# Advanced Cell Biology & Biotechnology

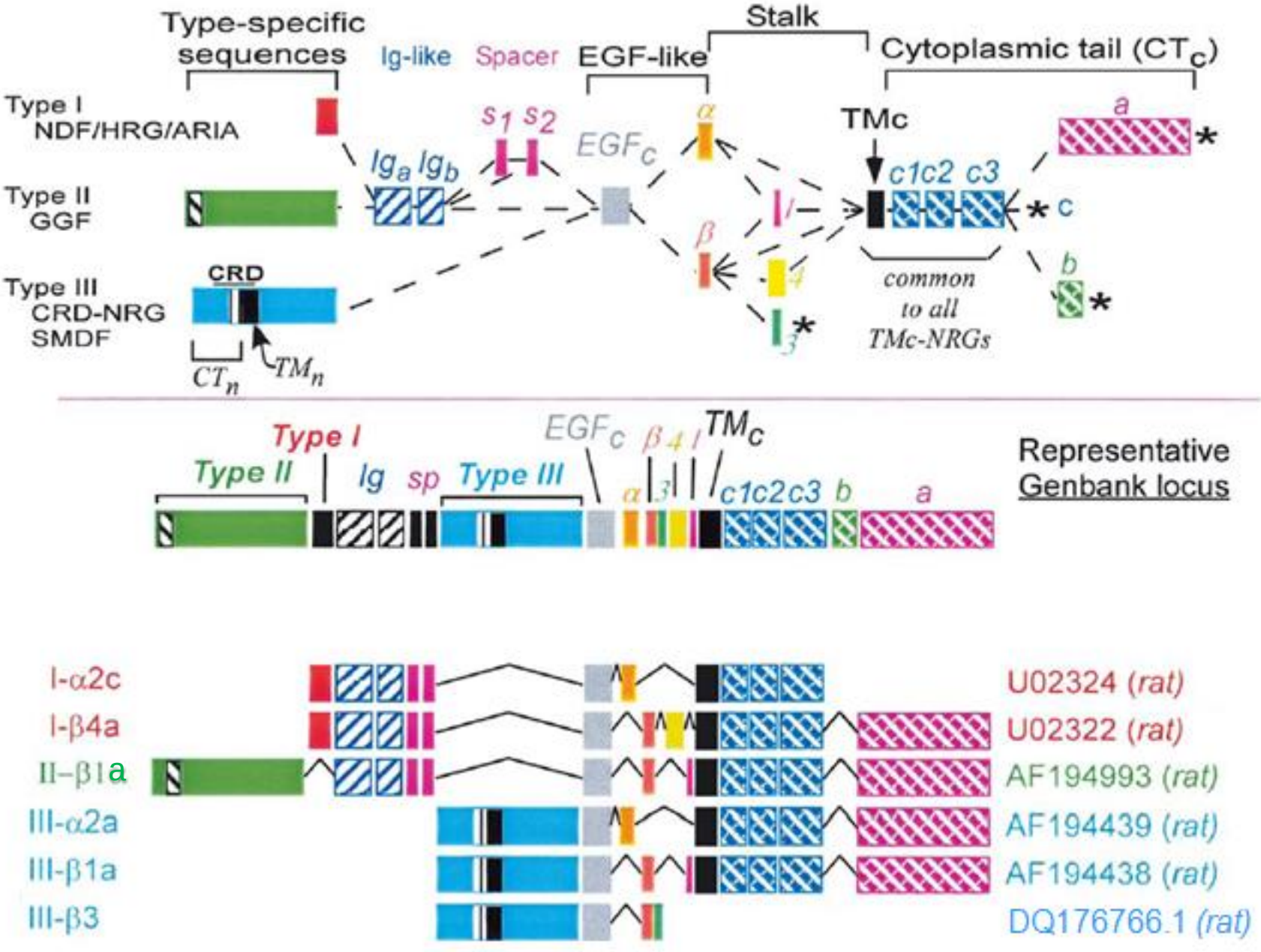
## Biotechnology Project Lab

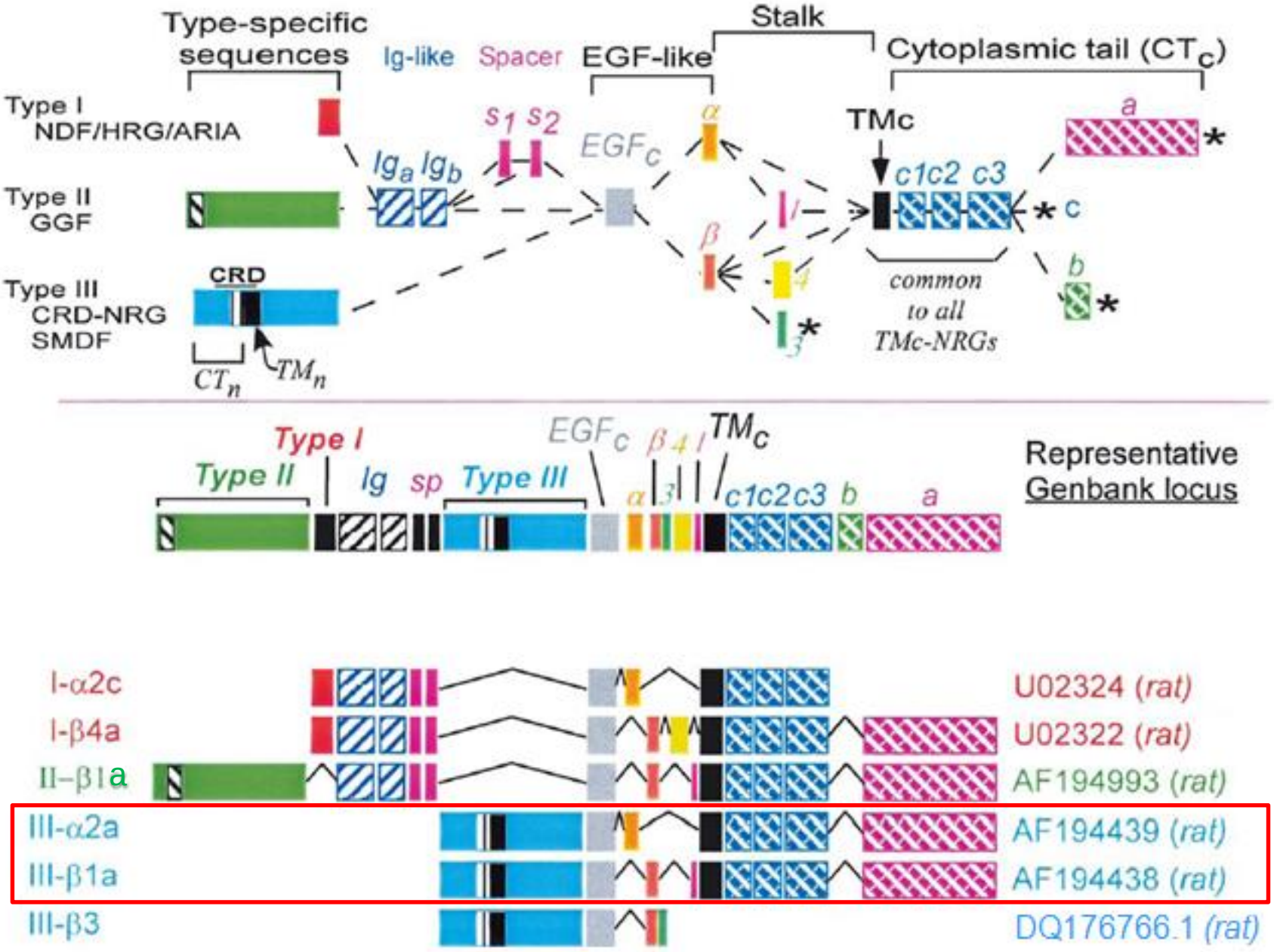
Giovanna Gambarotta
& Isabella Tarulli

The lecture is about to begin….

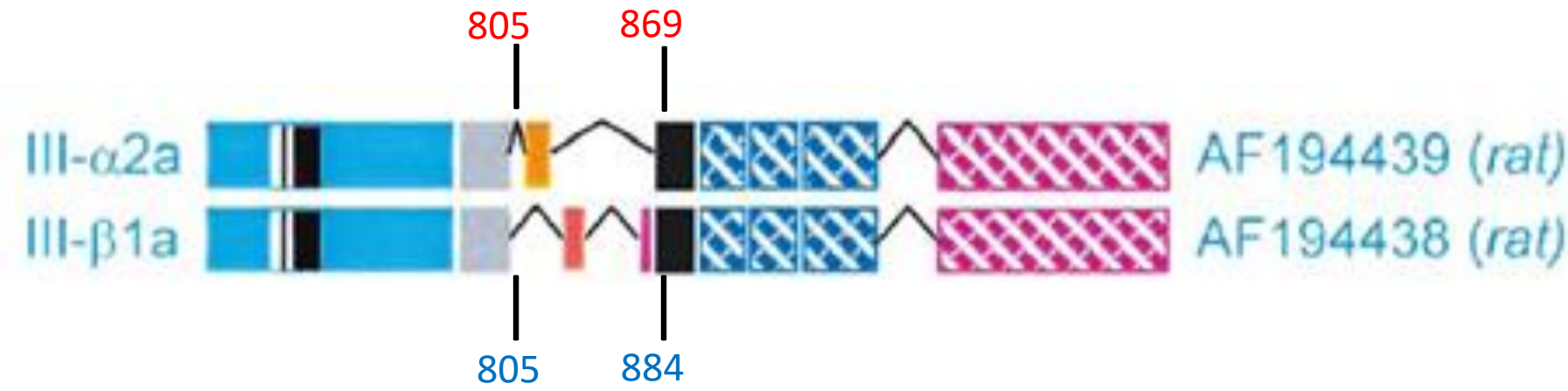
- Summary of the activity of the previous lesson
- Overview of the next lessons
- Welcome test
- RT-PCR for full length cloning or gene expression analysis

# Identification of specific domains





# 3’ end of the EGF-like domain + 5’ end of the transmembrane domain



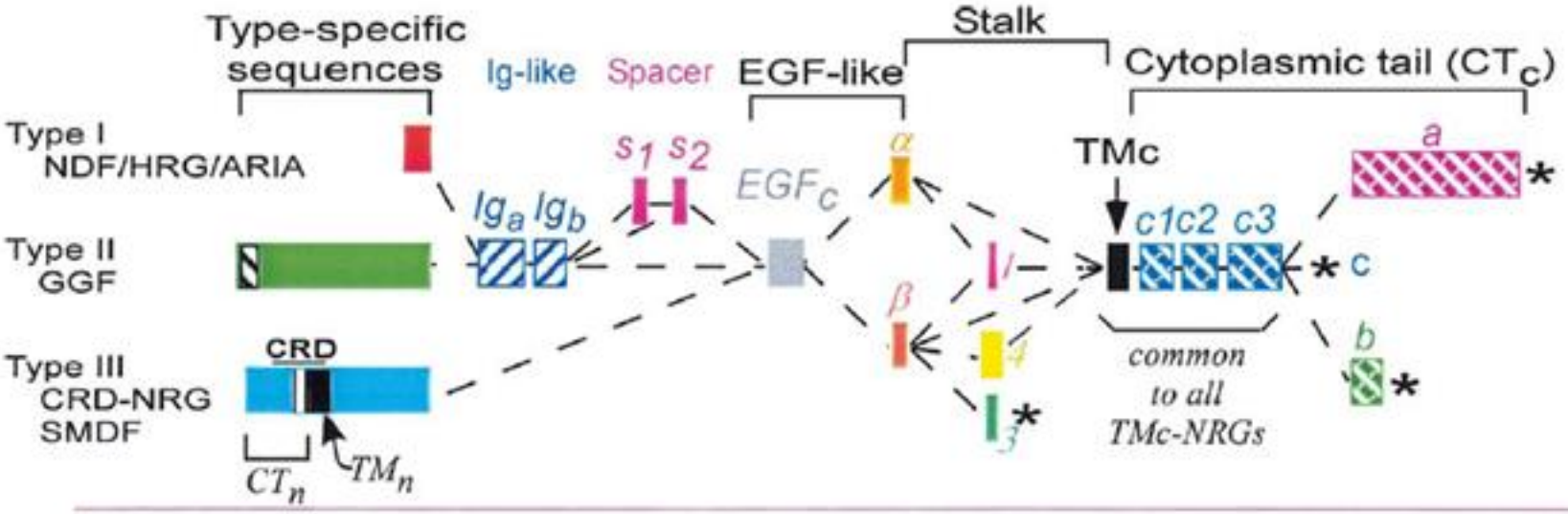

```
Query   661   CCTAAACTTTCCACATCAACATCCACGACTGGGACCAGCCATCTCATAAAGTGTGCGGAG   720
              ||||||||||||||||| ||||||||||||||||||||||||||||||| ||||||
Sbjct   661   CCTAAACTTTCCACATCGACATCCACGACTGGGACCAGCCATCTCATAAAGTGCGCGGAG   720

Query   721   AAGGAGAAAACTTTCTGTGTGAATGGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAAC   780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   721   AAGGAGAAAACTTTCTGTGTGAATGGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAAC   780

Query   781   CCGTCAAGATACTTGTGCAAGTGCC  805
              |||||||||||||||||||||||||
Sbjct   781   CCGTCAAGATACTTGTGCAAGTGCC  805
```

**Range 2: 884 to 2103** Graphics  ▼ Next Match  ▲ Previous Match  ▲ First Match

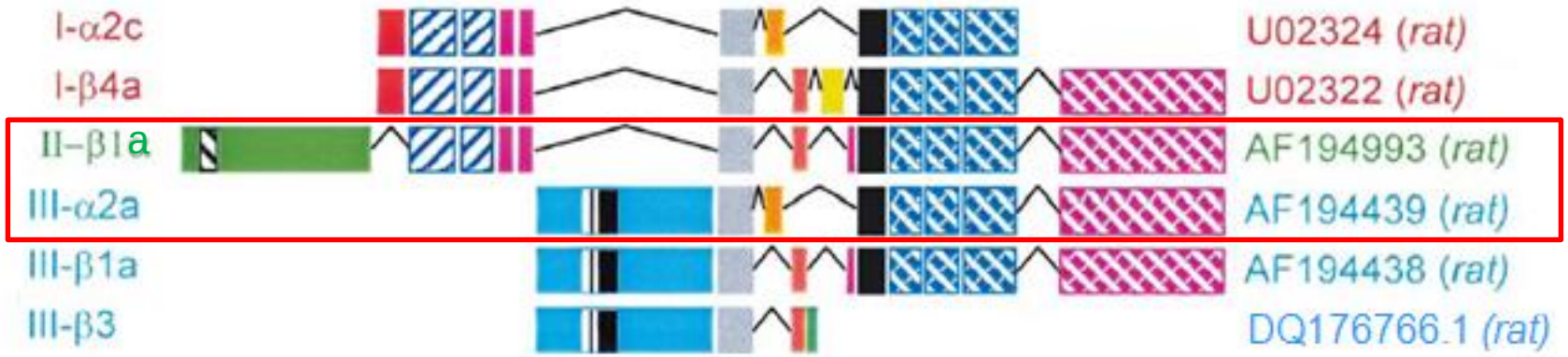
| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 2254 bits(1220) | 0.0 | 1220/1220(100%) | 0/1220(0%) | Plus/Plus |

```
Query   869   AAGCGGAGGAACTCTACCAGAAGAGGGTGCTGACAATTACTGGCATCTGTATCGCCCTGC   928
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   884   AAGCGGAGGAACTCTACCAGAAGAGGGTGCTGACAATTACTGGCATCTGTATCGCCCTGC   943

Query   929   TGGTGGTCGGCATCATGTGTGTGGTGGCCTACTGCAAAACCAAGAAGCAGCGGCAGAAGC   988
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   944   TGGTGGTCGGCATCATGTGTGTGGTGGCCTACTGCAAAACCAAGAAGCAGCGGCAGAAGC   1003

Query   989   TTCATGATCGGCTTCGGCAGAGTCTTCGGTCAGAACGGAGCAACCTGGTGAACATAGCGA   1048
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   1004  TTCATGATCGGCTTCGGCAGAGTCTTCGGTCAGAACGGAGCAACCTGGTGAACATAGCGA   1063

Query   1049  ATGGGCCTCACCACCCAAACCCGCCGCCAGAGAACGTGCAGCTGGTGAATCAATACGTAT   1108
              ............................................................
```

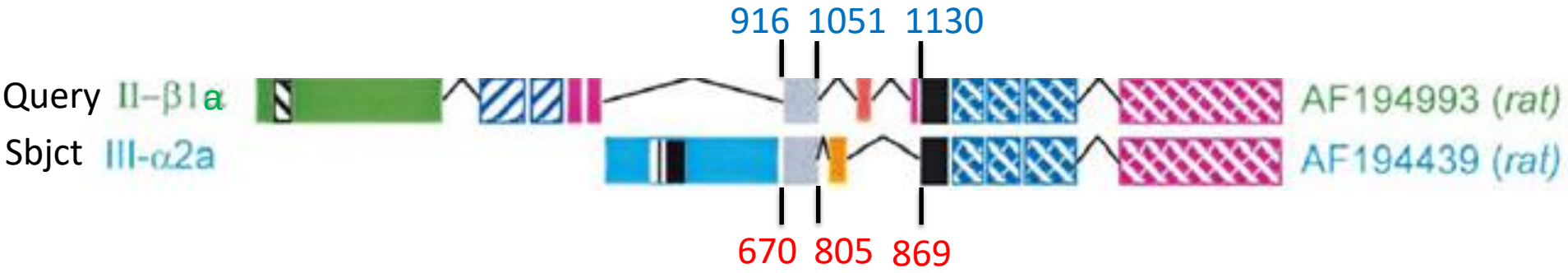
# 5’ and 3’ ends of the EGF-like domain







# 5’ and 3’ ends of the EGF-like domain



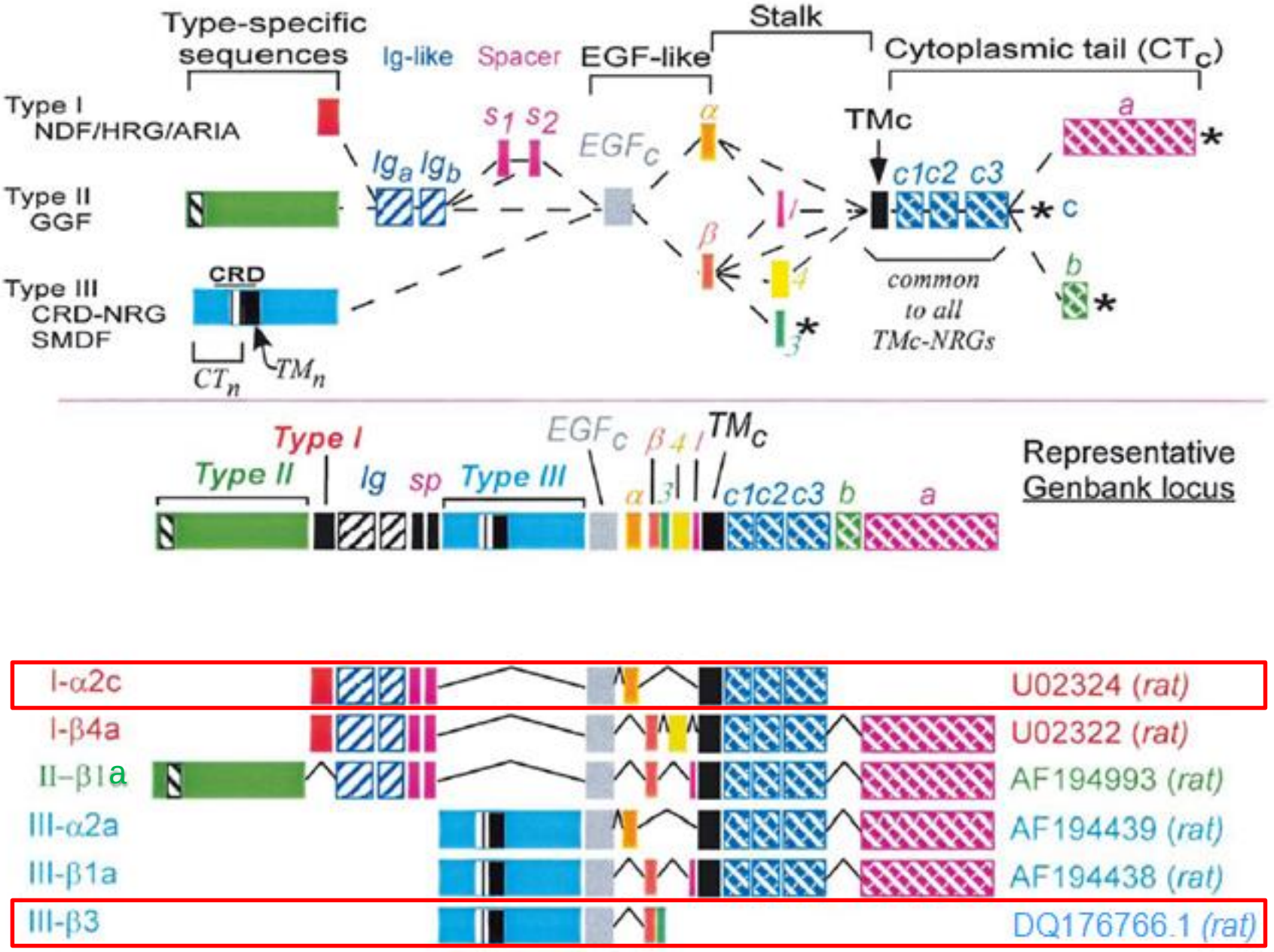

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 241 bits(130) | 1e-66 | 134/136(99%) | 0/136(0%) | Plus/Plus |

```
Query  916   TCCACATCGACATCCACGACTGGGACCAGCCATCTCATAAAGTGCGCGGAGAAGGAGAAA  975
             ||||||||  ||||||||||||||||||||||||||||||||||||  ||||||||||||||||
Sbjct  670   TCCACATCAACATCCACGACTGGGACCAGCCATCTCATAAAGTGTGCGGAGAAGGAGAAA  729

Query  976   ACTTTCTGTGTGAATGGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAACCCGTCAAGA  1035
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  730   ACTTTCTGTGTGAATGGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAACCCGTCAAGA  789

Query  1036  TACTTGTGCAAGTGCC 1051
             ||||||||||||||||
Sbjct  790   TACTTGTGCAAGTGCC 805
```

