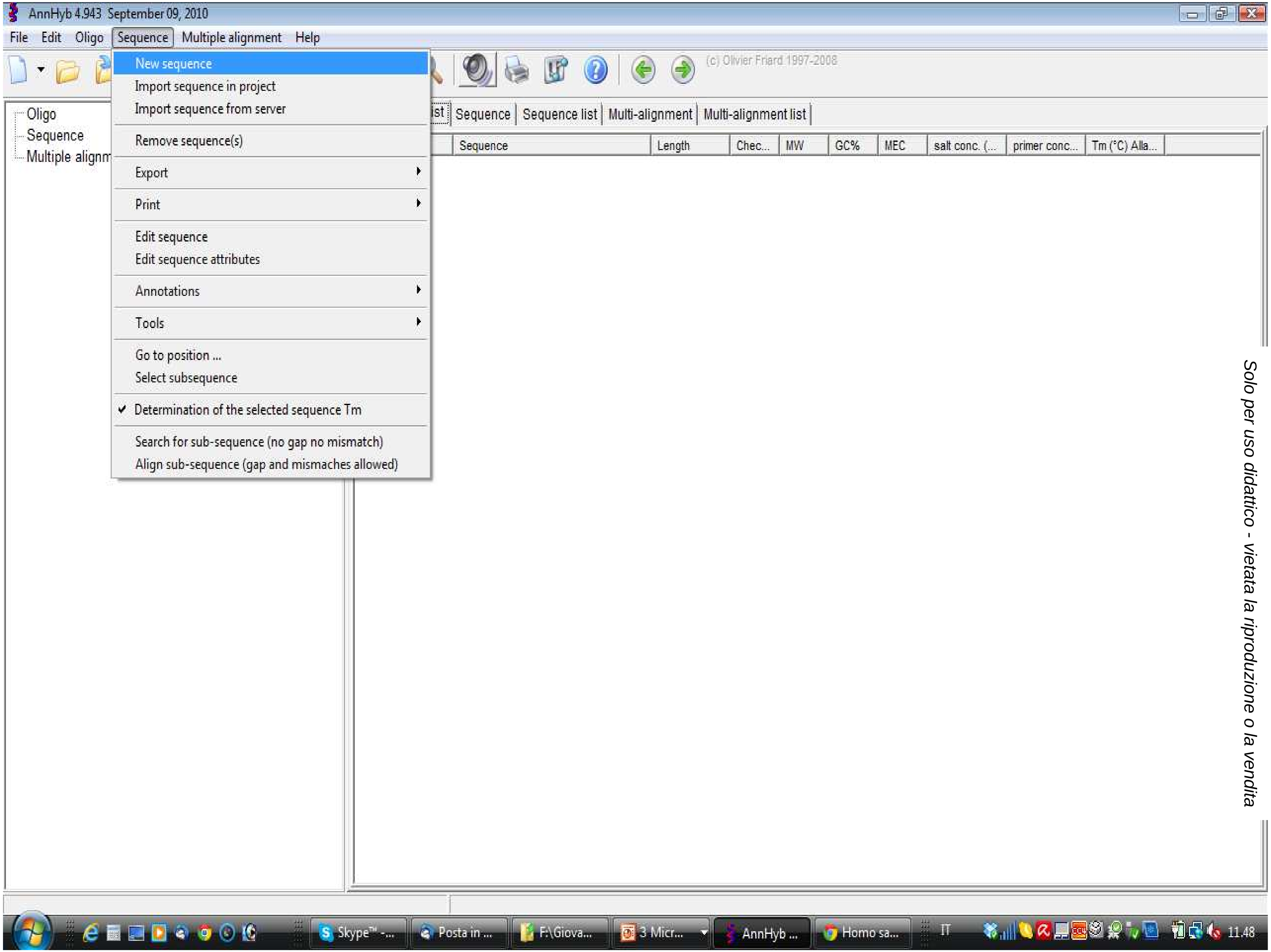
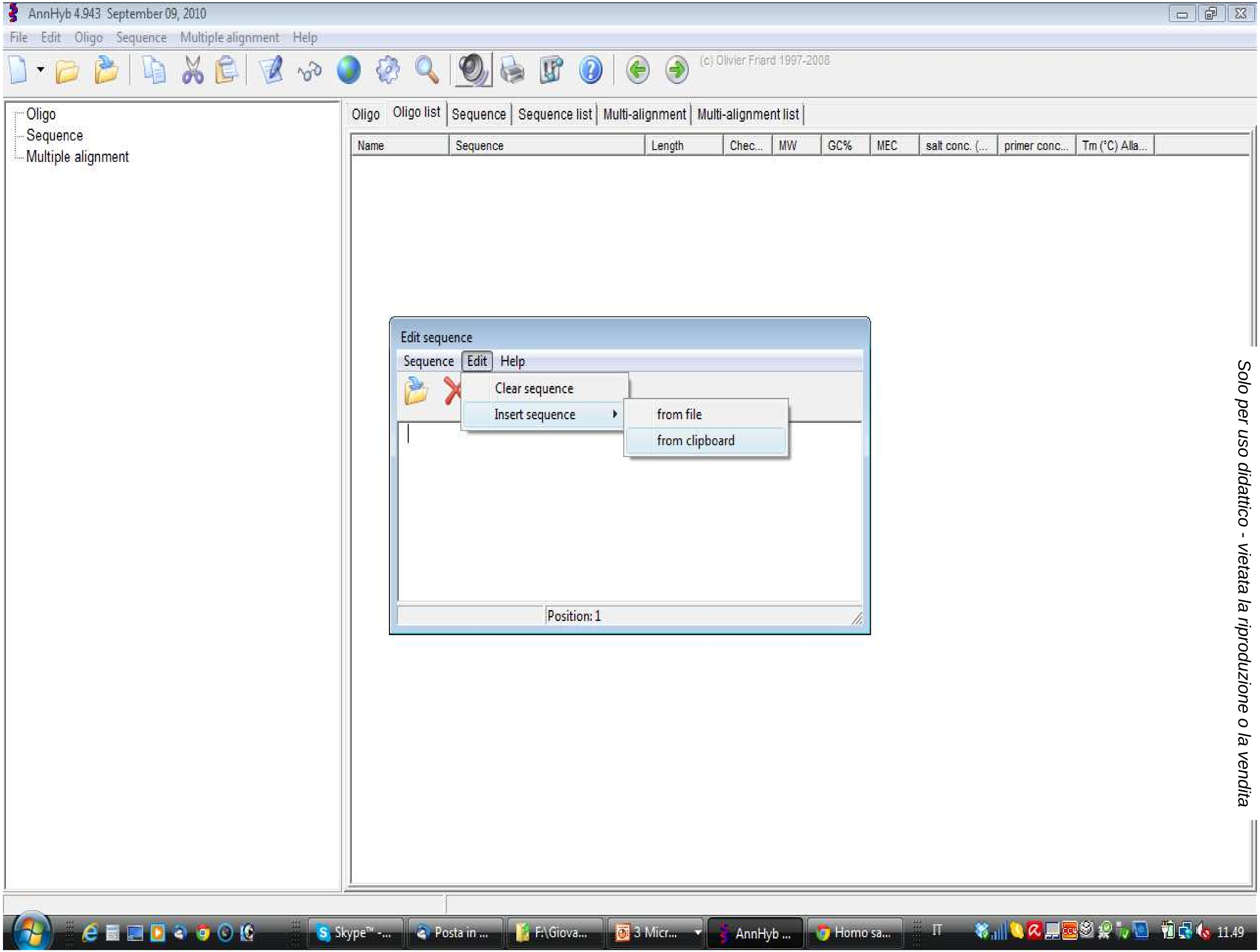
