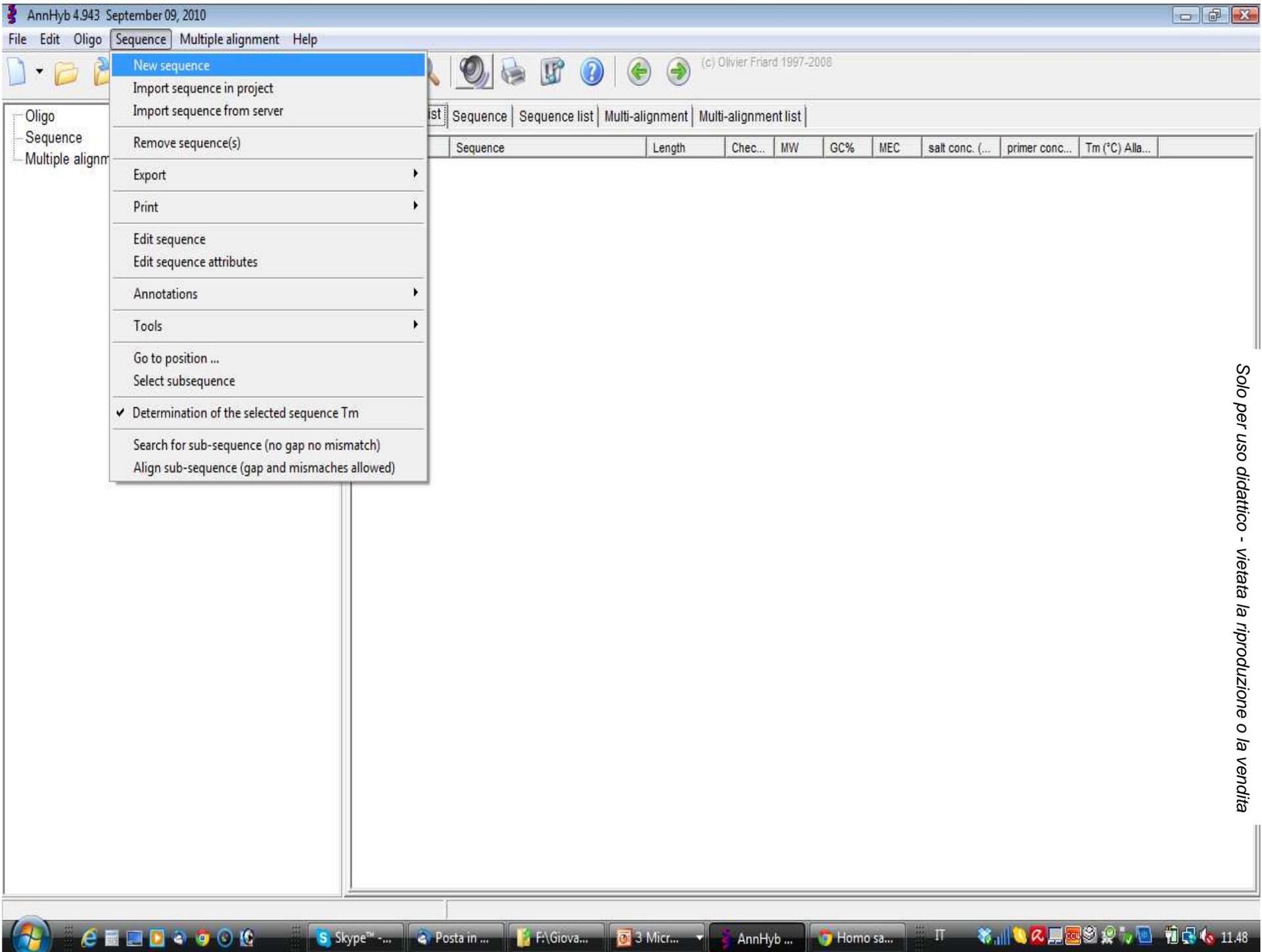
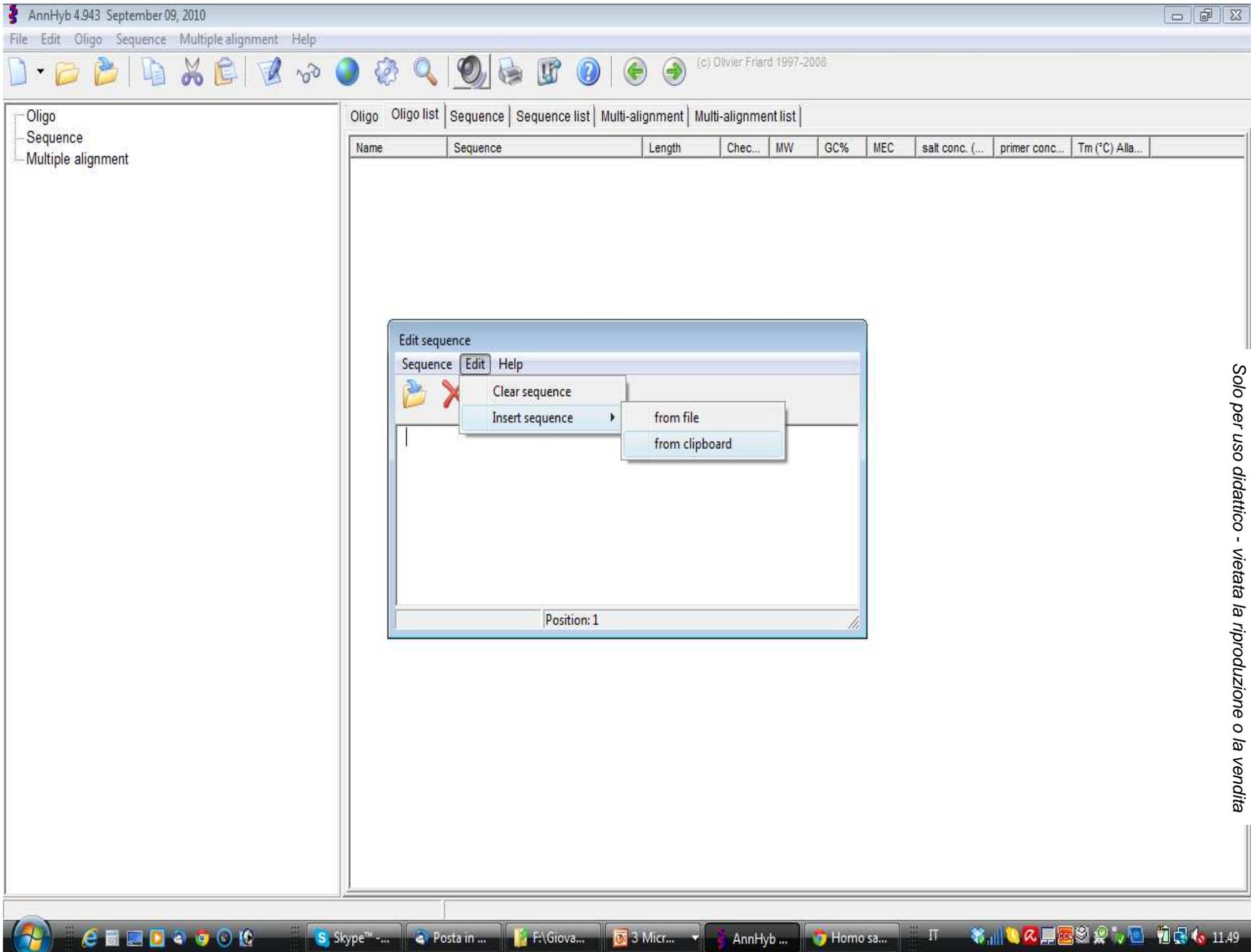
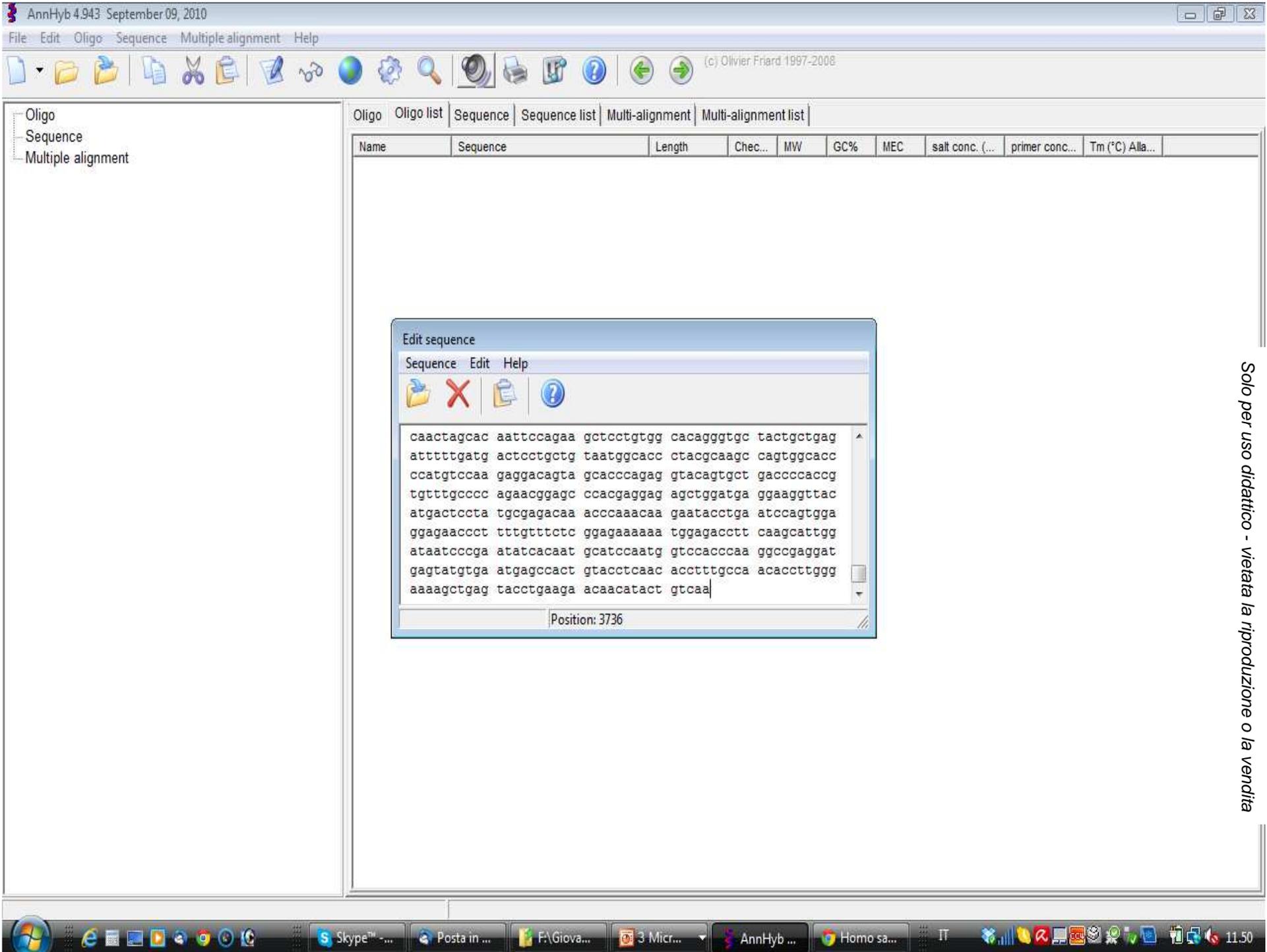