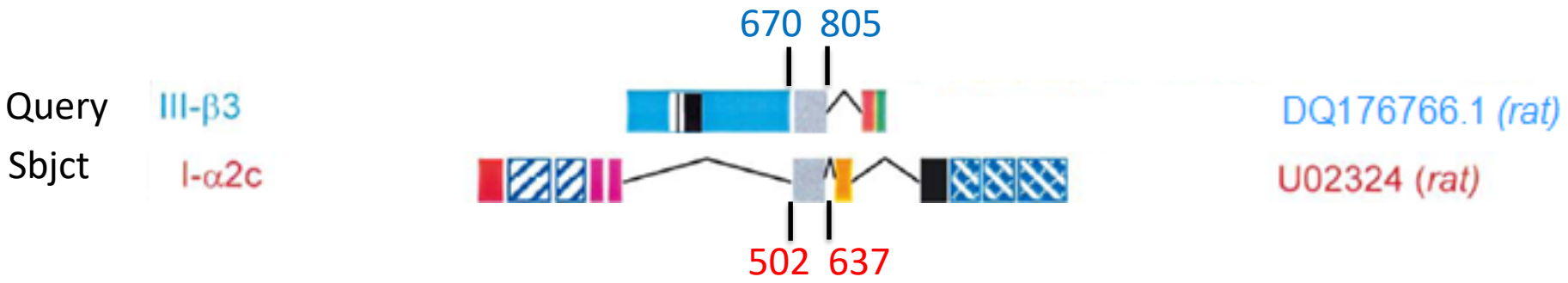
**Range 2: 869 to 2088** Graphics ▼ Next Match ▲ Previous Match ▲ First M

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 2254 bits(1220) | 0.0 | 1220/1220(100%) | 0/1220(0%) | Plus/Plus |

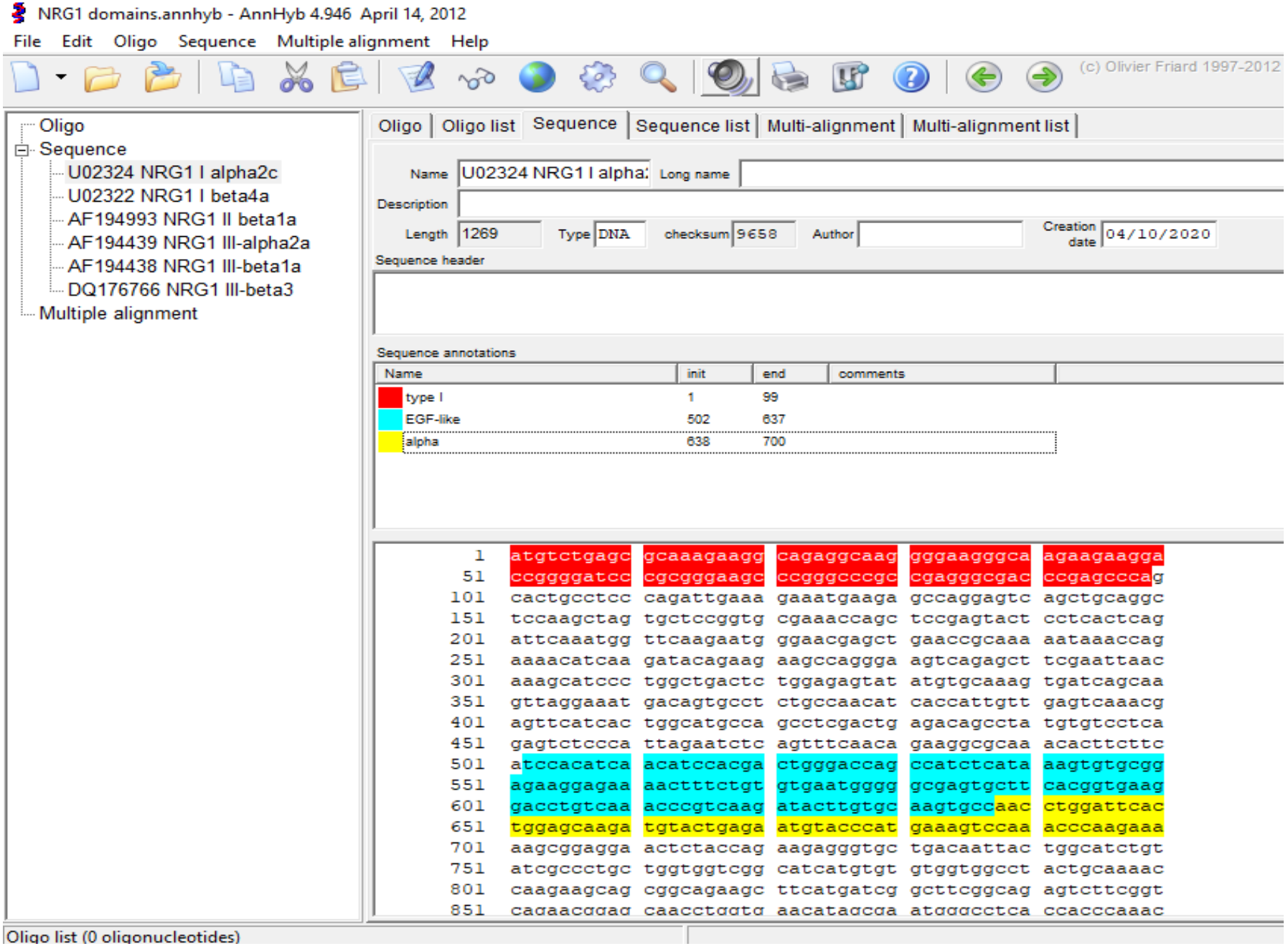
```
Query  1130  AAGCGGAGGAACTCTACCAGAAGAGGGTGCTGACAATTACTGGCATCTGTATCGCCCTGC  1189
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869   AAGCGGAGGAACTCTACCAGAAGAGGGTGCTGACAATTACTGGCATCTGTATCGCCCTGC  928

Query  1190  TGGTGGTCGGCATCATGTGTGTGGTGGCCTACTGCAAAACCAAGAAGCAGCGGCAGAAGC  1249
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  929   TGGTGGTCGGCATCATGTGTGTGGTGGCCTACTGCAAAACCAAGAAGCAGCGGCAGAAGC  988

Query  1250  TTCATGATCGGCTTCGGCAGAGTCTTCGGTCAGAACGGAGCAACCTGGTGAACATAGCGA  1309
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  989   TTCATGATCGGCTTCGGCAGAGTCTTCGGTCAGAACGGAGCAACCTGGTGAACATAGCGA  1048

Query  1310  ATGGGCCTCACCACCCAAACCCGCCGCCAGAGAACGTGCAGCTGGTGAATCAATACGTAT  1369
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1049  ATGGGCCTCACCACCCAAACCCGCCGCCAGAGAACGTGCAGCTGGTGAATCAATACGTAT  1108
```

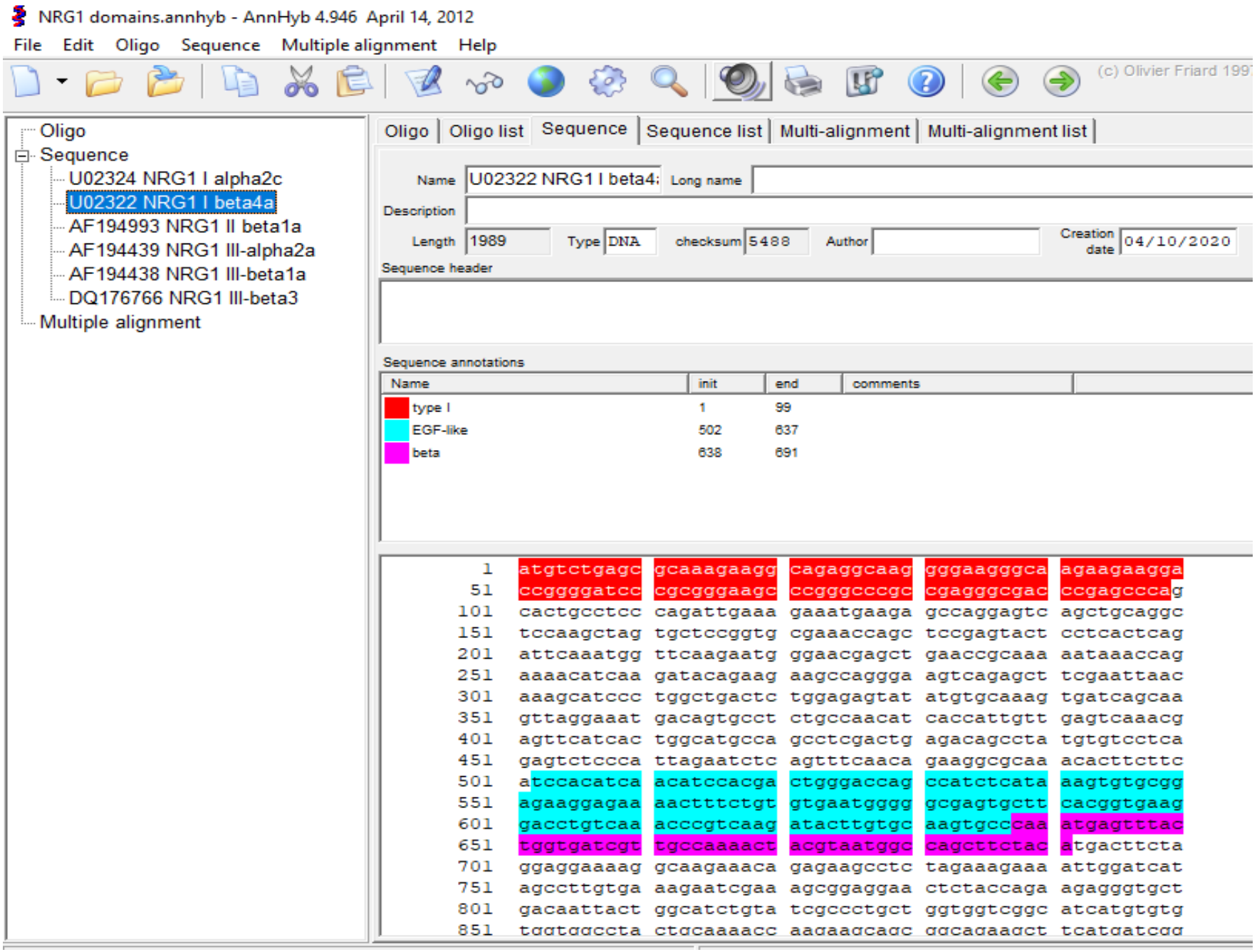


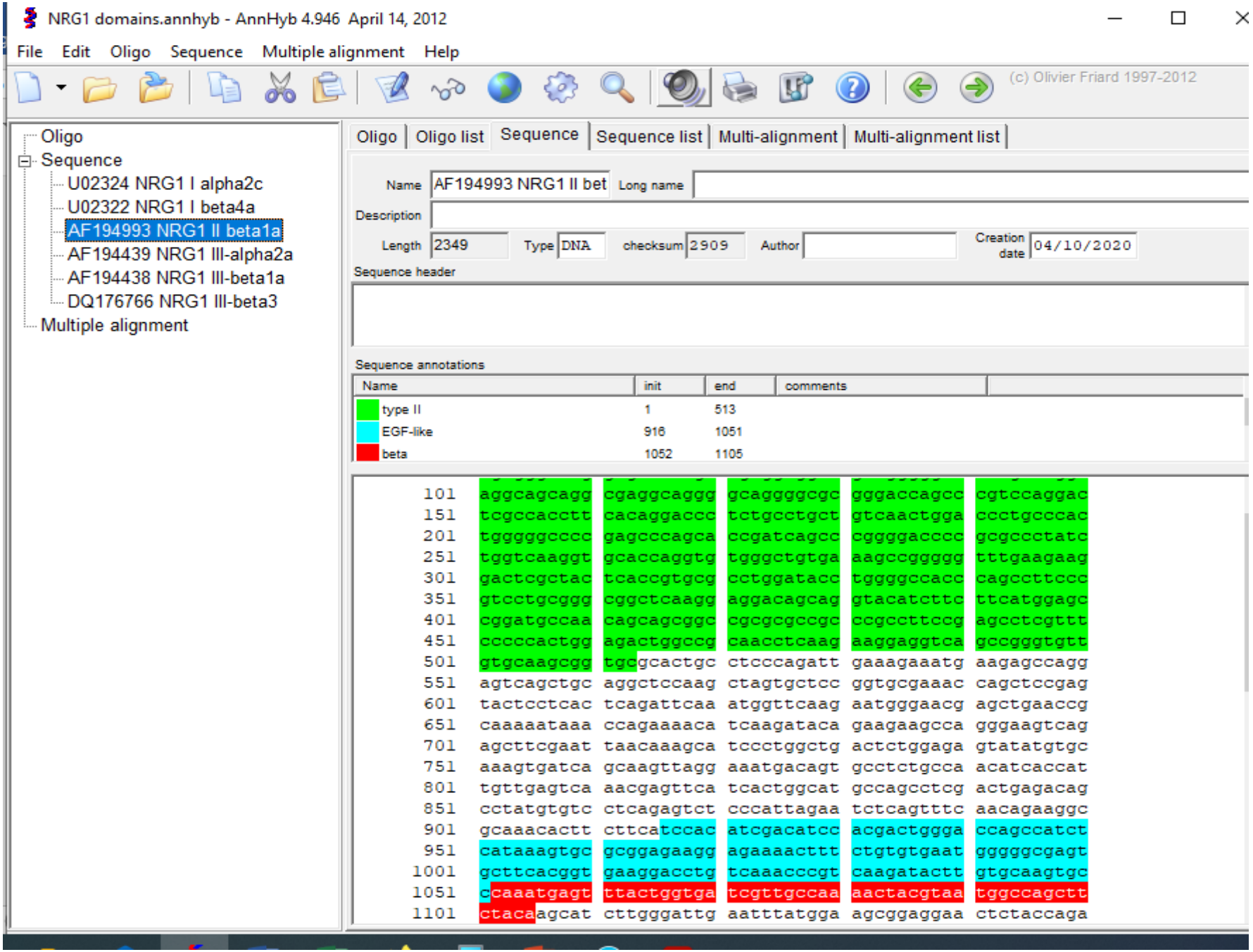
# 5’ and 3’ end of the EGF-like domain

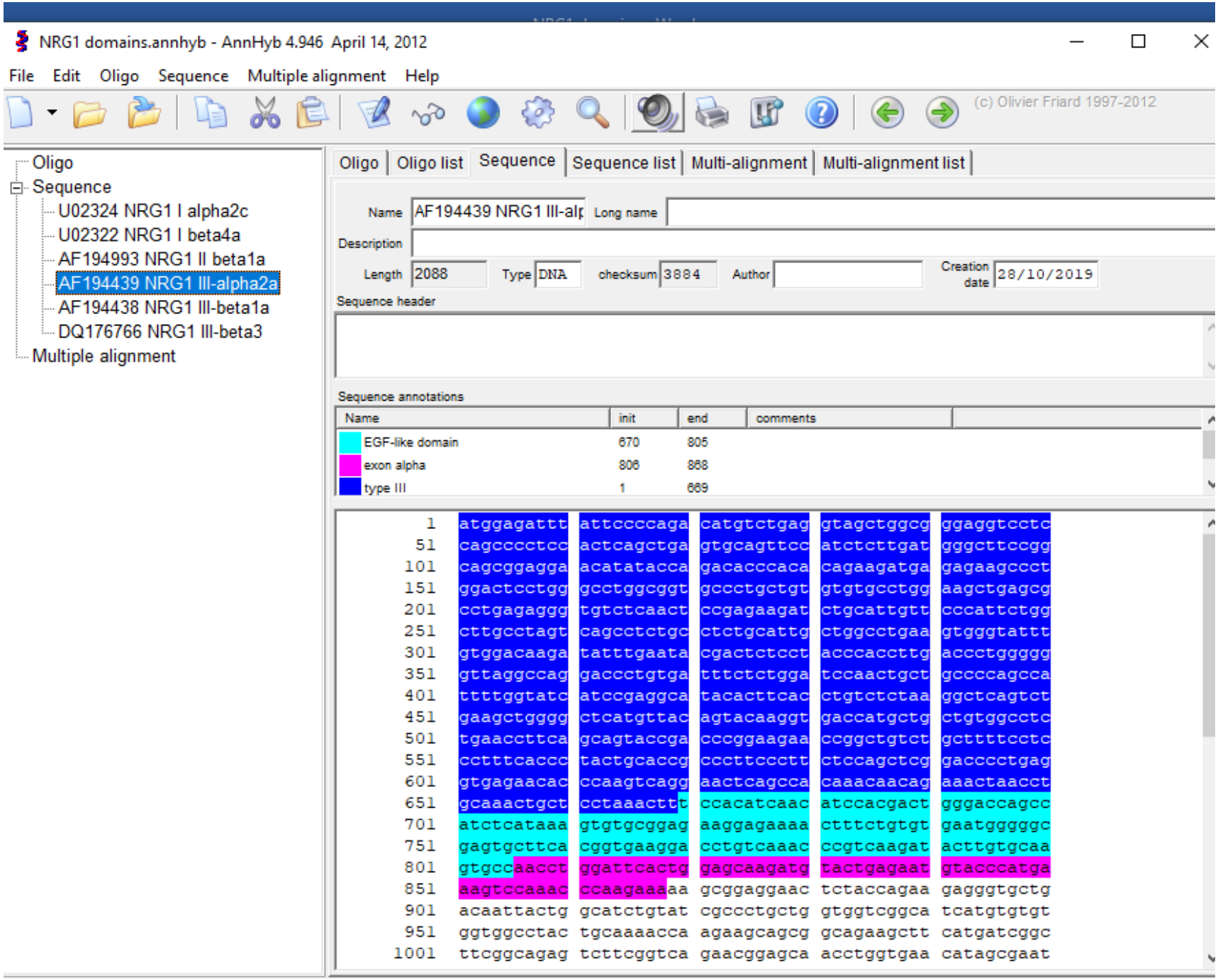


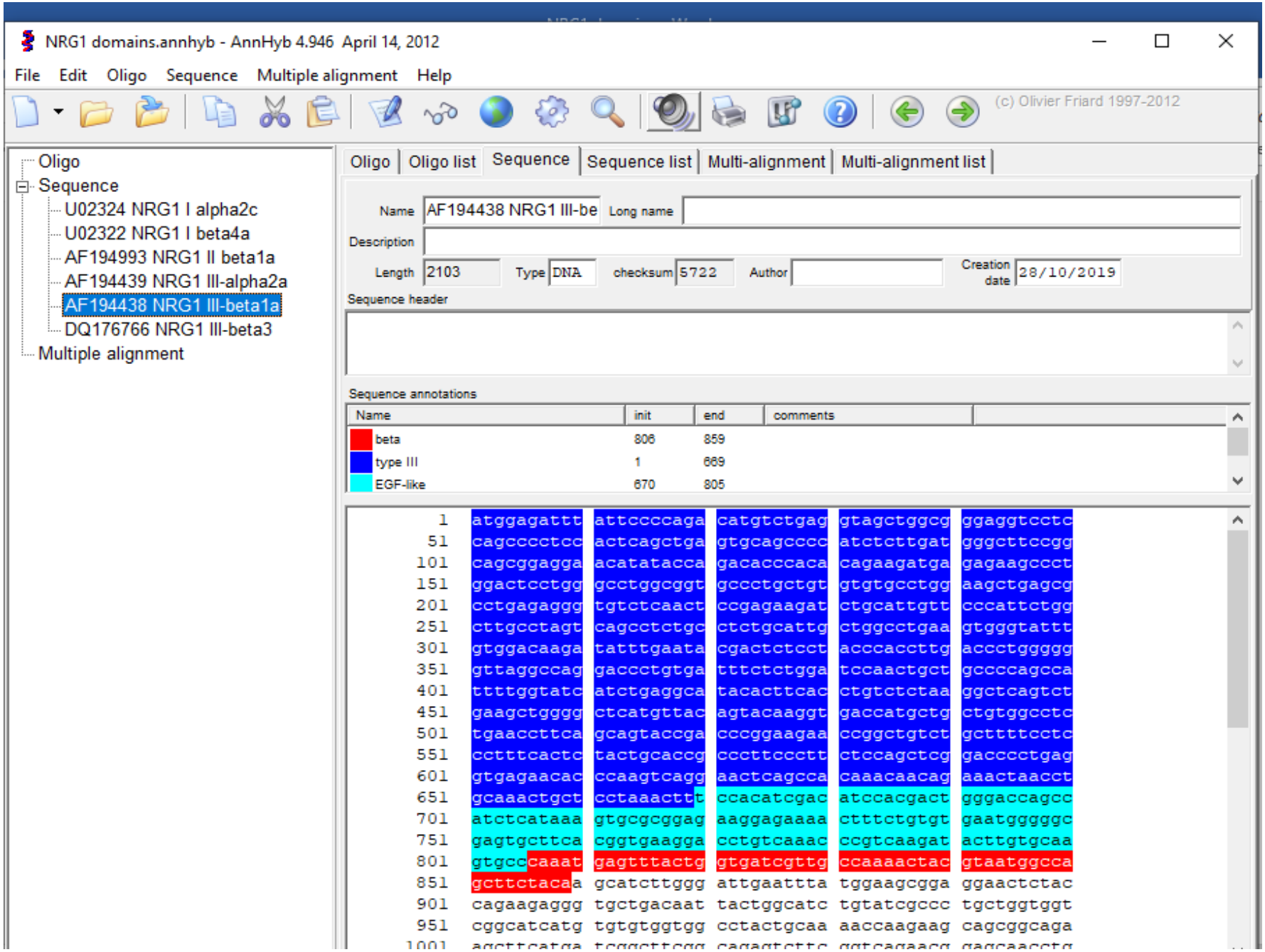

```
Query  670  TCCACATCAACATCCACGACTGGGACCAGCCATCTCATAAAGTGTGCGGAGAAGGAGAAA  729
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  502  TCCACATCAACATCCACGACTGGGACCAGCCATCTCATAAAGTGTGCGGAGAAGGAGAAA  561

Query  730  ACTTTCTGTGTGAATGGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAACCCGTCAAGA  789
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  562  ACTTTCTGTGTGAATGGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAACCCGTCAAGA  621

Query  790  TACTTGTGCAAGTGCC 805
           ||||||||||||||||
Sbjct  622  TACTTGTGCAAGTGCC 637
```