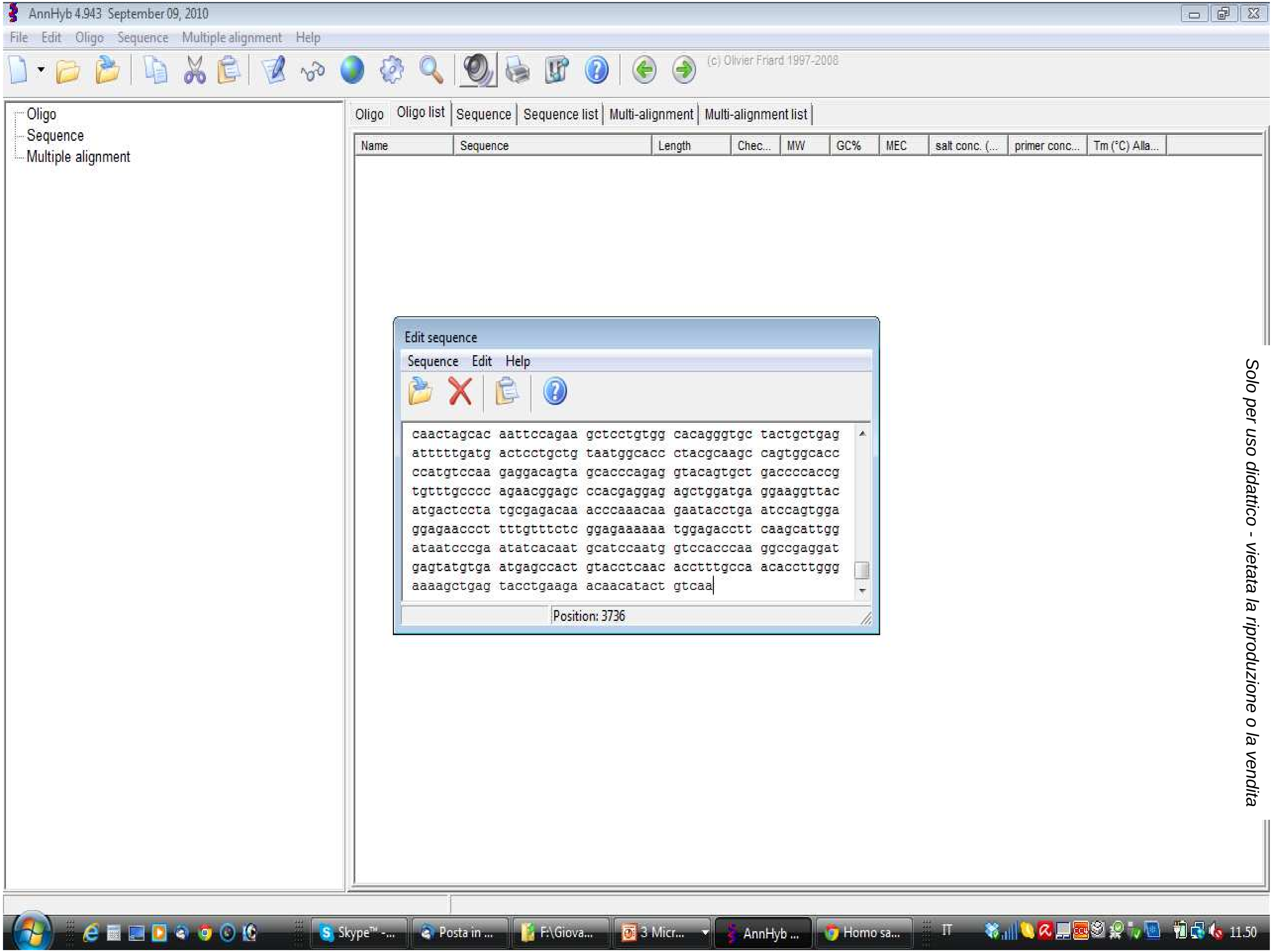
Come usare Annhyb