Come usare Annhyb

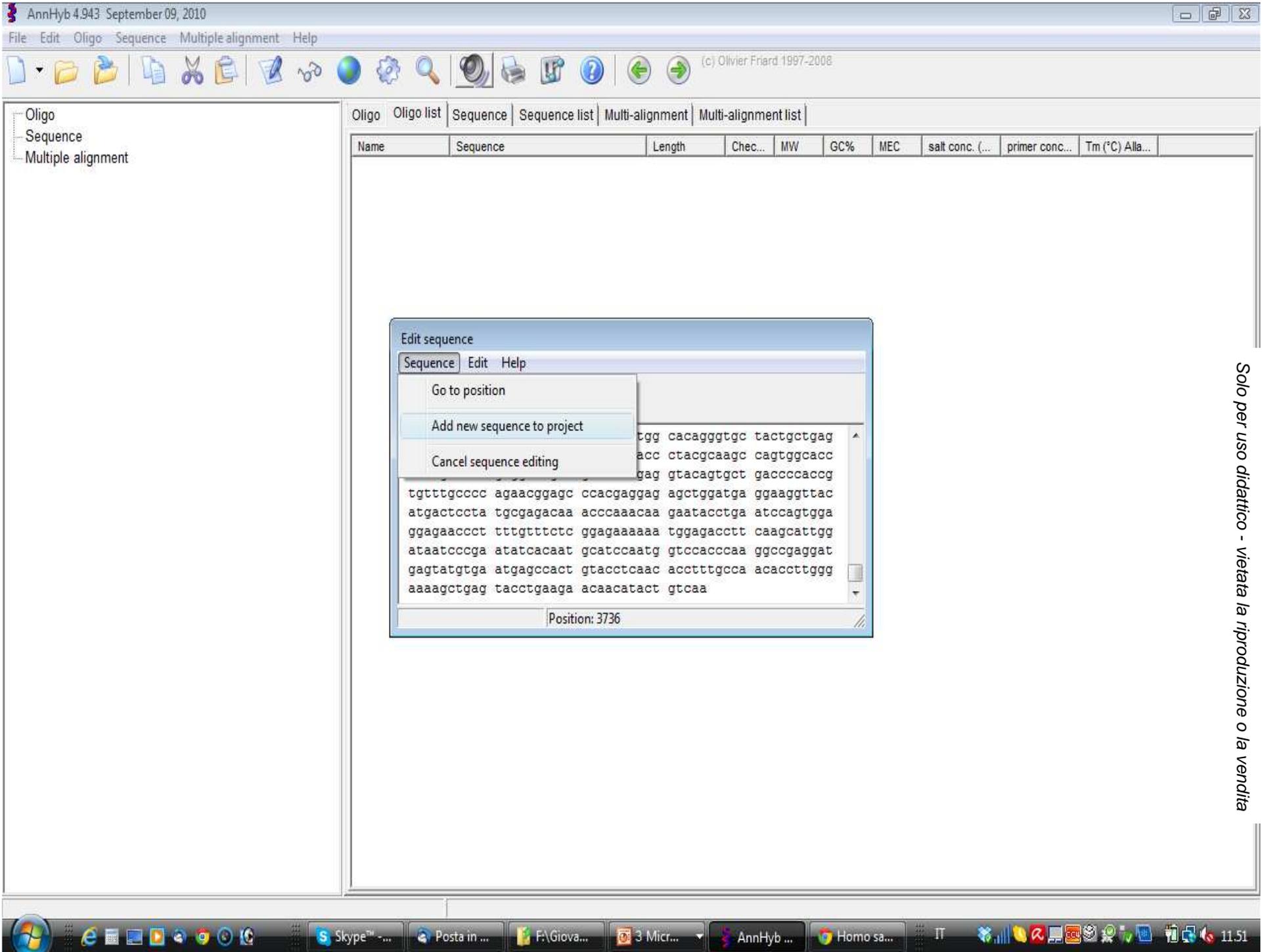


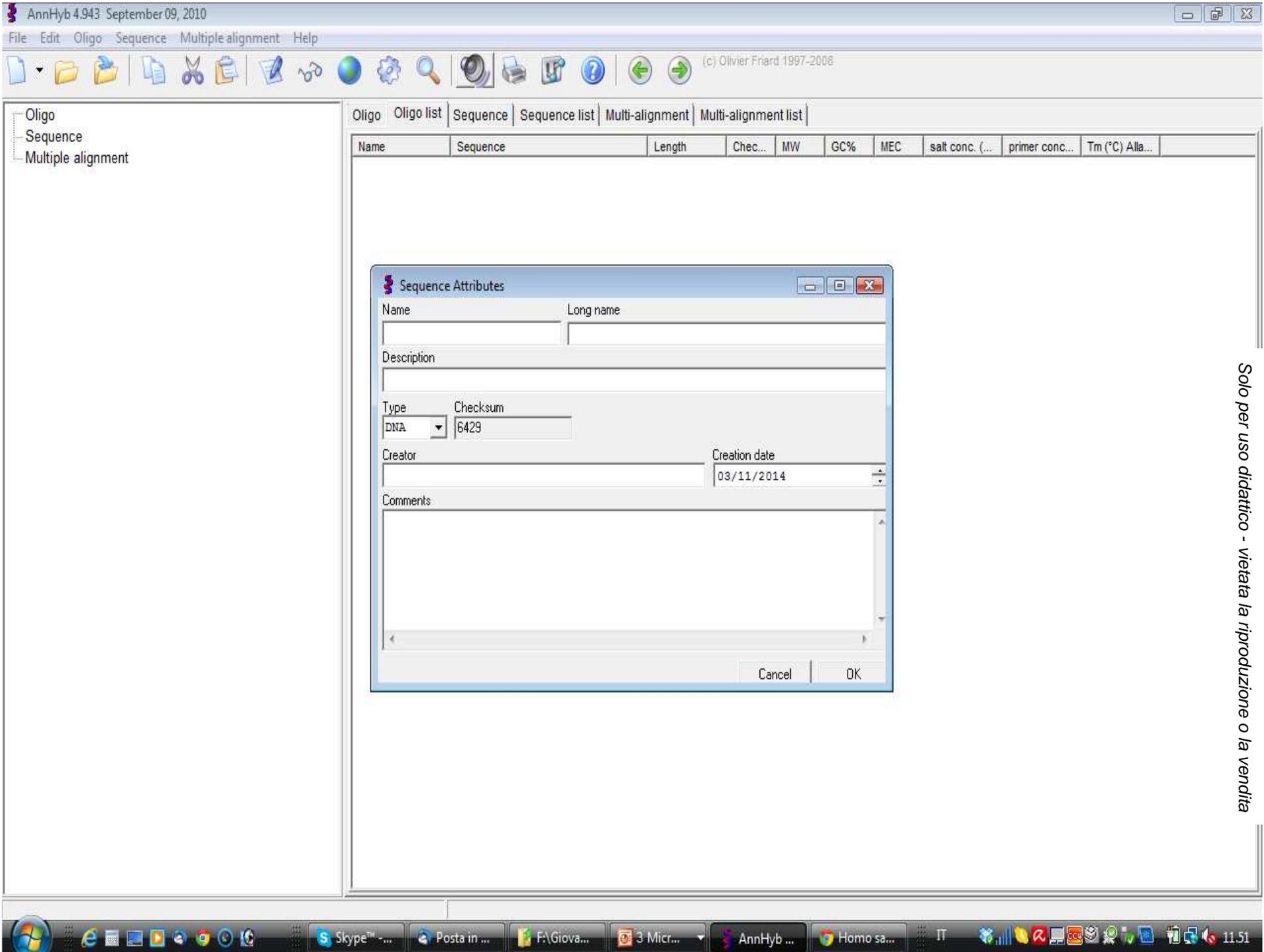


Solo per uso didattico - vietata la riproduzione o la vendita

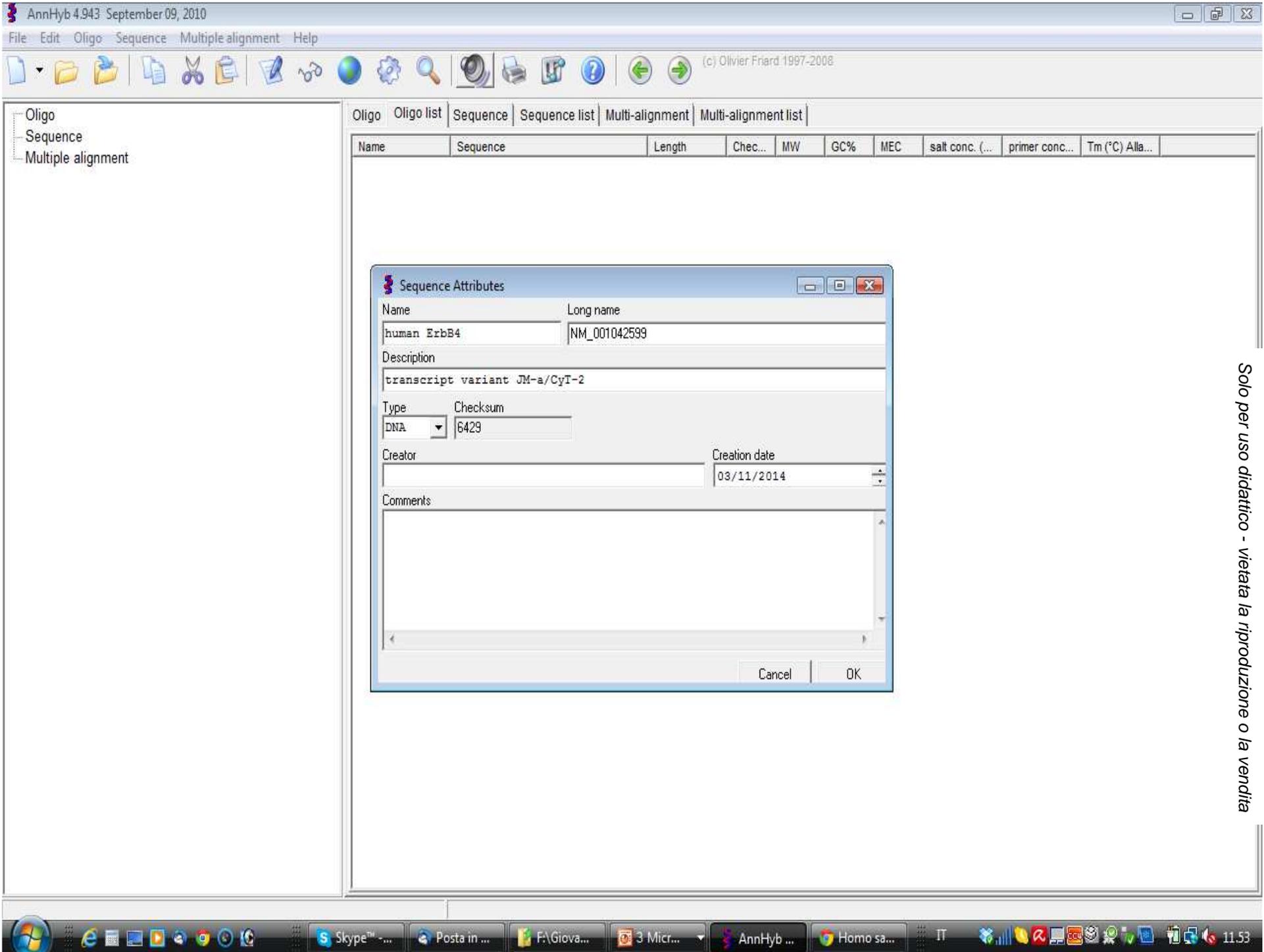


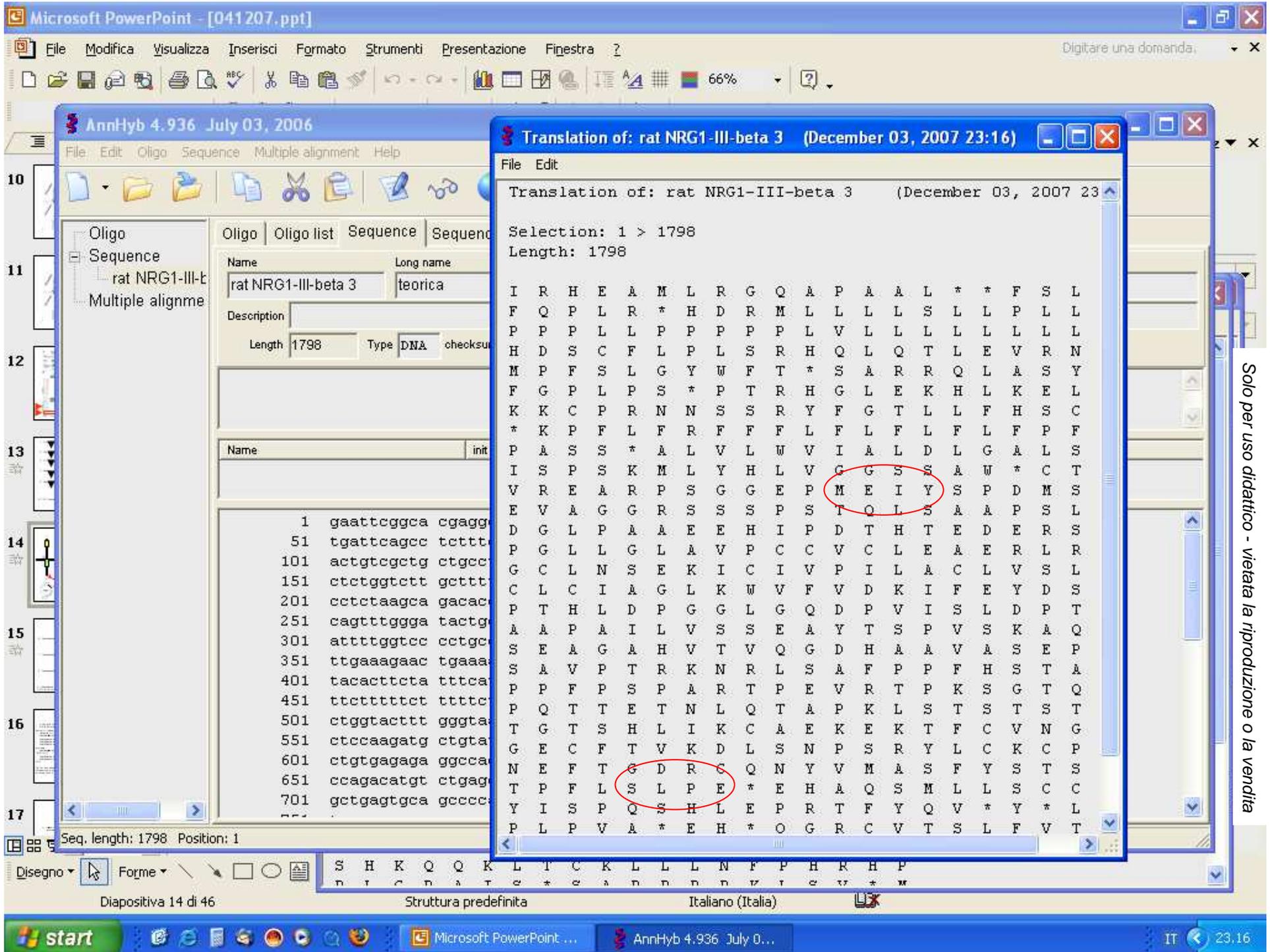
Solo per uso didattico - vietata la riproduzione o la vendita





Solo per uso didattico - vietata la riproduzione o la vendita





Translation of: rat NRG1-III-beta 3 (December 03, 2007 23:16)

File Edit

Translation of: rat NRG1-III-beta 3 (December 03, 2007 23:16)

Selection: 1 > 1798
Length: 1798

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P P F P S P A R T P E V R T P K S G T Q
P Q T T E T N L Q T A P K L S T S T S T
T G T S H L I K C A E K E K T F C V N G
G E C F T V K D L S N P S R Y L C K C P
N E F T G D R S Q N Y V M A S F Y S T S
T P F L S L P E * E H A Q S M L L S C C
Y I S P Q S H L E P R T F Y Q V * Y * L
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Solo per uso didattico - vietata la riproduzione o la vendita

sequenze.doc - Microsoft Word

File Modifica Visualizza Inserisci Formato Strumenti Tabella Finestra ?

ff_line Courier New 10 Italiano (Italia)

<Fare clic su Riconteggia> Riconteggia

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sequenze.doc - Microsoft Word

AnnHyb 4.943 September 09, 2010

File Edit Oligo Sequence Multiple alignment Help

ff_line + Courier Nt

Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name: Sequence1 Long name:

Description:

Length: 1163 Type: DNA checksum: 5082 Author: Creation date: 24/10/2010

Sequence header:

init end comments

Translation options

Amino acid code

1 character

3 characters

frame 1 frame 4

frame 2 frame 5

frame 3 frame 6

include nucleotide sequences

? Cancel Translate

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751 gagtgcctca cgggtaagga cctgtcaaac ccgtcaagat acttgtgcaa
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1001 catgagaaca ttaacaagc cgtgtgtgta cttctctggt tgtgactagt
1051 cggctcagag ttactaaata ggtgtgtgag gcccagagg tttctgaaa

Pg 3 Sez 1 3/3

Skype... Re: gi... Rattus... F:\Gio... 2 Mi... seque... AnnH...

Solo per uso didattico - vietata la riproduzione o la vendita

sequence.doc - Microsoft Wo
AnnHyb 4.943 September 09, 2010
File Edit Oligo Sequence Multiple alignment Help
ff_line + Courier Nt
Translation of: Sequence1 (October 24, 2013 22:52)
File Edit
Translation of: Sequence1 (October 24, 2013 22:52)
Selection: 1 > 1163
Length: 1163
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T Q L S A A P S L D G L P A A E E H I P