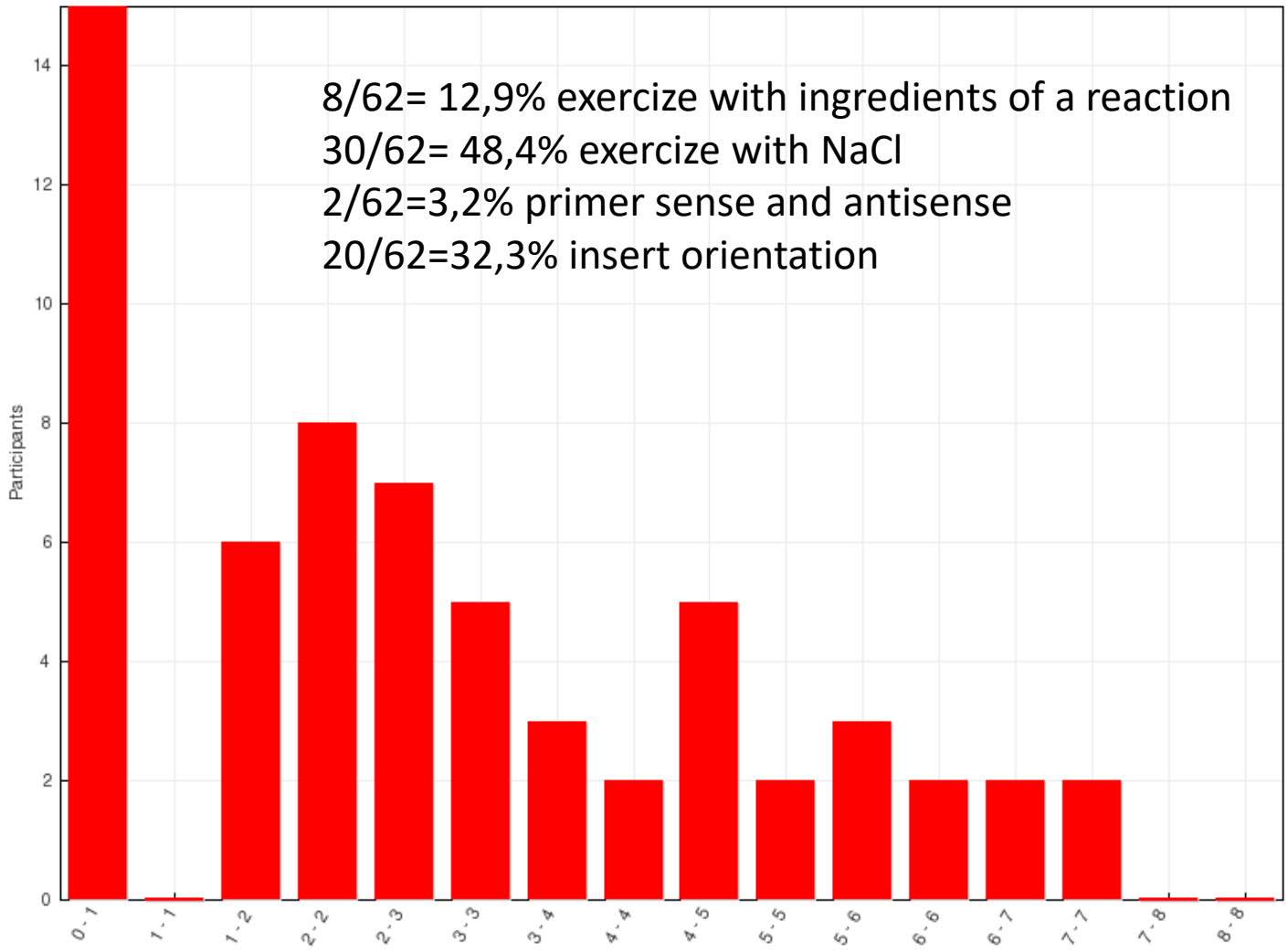

NRG1 domains.annhyb - AnnHyb 4.946 April 14, 2012
File Edit Oligo Sequence Multiple alignment Help
(c) Olivier Friard 1997-2012
Oligo
Sequence
U02324 NRG1 I alpha2c
U02322 NRG1 I beta4a
AF194993 NRG1 II beta1a
AF194439 NRG1 III-alpha2a
AF194438 NRG1 III-beta1a
DQ176766 NRG1 III-beta3
Multiple alignment
Oligo
Oligo list
Sequence
Sequence list
Multi-alignment
Multi-alignment list
Name U02324 NRG1 I alpha2
Long name
Description
Length 1269
Type DNA
checksum 9658
Author
Creation date 04/10/2020
Sequence header
Sequence annotations
Name
init
end
comments
type I 1 99
EGF-like 502 637
alpha 638 700
1 atgtctgagc gcaaagaagg cagaggcaag gggaagggca agaagaagga
51 ccggggatcc cgcgggaagc ccgggcccgc cgagggcgac ccgagcccag
101 cactgcctcc cagattgaaa gaaatgaaga gccaggagtc agctgcaggc
151 tccaagctag tgctccggtg cgaaaccagc tccgagtact cctcactcag
201 attcaaatgg ttcaagaatg ggaacgagct gaaccgcaaa aataaaccag
251 aaaacatcaa gatacagaag aagccaggga agtcagagct tcgaattaac
301 aaagcatccc tggctgactc tggagagtat atgtgcaaag tgatcagcaa
351 gttaggaaat gacagtgcct ctgccaacat caccattgtt gagtcaaacg
401 agttcatcac tggcatgcca gcctcgactg agacagccta tgtgtcctca
451 gagtctccca ttagaatctc agtttcaaca gaaggcgcaa acacttcttc
501 atccacatca acatccacga ctgggaccag ccatctcata aagtgtgcgg
551 agaaggagaa aactttctgt gtgaatgggg gcgagtgctt cacggtgaag
601 gacctgtcaa acccgtcaag atacttgtgc aagtgccaac ctggattcac
651 tggagcaaga tgtactgaga atgtacccat gaaagtccaa acccaagaaa
701 aagcggagga actctaccag aagagggtgc tgacaattac tggcatctgt
751 atcgccctgc tggtggtcgg catcatgtgt gtggtggcct actgcaaaac
801 caagaagcag cggcagaagc ttcatgatcg gcttcggcag agtcttcggt
851 cagaacggag caacctggtg aacatagcga atgggcctca ccacccaaac
Oligo list (0 oligonucleotides)


NRG1 domains.annhyb - AnnHyb 4.946 April 14, 2012
File Edit Oligo Sequence Multiple alignment Help
(c) Olivier Friard 199
Oligo
Sequence
U02324 NRG1 I alpha2c
U02322 NRG1 I beta4a
AF194993 NRG1 II beta1a
AF194439 NRG1 III-alpha2a
AF194438 NRG1 III-beta1a
DQ176766 NRG1 III-beta3
Multiple alignment
Oligo
Oligo list
Sequence
Sequence list
Multi-alignment
Multi-alignment list
Name
U02322 NRG1 I beta4:
Long name
Description
Length
1989
Type
DNA
checksum
5488
Author
Creation date
04/10/2020
Sequence header
Sequence annotations
Name
init
end
comments
type I
1
99
EGF-like
502
637
beta
638
691
1 atgtctgagc gcaaagaagg cagaggcaag gggaagggca agaagaagga
51 ccggggatcc cgcgggaagc ccgggcccgc cgagggcgac ccgagcccag
101 cactgcctcc cagattgaaa gaaatgaaga gccaggagtc agctgcaggc
151 tccaagctag tgctccggtg cgaaaccagc tccgagtact cctcactcag
201 attcaaatgg ttcaagaatg ggaacgagct gaaccgcaaa aataaaccag
251 aaaacatcaa gatacagaag aagccaggga agtcagagct tcgaattaac
301 aaagcatccc tggctgactc tggagagtat atgtgcaaag tgatcagcaa
351 gttaggaaat gacagtgcct ctgccaacat caccattgtt gagtcaaacg
401 agttcatcac tggcatgcca gcctcgactg agacagccta tgtgtcctca
451 gagtctccca ttagaatctc agtttcaaca gaaggcgcaa acacttcttc
501 atccacatca acatccacga ctgggaccag ccatctcata aagtgtgcgg
551 agaaggagaa aactttctgt gtgaatgggg gcgagtgctt cacggtgaag
601 gacctgtcaa acccgtcaag atacttgtgc aagtgcccaa atgagtttac
651 tggtgatcgt tgccaaaact acgtaatggc cagcttctac atgacttcta
701 ggaggaaaag gcaagaaaca gagaagcctc tagaaagaaa attggatcat
751 agccttgtga aagaatcgaa agcggaggaa ctctaccaga agagggtgct
801 gacaattact ggcatctgta tcgccctgct ggtggtcggc atcatgtgtg
851 tggtggccta ctgcaaaacc aagaagcagc ggcagaagct tcatgatcgg


NRG1 domains.annhyb - AnnHyb 4.946 April 14, 2012
File Edit Oligo Sequence Multiple alignment Help
(c) Olivier Friard 1997-2012
Oligo
Sequence
U02324 NRG1 I alpha2c
U02322 NRG1 I beta4a
AF194993 NRG1 II beta1a
AF194439 NRG1 III-alpha2a
AF194438 NRG1 III-beta1a
DQ176766 NRG1 III-beta3
Multiple alignment
Oligo Oligo list Sequence Sequence list Multi-alignment Multi-alignment list
Name AF194993 NRG1 II bet
Long name
Description
Length 2349
Type DNA
checksum 2909
Author
Creation date 04/10/2020
Sequence header
Sequence annotations
Name init end comments
type II 1 513
EGF-like 916 1051
beta 1052 1105
101 aggcagcagg cgaggcaggg gcaggggcgc gggaccagcc cgtccaggac
151 tcgccacctt cacaggaccc tctgcctgct gtcaactgga ccctgcccac
201 tgggggcccc gagcccagca ccgatcagcc cggggacccc gcgccctatc
251 tggtcaaggt gcaccaggtg tgggctgtga aagccggggg tttgaagaag
301 gactcgctac tcaccgtgcg cctggatacc tggggccacc cagccttccc
351 gtcctgcggg cggctcaagg aggacagcag gtacatcttc ttcatggagc
401 cggatgccaa cagcagcggc cgcgcgccgc ccgccttccg agcctcgttt
451 cccccactgg agactggccg caacctcaag aaggaggtca gccgggtgtt
501 gtgcaagcgg tgcgcactgc ctcccagatt gaaagaaatg aagagccagg
551 agtcagctgc aggctccaag ctagtgctcc ggtgcgaaac cagctccgag
601 tactcctcac tcagattcaa atggttcaag aatgggaacg agctgaaccg
651 caaaaataaa ccagaaaaca tcaagataca gaagaagcca gggaagtcag
701 agcttcgaat taacaaagca tccctggctg actctggaga gtatatgtgc
751 aaagtgatca gcaagttagg aaatgacagt gcctctgcca acatcaccat
801 tgttgagtca aacgagttca tcactggcat gccagcctcg actgagacag
851 cctatgtgtc ctcagagtct cccattagaa tctcagtttc aacagaaggc
901 gcaaacactt cttcatccac atcgacatcc acgactggga ccagccatct
951 cataaagtgc gcggagaagg agaaaacttt ctgtgtgaat gggggcgagt
1001 gcttcacggt gaaggacctg tcaaacccgt caagatactt gtgcaagtgc
1051 ccaaatgagt ttactggtga tcgttgccaa aactacgtaa tggccagctt
1101 ctacaagcat cttgggattg aatttatgga agcggaggaa ctctaccaga


NRG1 domains.annhyb - AnnHyb 4.946 April 14, 2012
File Edit Oligo Sequence Multiple alignment Help
(c) Olivier Friard 1997-2012
Oligo
Sequence
U02324 NRG1 I alpha2c
U02322 NRG1 I beta4a
AF194993 NRG1 II beta1a
AF194439 NRG1 III-alpha2a
AF194438 NRG1 III-beta1a
DQ176766 NRG1 III-beta3
Multiple alignment
Oligo
Oligo list
Sequence
Sequence list
Multi-alignment
Multi-alignment list
Name AF194439 NRG1 III-alp
Long name
Description
Length 2088
Type DNA
checksum 3884
Author
Creation date 28/10/2019
Sequence header
Sequence annotations
Name
init
end
comments
EGF-like domain 670 805
exon alpha 806 868
type III 1 669
1 atggagattt attccccaga catgtctgag gtagctggcg ggaggtcctc
51 cagcccctcc actcagctga gtgcagttcc atctcttgat gggcttccgg
101 cagcggagga acatatacca gacacccaca cagaagatga gagaagccct
151 ggactcctgg gcctggcggt gccctgctgt gtgtgcctgg aagctgagcg
201 cctgagaggg tgtctcaact ccgagaagat ctgcattgtt cccattctgg
251 cttgcctagt cagcctctgc ctctgcattg ctggcctgaa gtgggtattt
301 gtggacaaga tatttgaata cgactctcct acccaccttg accctggggg
351 gttaggccag gaccctgtga tttctctgga tccaactgct gccccagcca
401 ttttggtatc atccgaggca tacacttcac ctgtctctaa ggctcagtct
451 gaagctgggg ctcatgttac agtacaaggt gaccatgctg ctgtggcctc
501 tgaaccttca gcagtaccga cccggaagaa ccggctgtct gcttttcctc
551 cctttcaccc tactgcaccg cccttccctt ctccagctcg gacccctgag
601 gtgagaacac ccaagtcagg aactcagcca caaacaacag aaactaacct
651 gcaaactgct cctaaacttt ccacatcaac atccacgact gggaccagcc
701 atctcataaa gtgtgcggag aaggagaaaa ctttctgtgt gaatgggggc
751 gagtgcttca cggtgaagga cctgtcaaac ccgtcaagat acttgtgcaa
801 gtgccaacct ggattcactg gagcaagatg tactgagaat gtacccatga
851 aagtccaaac ccaagaaaaa gcggaggaac tctaccagaa gagggtgctg
901 acaattactg gcatctgtat cgccctgctg gtggtcggca tcatgtgtgt
951 ggtggcctac tgcaaaacca agaagcagcg gcagaagctt catgatcggc
1001 ttcggcagag tcttcggtca gaacggagca acctggtgaa catagcgaat


NRG1 domains.annhyb - AnnHyb 4.946 April 14, 2012
File Edit Oligo Sequence Multiple alignment Help
(c) Olivier Friard 1997-2012
Oligo
Sequence
U02324 NRG1 I alpha2c
U02322 NRG1 I beta4a
AF194993 NRG1 II beta1a
AF194439 NRG1 III-alpha2a
AF194438 NRG1 III-beta1a
DQ176766 NRG1 III-beta3
Multiple alignment
Oligo
Oligo list
Sequence
Sequence list
Multi-alignment
Multi-alignment list
Name AF194438 NRG1 III-be
Long name
Description
Length 2103
Type DNA
checksum 5722
Author
Creation date 28/10/2019
Sequence header
Sequence annotations
Name
init
end
comments
beta 806 859
type III 1 669
EGF-like 670 805
1 atggagattt attccccaga catgtctgag gtagctggcg ggaggtcctc
51 cagcccctcc actcagctga gtgcagcccc atctcttgat gggcttccgg
101 cagcggagga acatatacca gacacccaca cagaagatga gagaagccct
151 ggactcctgg gcctggcggt gccctgctgt gtgtgcctgg aagctgagcg
201 cctgagaggg tgtctcaact ccgagaagat ctgcattgtt cccattctgg
251 cttgcctagt cagcctctgc ctctgcattg ctggcctgaa gtgggtattt
301 gtggacaaga tatttgaata cgactctcct acccaccttg accctggggg
351 gttaggccag gaccctgtga tttctctgga tccaactgct gccccagcca
401 ttttggtatc atctgaggca tacacttcac ctgtctctaa ggctcagtct
451 gaagctgggg ctcatgttac agtacaaggt gaccatgctg ctgtggcctc
501 tgaaccttca gcagtaccga cccggaagaa ccggctgtct gcttttcctc
551 cctttcactc tactgcaccg cccttccctt ctccagctcg gacccctgag
601 gtgagaacac ccaagtcagg aactcagcca caaacaacag aaactaacct
651 gcaaactgct cctaaacttt ccacatcgac atccacgact gggaccagcc
701 atctcataaa gtgcgcggag aaggagaaaa ctttctgtgt gaatgggggc
751 gagtgcttca cggtgaagga cctgtcaaac ccgtcaagat acttgtgcaa
801 gtgccaaat gagtttactg gtgatcgttg ccaaaactac gtaatggcca
851 gcttctacaa gcatcttggg attgaattta tggaagcgga ggaactctac
901 cagaagaggg tgctgacaat tactggcatc tgtatcgccc tgctggtggt
951 cggcatcatg tgtgtggtgg cctactgcaa aaccaagaag cagcggcaga


8/62= 12,9% exercize with ingredients of a reaction
30/62= 48,4% exercize with NaCl
2/62=3,2% primer sense and antisense
20/62=32,3% insert orientation
Participants
0
2
4
6
8
10
12
14
0 - 1
1 - 1
1 - 2
2 - 2
2 - 3
3 - 3
3 - 4
4 - 4
4 - 5
5 - 5
5 - 6
6 - 6
6 - 7
7 - 7
7 - 8
8 - 8

## Question 3

Correct Mark 3.0 out of 3.0

You have to amplify ALL the following sequence, from the first to the last nucleotide; please design the FORWARD (=SENSE) primer, 10 base long and the REVERSE (=ANTISENSE) primer, 10 base long;

5′-TGCTATCTGCATGCTTAAGGCGCTAGGCTGACCTGAATCGTAGGCTAGCTAGCTAGCTAGCTCAGATAACCT-3
3′-ACGATAGACGTACGAATTCCGCGATCCGACTGGACTTAGCATCCGATCGATCGATCGATCGAGTCTATTGGA-5′

write only nucleotides, not symbols or numbers.

sense:

antisense:

## Question 3

Correct Mark 3.0 out of 3.0

You have to amplify ALL the following sequence, from the first to the last nucleotide; please design the FORWARD (=SENSE) primer, 10 base long and the REVERSE (=ANTISENSE) primer, 10 base long;

```
5′-TGCTATCTGCATGCTTAAGGCGCTAGGCTGACCTGAATCGTAGGCTAGCTAGCTAGCTAGCTCAGATAACCT-3
3′-ACGATAGACGTACGAATTCCGCGATCCGACTGGACTTAGCATCCGATCGATCGATCGATCGAGTCTATTGGA-5′
```

write only nucleotides, not symbols or numbers.

sense: TGCTATCTGC ✓

antisense: AGGTTATCTG ✓

# PROJECT OVERVIEW

Genebank sequence analysis

↓

primer design

↓

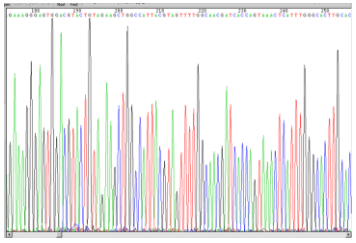
RT-PCR

↓

cloning in the vector pCRII-blunt

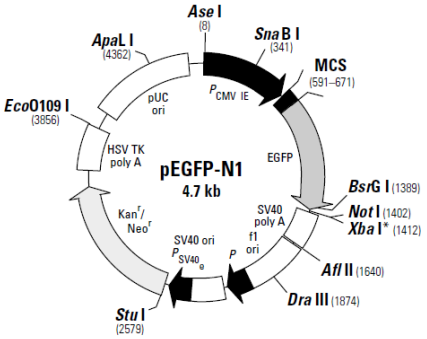
↓

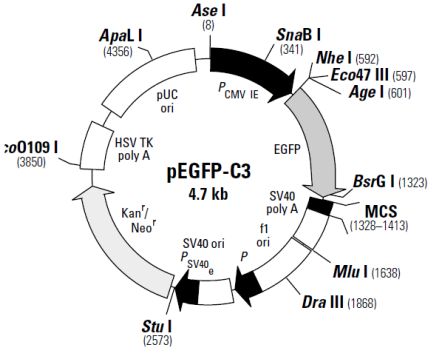
sequence analysis

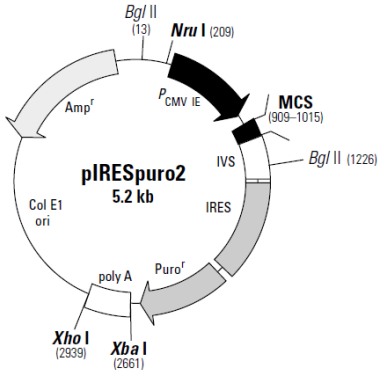


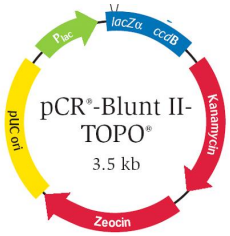
- real time PCR
- protein quantification