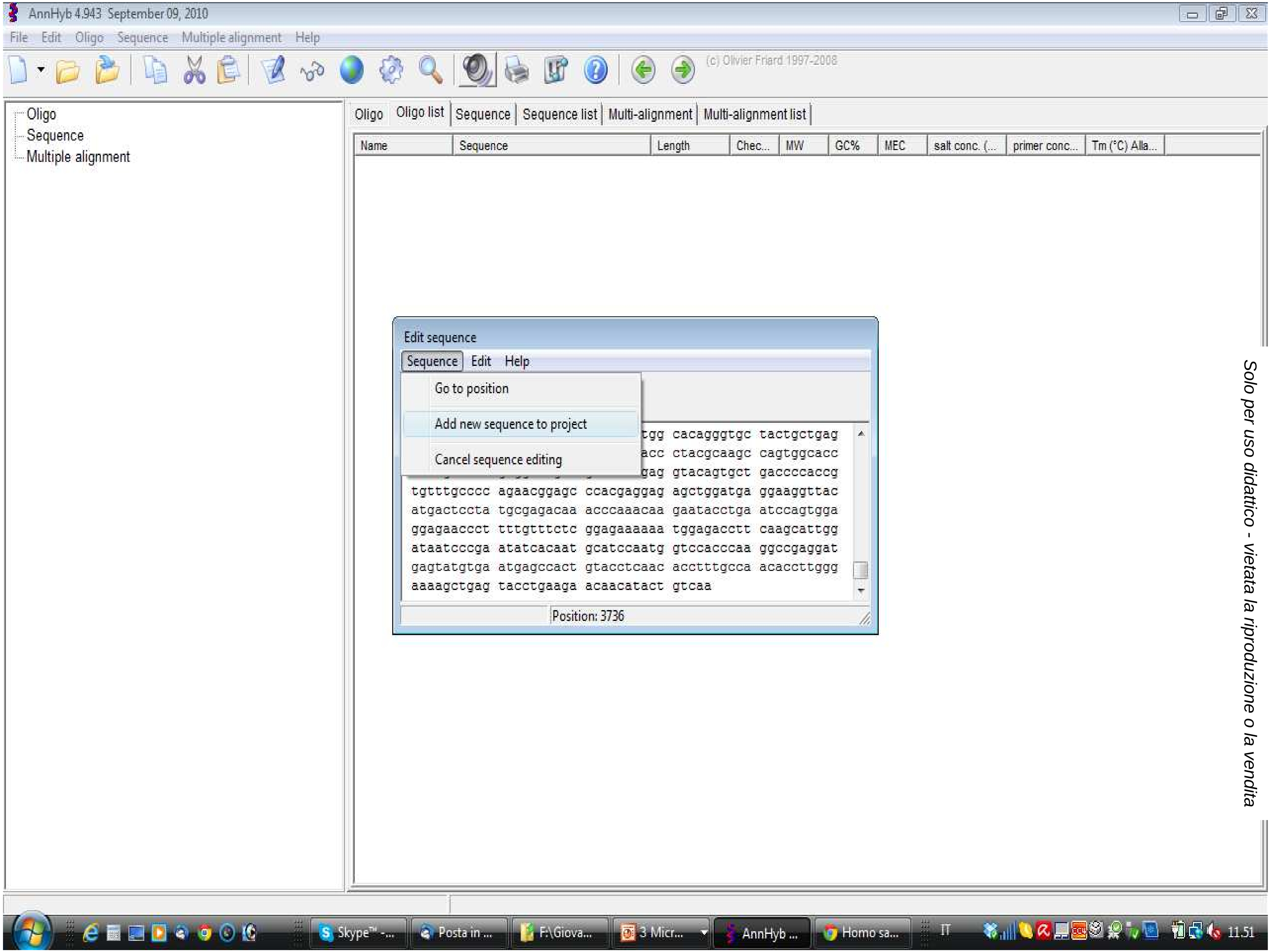

AnnHyb 4.943 September 09, 2010
File
Edit
Oligo
Sequence
Multiple alignment
Help
(c) Olivier Friard 1997-2008
New sequence
Import sequence in project
Import sequence from server
Remove sequence(s)
Export
Print
Edit sequence
Edit sequence attributes
Annotations
Tools
Go to position ...
Select subsequence
✓ Determination of the selected sequence Tm
Search for sub-sequence (no gap no mismatch)
Align sub-sequence (gap and mismaches allowed)
Oligo
Sequence
Multiple alignm
Sequence
Sequence list
Multi-alignment
Multi-alignment list
Sequence
Length
Chec...
MW
GC%
MEC
salt conc. (...
primer conc...
Tm (°C) Alla...
Skype™ -...
Posta in ...
F:\Giova...
3 Micr...
AnnHyb ...
Homo sa...
IT
11.48


AnnHyb 4.943 September 09, 2010
File Edit Oligo Sequence Multiple alignment Help
(c) Olivier Friard 1997-2008
Oligo
Sequence
Multiple alignment
Oligo | Oligo list | Sequence | Sequence list | Multi-alignment | Multi-alignment list
Name | Sequence | Length | Chec... | MW | GC% | MEC | salt conc. (... | primer conc... | Tm (°C) Alla...
Edit sequence
Sequence Edit Help
Clear sequence
Insert sequence
from file
from clipboard
Position: 1
Skype™ -...
Posta in ...
F:\Giova...
3 Micr...
AnnHyb ...
Homo sa...
IT
11.49