D T H T E D E R S P G L L G L A V P C C
V C L E A E R L R G C L N S E K I C I V
P I L A C L V S L C L C I A G L K W V F
V D K I F E Y D S P T H L D P G G L G Q
D P V I S L D P T A A P A I L V S S E A
Y T S P V S K A Q S E A G A H V T V Q G
D H A A V A S E P S A V P T R K N R L S
A F P P F H S T A P P F P S P A R T P E
V R T P K S G T Q P Q T T E T N L Q T A
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K E K T F C V N G G E C F T V K D L S N
P S R Y L C K C P N E F T G D R C Q N Y
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Pg 3 Sez 1 3/3

Skype... Re: gi... Rattus... F: Gio... 2 Mi... seque... AnnH... IT

Digitare una domanda.

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Solo per uso didattico - vietata la riproduzione o la vendita

Come disegnare primers con Annhyb

Microsoft
File Edit Oligo Sequence Multiple alignment Help
 (c) Olivier Friard 1997-2008

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 8
 Disegno

Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name: Sequence1 Long name:
 Description:
 Length: 5712 Type: DNA checksum: 9812 Author: Creation date: 27/10/2012

Sequence header:
 Sequence annotations:

Name	init	end	comments

1 gaattcgca cgaggcgatg ctcagagggc
 51 tgattcagcc tctttcagcc gctgcgttaa
 101 actgtogctg ctgcctctcc tgccgcgcgc
 151 **ctctgggtctt gcttttgctt tta**ctctcc
 201 cctctaagca gacaccagct tcagacgctt gaggtgagaa acatgccttt
 251 cagtttggga tactggttta cttaatoggc tagggggcag cttgottcct
 301 attttggcc cctgccttct tgaccaacco ggcatggttt ggagaagcat
 351 ttgaaagAAC tgaaaaagtG tcccagaaac aacagctcaa gatatttcgg
 401 tacacttcta ttccatagtt gctagaagcc ctttcttttt cgtttttttt
 451 tctttttct tttcttttt ctttttccct tctctgcttc ctactaagct
 501 ctggtacttt gggtaattgc cttggacttg ggtgccttat cgatttcccc
 551 ctccaagatg ctgtatcatt tggttggggg gagctctgcg tggtaatgca
 601 ctgtgagaga ggccaggcct tctggagggt agccgatgga gatttattcc
 651 ccagacatgt ctgaggtagc tggcgggagg tccctccagcc cctccactca
 701 gctgagtgca gcccactctc ttgatgggct tccggcagcg gaggaacata
 751 taccagacac ccacacagaa gatgagagaa gccctggaact cctgggctctg
 801 gcggtgcctc gtgtgtgtg cctggaagct gagcgcctga gagggtgtct
 851 caactccgag aagatctgca ttgttcccat tctggcttgc ctagtacgcc
 901 tctgcctctg cattgtctgc ctgaaagtgg tatttgtgga caagatattt
 951 gaatacgaact ctccatccca ccttgacctt ggggggttag gccaggagccc
 1001 tgtgatttct ctggatccaa ctgctgcccc agccattttg gtatcatctg

Seq. length: 5712 Selection: 151 - 174 (24 bp) Tm= 55.0°C (Allawi, 1997)

Confirm
 ? Copy only nucleotides?

