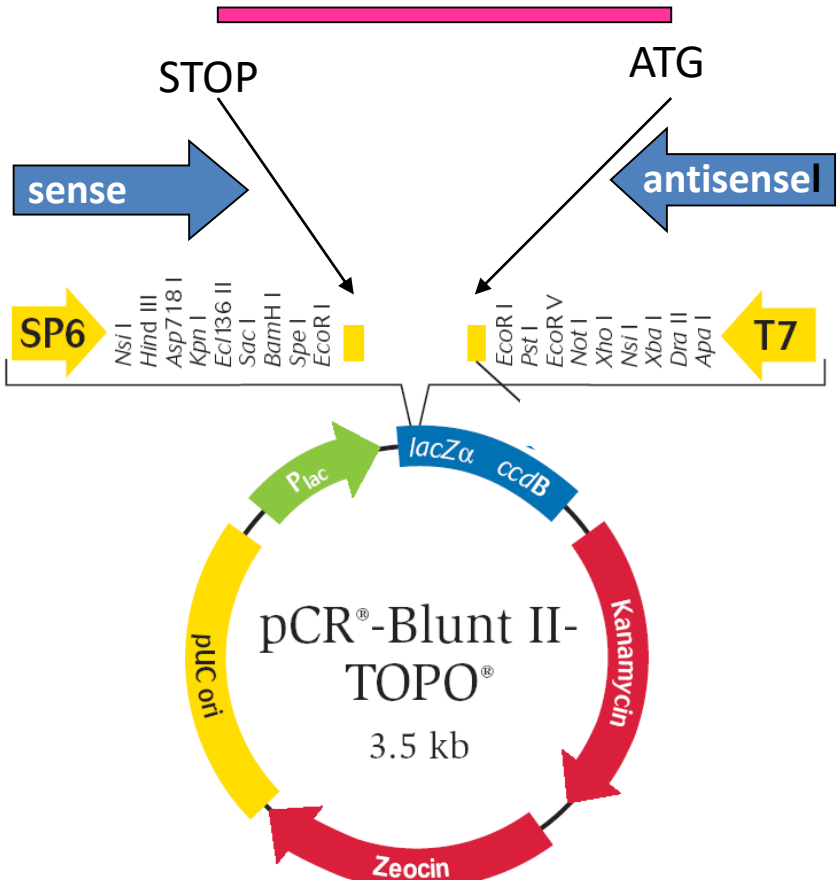
INSERT cloned in SENSE orientation

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



INSERT cloned in ANTISENSE orientation

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



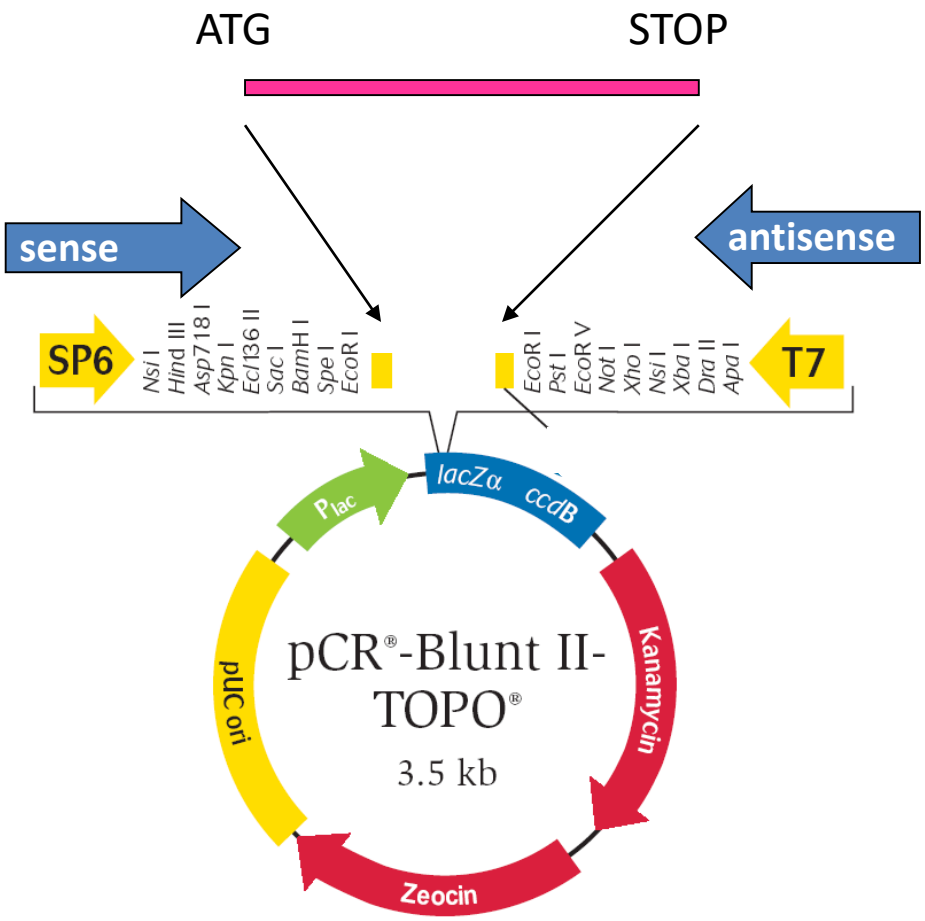
SENSE ORIENTATION

sense

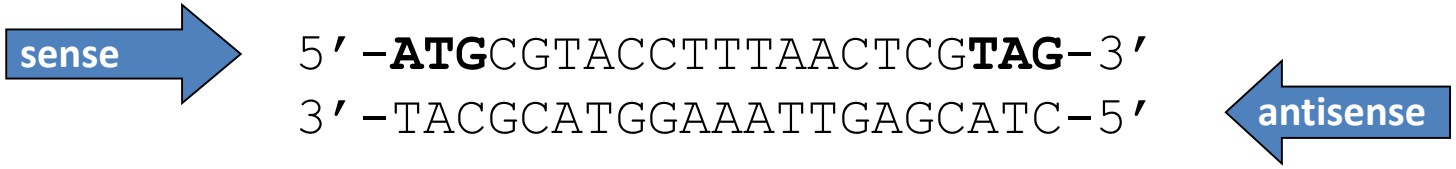
5′–**ATG**CGTACCTTTAACTCG**TAG**–3′

3′–TACGCATGGAAATTGAGCATC–5′

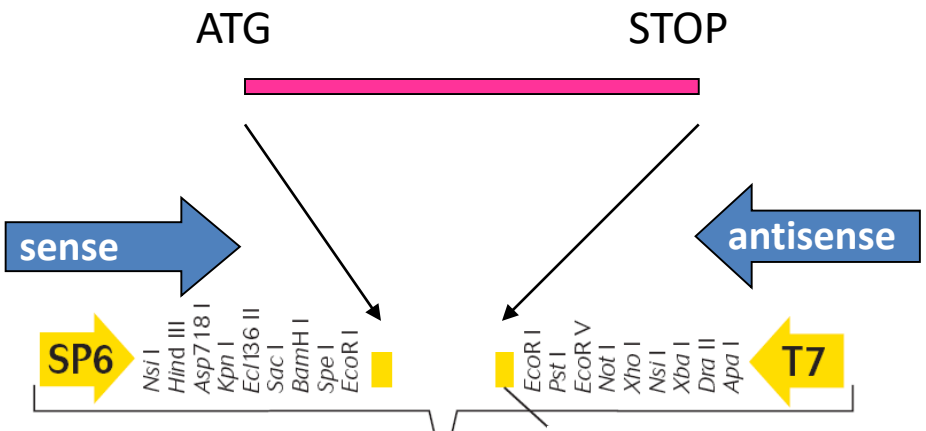
antisense



SENSE ORIENTATION



SENSE: NNNNNNATGCGTACCTTTAACTCGTAGNNNNNNN