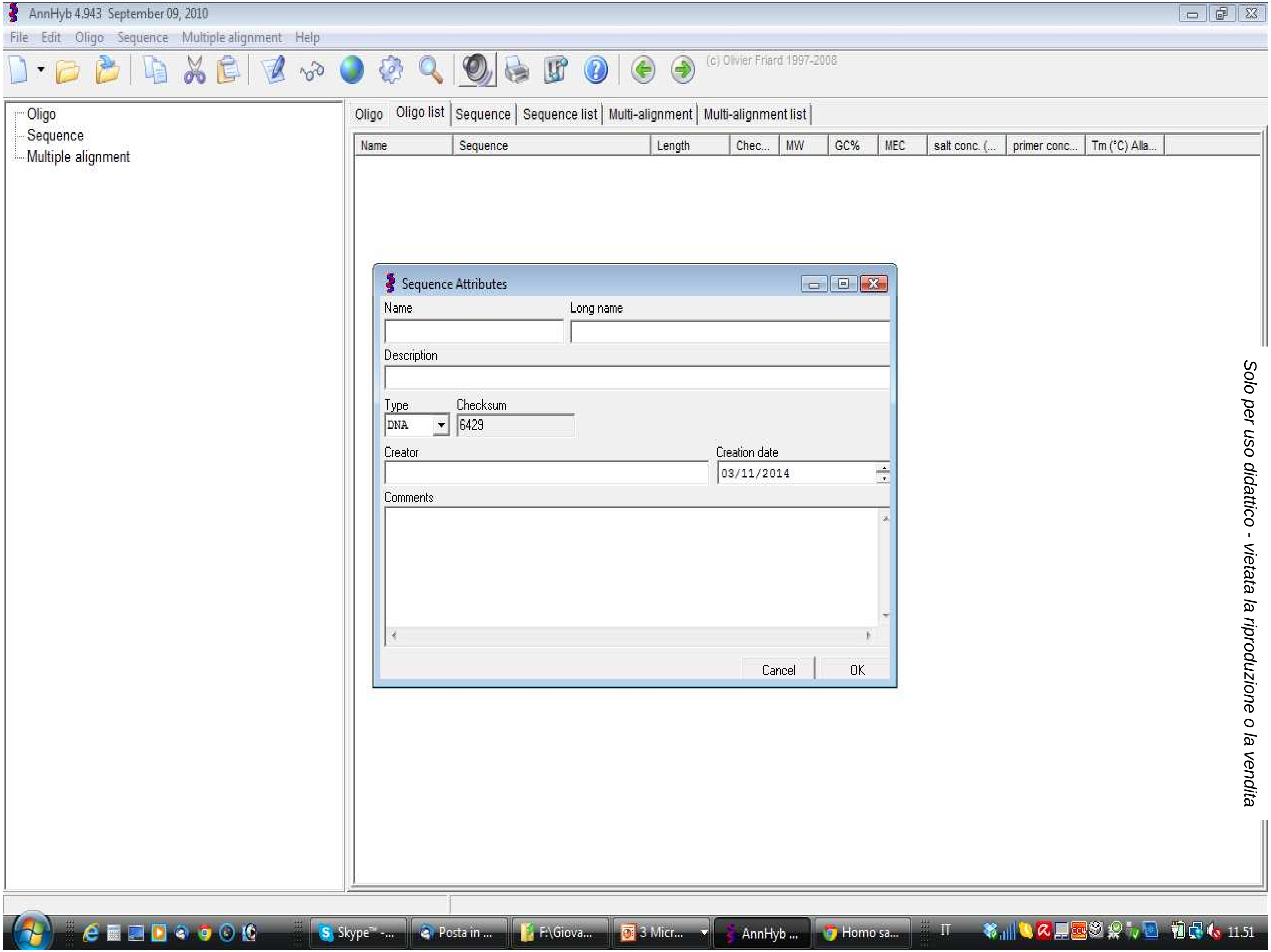
```
caactagcac aattccagaa gctcctgtgg cacagggtgc tactgctgag
atttttgatg actcctgctg taatggcacc ctacgcaagc cagtggcacc
ccatgtccaa gaggacagta gcacccagag gtacagtgct gaccccaccg
tgtttgcccc agaacggagc ccacgaggag agctggatga ggaaggttac
atgactccta tgcgagacaa acccaaacaa gaatacctga atccagtgga
ggagaaccct tttgtttctc ggagaaaaaa tggagacctt caagcattgg
ataatcccga atatcacaat gcatccaatg gtccacccaa ggccgaggat
gagtatgtga atgagccact gtacctcaac acctttgcca acaccttggg
aaaagctgag tacctgaaga acaacatact gtcaa
```

Position: 3736



```
                                  tgg cacagggtgc tactgctgag
                                  acc ctacgcaagc cagtggcacc
                                  gag gtacagtgct gaccccaccg
tgtttgcccc agaacggagc ccacgaggag agctggatga ggaaggttac
atgactccta tgcgagacaa acccaaacaa gaatacctga atccagtgga
ggagaaccct tttgtttctc ggagaaaaaa tggagacctt caagcattgg
ataatcccga atatcacaat gcatccaatg gtccacccaa ggccgaggat
gagtatgtga atgagccact gtacctcaac acctttgcca acaccttggg
aaaagctgag tacctgaaga acaacatact gtcaa
```