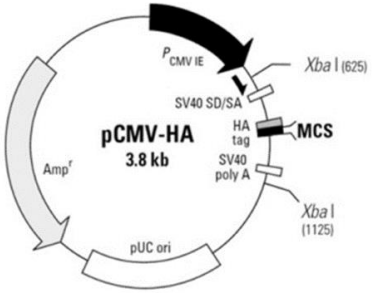
subcloning in different expression vectors

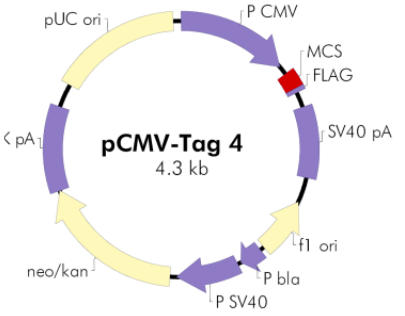


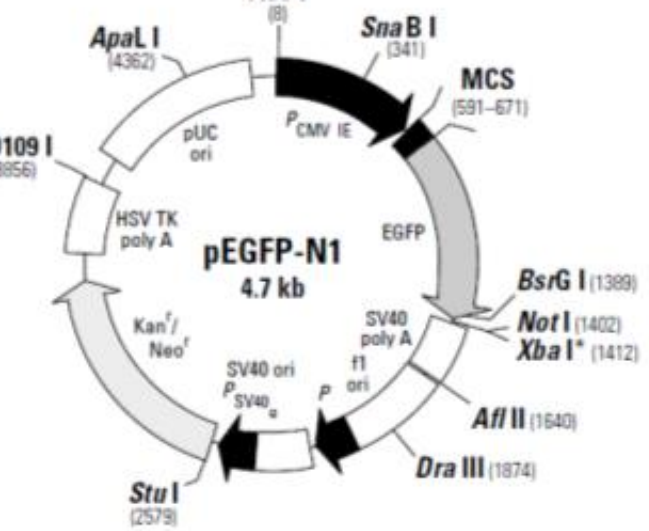



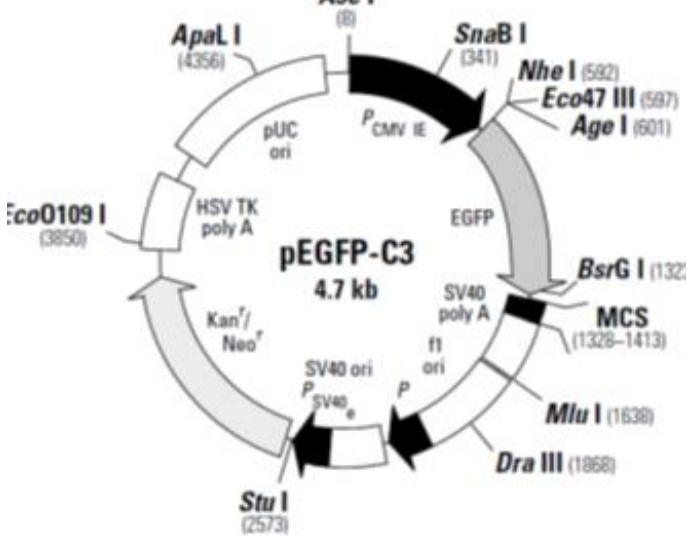



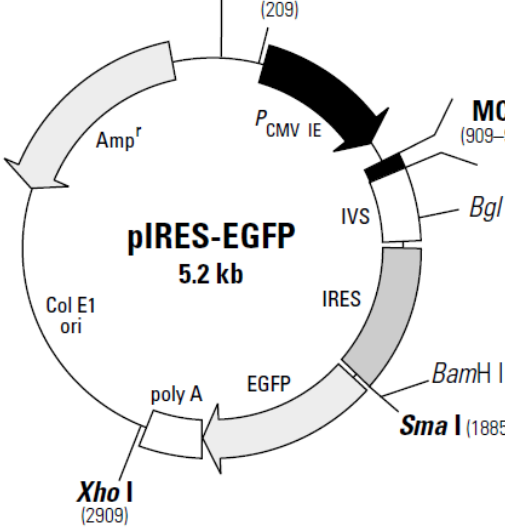



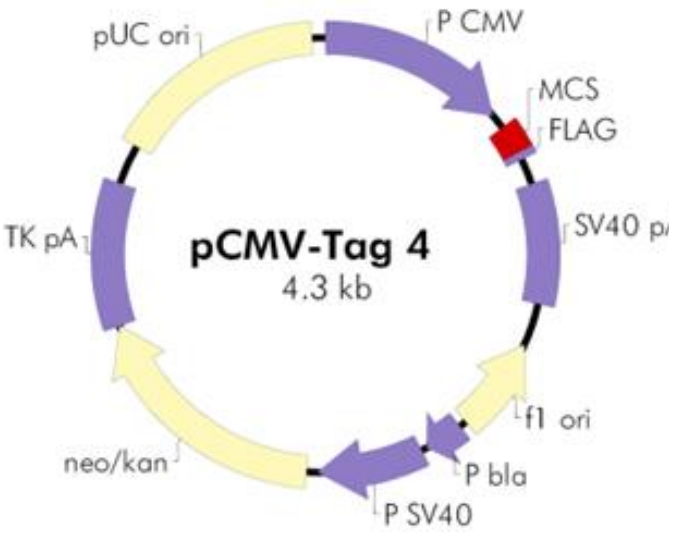



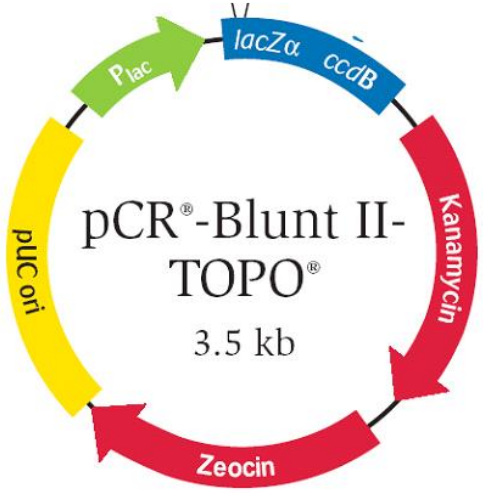



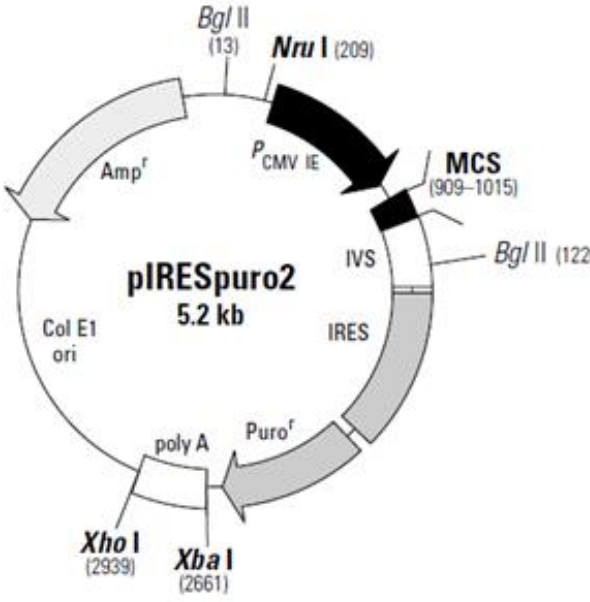

ApaL I
(4362)
SnaB I
(341)
MCS
(591–671)
pUC ori
P CMV IE
HSV TK poly A
EGFP
pEGFP-N1
4.7 kb
BsrG I (1389)
Not I (1402)
Xba I* (1412)
Kanr/ Neor
SV40 poly A
SV40 ori
f1 ori
Afl II (1640)
Dra III (1874)
Stu I (2579)

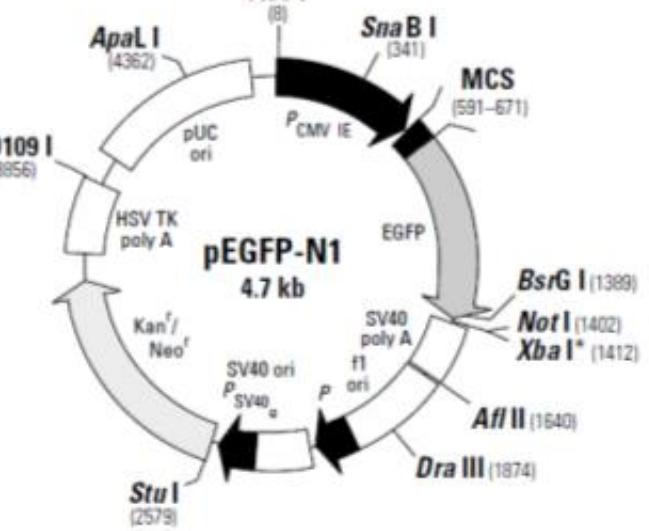

ApaL I
(4356)
SnaB I
(341)
Nhe I (592)
Eco47 III (597)
Age I (601)
pUC ori
P CMV IE
HSV TK poly A
EGFP
pEGFP-C3
4.7 kb
BsrG I (132
MCS
(1328–1413)
Kanr/ Neor
SV40 poly A
SV40 ori
f1 ori
Mlu I (1638)
Dra III (1868)
Stu I (2573)

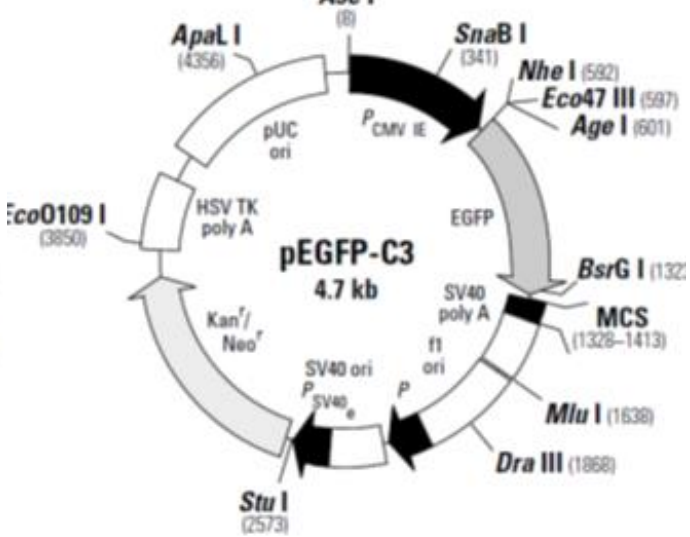

(209)
Ampr
P CMV IE
IVS
pIRES-EGFP
5.2 kb
Col E1 ori
IRES
poly A
EGFP
Xho I
(2909)

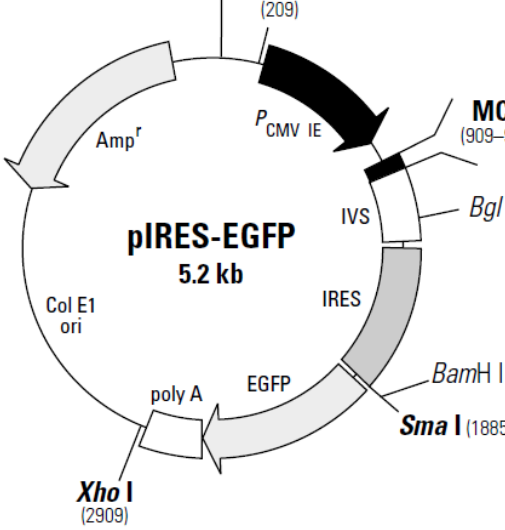

pUC ori
P CMV
MCS
FLAG
TK pA
pCMV-Tag 4
4.3 kb
neo/kan
f1 ori
P bla
P SV40


lacZα
ccdB
Plac
pUC ori
pCR®-Blunt II-TOPO®
3.5 kb
Kanamycin
Zeocin

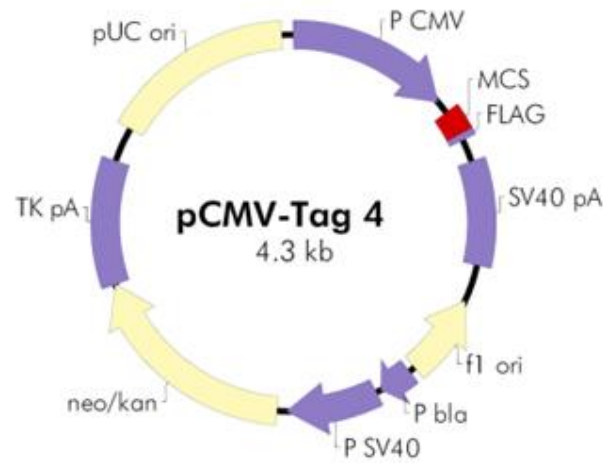

Bgl II
(13)
Nru I (209)
Ampr
P CMV IE
MCS
(909–1015)
IVS
pIRESpuro2
5.2 kb
Col E1 ori
IRES
poly A
Puror
Xho I
(2939)
Xba I
(2661)

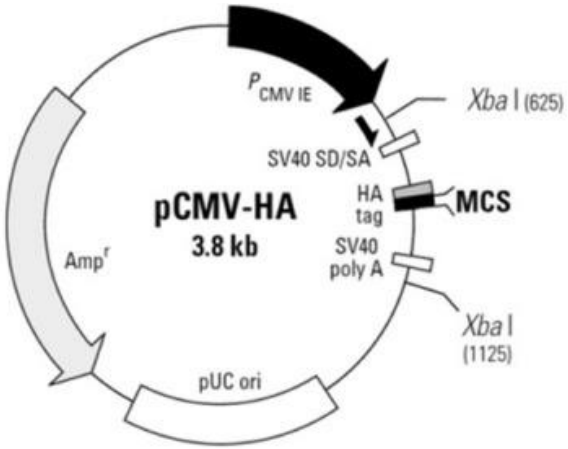


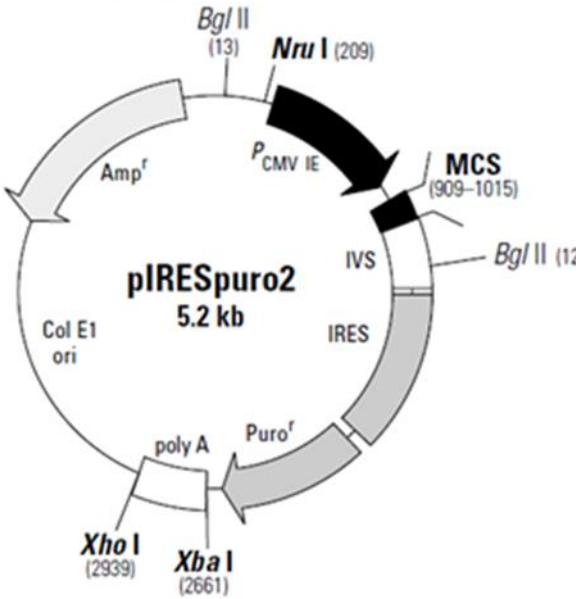



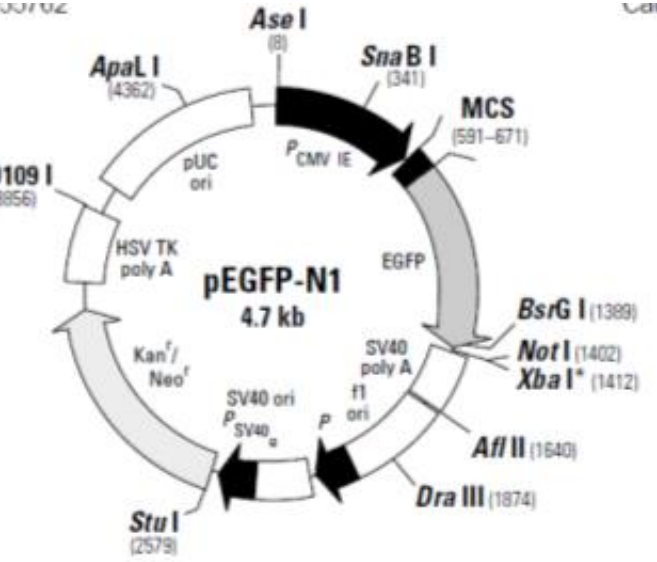



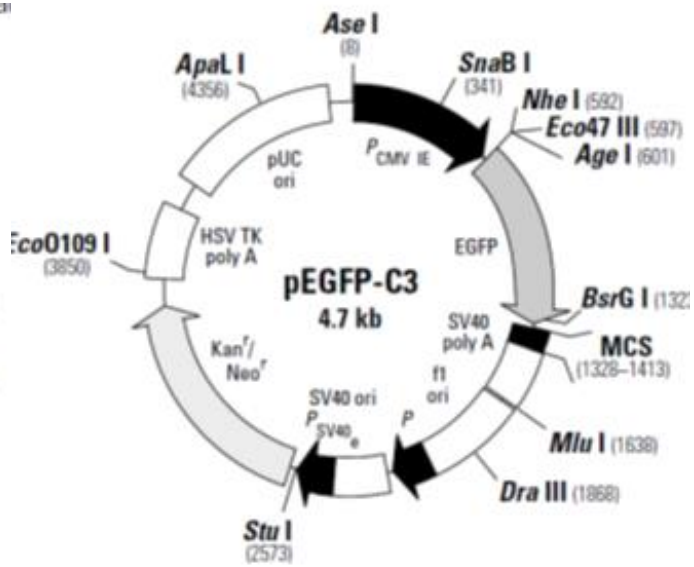

The internal ribosome entry site (**IRES**) of the encephalomyocarditis virus (ECMV), permits the translation of two open reading frames from one messenger RNA.

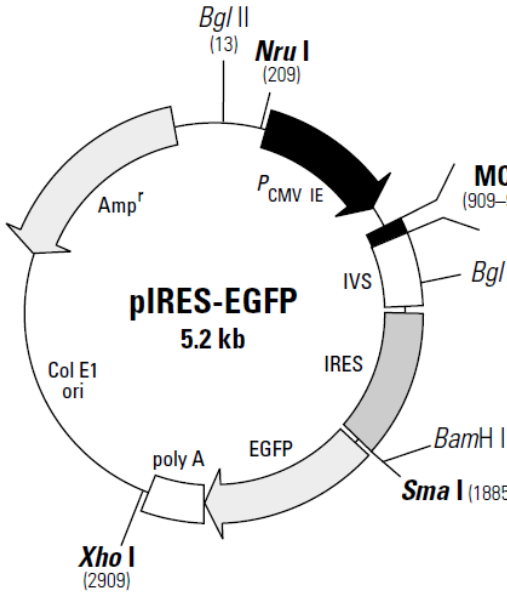


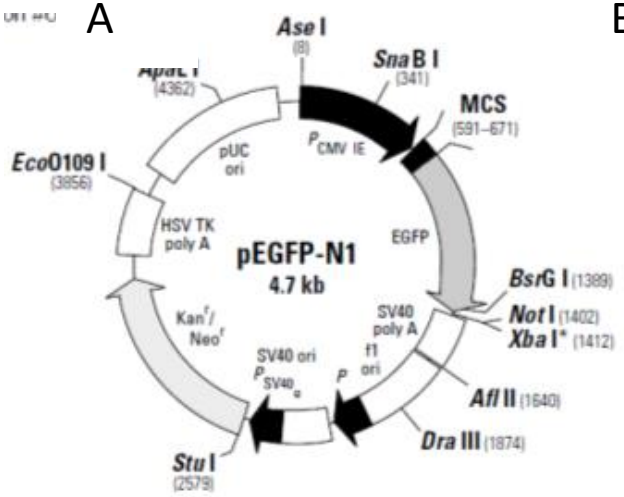


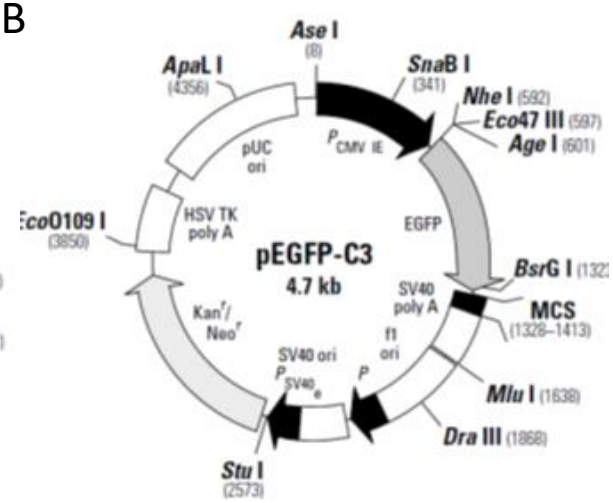




Which is the difference among the recombinant proteins obtained with these three expression vectors?