SENSE ORIENTATION

sense

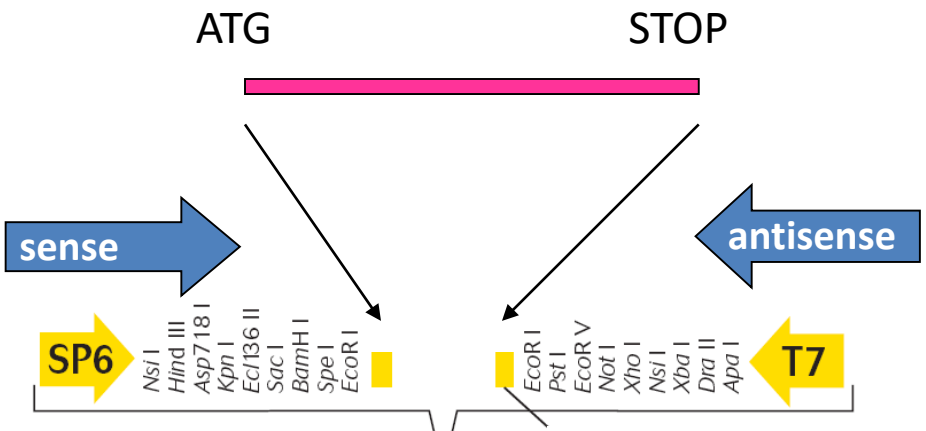
5′-**ATG**CGTACCTTTAACTCG**TAG**-3′

3′-TACGCATGGAAATTGAGCATC-5′

antisense

SENSE: NNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN

ANTISENSE: NNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN



SENSE ORIENTATION

200bp 100bp

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

3′-TACGCATGGAAATTGAGCATC-5′ antisense

SENSE: NNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNN

ANTISENSE: NNNNNNCTACGAGTTAAAGGTACGCATNNNNNN

BLAST:

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

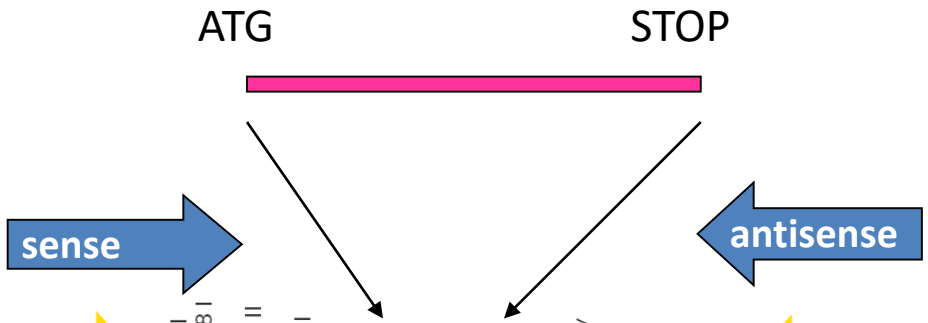
||||||||||||||||||||

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

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

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

Subjct Antisense: 121 **ATG**CGTACCTTTAACTCG**TAG** 101



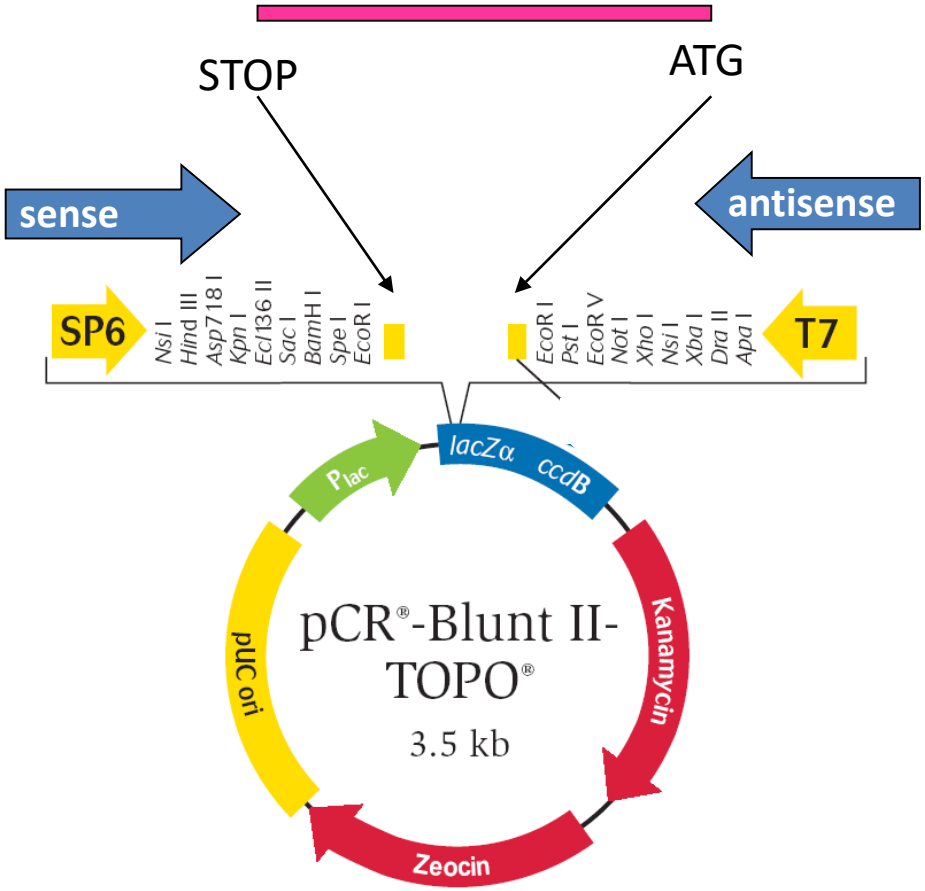
ANTISENSE ORIENTATION

sense

5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

antisense



ANTISENSE ORIENTATION

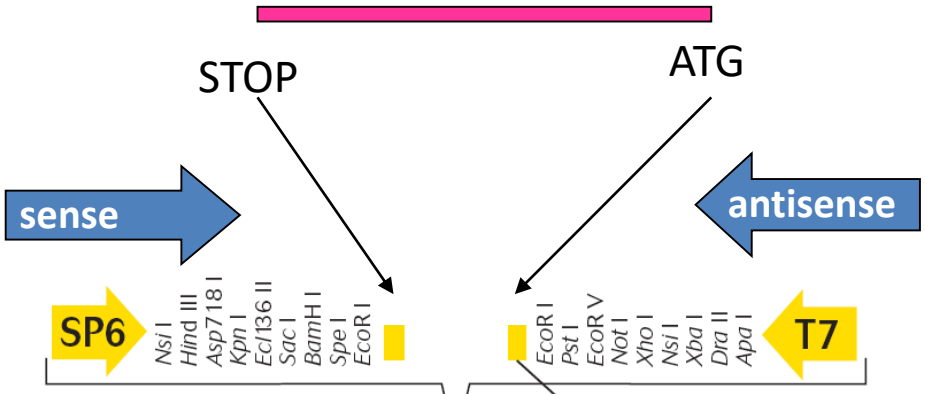
sense

5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

antisense

SENSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN



ANTISENSE ORIENTATION

sense

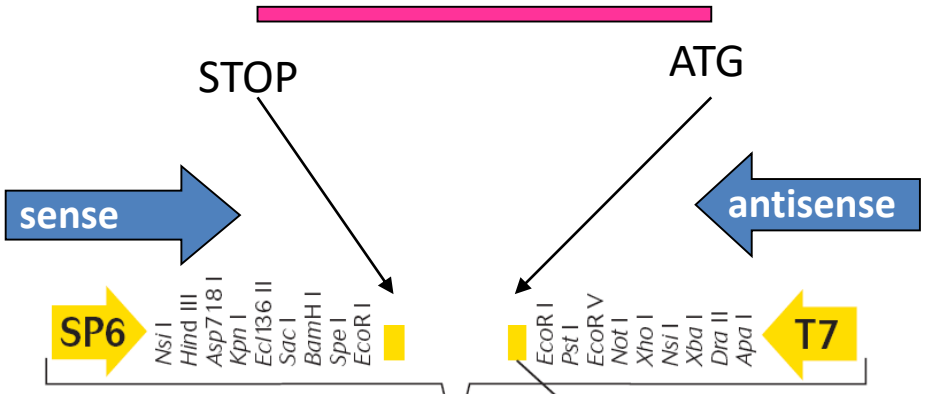
5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

antisense

SENSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN

ANTISENSE: NNNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN



ANTISENSE ORIENTATION

200bp 100bp

sense

5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

antisense

SENSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN

ANTISENSE: NNNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN

BLAST:

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

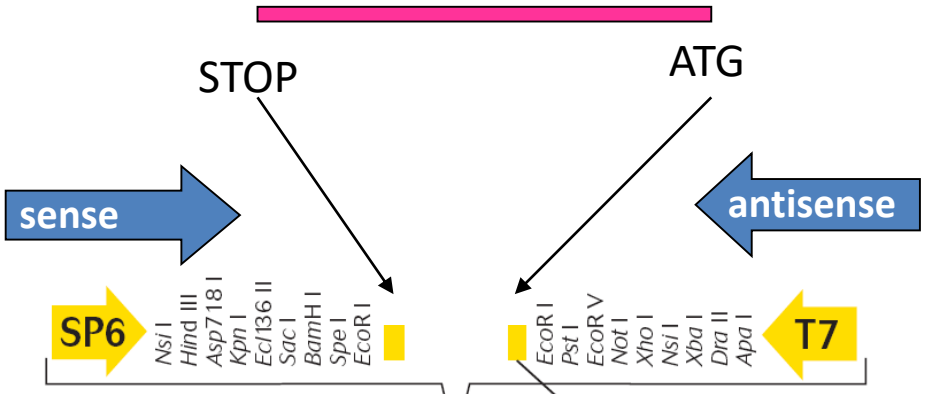
||||||||||||||||||||

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

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

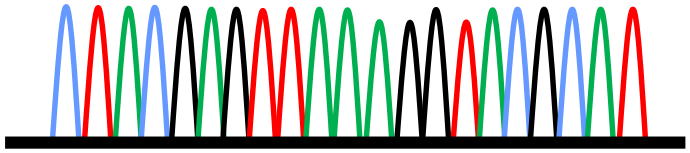
||||||||||||||||||||

Subjct Antisense: 101 **ATG**CGTACCTTTAACTCG**TAG** 121

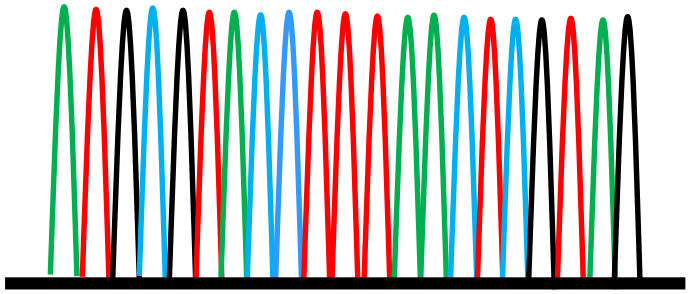


ANTISENSE ORIENTATION

SENSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN



ANTISENSE: NNNNNNNATGCGTACCTTTAACTCGTAGNNNNNNN



SENSE: NNNNNNNCTACGAGTTAAAGGTACGCATNNNNNNN

ANTISENSE: NNNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN

C -> T

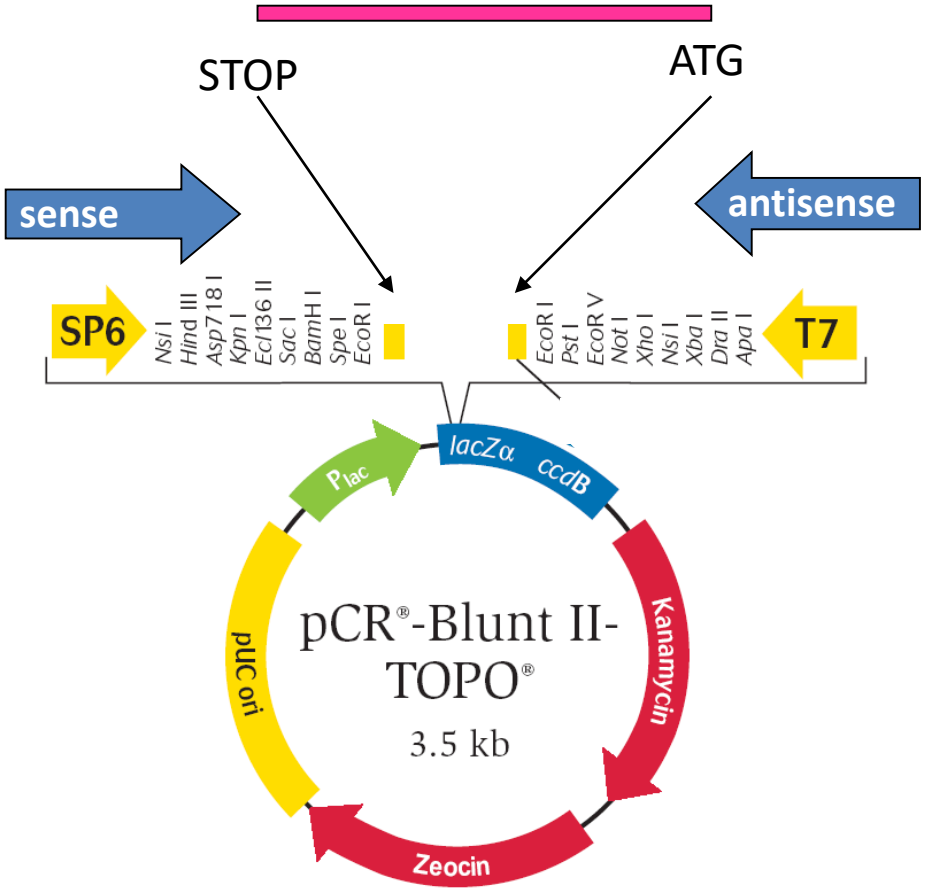
MUTATED ANTISENSE ORIENTATION

sense

5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