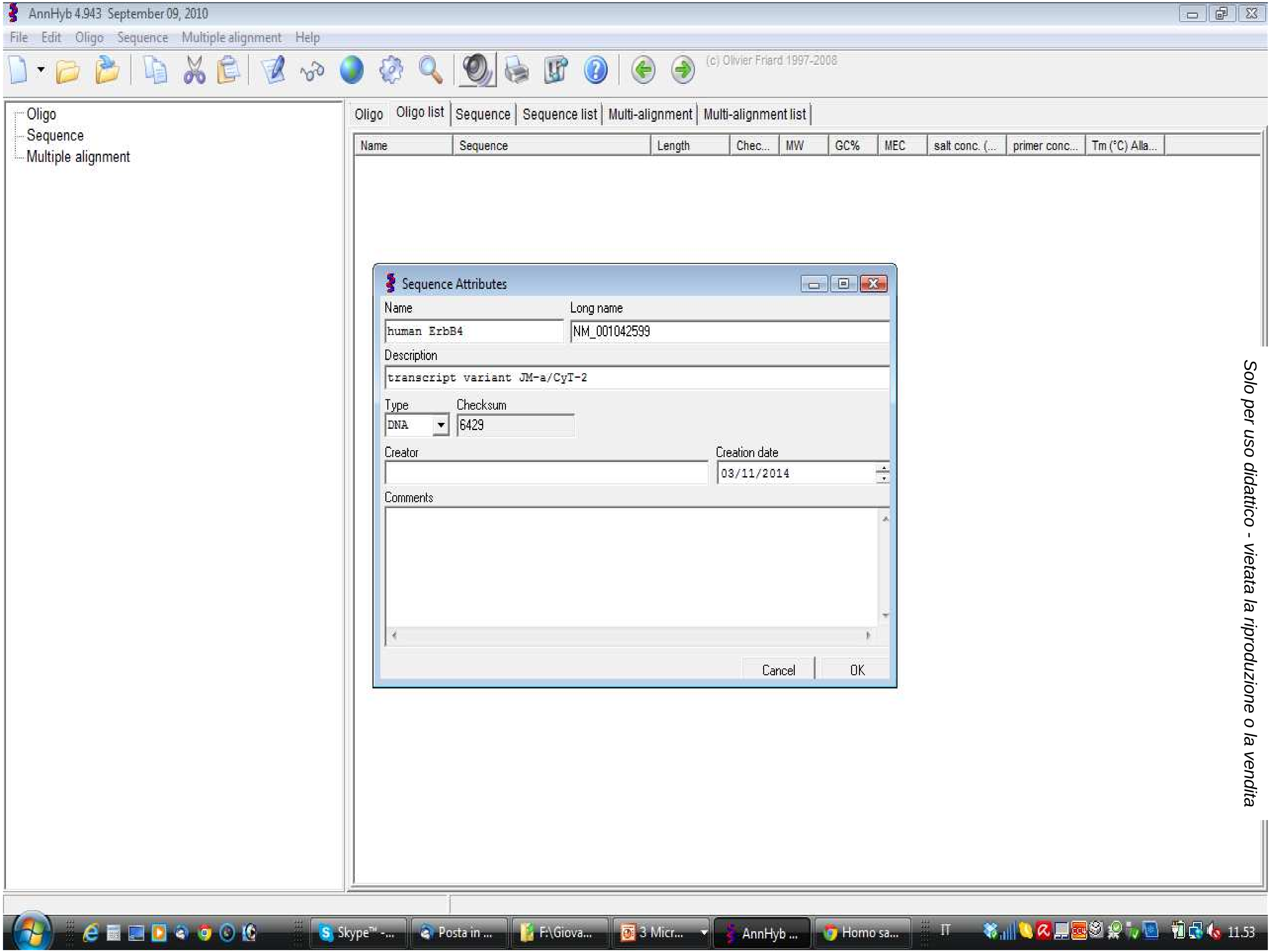
Position: 3736



Type: DNA

Checksum: 6429

Creation date: 03/11/2014



Name: human ErbB4

Long name: NM_001042599

Description: transcript variant JM-a/CyT-2

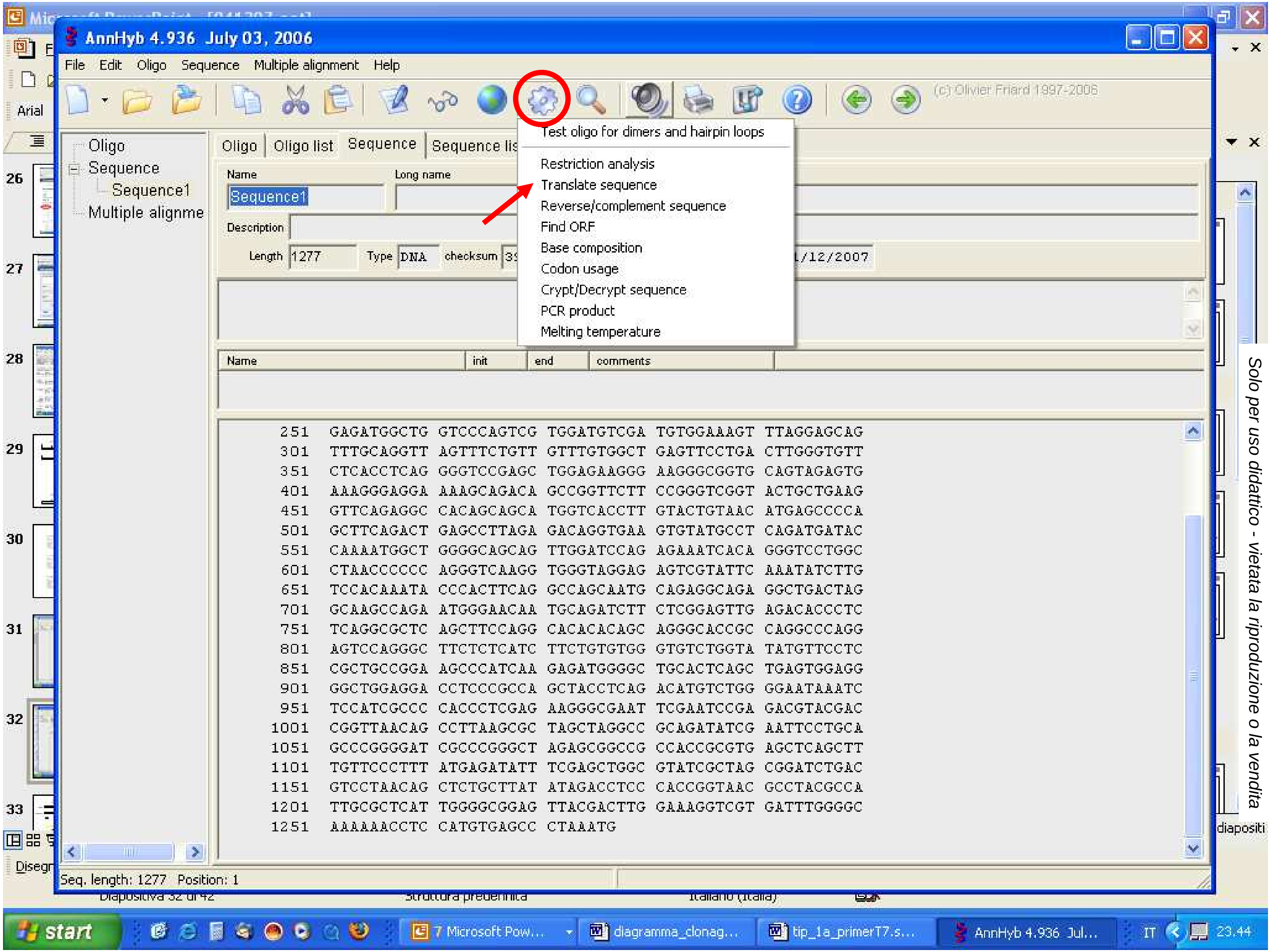
Type: DNA

Checksum: 6429

Creator:

Creation date: 03/11/2014

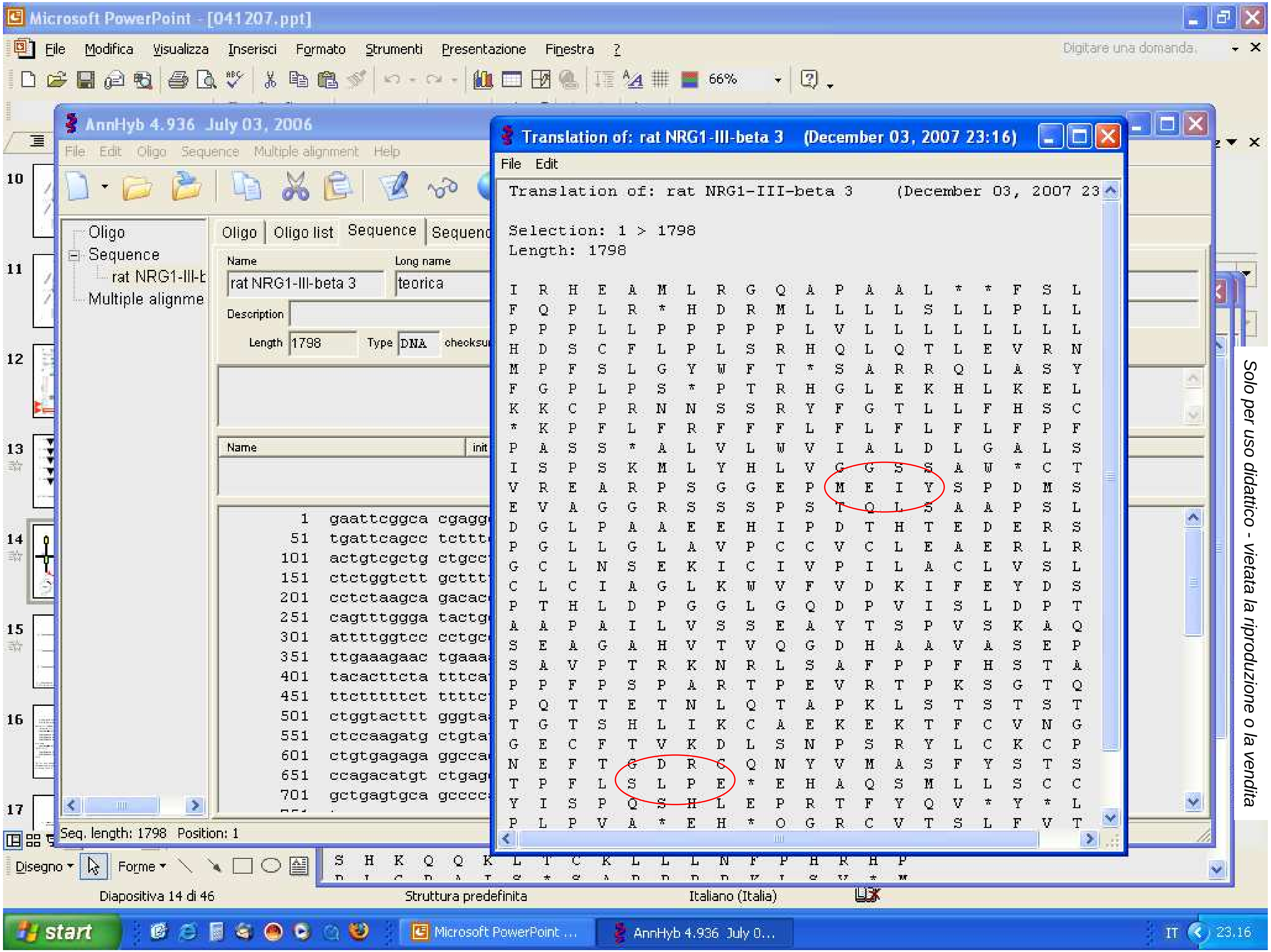
Comments:



Name: Sequence1
Long name:
Description:
Length: 1277 Type: DNA checksum: 39 … /12/2007

```
  251  GAGATGGCTG GTCCCAGTCG TGGATGTCGA TGTGGAAAGT TTAGGAGCAG
  301  TTTGCAGGTT AGTTTCTGTT GTTTGTGGCT GAGTTCCTGA CTTGGGTGTT
  351  CTCACCTCAG GGGTCCGAGC TGGAGAAGGG AAGGGCGGTG CAGTAGAGTG
  401  AAAGGGAGGA AAAGCAGACA GCCGGTTCTT CCGGGTCGGT ACTGCTGAAG
  451  GTTCAGAGGC CACAGCAGCA TGGTCACCTT GTACTGTAAC ATGAGCCCCA
  501  GCTTCAGACT GAGCCTTAGA GACAGGTGAA GTGTATGCCT CAGATGATAC
  551  CAAAATGGCT GGGGCAGCAG TTGGATCCAG AGAAATCACA GGGTCCTGGC
  601  CTAACCCCCC AGGGTCAAGG TGGGTAGGAG AGTCGTATTC AAATATCTTG
  651  TCCACAAATA CCCACTTCAG GCCAGCAATG CAGAGGCAGA GGCTGACTAG
  701  GCAAGCCAGA ATGGGAACAA TGCAGATCTT CTCGGAGTTG AGACACCCTC
  751  TCAGGCGCTC AGCTTCCAGG CACACACAGC AGGGCACCGC CAGGCCCAGG
  801  AGTCCAGGGC TTCTCTCATC TTCTGTGTGG GTGTCTGGTA TATGTTCCTC
  851  CGCTGCCGGA AGCCCATCAA GAGATGGGGC TGCACTCAGC TGAGTGGAGG
  901  GGCTGGAGGA CCTCCCGCCA GCTACCTCAG ACATGTCTGG GGAATAAATC
  951  TCCATCGCCC CACCCTCGAG AAGGGCGAAT TCGAATCCGA GACGTACGAC
 1001  CGGTTAACAG CCTTAAGCGC TAGCTAGGCC GCAGATATCG AATTCCTGCA
 1051  GCCCGGGGAT CGCCCGGGCT AGAGCGGCCG CCACCGCGTG AGCTCAGCTT
 1101  TGTTCCCTTT ATGAGATATT TCGAGCTGGC GTATCGCTAG CGGATCTGAC
 1151  GTCCTAACAG CTCTGCTTAT ATAGACCTCC CACCGGTAAC GCCTACGCCA
 1201  TTGCGCTCAT TGGGGCGGAG TTACGACTTG GAAAGGTCGT GATTTGGGGC
 1251  AAAAAACCTC CATGTGAGCC CTAAATG
```