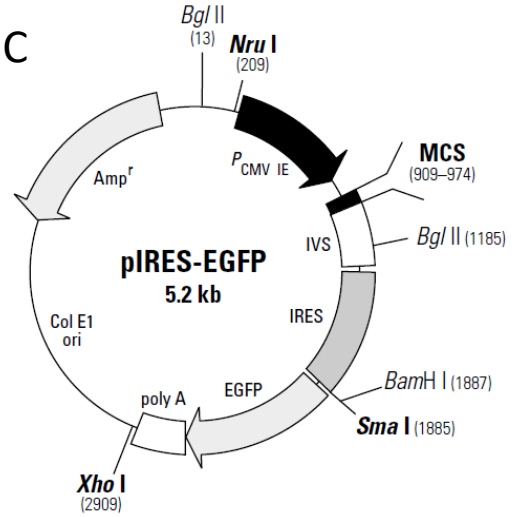
A




B




C




1- N- -C

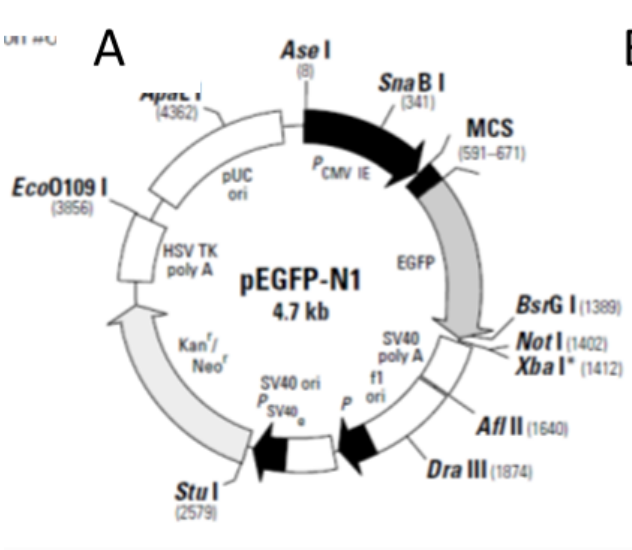
2- N- -C N- -C

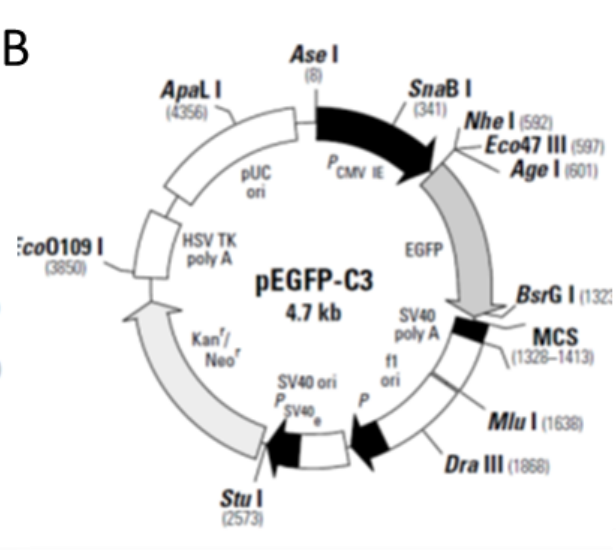
3- N- -C

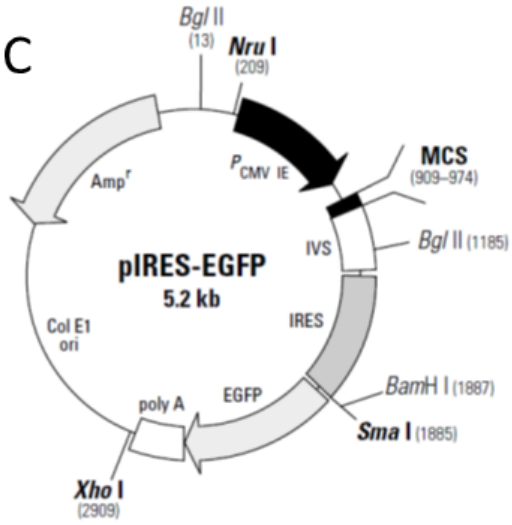
**PROTEIN OF INTEREST**

**EGFP**

**wooclap**



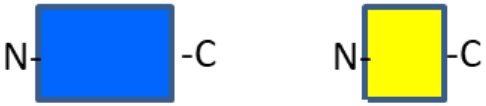





3-

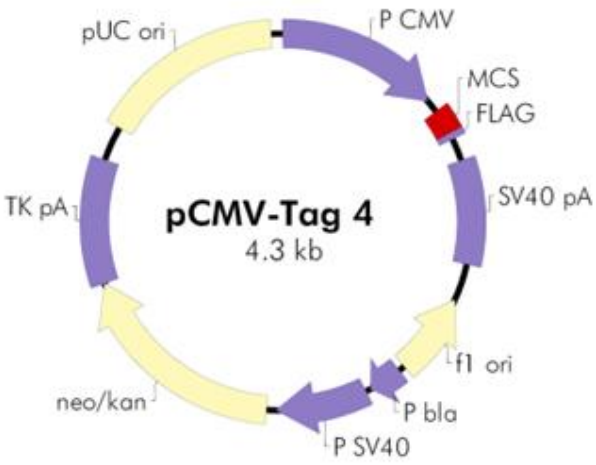

1-

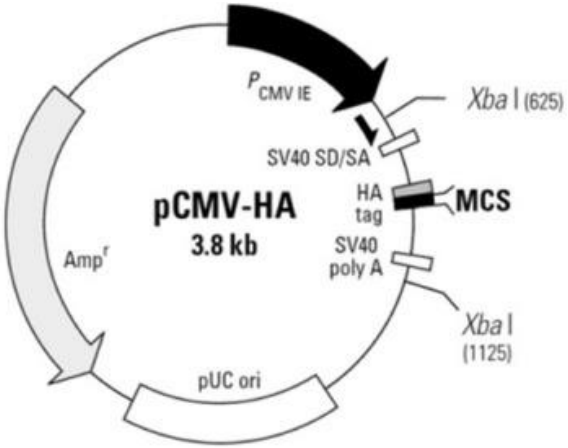


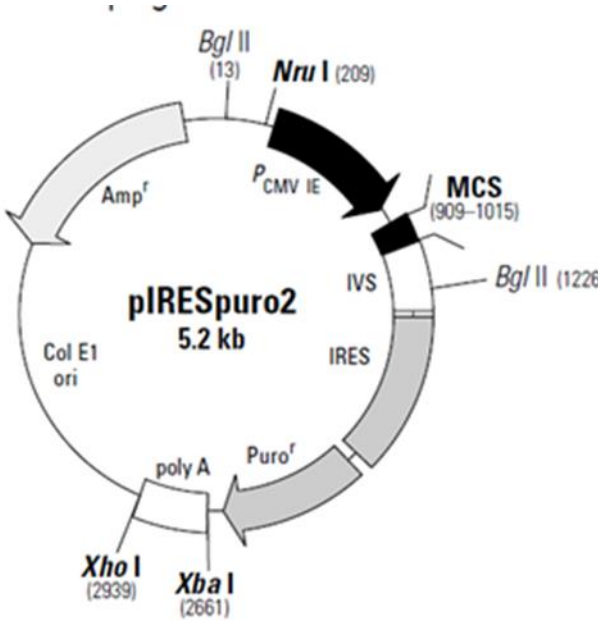

2-

**PROTEIN OF INTEREST**

**EGFP**

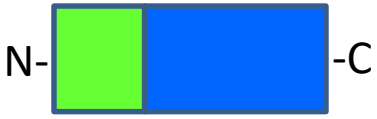


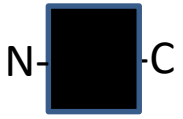





**PROTEIN OF INTEREST**
**FLAG**




**PROTEIN OF INTEREST**
**HA**

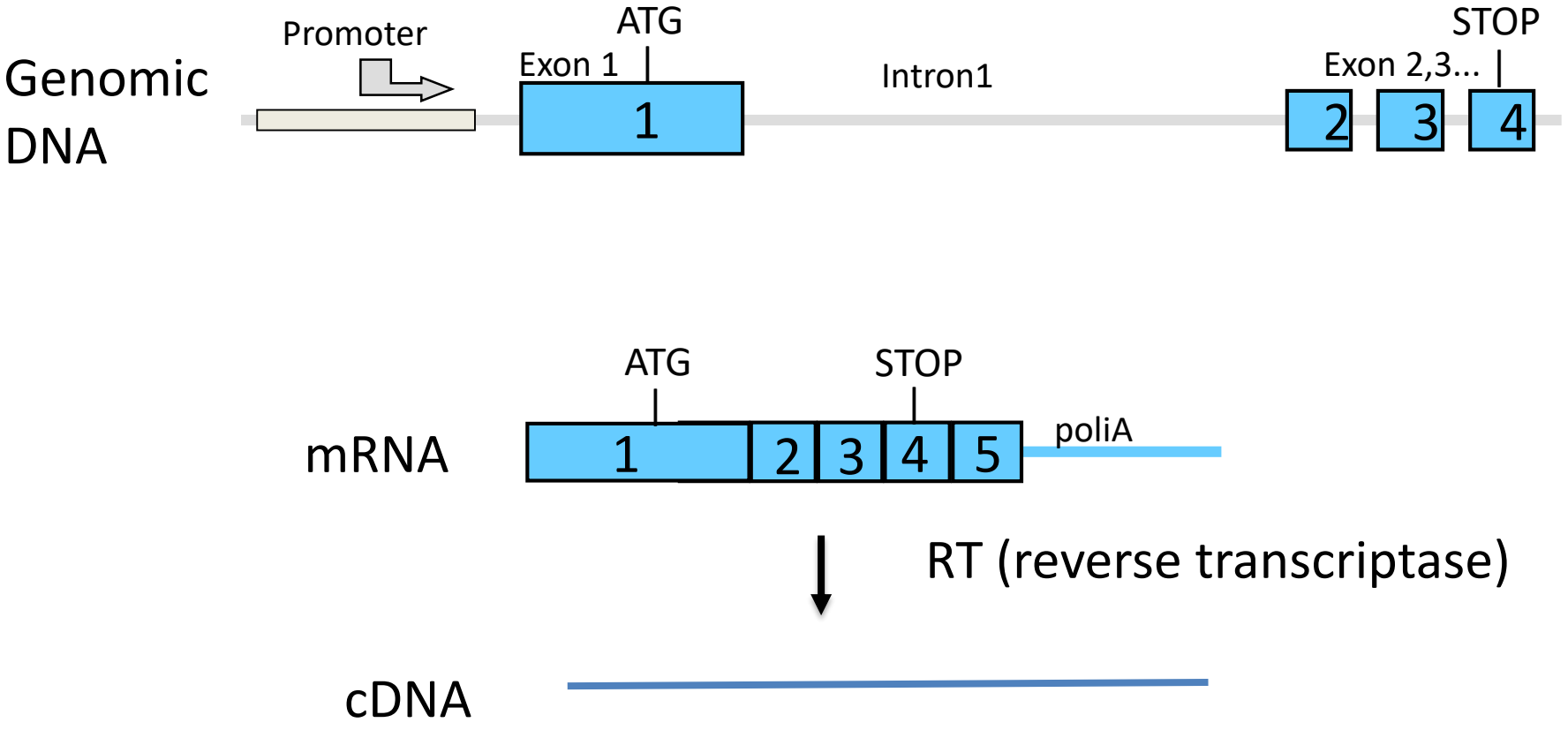

**PROTEIN OF INTEREST**
**PUROMYCIN RESISTANCE**

# RT-PCR amplification

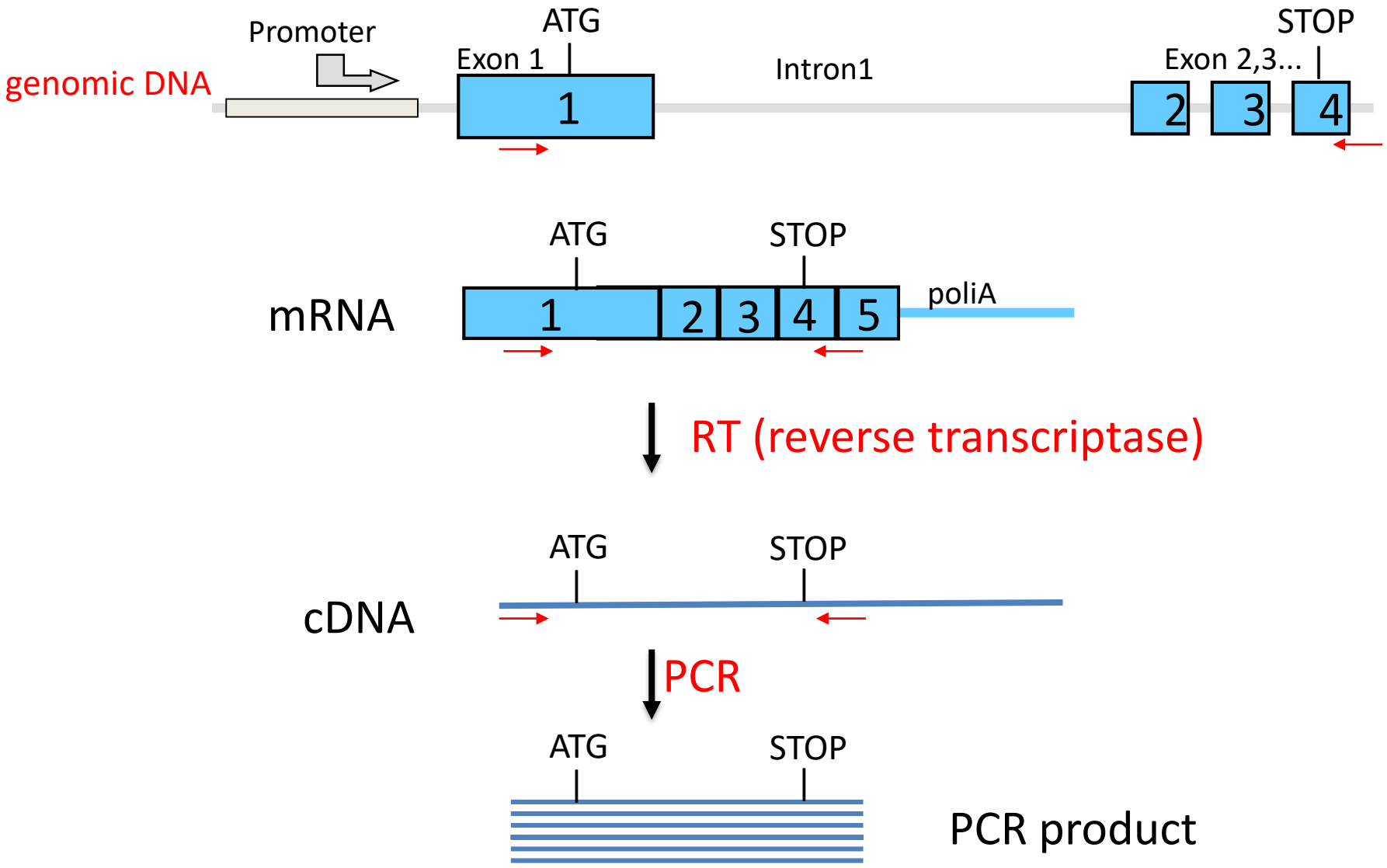
**1** - to clone the **full length cDNA** to express the protein

**2** -to verify/quantify  the **expression of a gene/specific isoform**

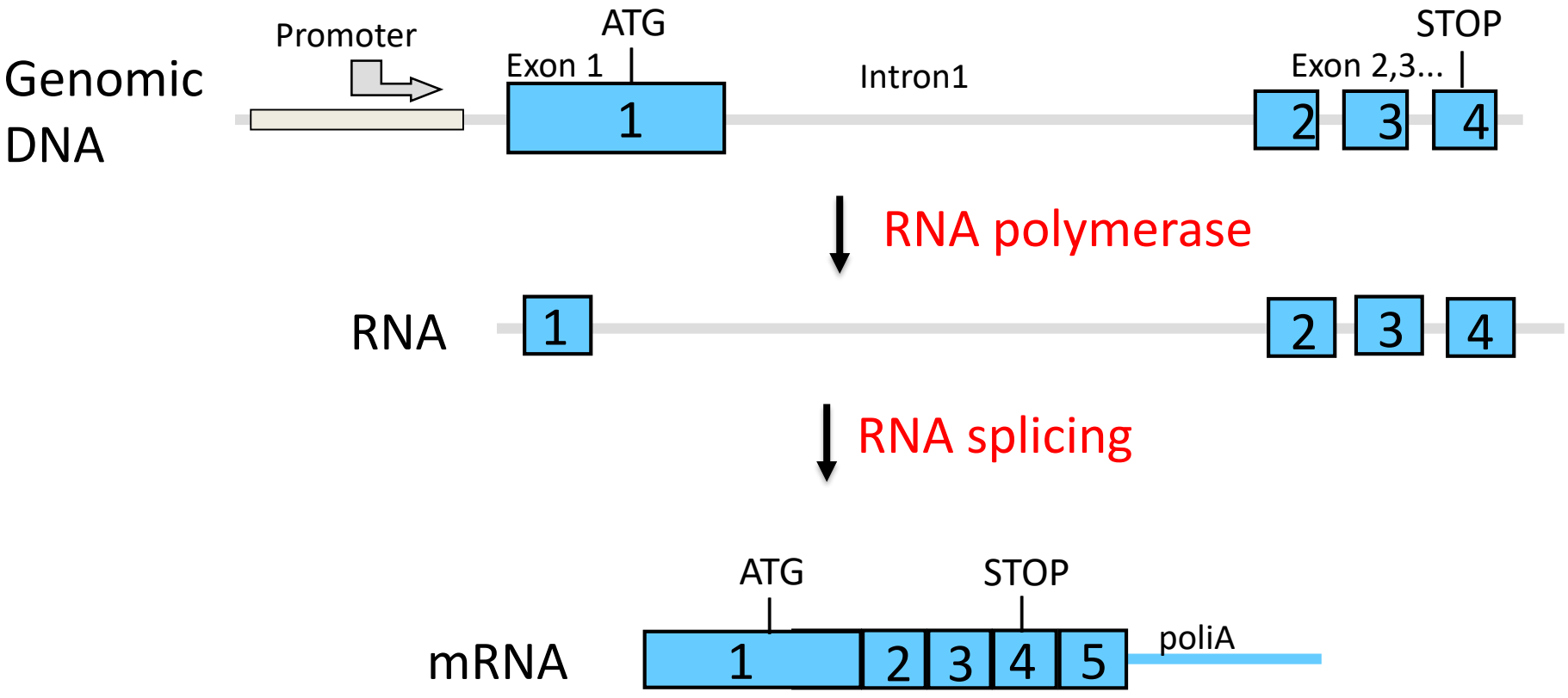
**1** – where do you put primers if you want to amplify & clone the full length cDNA to express the corresponding full length protein ?



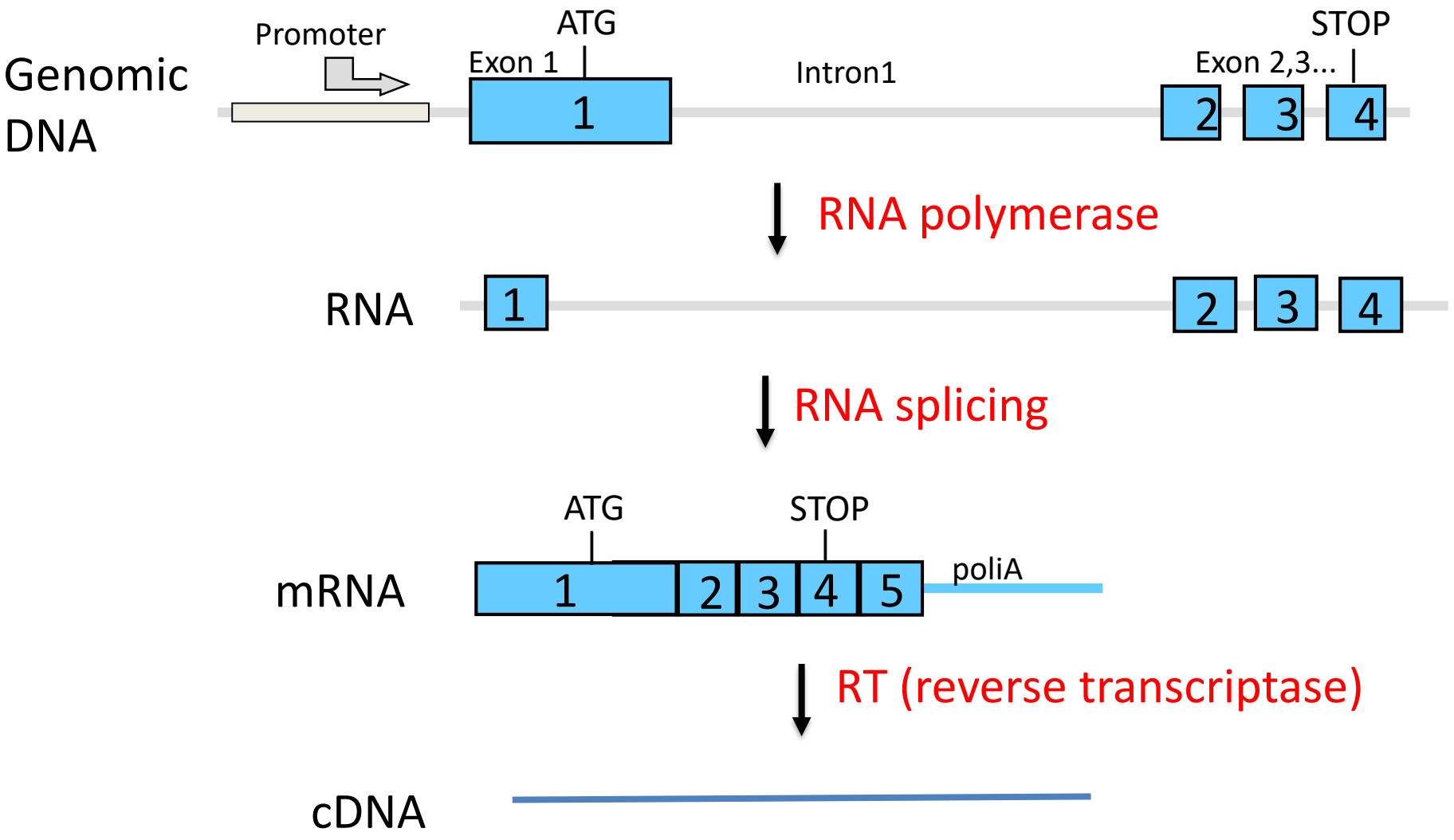
**1** – where do you put primers if you want to amplify & clone the full length cDNA to express the corresponding full length protein ?