antisense



C -> T

MUTATED ANTISENSE ORIENTATION

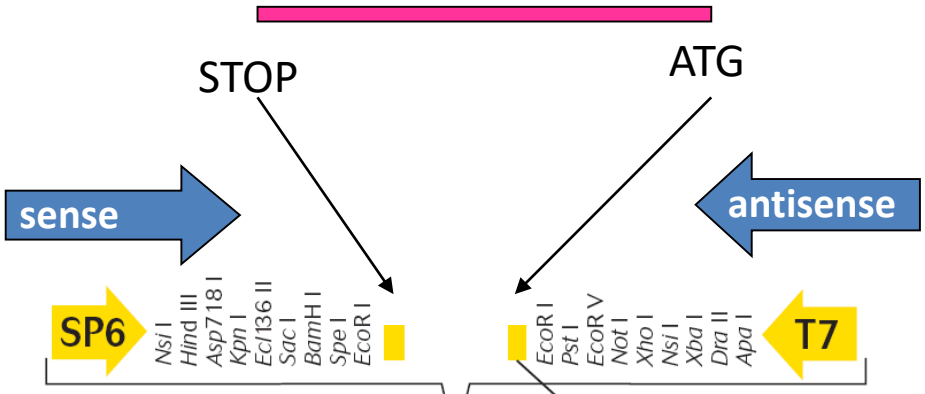
sense

5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

antisense

SENSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN



C -> T

MUTATED ANTISENSE ORIENTATION

sense

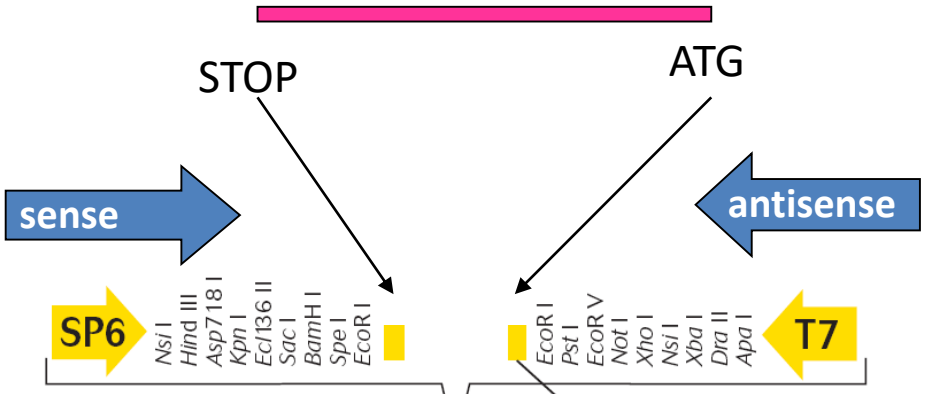
5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

antisense

SENSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN

ANTISENSE: NNNNNNN**ATG**CGTACTTTTAACTCG**TAG**NNNNNNN



200bp C -> T 100bp

MUTATED ANTISENSE ORIENTATION

sense

5′-CTACGAGTTAAAAGTACGCAT-3′

3′-**GAT**GCTGAATTTTCATGC**GTA**-5′

antisense

SENSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN

ANTISENSE: NNNNNNN**ATG**CGTACTTTTAACTCG**TAG**NNNNNNN

BLAST:

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

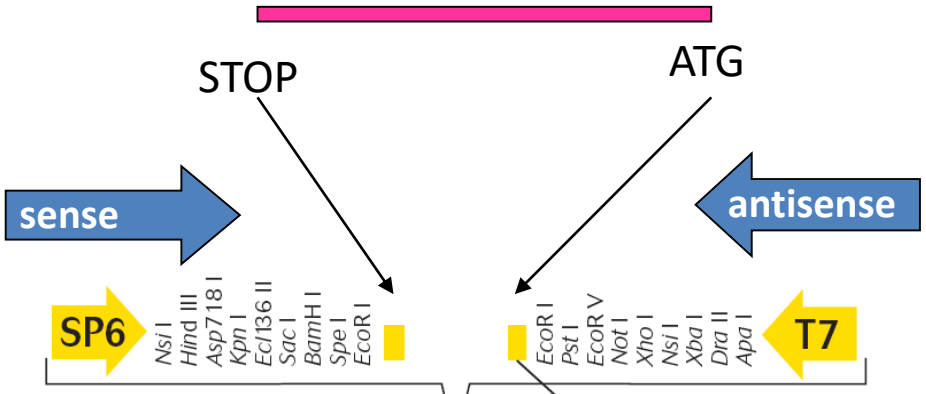
||||||||| ||||||||||||

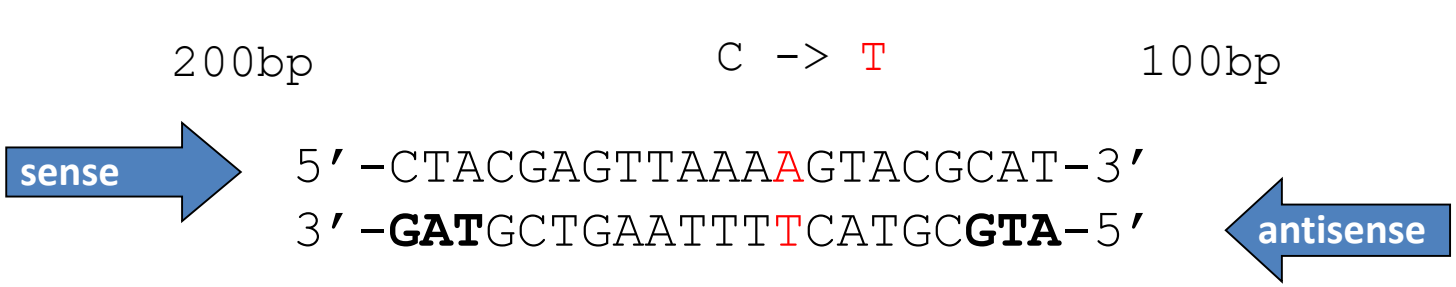
Subjct Sense: 221 **ATG**CGTACTTTTAACTCG**TAG** 201

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

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

Subjct Antisense: 101 **ATG**CGTACTTTTAACTCG**TAG** 121





SENSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN

ANTISENSE: NNNNNNN**ATG**CGTACTTTTAACTCG**TAG**NNNNNNN

BLAST:

```
Query:                1 ATGCGTACCTTTAACTCGTAG 21
                        ||||||||  ||||||||||||
Subjct Sense:       221 ATGCGTACTTTTAACTCGTAG 201

Query:                1 ATGCGTACCTTTAACTCGTAG 21
                        ||||||||  ||||||||||||
Subjct Antisense:   101 ATGCGTACTTTTAACTCGTAG 121
```

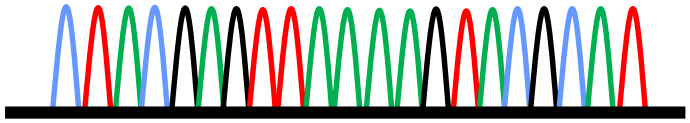
Identify the mutation sequence on Query, Sense and antisense:

Query	Sense	Antisense
9 (C)	213 (A)	109 (T)