Seq. length: 1277 Position: 1



Name: rat NRG1-III-beta 3

Long name: teorica

Description:

Length: 1798 Type: DNA checksu

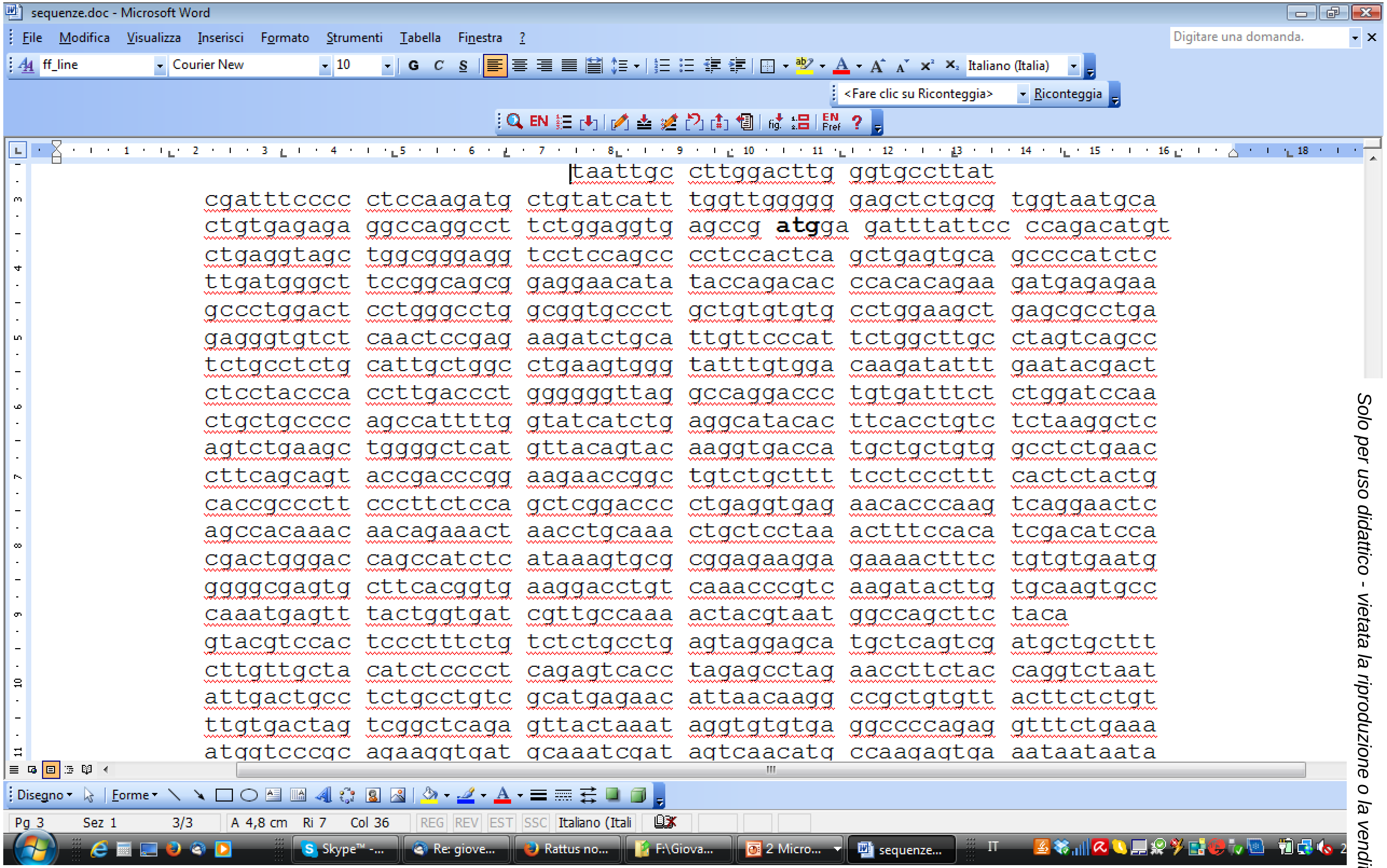
```
  1   gaattcggca cgagg
 51   tgattcagcc tcttt
101   actgtcgctg ctgcc
151   ctctggtctt gcttt
201   cctctaagca gacac
251   cagtttggga tactg
301   atttggtcc  cctgc
351   ttgaaagaac tgaaa
401   tacacttcta tttca
451   ttctttttct ttttc
501   ctggtacttt gggta
551   ctccaagatg ctgta
601   ctgtgagaga ggcca
651   ccagacatgt ctgag
701   gctgagtgca gcccc
```