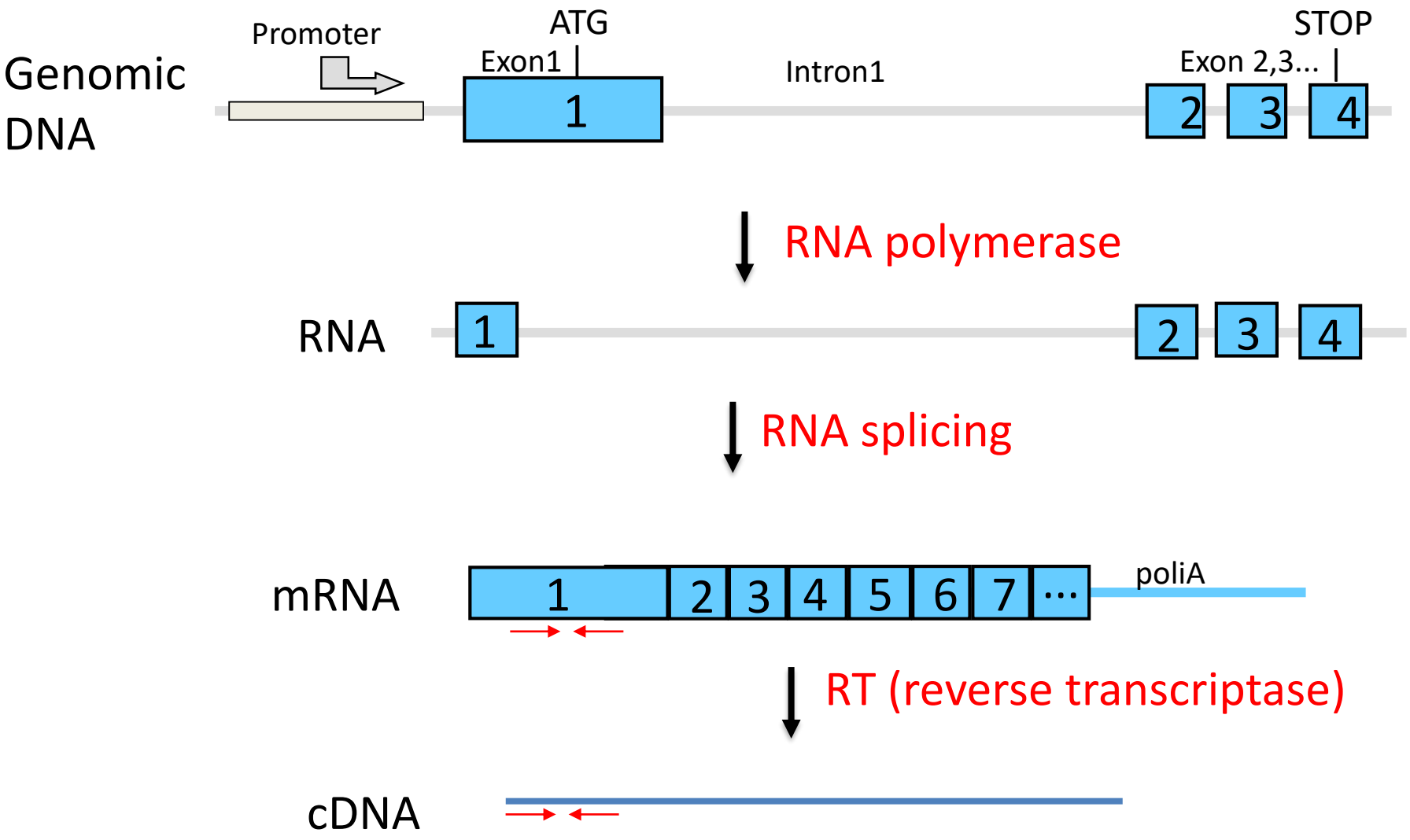
**2** – where do you put primers if you want to verify & quantify the expression of a gene?



**2** – where do you put primers if you want to verify & quantify the expression of a gene?

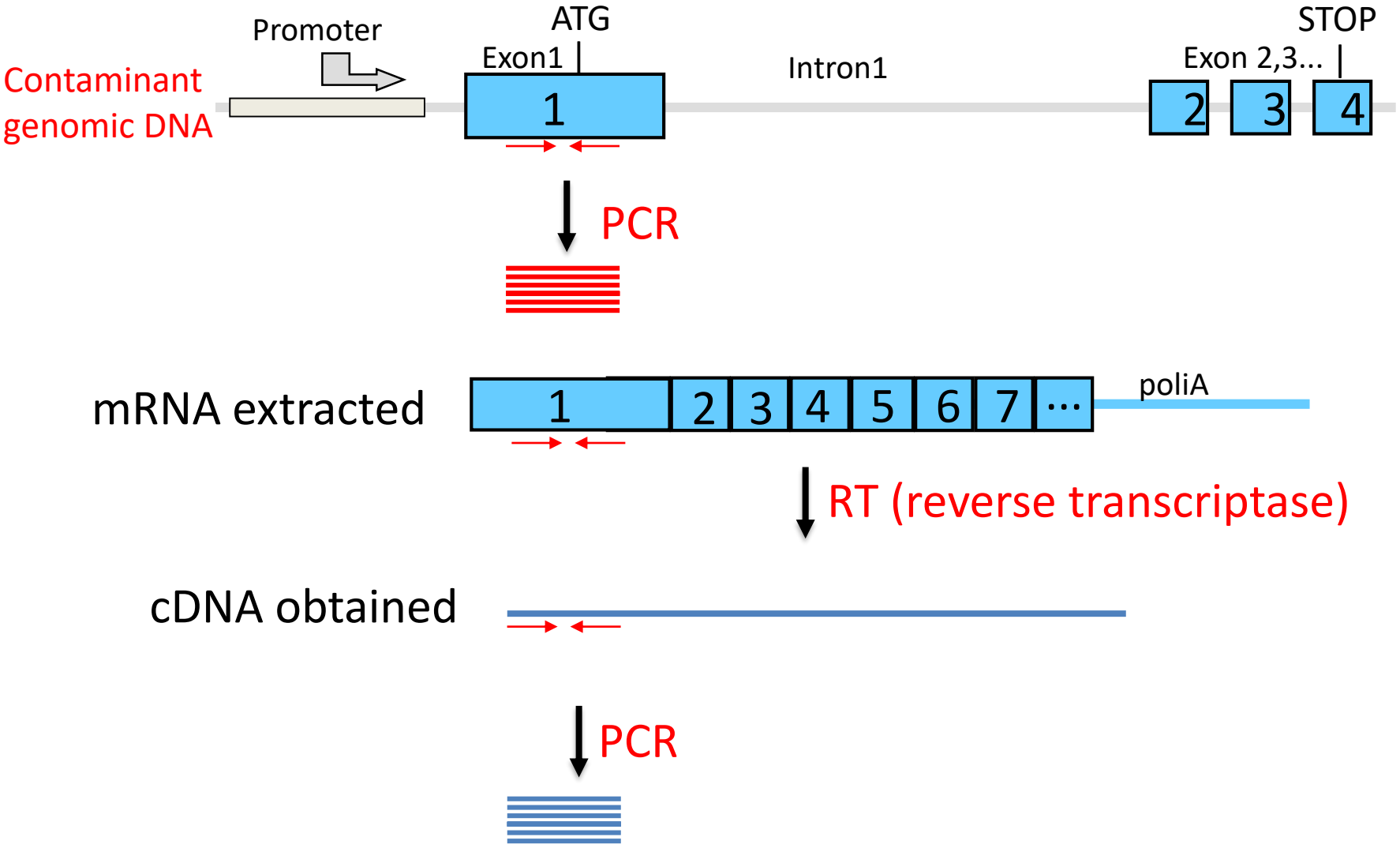


**2** – where do you put primers if you want to verify & quantify the expression of a gene?

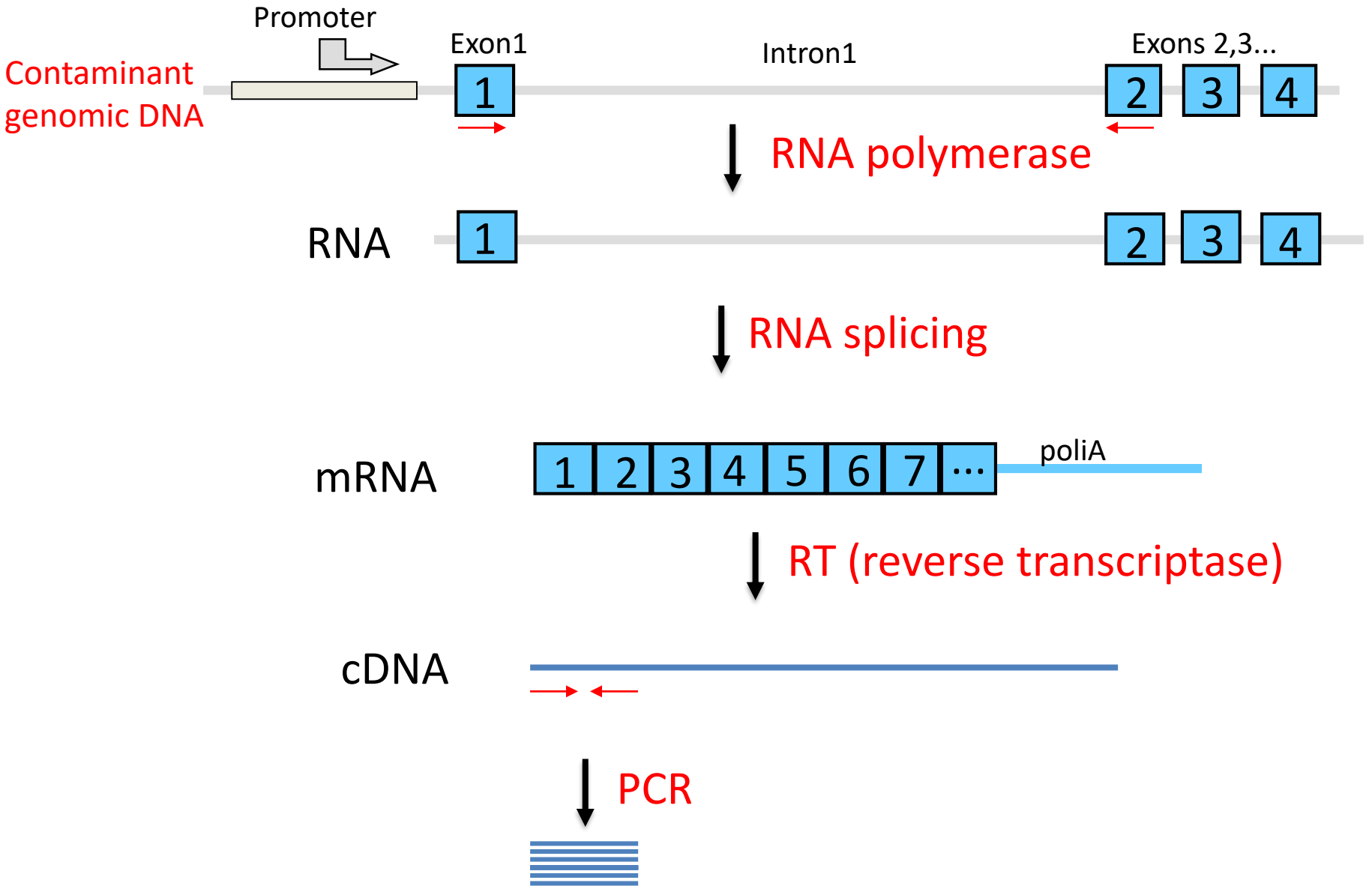


• sometime you have genomic DNA contamination.

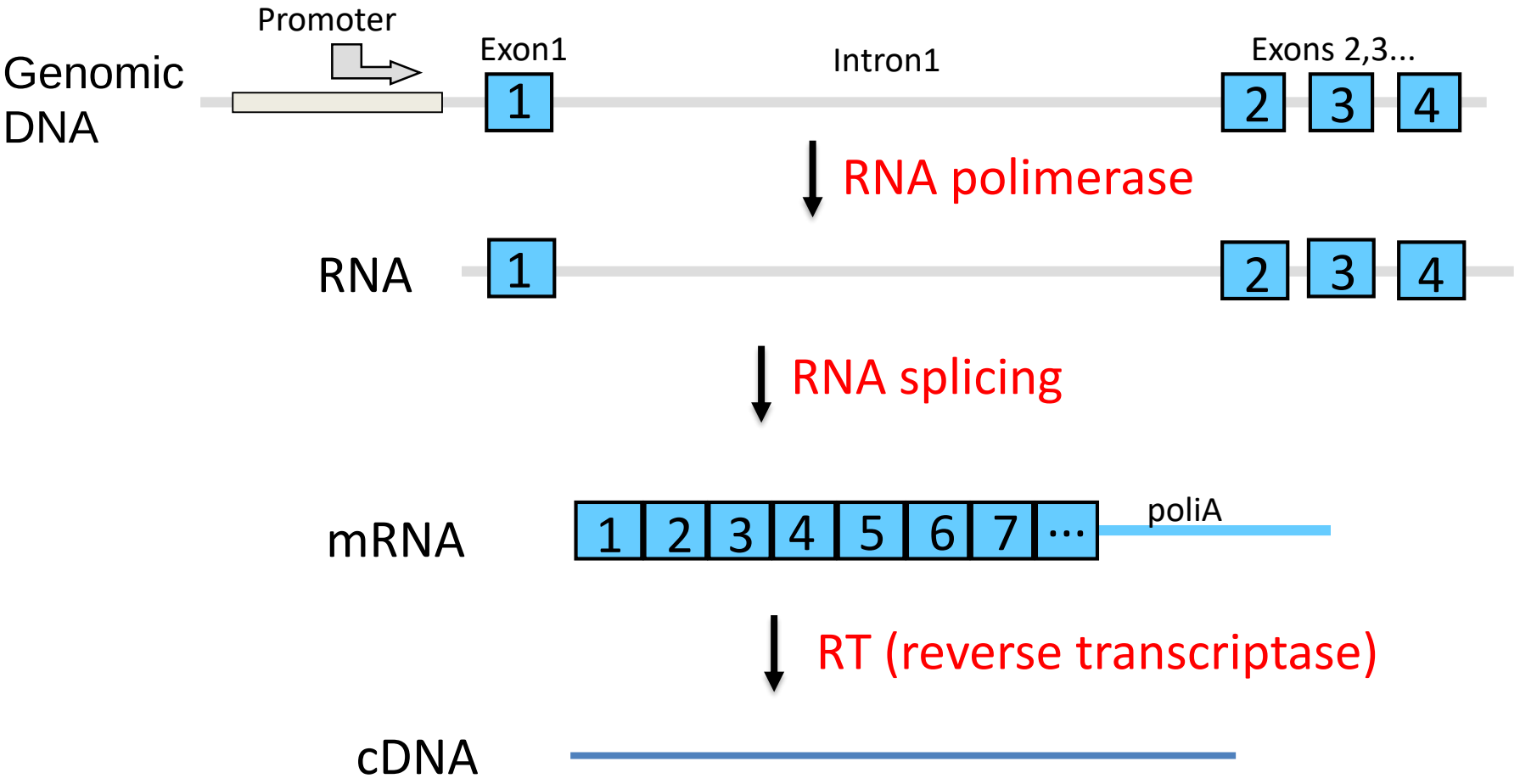
How can you amplify only cDNA and not contaminant genomic DNA?



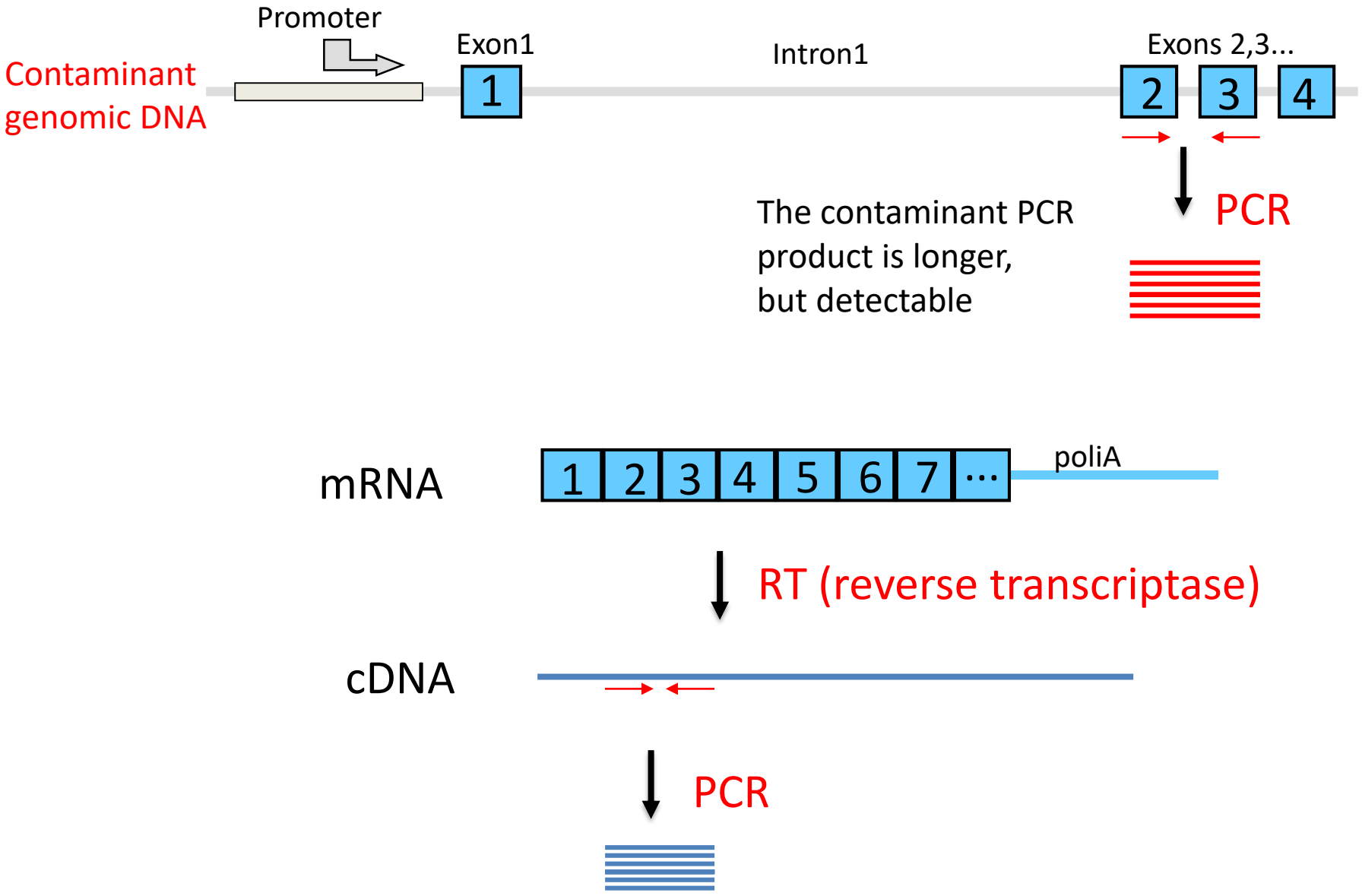
# 1- one strategy could be to design primers on different exons separated by a big intron (≥1000 bp)



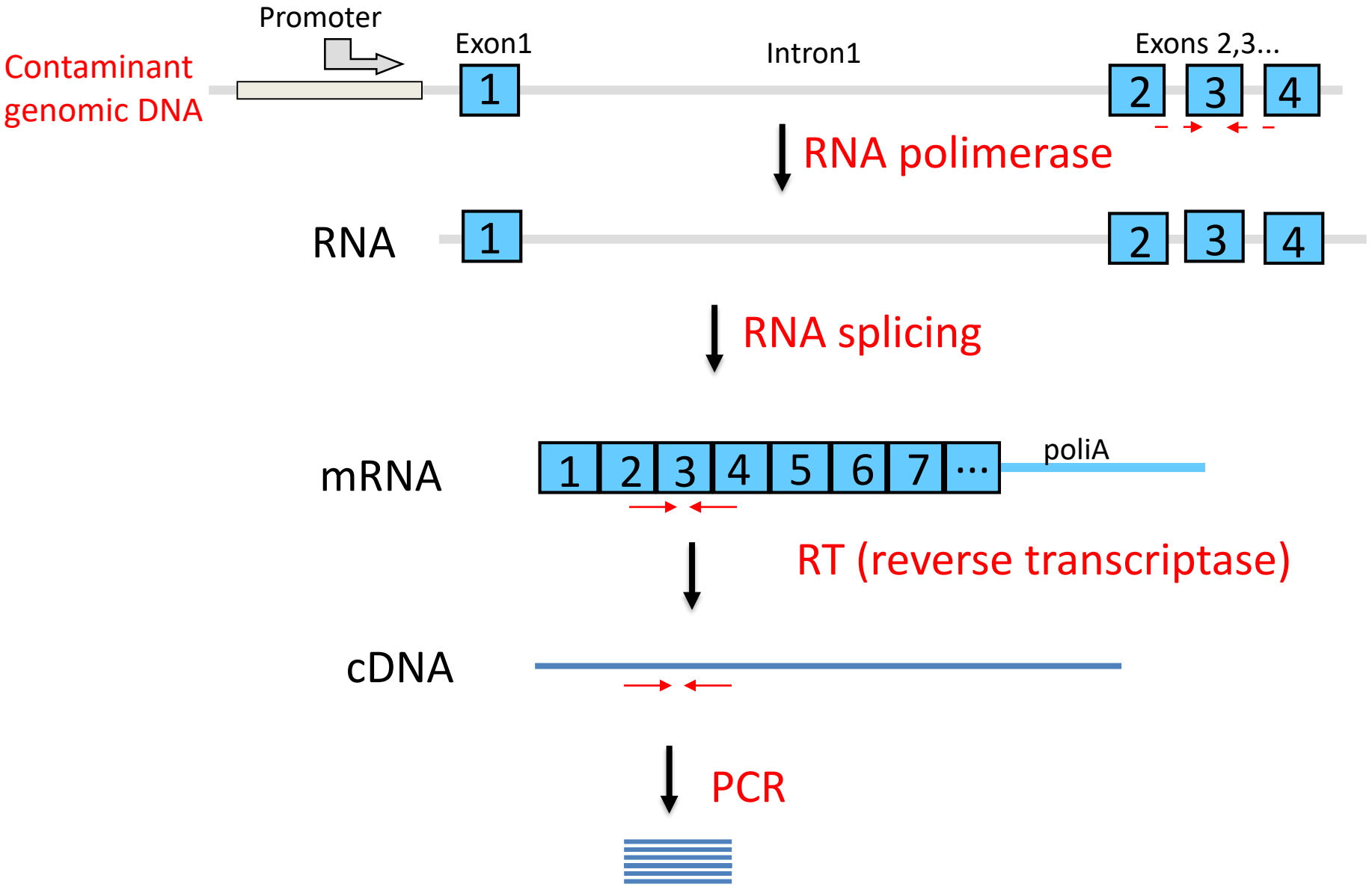
• which strategy could you use if intron is too small (<1000bp)?