MUTATED ANTISENSE ORIENTATION

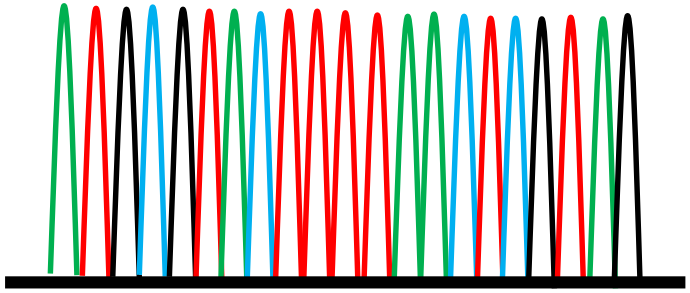
213

SENSE: NNNNNNNCTACGAGTTAAA**A**GTACGCATNNNNNNN



109

ANTISENSE: NNNNNNNATGCGTAC**T**TTTAACTCGTAGNNNNNNN



SENSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN

ANTISENSE: NNNNNNN**ATG**CGTACTTTTAACTCG**TAG**NNNNNNN

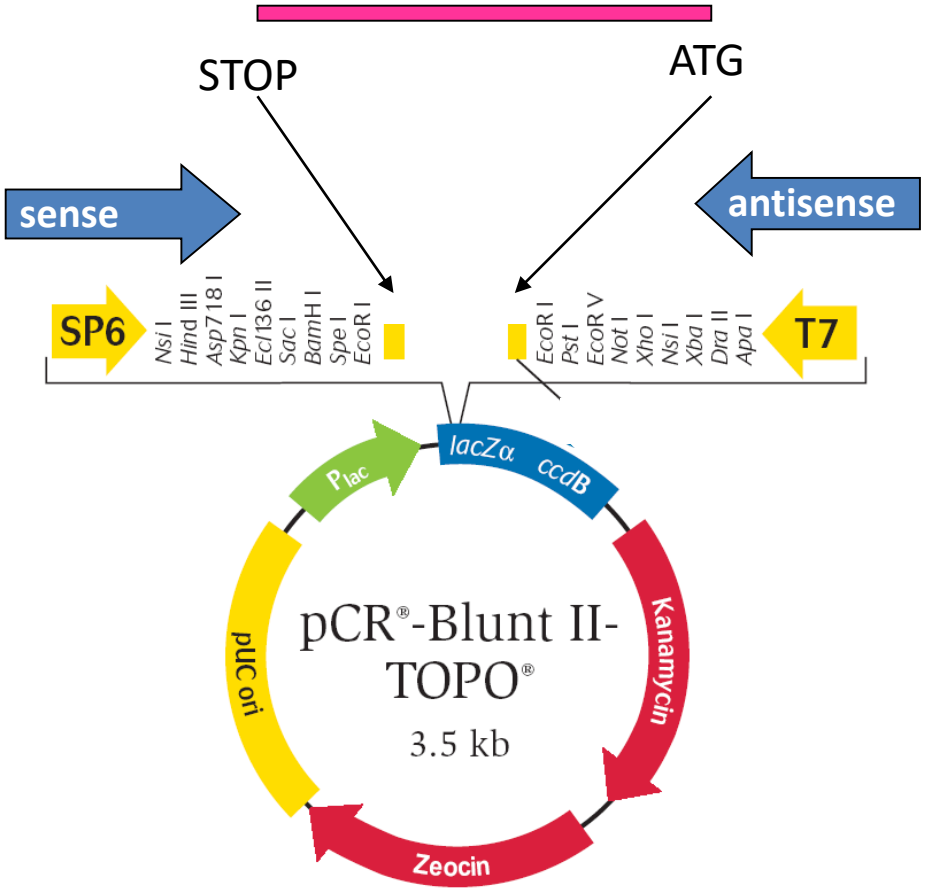
ANTISENSE OK WITH A SEQUENCE MISTAKE

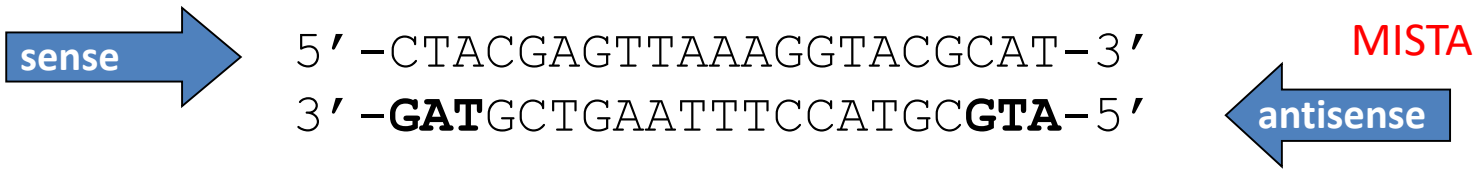
sense

5′-CTACGAGTTAAAGGTACGCAT-3′

3′-**GAT**GCTGAATTTCCATGC**GTA**-5′

antisense





ANTISENSE OK WITH A SEQUENCE MISTAKE

```
SENSE:       NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN
ANTISENSE:   NNNNNNNATGCGTACCTTTAACTCGTAGNNNNNNN
```

BLAST:

```
Query:                1 ATGCGTACCTTTAACTCGTAG 21
                        ||||||||  ||||||||||||
Subjct Sense   :    221 ATGCGTACTTTTAACTCGTAG 201

Query:                1 ATGCGTACCTTTAACTCGTAG 21
                        ||||||||||||||||||||
Subjct Antisense:   101 ATGCGTACCTTTAACTCGTAG 121
```

Identify the mutation sequence on Query, Sense and antisense:

Query	Sense	Antisense
9 (C)	213 (A)	109 (C)