2 Skype LA PARO... NCBI Sequ... F:\Giovan... 3 Micros... AnnHyb 4... IT 17.59

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Microsoft **Oligo** Sequence Multiple alignment Help

File Edit **Oligo** Sequence Multiple alignment Help

Sequence | Sequence list | Multi-alignment | Multi-alignment list

ce1 Long name

Type DNA checksum 9812 Author Creation date 27/10/2012

Name init end comments

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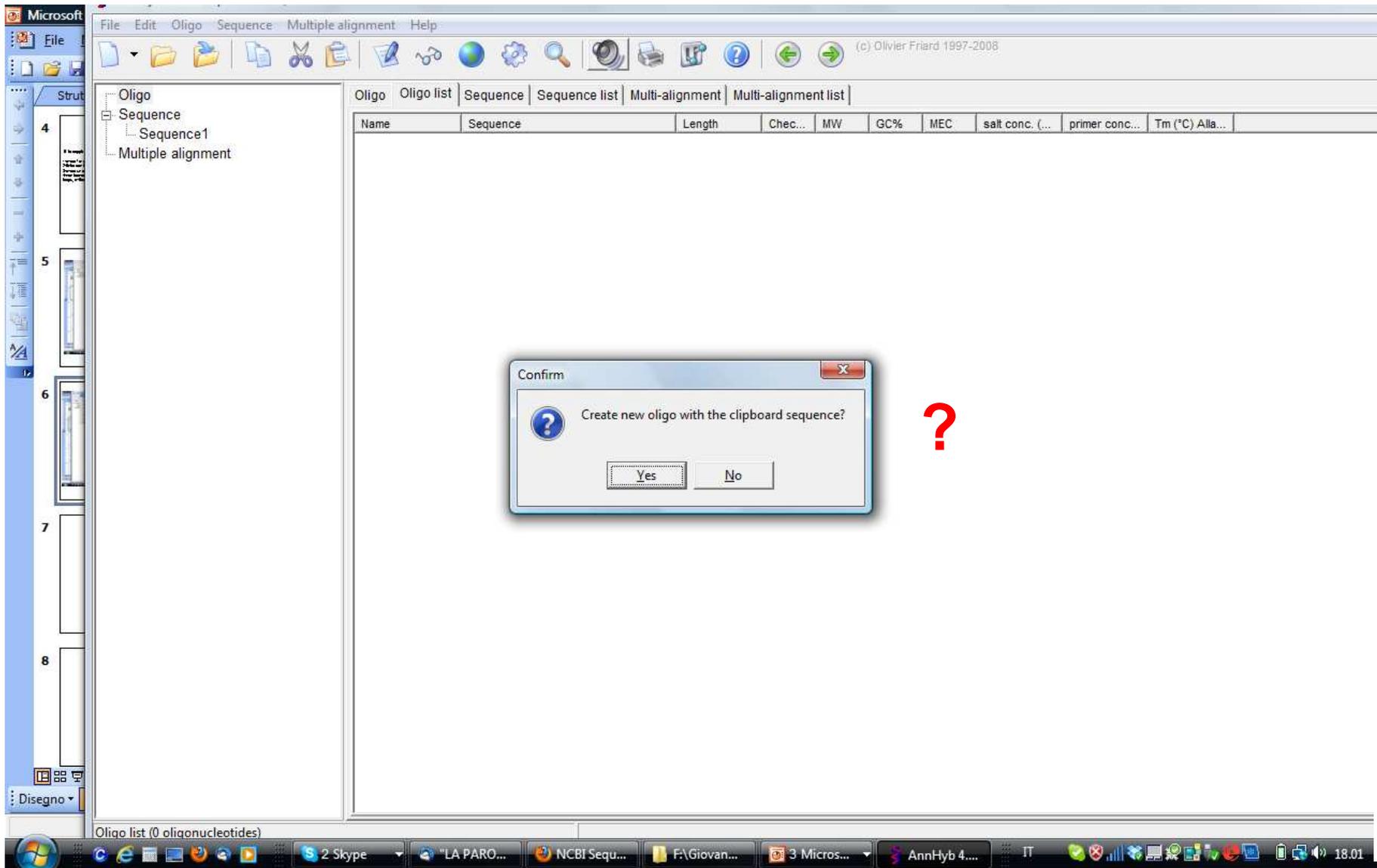
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51 tgattcagcc ttttcagcc gctgcgttaa cacgacagga tgctgttgct
101 actgtgctg ctgcctctcc tgcgcgcgcc gctgctgccg ccgcccctcc
151 ctctgggtctb gcttttgctb ttacttctcc tgcattgacac ttgttttctt
201 cctctaagca gacaccagct tcagacgctt gaggtagaaa acatgccttt
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301 attttggtcc cctgcctctc tgaccaaccc ggcatggttt ggagaagcat
351 ttgaaagaac tgaaaaagtg tcccagaaac aacagctcaa gatatttcgg
401 tacacttcta tttcatagtt gctagaagcc ctttcttttt cgtttttttt
451 tttcttttct ttttcttttt ctttttcctt ttctgcttc ctocaaagt
501 ctggtacttt gggtaatgct cttggacttg ggtgccttat cgatttcccc
551 ctccaagatg ctgtatcatt tgggtggggg gagctctgcg tggtaatgca
601 ctgtgagaga ggccaggcct tctggaggtg agccgatgga gatttatcc
651 ccagacatgt ctgaggtagc tggcgggagg tccctccagcc cctccaactca
701 gctgagtgca gccccatctc ttgatgggct tccggcagcg gaggaacata
751 taccagacac ccacacagaa gatgagagaa gccctggact cctgggctcg
801 gcggtgccct gctgtgtgtg cctggaagct gagcgcctga gaggggtgtct
851 caactccgag aagatctgca ttgttcccat tctggcttgc ctagtacgcc
901 tctgcctctg cattgctggc ctgaagtggg tatttgtgga caagatattt
951 gaatacgaact ctctaccca ccttgacctt ggggggttag gccaggaccc
1001 tgtgatttct ctggatccaa ctgctgcccc agccattttg gtatcatctg

```

Seq. length: 5712 Selection: 151 - 174 (24 bp) Tm= 55.0°C (Allawi, 1997)

2 Skype "LA PARO..." NCBI Sequ... F:\Giovan... 3 Micros... AnnHyb 4.... IT 18.00

Solo per uso didattico - vietata la riproduzione o la vendita



Solo per uso didattico - vietata la riproduzione o la vendita

Microsoft
File Edit Oligo Sequence Multiple alignment Help

File

Strut

- Oligo
 - Oligo1-sense
 - Sequence
 - Sequence1
 - Multiple alignment

Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name: Oligo1-sense [notes] [None]

Seq. 5' ctcctggtcttgc ttt tgc ttt tac
Insert the sequence of oligo

IUB seq. 5' CTC TGG TCT TGC TTT TGC TTT TAC

rev. 5' GTAAAAGCAAAGCAAGACCAGAG

comp.

Length	MW	GC%	GCG checksum	Molar ext. coef.	
24	b	7320	41.6%	3134	198360 l/mol (at 280 nm)

Salt cc: 50 mM Primers cc: 250 nM Tm: 55.0 °C

Alignment results Allawi, 1997

Seq. name	sense	init	end	score	alignment	Sequence target	Color ...

Disegno

Insert the sequence of oligo

2 Skype "LA PAROLA A... Corso: Biologi... 3 Microsoft ... AnnHyb 4.943 ... IT 18.28

Microsoft Oligo

File Edit Oligo Sequence Multiple alignment Help

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Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name **Oligo1** notes None

Seq. 5'

IUB seq. 5'

rev. 5'

comp.

Length MW GC% GCG checksum Molar ext. coef.
0 b 0 0 0 /mol (at 280 nm)

Salt cc 50 mM Primers cc 250 nM Tm °C

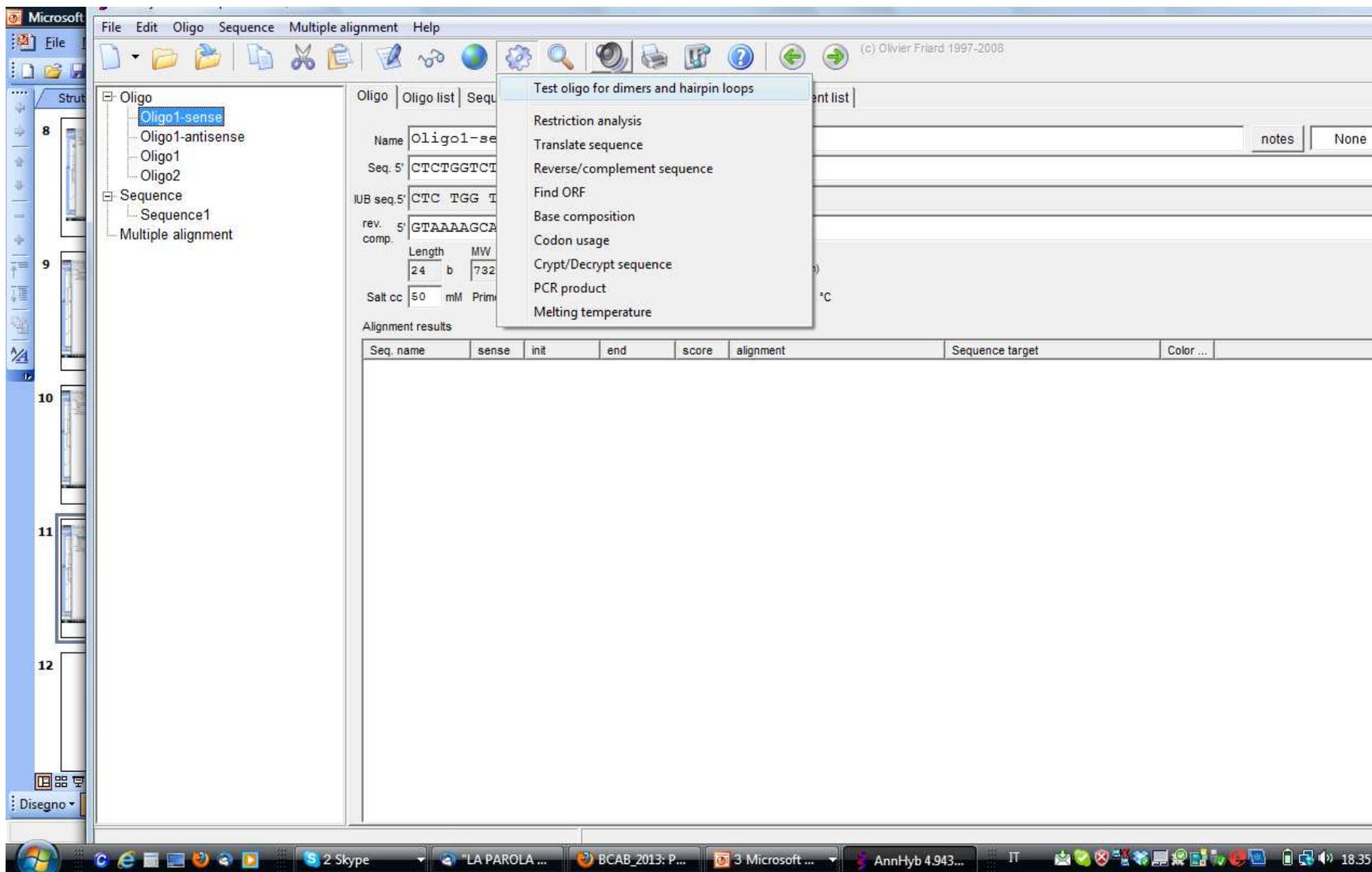
Alignment results
Allawi, 1997

Seq. name	sense	init	end	score	alignment	Sequence target	Color ...
-----------	-------	------	-----	-------	-----------	-----------------	-----------

primer senso
(create oligo with the clipboard sequence)

primer antisenso
(DO NOT create oligo with the clipboard sequence, dovete fare "copia e incolla" in questa casella)

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Solo per uso didattico - vietata la riproduzione o la vendita

Microsoft
File Edit Oligo Sequence Multiple alignment Help
File (c) Olivier Friard 1997-2008

Strut

- 8 Oligo
 - Oligo1-sense
 - Oligo1-antisense
 - Oligo1
 - Oligo2
- 9 Sequence
 - Sequence1
- 10 Multiple alignment
- 11
- 12

Disegno

Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name: notes None

Seq. 5'

IUB seq. 5'

rev. 5'

comp.

Length	MW	GC%	GCG checksum	Molar ext. coef.
24 b	7320	41.6%	3134	198360 $\mu\text{mol (at 260 nm)}$

Salt cc mM Primers cc nM Tm °C

Alignment results Allawi, 1997

Seq. name	sense	init	end	score	alignment	Sequence target	Color ...

Primer dimers and Hairpin loops

Oligo1-sense: CTCTGGTCTTGCTTTTGCTTTTAC
 Oligo1-sense : CTCTGGTCTTGCTTTTGCTTTTAC
 Oligo1-antisense : TGCCGCTAGCCGATTAAG

Dimers < > Max