• which strategy could you use if intron is too small (<1000bp)?

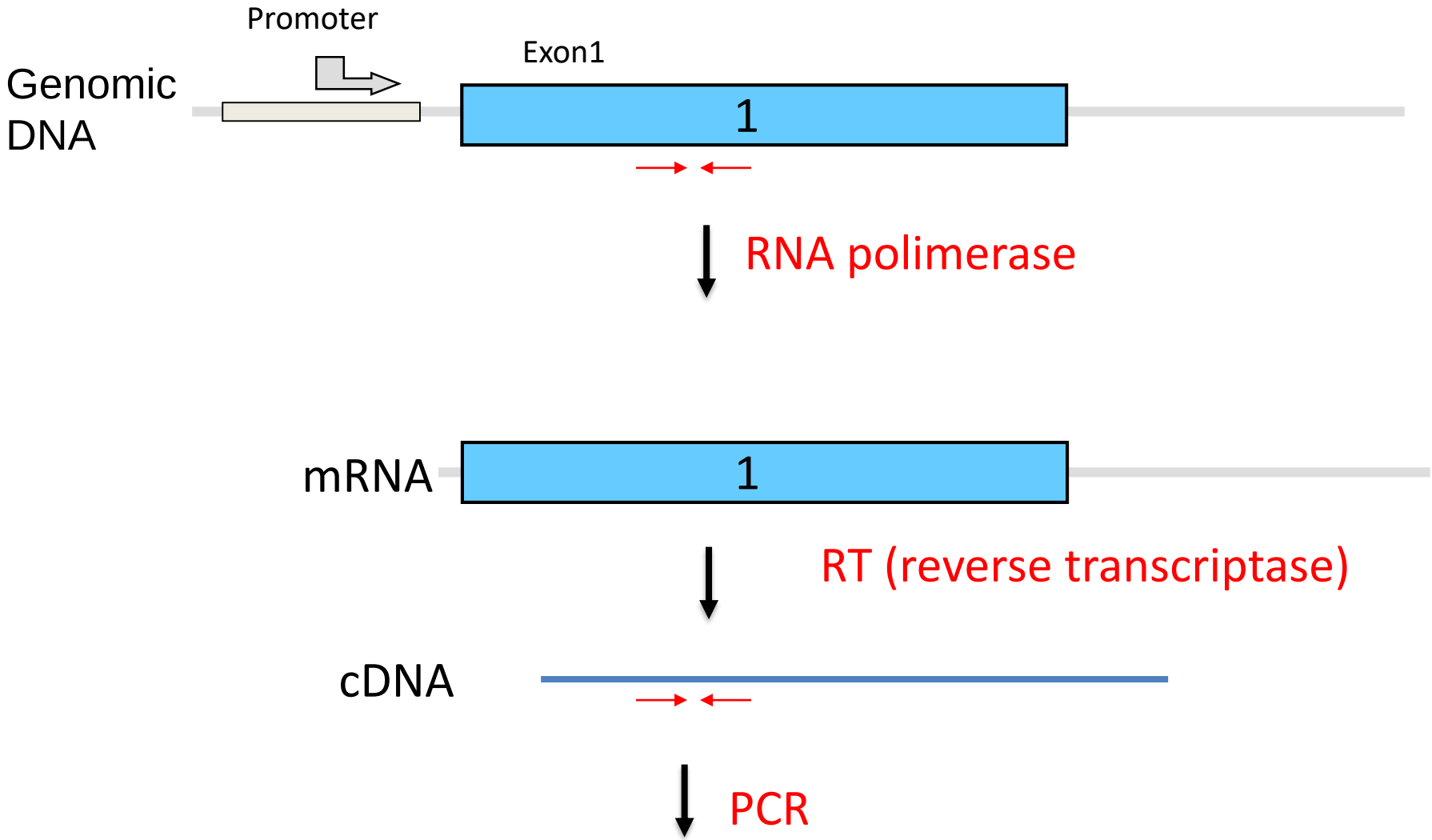


# 2 - if introns are too small (< 1000 bp) a good strategy could be to design primers across two exons



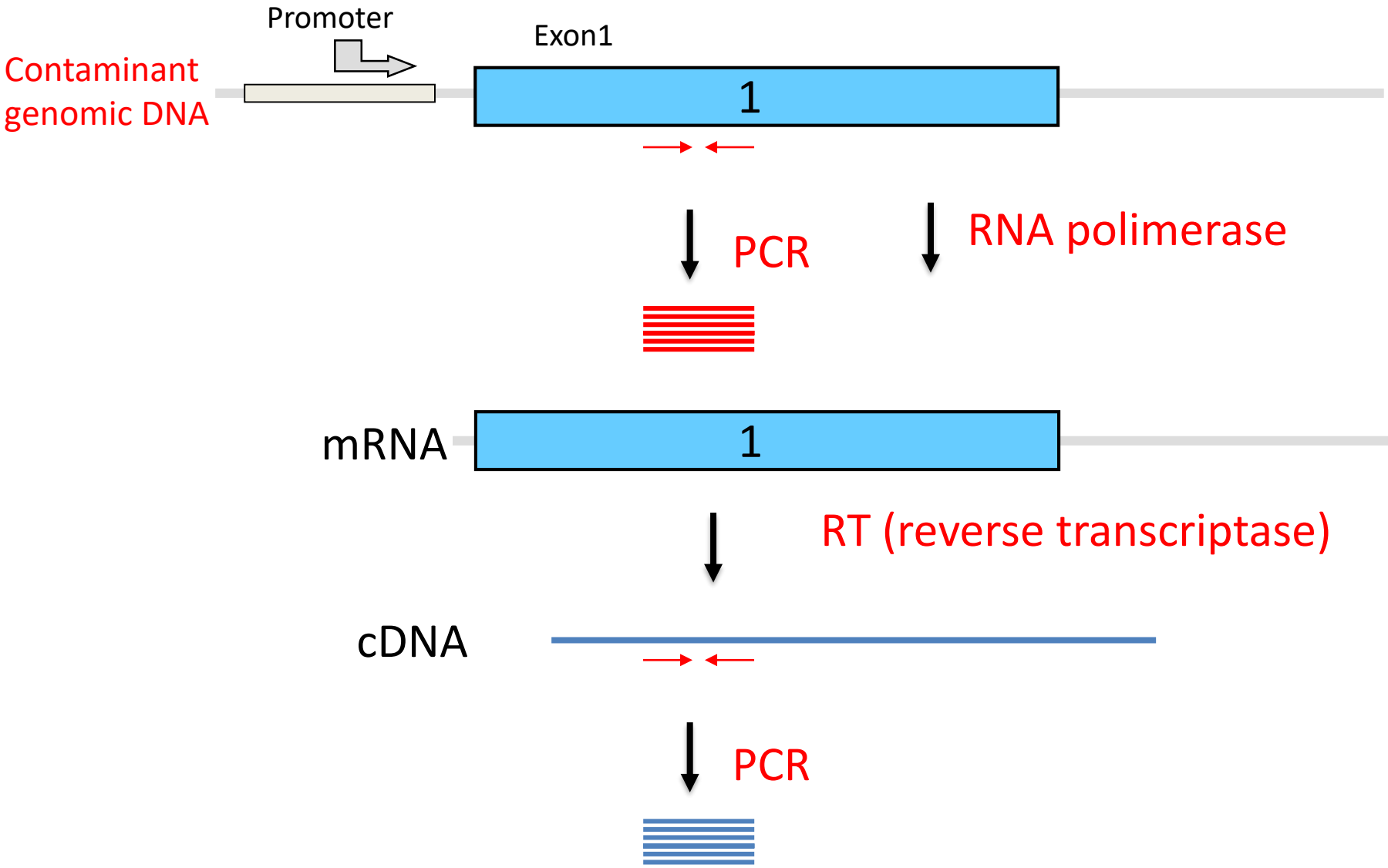
- what happens if you have a single exon?
- or if you have different isoforms sharing a single exon

(and you have to put primers in the same exon to quantify all of them)?



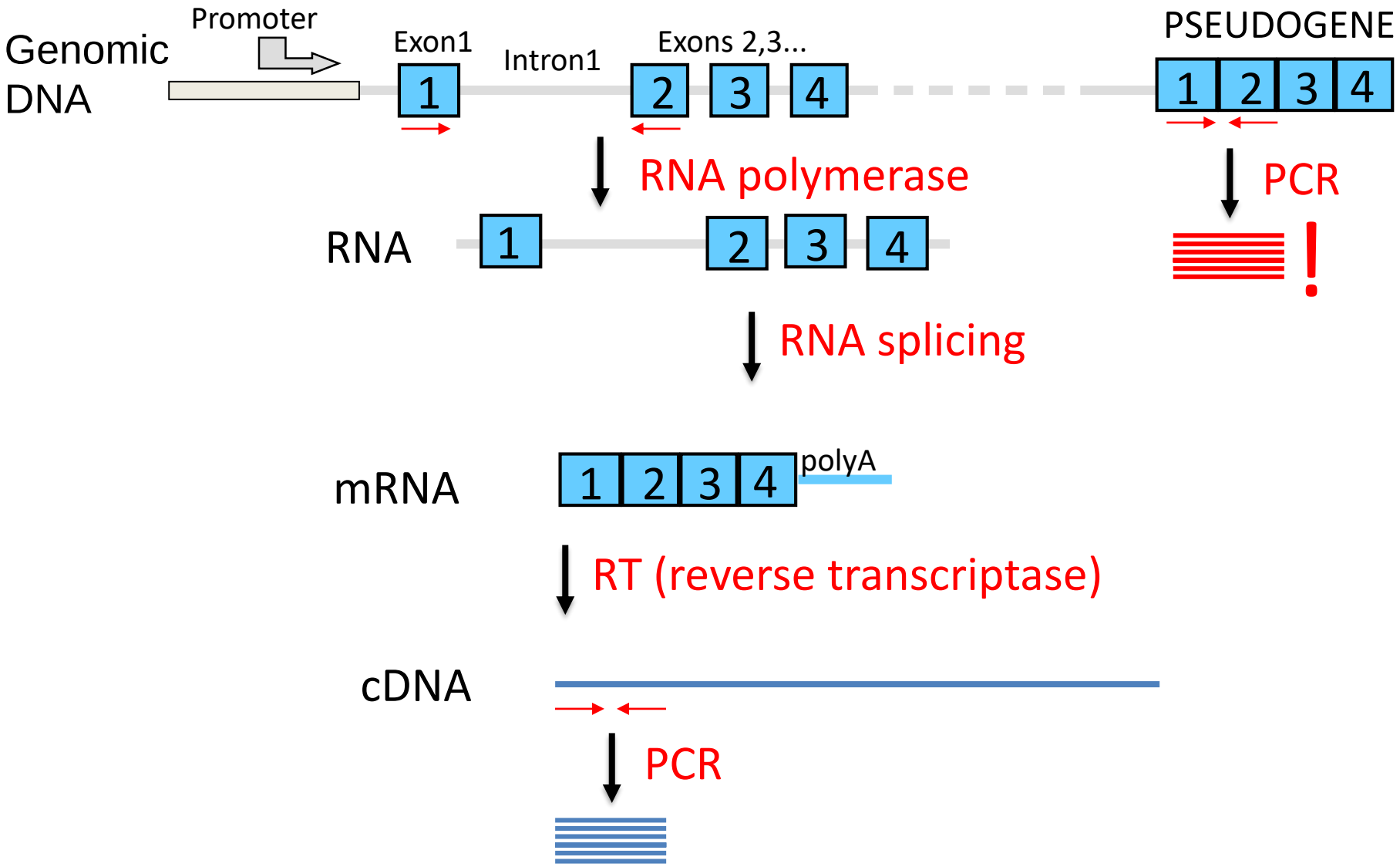
- if you design primers in a single exon,

you need to insert a specific control in your experiment,
to quantify possible genomic DNA contamination.



- What happen if you have **pseudogenes**?
- What are pseudogenes?

**Pseudogenes** are cDNA copies inside genomic DNA.
If you have genomic DNA contamination, you can amplify pseudogenes even if your primers are on different exons, separated by a big intron.



If you put primers on a single exon or you have pseudogenes, you have to insert a control to quantify genomic DNA contamination.

**Which control?**

# RT

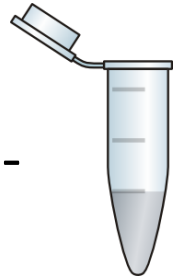
Which control can you do to verify that RNA and the reverse transcription ingredients are not contaminated by DNA (genomic, plasmidic, amplification products)?

| | stock | 1 sample | RT negative control | 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 | | 0,5mM |
| random primers | 50 µM | µl | | 5 µM |
| RT | 100u/µl | µl | | 200u/reaction |
| RNAsin | 33u/µl | µl | | 33u/reaction |
| Water to 25µl | | µl | | |
| Tot | | 25 µl | 25 µl | |

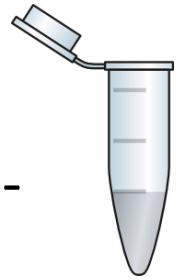


The negative control is called....

# Reverse Transcription

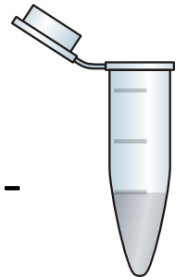
Tube 1 - 

RT on the sample that you have to analyse

Tube 2 - 

control “RT-”: all the ingredients, except the reverse transcriptase (RT-) to detect genomic DNA contamination

* **IF** you find contamination, you can insert an extra control with **all ingredients except RNA** to detect contamination due to plasmidic DNA or previous amplification.

Tube * - 

Usually this control (reagents, without RNA) is not carried out.

# PCR reaction

Which control can you do to verify that the PCR reaction is not contaminated by DNA (plasmidic, amplification products)?

| **STOCK** | RT+ | RT - | PCR negative ctr | final conc |
|---|---|---|---|---|
| cDNA | 1 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 | 50 µl | | |

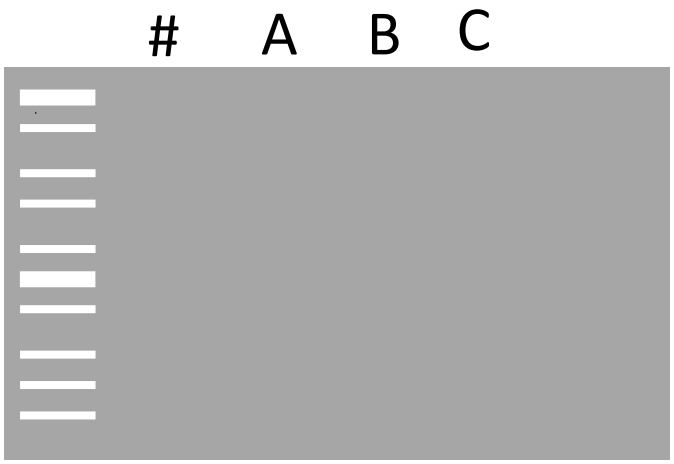
# PCR reaction

Which control can you do to verify that the PCR reaction is not contaminated by DNA (plasmidic, amplification products)?

| **STOCK** | RT+ | RT - | PCR negative ctr | final conc |
|---|---|---|---|---|
| cDNA | 1 µl RT+ | 1 µl RT- | | |
| 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 | 50 µl | | |

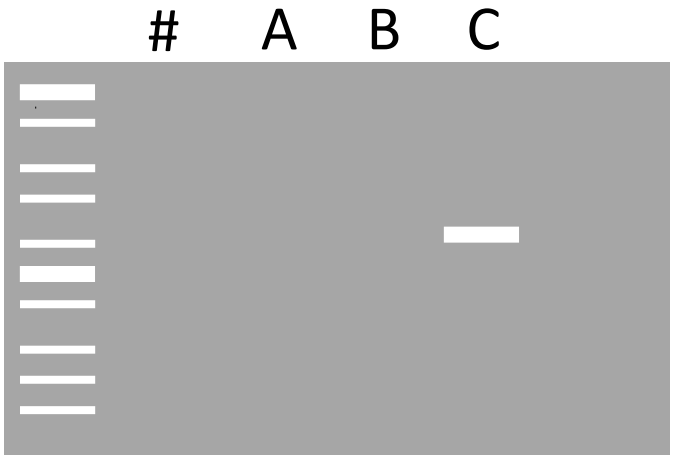
-**#-** sample
-**A-** RT reaction negative control (RT-)
-**B-** PCR reaction negative control
-**C-** which control is missing?

- which result do you expect?




PCR for the gene of interest

-**#-** sample
-**A-** RT reaction negative control (RT-)
-**B-** PCR reaction negative control
-**C-** positive control



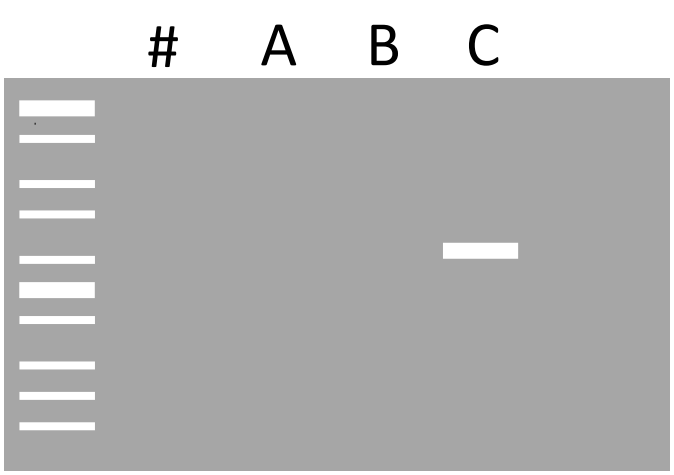
PCR for the gene of interest

The positive control for your gene of interest is useful to be sure that the negative results are really negative results and not just PCR technical problems (that is: the primers are working well, the mix is ok).

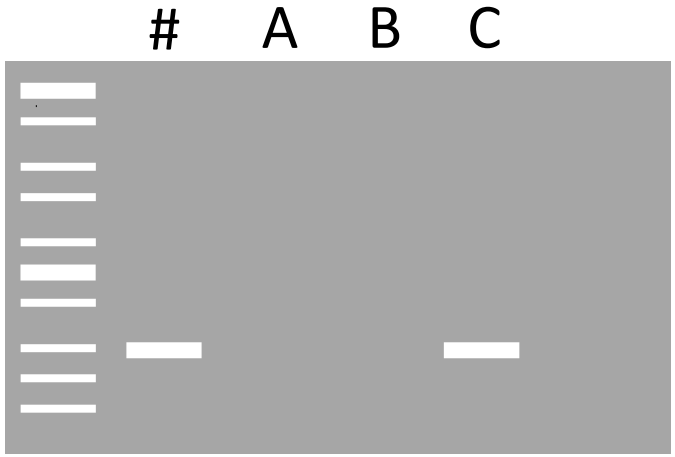
- which control can you do to verify that the RNA is good and correctly retro-transcribed?

(that is, that the negative results are really negative results and not just technical problems...)

-**#-** sample
-**A-** RT reaction negative control (RT-)
-**B-** PCR reaction negative control
-**C-** positive control




PCR for the gene of interest




housekeeping gene

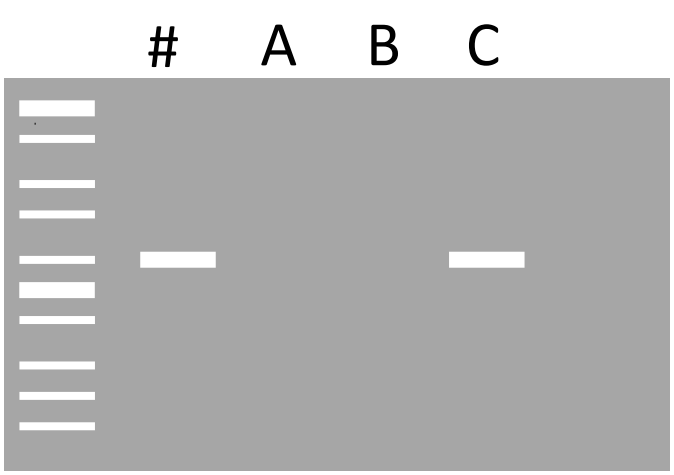
-You can amplify an housekeeping gene (TBP, actin, GAPDH, ….).
-You expect that the housekeeping gene is expressed also by those samples that do not express your gene of interest.