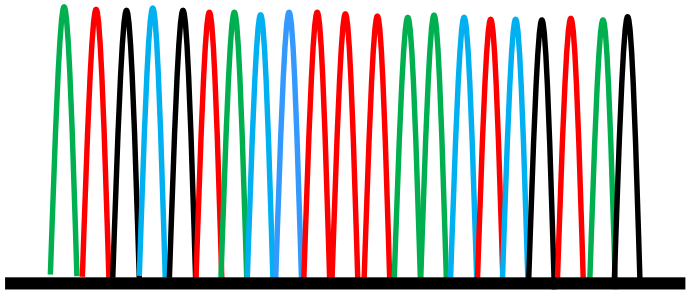
ANTISENSE OK WITH A SEQUENCE MISTAKE

213

SENSE: NNNNNNNCTACGAGTTAAA**A**GTACGCATNNNNNNN

109

ANTISENSE: NNNNNNNATGCGTAC**C**TTTAACTCGTAGNNNNNNN



SENSE: NNNNNNNCTACGAGTTAAAAGTACGCATNNNNNNN

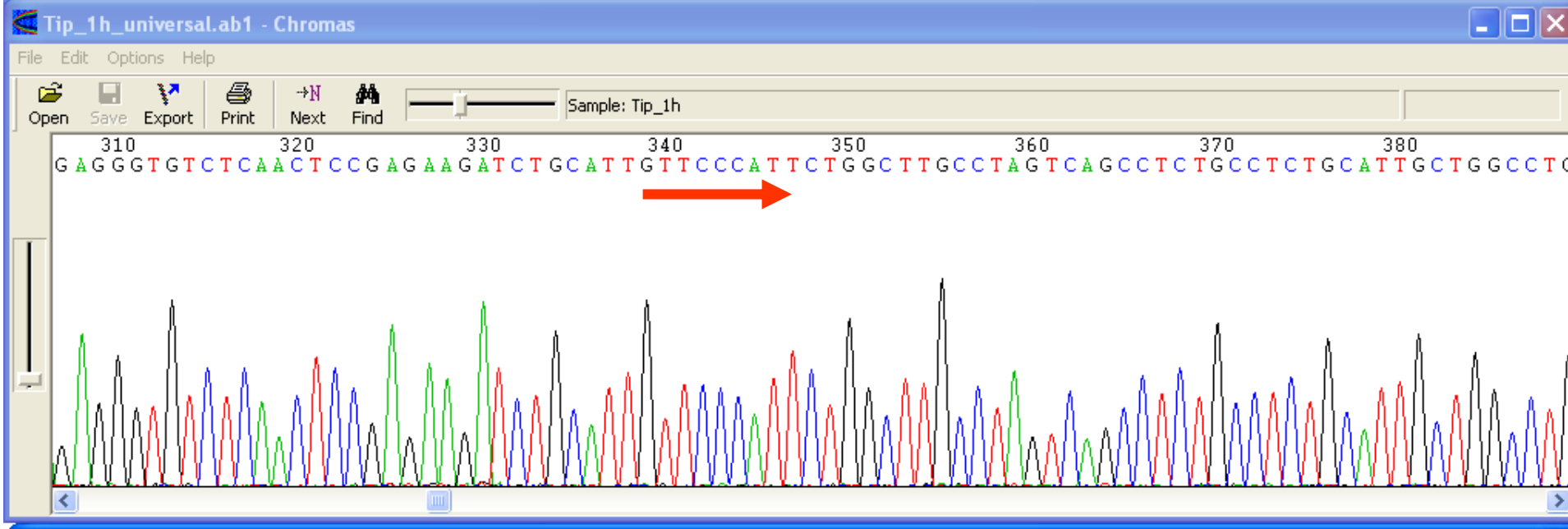
ANTISENSE: NNNNNNN**ATG**CGTACCTTTAACTCG**TAG**NNNNNNN

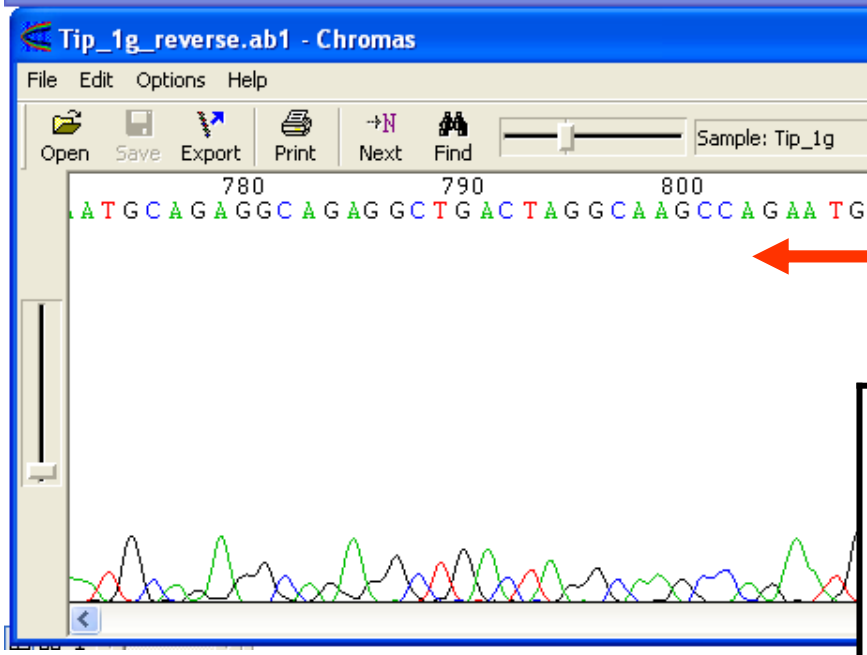
EXERCISE # 2

- you have to identify the peak corresponding to a specific mutated nucleotide in the electropherograms c/d or g/h to verify if it is really mutated.

prepare 1 slide:

- showing the two peaks corresponding to that specific mutated nucleotide in the electropherogram of the sequence obtained with primer sense and in the electropherogram of the sequence obtained with primer antisense





Sequence in NRG1IIIβ3	Position on sequence with primer antisense (h)	Position on sequence with primer sense (g)
Es: 228 (G)	339 (G)	813 (C)

To analyze electropherograms.ab1 you have to use the program Chromas; in this way, you can identify the peak corresponding to a specific nucleotide

http://www.technelysium.com.au/chromas.html