```

CTCTGGTCTTGCTTTTGCTTTTAC
 |   |   |   |
CAITTTGTTTTCTTCTGGTCTC
    
```

Hairpin loops < > Max

```

      CTCTGGTCT
      |   |   T
CAITTTGTTTTCTG
    
```

? Save Close

2 Skype "LA PAROLA ... BCAB_2013: P... 3 Microsoft ... AnnHyb 4.943... IT 18.37

Solo per uso didattico - vietata la riproduzione o la vendita

Microsoft **File Edit Oligo Sequence Multiple alignment Help** (c) Olivier Friard 1997-2008

Strut

- Oligo
 - Oligo1-sense
 - Oligo1-antisense
 - Oligo1
 - Oligo2
- Sequence
 - Sequence1
 - Multiple alignment

Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name: notes None

Seq. 5'

IUB seq.5'

rev. 5'

comp.

Length	MW	GC%	GCG checksum	Molar ext. coef.
24 b	7320	41.6%	3134	198360 /mol (at 260 nm)
Salt cc	50 mM	Primers cc	250 nM	Tm
				55.0 °C

Alignment results Allawi, 1997

Seq. name	sense	init	end	score	alignment	Sequence target	Color ...
-----------	-------	------	-----	-------	-----------	-----------------	-----------

Primer dimers and Hairpin loops

Oligo1-sense: CTCTGGTCTTGCTTTTGCTTTTAC
 Oligo1-sense : CTCTGGICTTGCTTTTGCTTTTAC
 Oligo1-antisense : TGCCGCCTAGCCGATTAAG

Dimers < > Max

```

CTCTGGTCTTGCTTTTGCTTTTAC
  ||  ||
GAATTAGCCGATCCGCCGT
    
```

Hairpin loops < > Max

```

CTCTGGIC
 | | | T
CATTTTGGTTTTCGT
    
```

? Save Close

Disegno

Windows Taskbar: 2 Skype, Statali, Tfr e d..., BCAB_2013: Pr..., 3 Microsoft..., AnnHyb 4.943 ..., IT, 18.38

Solo per uso didattico - vietata la riproduzione o la vendita

Microsoft **Oligo** Sequence Multiple alignment Help

File Edit **Oligo** Sequence Multiple alignment Help

Strutture: 10, 11, 12, 13, 14

Disegno

Sequence | Sequence list | Multi-alignment | Multi-alignment list

Sequence	Length	Chec...	MW	GC%	MEC	salt conc. (...)	primer conc...	Tm (°C) Alla...
CTCTGGTCTTGCTTTTGTCTTTTAC	24	3134	7320	41.7	198360	50	250	55.0
TGCCGCCTAGCCGATTAAG	19	3490	5851	57.9	179190	50	250	56.5

Oligo menu items:

- New oligo
- Import oligo in project
- Remove oligo(s)
- Export
- Print
- Test oligo for dimers and hairpin loops
- Quick search oligo in sequences
- Align oligo with sequences**
- Quick search oligo in multiple alignment
- Align oligo in multiple alignment

Oligo list (2 oligonucleotides)

Taskbar: 2 Skype, Statali, Tfr e d..., BCAB_2013: Pr..., 3 Microsoft ..., AnnHyb 4.943 ...

AnnHyb 4.943 September 09, 2010

File Edit Oligo Sequence Multiple alignment Help

(c) Olivier Friard 1997-2008

Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name	Sequence	Length	Chec...	MW	GC%	MEC	salt conc. (...)	primer conc...	Tm (°C) Alla...
<input checked="" type="checkbox"/> Oligo1-sen...	CTCTGGTCTTGCTTTTGCTTTTAC	24	3134	7320	41.7	198360	50	250	55.0
<input checked="" type="checkbox"/> Oligo1-anti...	TGCCGCCTAGCCGATTAAG	19	3490	5851	57.9	179190	50	250	56.5

Windows taskbar: 2 Skype, Statal, Tfr e d..., BCAB_2013: Pr..., 3 Microsoft ..., AnnHyb 4.943 ...

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Microsoft PowerPoint - [291012.ppt]

AnnHyb 4.943 September 09, 2010

File Edit Oligo Sequence Multiple alignment Help

(c) Olivier Friard, 1997-2008

Oligo Oligo list Sequence Sequence list Multi-alignment Multi-alignment list

Name: Sequence1 Long name:

Description:

Length: 5712 Type: DNA checksum: 9812 Author: Creation date: 27/10/2012

Sequence header:

Sequence annotations

Name	init	end	comments
■ Oligo1-sense	151	174	found oligo score: 100 sense: ...
■ Oligo1-antisense	271	289	found oligo score: 100 sense: ...

```

1  gaattcggca cgaggcgatg ctacaggggc aggcacctgc tgctctgtaa
51  tgattcagcc tctttcagcc gctgogtaa cacgacagga tgctgttgct
101 actgtcgctg ctgcctctcc tgccgcggcc gctgctgccg ccgcccctc
151 ctctgggtctt gcttttgctt ttacttctcc tgcatgacag ttgttttctt
201 cctctaagca gacaccagct tcagacgctt gaggtgagaa acatgccttt
251 cagtttggga tactggttta cctaatcggc tagggcgagc cttgcttctt
301 attttggtcc cctgccttct tgaccaaccg gccatgggtt ggagaagcat
351 ttgaaagaac tgaaaaagtg tcccagaaac aacagctcaa gatatttcgg
401 tacacttcta ttccatagtt gctagaagcc ctttcttttt cgtttttttt
451 ttctttttct ttttcttttt ctttttctt ttctctgttc ctctaagct
501 ctggtacttt gggtaattgc ctggacttg ggtgccttat cgatttcccc
551 ctccaagatg ctgtatcatt tgggtggggg gagctctgcg ttgtaatgca
601 ctgtgagaga ggccaggcct tctggaggtg agccgatgga gatttattcc
651 ccagacatgt ctgaggtagc tggcgggagg tcctccagcc cctccactca
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751 taccagacac ccacacagaa gatgagagaa gccctggact cctgggcctg
801 gcggtgcctc gctgtgtgtg cctggaagct gagcgctgga gaggggtgtc
851 caactccgag aagatctgca ttgttccat tctggcttgc ctagtccagc
901 tctgcctctg cattgctggc ctgaaagtgg tattttgtga caagatatt
951 gaatacgact ctctaccoca ccttgacctt ggggggttag gccaggacc

```

Statali, Tfr e d... BCAB_2013: Pr... 3 Microsoft ... AnnHyb 4.943 ... Skype™ - giov... IT 18.49

AnnHyb 4.943 September 09, 2010

File Edit Oligo Sequence Multiple alignment Help

(c) Olivier Friard 1997-2008

Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list

Name: Sequence1 Long name:

Description:

Length: 5712 Type: DNA checksum: 9812 Author: Creation date: 27/10/2012

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Sequence annotations

Name	init	end	comments
Oligo1-sense	151	174	found oligo score: 100 sense: ...
Oligo1-antisense	271	289	found oligo score: 100 sense: ...