-**#-** sample

-**A-** RT reaction negative control (RT-)

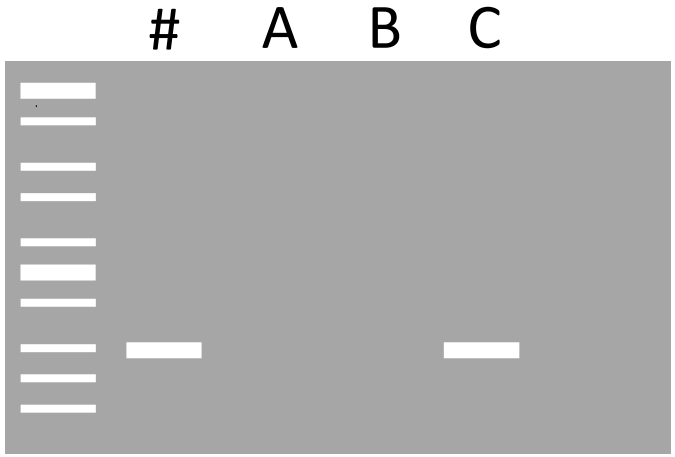
-**B-** PCR reaction negative control

-**C-** positive control




PCR for the gene of interest

?




PCR for the housekeeping gene

-**#-** sample

-**A-** RT reaction negative control (RT-)

-**B-** PCR reaction negative control

-**C-** positive control


PCR for the gene of interest

?

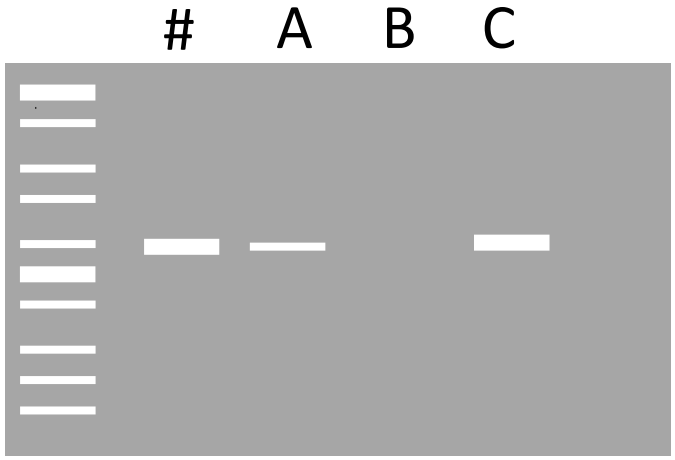

PCR for the housekeeping gene

-**#-** sample

-**A-** RT reaction negative control (RT-)

-**B-** PCR reaction negative control

-**C-** positive control




?

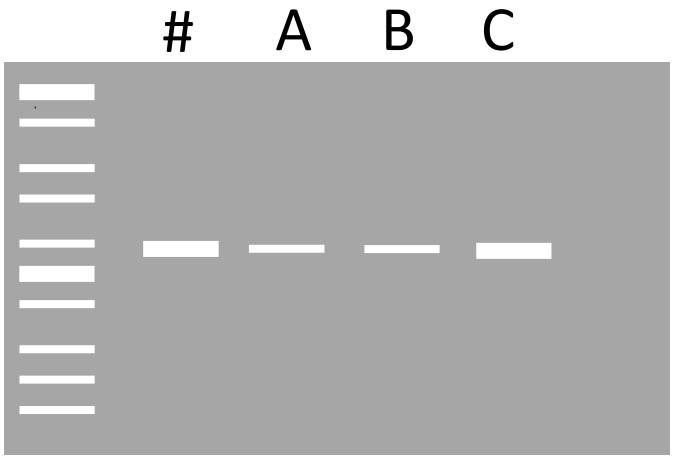
PCR for the gene of interest

-**#-** sample

-**A-** RT reaction negative control (RT-)

-**B-** PCR reaction negative control

-**C-** positive control




?

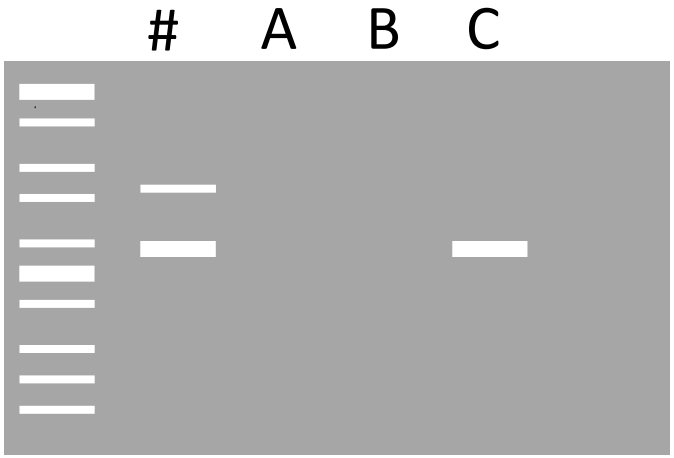
PCR for the gene of interest

-**#-** sample

-**A-** RT reaction negative control (RT-)

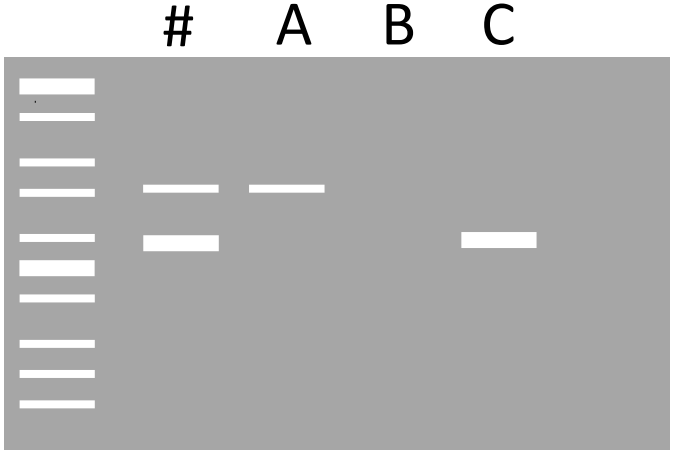
-**B-** PCR reaction negative control

-**C-** positive control



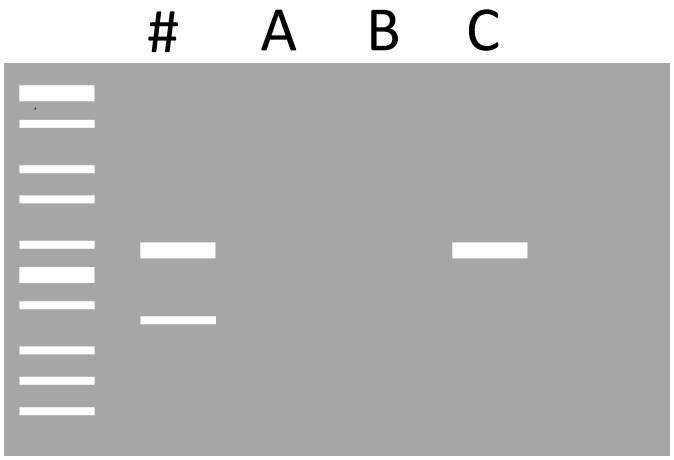

?

PCR for the gene of interest




?

PCR for the gene of interest




?

PCR for the gene of interest

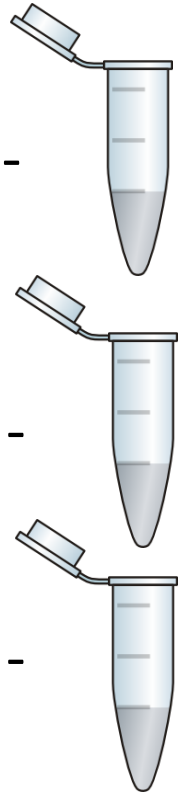
# PCR for the gene of interest

| **STOCK** | Sample # | CTR A | CTR B | CTR C | MIX 4 samples | **Conc. finale** |
|---|---|---|---|---|---|---|
| cDNA | µl | | | | | |
| 5 x buffer Taq | µl | | | | | -> 1x |
| Sense primer gene target 10 µM | µl | | | | | -> 200 nM |
| Antisense primer gene target 10 µM | µl | | | | | -> 200 nM |
| 100% glycerol | µl | | | | | -> 5% |
| 10mM dNTPs | µl | | | | | -> 200 µM |
| Taq polimerase 1u/ul | µl | | | | | -> 1u |
| $H_2O$ | µl | | | | | |
| totale | 50 µl | | | | | |

# PCR for the housekeeping gene (HKG)

| STOCK | Sample # | CTR A | CTR B | CTR C | MIX 4 samples | **Conc. finale** |
|---|---|---|---|---|---|---|
| cDNA | µl | | | | | |
| 5 x buffer Taq | µl | | | | | -> 1x |
| Sense primer HKG 10 µM | µl | | | | | -> 200 nM |
| Antisense primer HKG 10 µM | µl | | | | | -> 200 nM |
| 100% glycerol | µl | | | | | -> 5% |
| 10mM dNTPs | µl | | | | | -> 200 µM |
| Taq polimerase 1u/ul | µl | | | | | -> 1u |
| $H_2O$ | µl | | | | | |
| totale | 50 µl | | | | | |

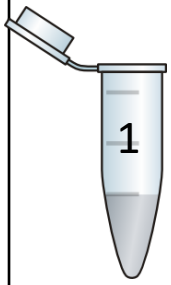
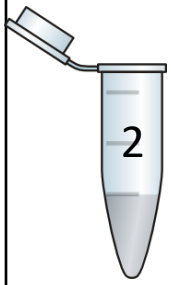
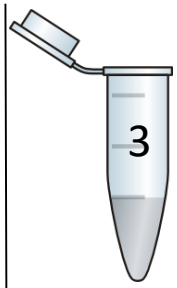
# Reverse Transcription



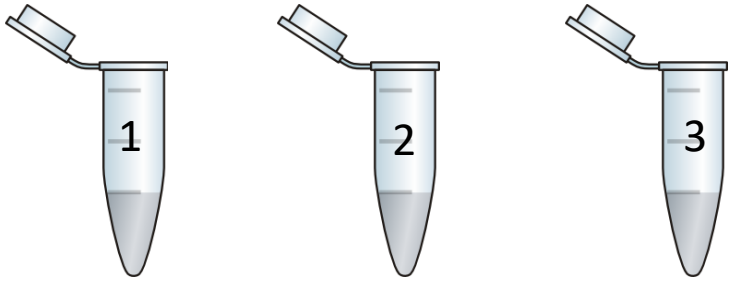
Tube 1 - RT on the sample that you have to analyse

Tube 2 - control “RT-”: all the ingredients, except the reverse transcriptase (RT-)

Tube 3 - “positive control”: RT on RNA extracted from a sample expressing the gene of interest

| |  “RT+” |  “RT-” |  “control +” | | MIX x 3 | Final concentration |
|---|---|---|---|---|---|---|
| RNA | µl | | – | | | 1 µg |
| o | | | | | | |
| $H_2O$ | µl | | | | | |
| Buffer 5x | µl | | | | | 1x |
| BSA 1 µg/µl | µl | | | | | 0.1 µg/µl |
| Triton 1% | µl | | | | | 0.05% |
| dNTPs 10mM | µl | | | | | 0,5mM |
| Random primers 50µM | µl | | | | | 5 µM |
| RT 100u/µl | µl | – | | | | 200u/25µl |
| RNAsin 33u/µl | µl | | | | | 33u/25µl |
| water to 25µl | µl | | | | | |
| Total | 25 µl | 25 µl | | | | |

# RT-PCR




"RT+" "RT-" "control +"

RT 25 µl

take 1 µl cDNA and amplify by PCR

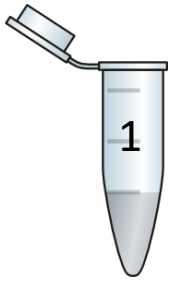
1 µl 1 µl 1 µl


PCR 50 µl

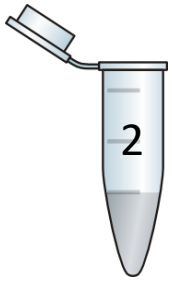
+ 49 µl mix PCR

# PCR

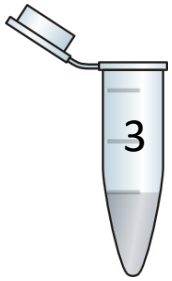
| **STOCK** |  |  |  |  | | MIX x 4 | |
|---|---|---|---|---|---|---|---|
| RT<br>o<br>$H_2O$ | 1 µl #1<br>– | 1 µl #2<br>– | 1µl #3<br>– | –<br>1 µl | | | |
| 5 x buffer Pfu | µl | | | | | | -> 1x |
| Sense primer 10 µM | µl | | | | | | -> 200 nM |
| Antisense primer 10 µM | µl | | | | | | -> 200 nM |
| 100% glicerolo | µl | | | | | | -> 5% |
| 10mM dNTPs | µl | | | | | | -> 200 µM |
| Taq polimerasi 1u/ul | µl | | | | | | -> 1u/50µl |
| $H_2O$ | µl | | | | | | |
| total | 50 µl | | | | | | |




"RT+"




"RT-"




"control +"

25 µl
REVERSE TRANSCRIPTION

1 µl ↓ 1 µl ↓ 1 µl ↓ ↓ 1 µl $H_2O$


GENE X
PCR

1 µl ↓ 1 µl ↓ 1 µl ↓ ↓ 1 µl $H_2O$


GENE Y
PCR

# Exercizes

(as soon as you finish, we will discuss your results)


1-find the length of the intron between domain EGF-like and domain alpha

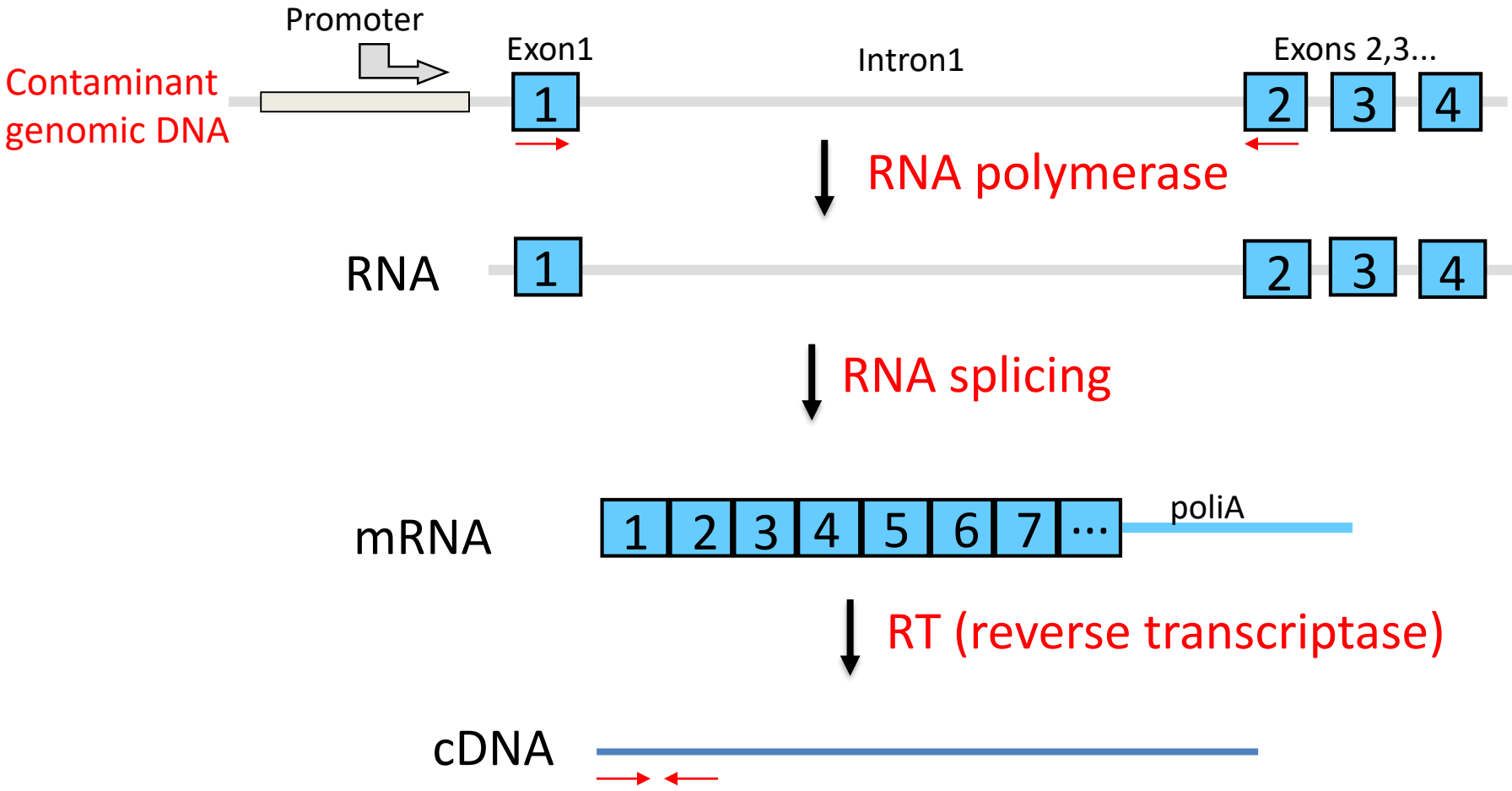
2-find the length of the intron between domain EGF-like and domain beta

3-find coding exons (number and length) and introns (length) of Rattus norvegicus TATA box binding protein (Tbp) mRNA, complete cds (NM_001004198.1) to decide where to design primers.

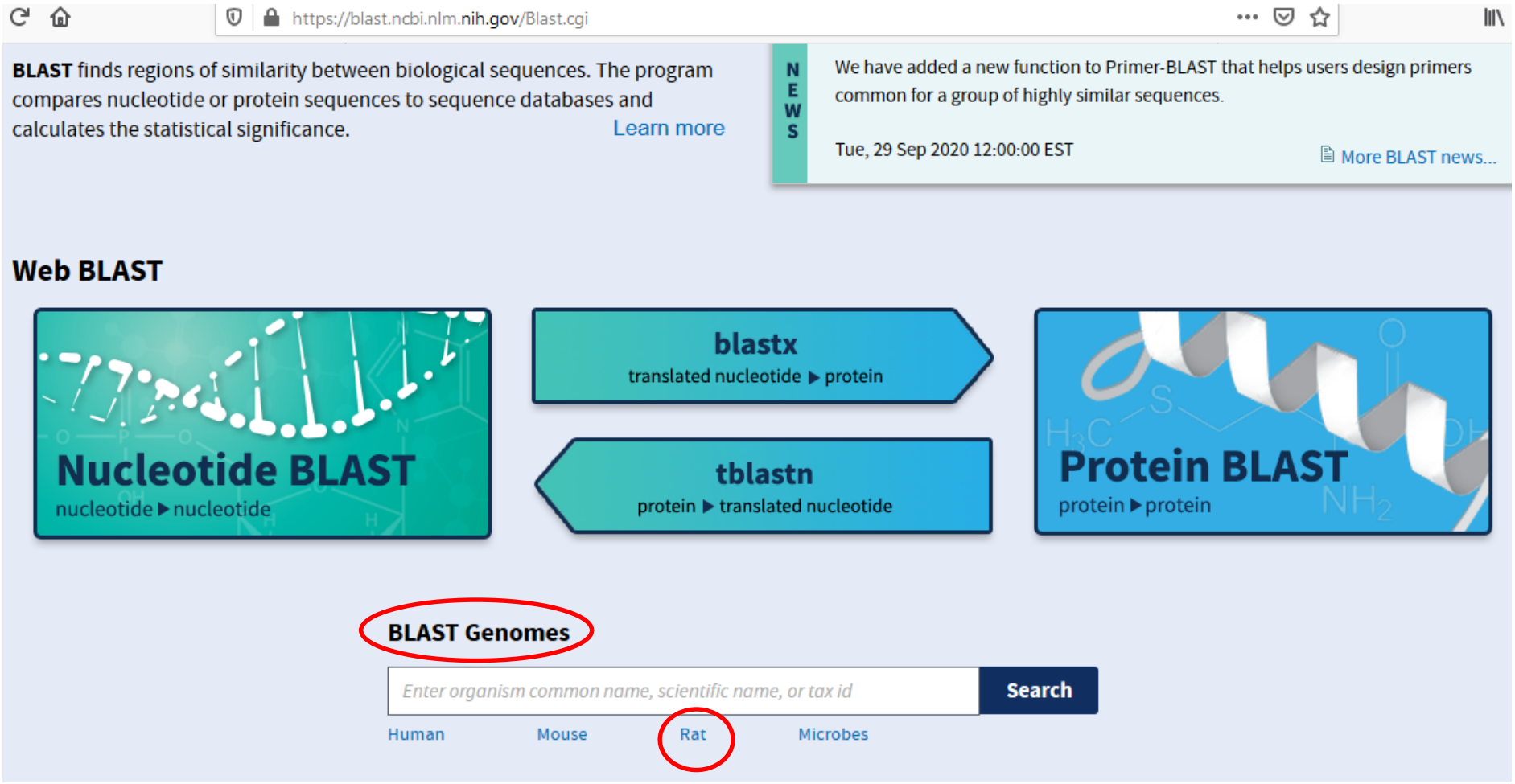
4-find coding exons (number and length) and introns (length) of Rattus norvegicus glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, complete cds (AF106860.2) to decide where to design primer

5-if you still have time, you can start to prepare primers for full length cloning, using annhyb or primer3.

- To design primers on different exons separated by a big intron (≥1000 bp) you need to know the exon-intron map and length




- How do you identify exons and introns in the genomic DNA?


https://blast.ncbi.nlm.nih.gov/Blast.cgi
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
We have added a new function to Primer-BLAST that helps users design primers common for a group of highly similar sequences.
Tue, 29 Sep 2020 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
BLAST Genomes
Enter organism common name, scientific name, or tax id
Search
Human
Mouse
Rat
Microbes