```

1  gaattcggca cgaggcgatg ctcagagggc aggcacctgc tgctctgtaa
51  tgattcagcc ttttcagcc gctgcgtaa cacgacagga tgctgttgc
101 actgtcgtg ctgcctctcc tgccgcgcc gctgctgcc ccgcgcctc
151 ctctgggtctt gcttttgctt ttactttctcc tgcattgacag ttgttttctt
201 cctctaagca gacaccagct tcagacgctt gaggtgagaa acatgccttt
251 cagtttggga tactggttta cttaatcggc tagggcgca g cttgcttct
301 attttggtcc cctgccttct tgaccaacc ggcattggtt ggagaagcat
351 ttgaaagaac tgaaaaagt tccagaaac aacagctcaa gatatttcg
401 tacacttcta tttcatagt gctagaagcc ctttctttt cgttttttt
451 ttctttttct tttcttttt ctttttctt ttctgcttc ctctaaagt
501 ctggtacttt ggtaattgc cttggacttg ggtgcctat cgattcccc
551 ctccaagatg ctgtatcatt tggttgggg gagctctgcg ttgtaaatgca
601 ctgtgagaga ggccaggcct totggagtg agccgatgga gattattcc
651 ccagacatgt ctgaggtagc tggcgggagg tcctccagcc cctccactca
701 gctgagtgca gcccatctc ttgatgggct tcggcagcg gaggaacata
751 taccacacac ccacacaqaa qatqaaqaa qcctqact cctqaaqact

```

Seq. length: 5712 Selection: 151 - 289 (139 bp) Tm= 75.2°C (Howley, 1979)

Solo per uso didattico - vietata la riproduzione o la vendita

PROGETTO DETTAGLIATO BIOTECNOLOGIE CELLULARI

- 1 - Studio delle sequenze presenti in banca dati riguardanti la NRG1-typelll-beta 3
- 2 - Scelta dei primers per amplificare la NRG1-typelll-beta 3 di ratto (tenendo conto del fatto che poi la cloneremo *in frame* in un vettore esprime la GFP).**
- 3 - RT-PCR
- 4 - Clonaggio di NRG1-typelll-beta 3 in vettore pCR-bluntll-TOPO
- 5 - Sequenziamento
- 6 - Subclonaggio in vettore di espressione pEGFP-C3 (proteina ibrida NRG-EGFP)
- 7 - Subclonaggio in vettore di espressione pIRES-puro2
- 8 - Subclonaggio in vettore di espressione con coda FLAG per identificazione della proteina
- 9 - Subclonaggio di NRG1-typelll-beta3 in vettore virale adenoassociato pAAV-MCS
- 10 - Subclonaggio di NRG1-typelll-beta3-FLAG in vettore virale adenoassociato pAAV-MCS

NB Per ogni clonaggio e sub-clonaggio studio delle digestioni enzimatiche che consentono di verificare il corretto orientamento dell'inserto, con previsione delle bande attese in caso di clonaggio senso o antisense

Sensory and Motor Neuron-derived Factor Is a Transmembrane Heregulin That Is Expressed on the Plasma Membrane with the Active Domain Exposed to the Extracellular Environment*

(Received for publication, March 12, 1998, and in revised form, August 7, 1998)

Allen Schroering and David J. Carey‡

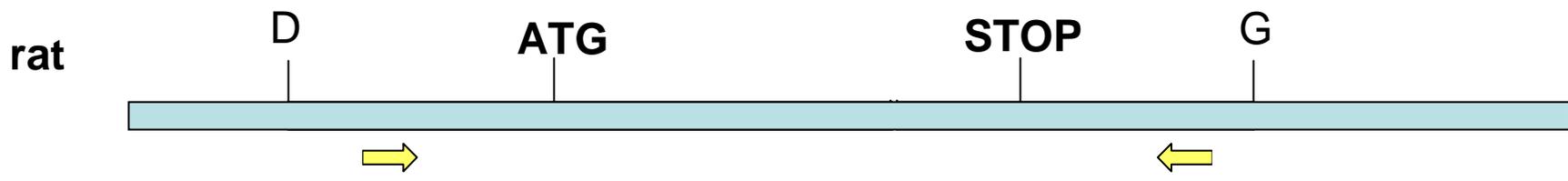
From the Weis Center for Research, Penn State College of Medicine, Danville, Pennsylvania 17822-2613

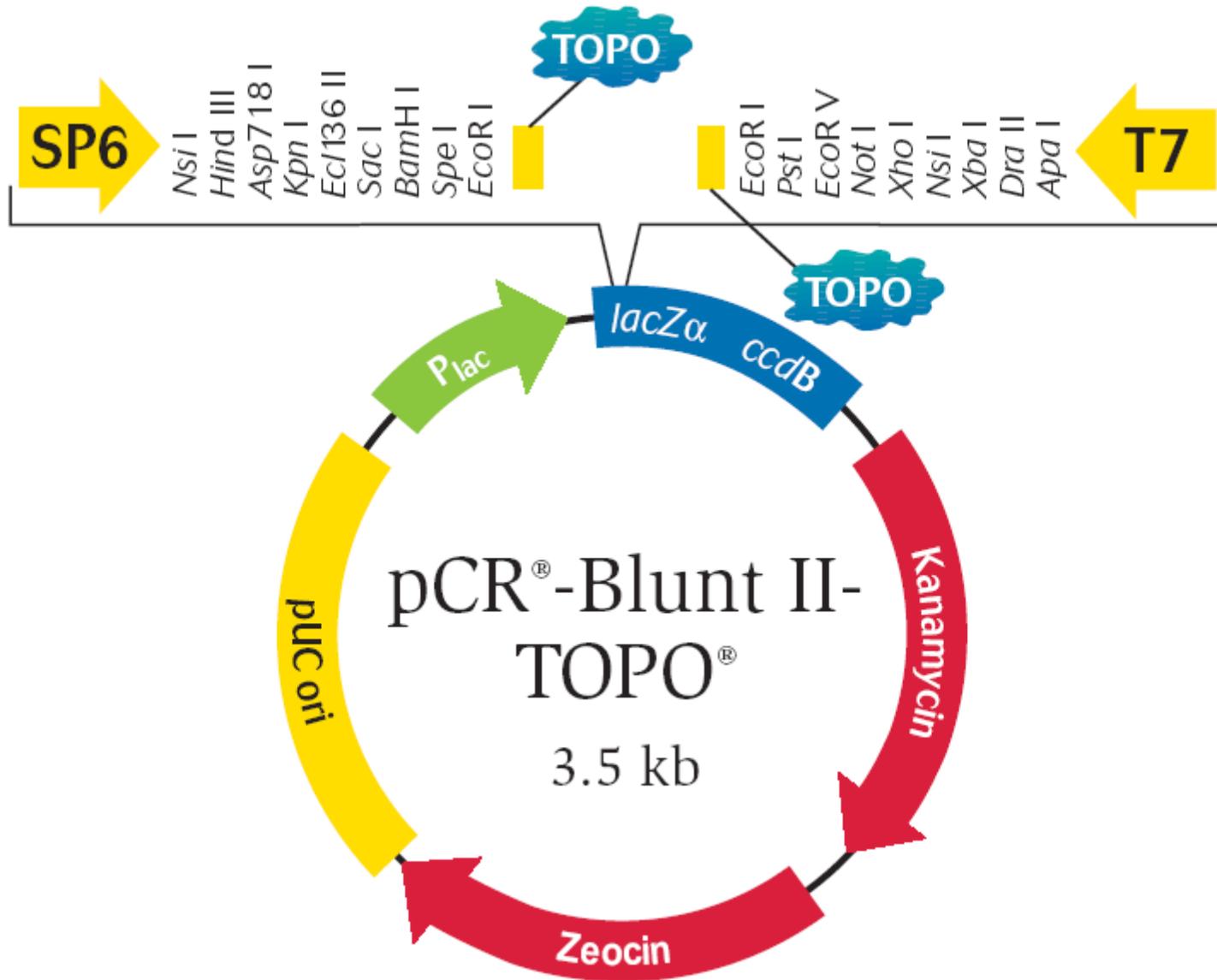
EXPERIMENTAL PROCEDURES

Cloning and Expression of SMDF—Human SMDF cDNA was cloned by nested PCR amplification from a human brain stem cDNA library (LMG2, American Type Culture Collection, 37432). The primers were based on the published human SMDF cDNA sequence (13) and are shown in Table I. Conditions for PCR reactions were 94 °C for 1 min (denaturation), 60 °C for 2 min (annealing) and 72 °C for 10 min (extension) for 30 cycles. The resulting PCR product was cloned into pCRII (Invitrogen). The insert was excised from this vector by digestion with *EcoRI* and subcloned into the plasmid pCMVneo. For the addition of FLAG epitope tags, the insert was reamplified by PCR using primers that overlapped the ends of the protein coding sequence and were extended on their 5'-ends by sequence coding for the FLAG epitope sequence. These products were cloned into the T/A expression plasmid pCR3.1 (Invitrogen). Sequences of expression plasmids were verified by DNA sequence analysis.

TABLE I
Primers used for PCR amplification of human SMDF

Primer	Sequence
First round	
Sense	GCCTCTGCGTGGTAATGGAC
Antisense	AATGTTCTCATGCGACAGGC
Second round	
Sense	CTTCTGGAGGTGAGCCGATG
Antisense	AAGCAGCACCAACTGAGCAT





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<http://www.invitrogen.com/>

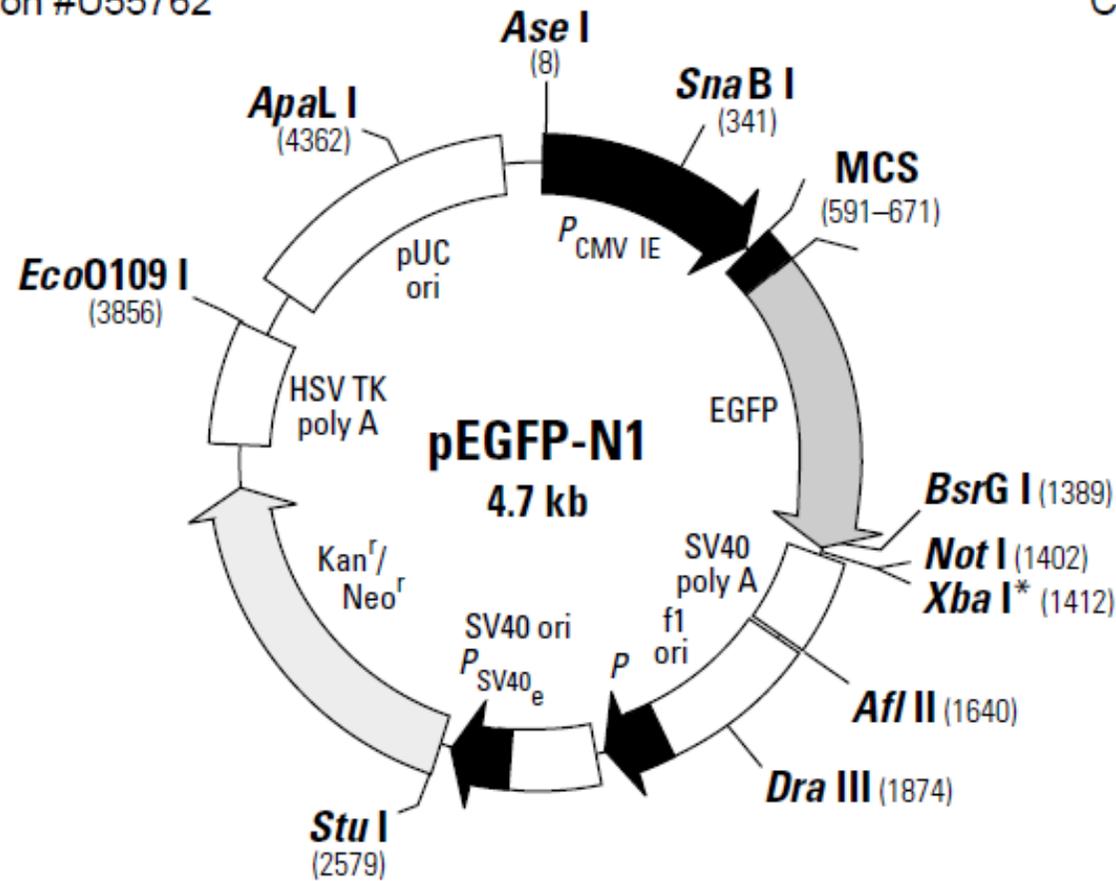
Vettore di clonaggio del prodotto di RT-PCR

pEGFP-N1 Vector Information

GenBank Accession #U55762

PT3027-5

Catalog #6085-1



591 601 611 621 631 641 651 661 671 **EGFP** →

G CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC GCC ACC ATG GTG
Nhe I Eco47 III Bgl II Xho I Sac I Hind III EcoR I Pst I Sal I Kpn I Acc I Asp718 I Sac II Apa I Bsp120 I Xma I BamH I Age I Sma I

pEGFP-C3 Vector Information

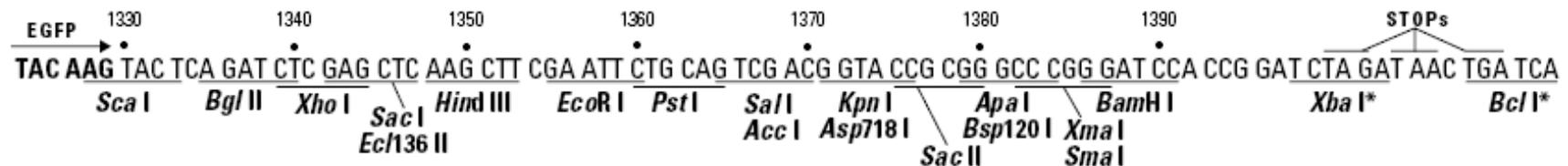
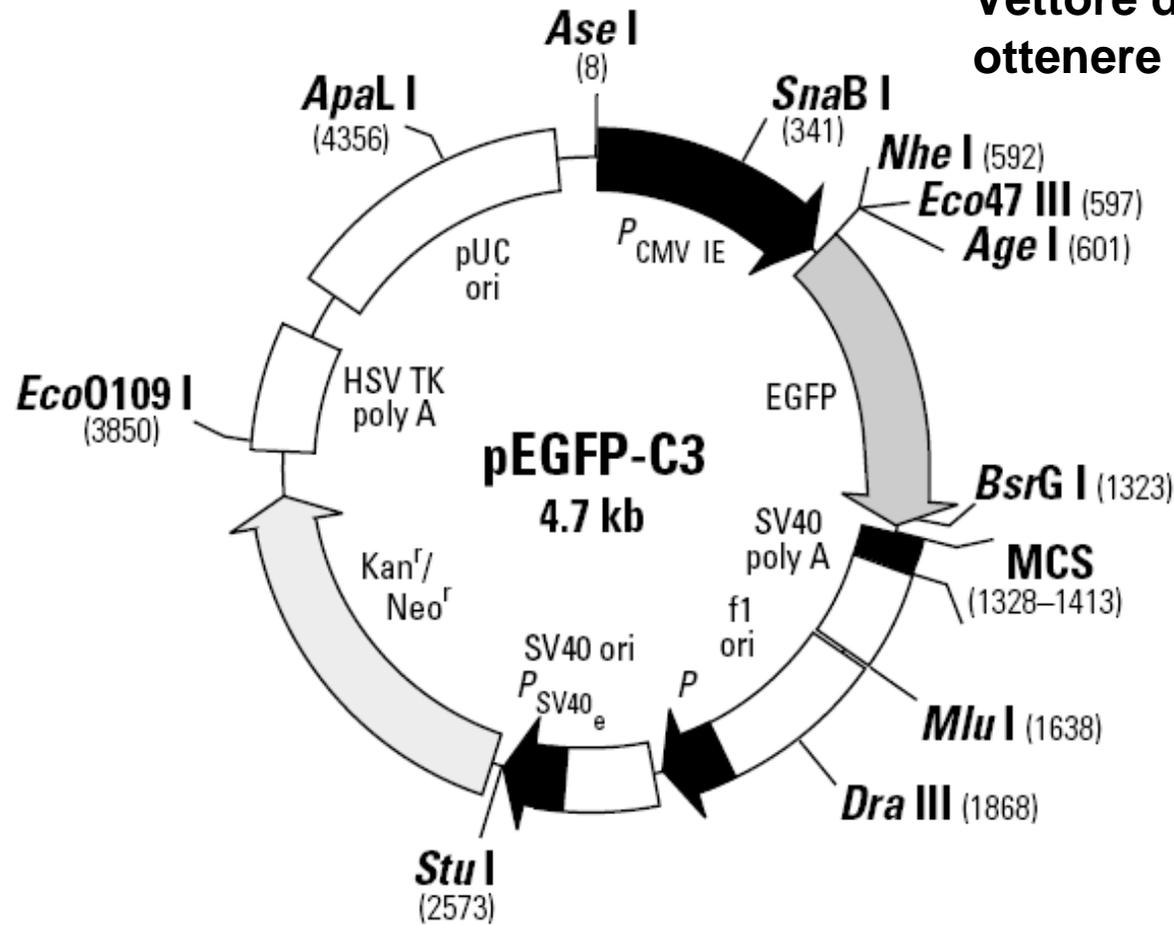
GenBank Accession #: U57607

Clontech

PT3052-5

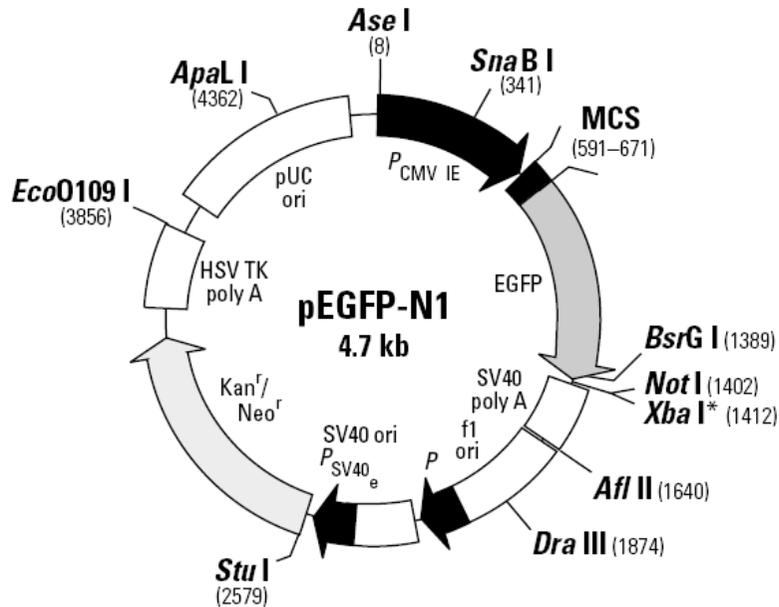
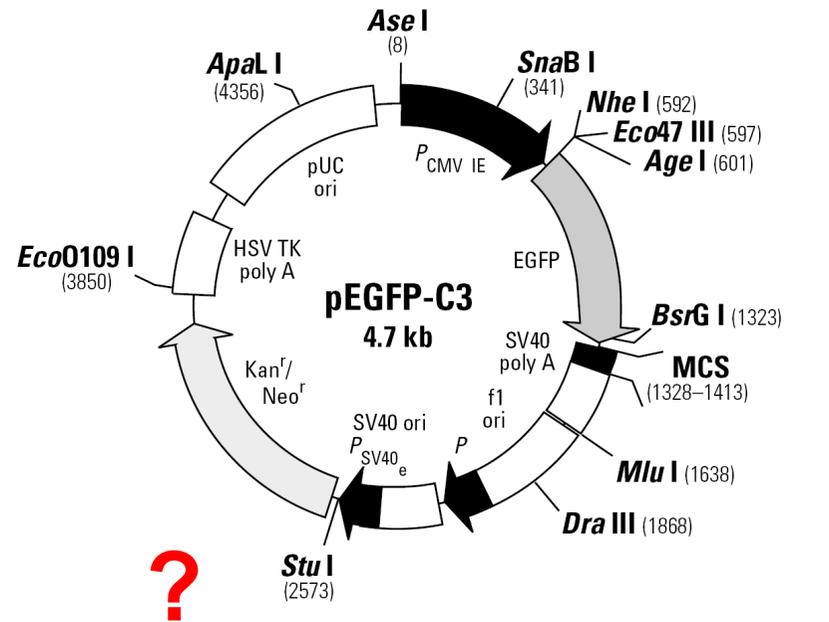
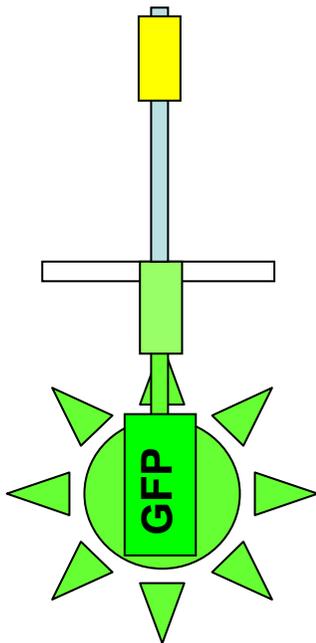
Catalog #6082-1

**Vettore di espressione per
ottenere proteina ibrida**

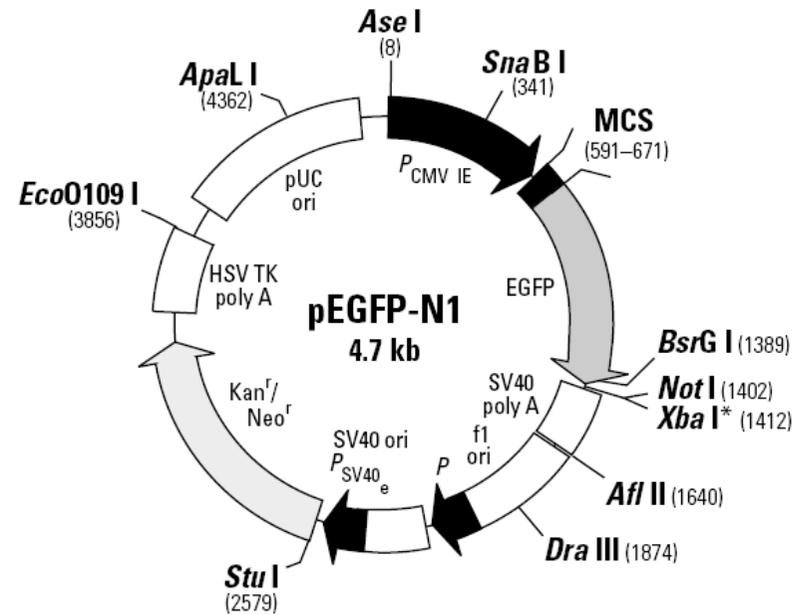
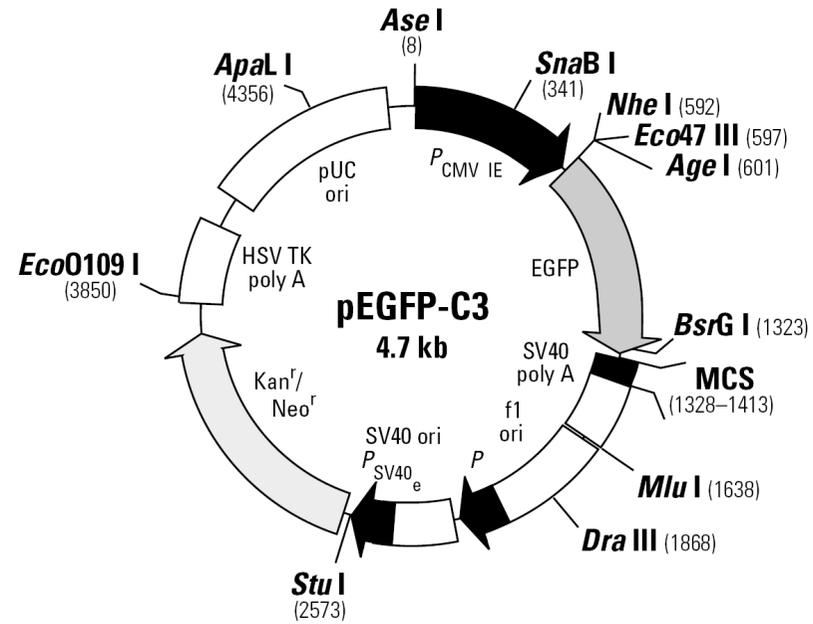
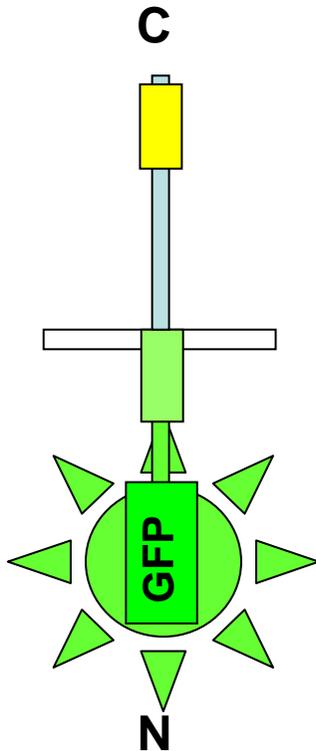


Solo per uso didattico - vietata la riproduzione o la vendita

- Quale vettore devo usare per avere la GFP nella regione citoplasmatica?



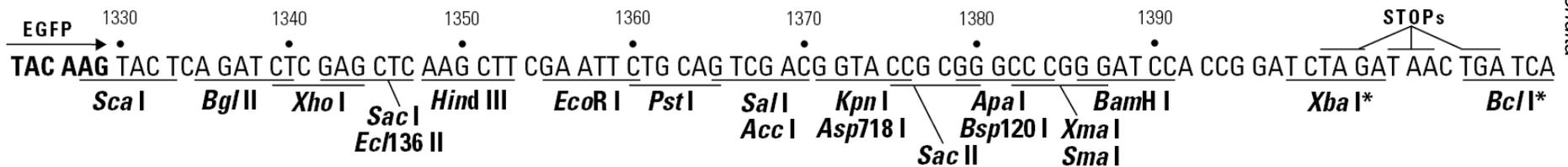
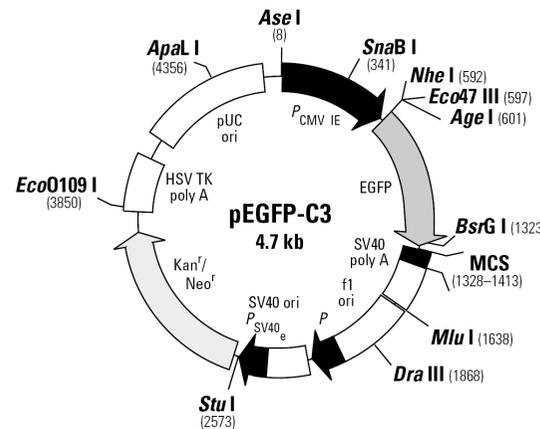
- Quale vettore devo usare per avere la GFP nella regione citoplasmatica?



* per semplicità non faremo due cicli di PCR, ossia una PCR seguita da una “nested” PCR, bensì una sola reazione: scegliete dunque una sola coppia di primers per amplificare

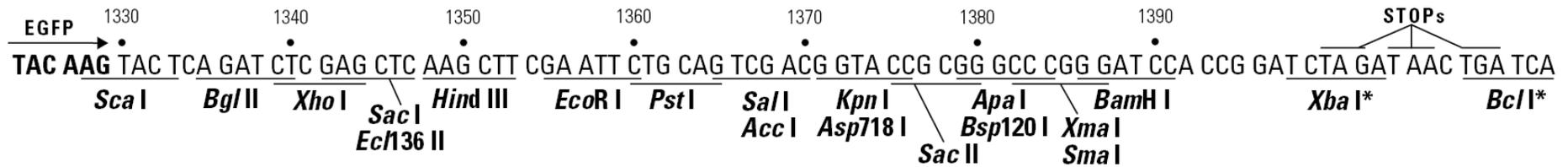
* tenete conto del fatto che la NRG1-III-beta 3 andrà poi clonata in frame a valle di EGFP nel vettore pEGFP-C3: nel primer al 5' inserite un sito di restrizione che faciliti il clonaggio e mantenga il frame di lettura

* i siti di restrizione devono essere entrambi compatibili con il multiple cloning site del vettore pEGFP-C3 e non devono tagliare la NRG1...



Solo per uso didattico - vietata la riproduzione o la vendita

*** Attenzione al frame di lettura ***



Esempio: decido di inserire il sito EcoRI nel primer a monte :



EGFP

TAC AAG TAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG

Dovrò fare attenzione alle triplette codificanti la NRG1 e fare in modo che il sito per l'enzima di restrizione sia inserito nel *primer* in modo da mantenere le triplette di EGFP *in frame* con l'ATG della NRG1 (l'ATG può anche non essere compreso nel primer, bensì molte basi dopo:

Es: 5'- **GA ATT C**  NN NNN NNN NNN ATG NNN NN-3'

Così otterrò:

TAC AAG TAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CNN NNN NNN NNN ATG NNN

EGFP

linker

NRG1

* i siti di restrizione che metterete **non** devono tagliare **né** dentro l'inserto, **né** il vettore per il sub-clonaggio (fate l'analisi della mappa di restrizione dei vettori e dell'inserto con *neb cutter*: fate la lista degli enzimi che non tagliano e cercate fra questi quelli presenti nell'MCS del vettore con la GFP)

* le coppie di primers devono soddisfare i seguenti criteri:

- 1- avere T_m simile (circa 55°C calcolati col metodo Allawi)
- 2- finire con G o C
- 3- avere un contenuto di G e C pari almeno al 50%
- 4- non formare strutture secondarie (con *Annhyb: tools* -> test for dimers and hairpin loops, evitare 5 tacche di seguito)

TAC AAG TAC TCA GAT CTC GAG CTC AAG CTT **CGA ATT CNN** NNN NNN NNN **ATG** NNN
EGFP linker NRG1

* provate a tradurre in aminoacidi le triplette fra l'EGFP e la NRG1: non devono essere presenti STOP

Per l'analisi di restrizione e l'identificazione degli ORF potete utilizzare il sito NEB cutter:

<http://tools.neb.com/NEBcutter2/index.php>

Per tradurre una sequenza nucleotidica in aminoacidi potete utilizzare Annhyb, ma questo programma è consigliato soprattutto per disegnare i primers (verifica che i due primers non formino loop, omodimeri o eterodimeri, 5 tacche successive indicano un legame forte)

<http://www.bioinformatics.org/annhyb/>

NEBcutter V2.0 - Mozilla Firefox

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<http://tools.neb.com/NEBcutter2/index.php>

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neb cutter

NEBcutter V2.0



NEBcutter V2.0

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This tool will take a DNA sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 KBases.**

KBases.

[What's new in V2.0](#)

Local sequence file: [Browse...](#)

GenBank number: [Browse GenBank](#)

or paste in your DNA sequence: *(plain or FASTA format)*

Standard sequences:

Plasmid vectors

Viral + phage

The sequence is: Linear Circular

Enzymes to use:

- NEB enzymes
- All commercially available specificities
- All specificities
- All + defined oligonucleotide sequences
- Only defined oligonucleotide sequences

[define oligos](#)

Minimum ORF length to display: a.a.

Name of sequence: *(optional)*

Earlier projects:

Note: Your earlier projects will be deleted 2 days after they were last accessed. You need to have cookies enabled in your browser for this feature to work.

Disable NEBcutter cookies

Solo per uso didattico - vietata la riproduzione o la vendita

Done

start

2 Microsoft Office P... 3 Firefox 2 Esplora risorse NCBI Sequence View... Posta in arrivo per gi... IT 14.09