Seq. length: 1798 Position: 1

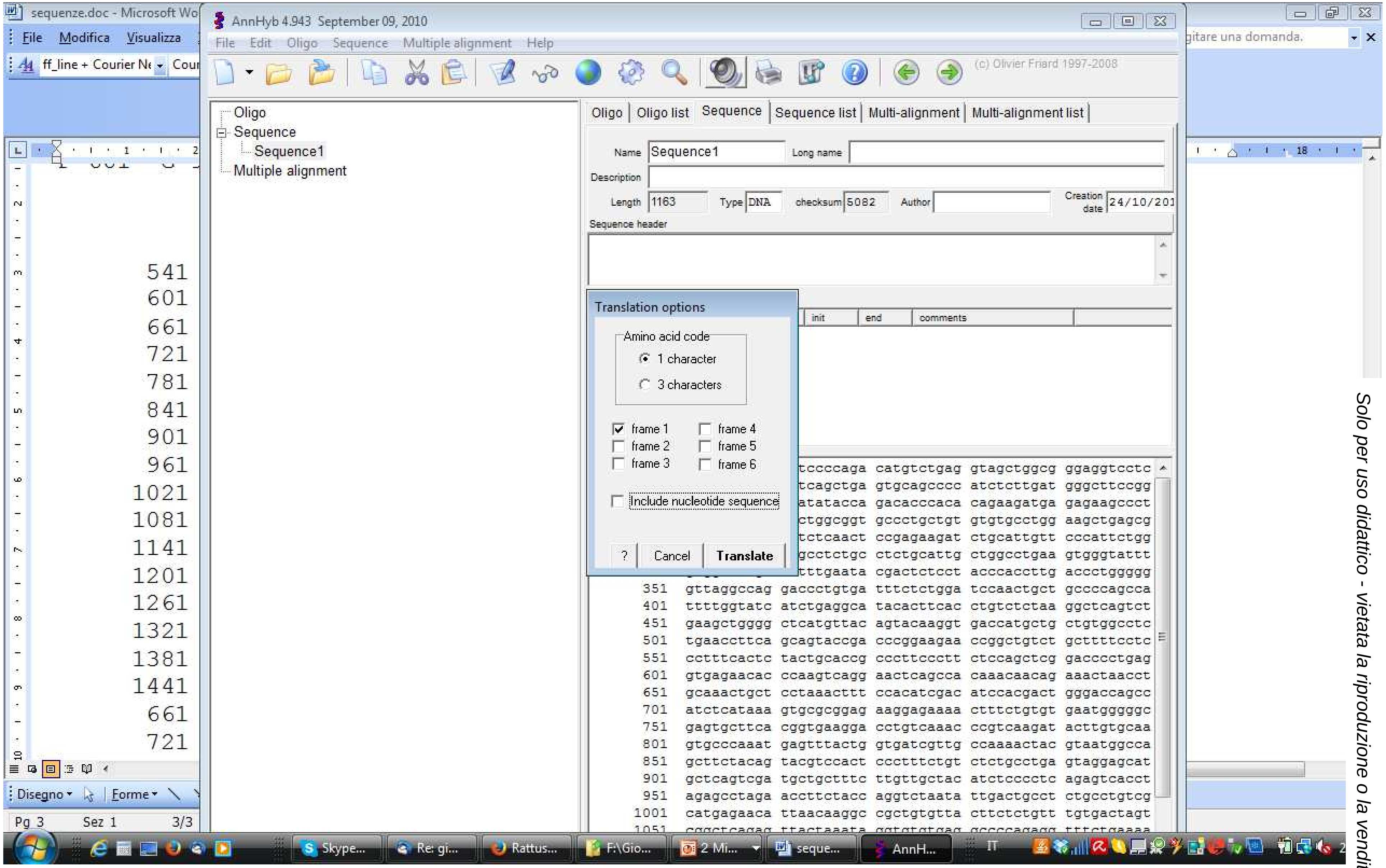
Translation of: rat NRG1-III-beta 3 (December 03, 2007 23:16)

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

Selection: 1 > 1798
Length: 1798

I  R  H  E  A  M  L  R  G  Q  A  P  A  A  L  *  *  F  S  L
F  Q  P  L  R  *  H  D  R  M  L  L  L  L  S  L  L  P  L  L
P  P  P  L  L  P  P  P  P  P  L  V  L  L  L  L  L  L  L  L
H  D  S  C  F  L  P  L  S  R  H  Q  L  Q  T  L  E  V  R  N
M  P  F  S  L  G  Y  W  F  T  *  S  A  R  R  Q  L  A  S  Y
F  G  P  L  P  S  *  P  T  R  H  G  L  E  K  H  L  K  E  L
K  K  C  P  R  N  N  S  S  R  Y  F  G  T  L  L  F  H  S  C
*  K  P  F  L  F  R  F  F  F  L  F  L  F  L  F  L  F  P  F
P  A  S  S  *  A  L  V  L  W  V  I  A  L  D  L  G  A  L  S
I  S  P  S  K  M  L  Y  H  L  V  G  G  S  S  A  W  *  C  T
V  R  E  A  R  P  S  G  G  E  P  M  E  I  Y  S  P  D  M  S
E  V  A  G  G  R  S  S  S  P  S  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  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  C  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
P  L  P  V  A  *  E  H  *  Q  G  R  C  V  T  S  L  F  V  T
```

```
S  H  K  Q  Q  K  L  T  C  K  L  L  L  N  F  P  H  R  H  P
```



taattgc cttggacttg ggtgccttat
cgatttcccc ctccaagatg ctgtatcatt tggttggggg gagctctgcg tggtaatgca
ctgtgagaga ggccaggcct tctggaggtg agccg **atg**ga gatttattcc ccagacatgt
ctgaggtagc tggcgggagg tcctccagcc cctccactca gctgagtgca gccccatctc
ttgatgggct tccggcagcg gaggaacata taccagacac ccacacagaa gatgagagaa
gccctggact cctgggcctg gcggtgccct gctgtgtgtg cctggaagct gagcgcctga
gagggtgtct caactccgag aagatctgca ttgttcccat tctggcttgc ctagtcagcc
tctgcctctg cattgctggc ctgaagtggg tatttgtgga caagatattt gaatacgact
ctcctaccca ccttgaccct ggggggttag gccaggaccc tgtgatttct ctggatccaa
ctgctgcccc agccattttg gtatcatctg aggcatacac ttcacctgtc tctaaggctc
agtctgaagc tggggctcat gttacagtac aaggtgacca tgctgctgtg gcctctgaac
cttcagcagt accgacccgg aagaaccggc tgtctgcttt tcctcccttt cactctactg
caccgccctt cccttctcca gctcggaccc ctgaggtgag aacacccaag tcaggaactc
agccacaaac aacagaaact aacctgcaaa ctgctcctaa actttccaca tcgacatcca
cgactgggac cagccatctc ataaagtgcg cggagaagga gaaaactttc tgtgtgaatg
ggggcgagtg cttcacggtg aaggacctgt caaacccgtc aagatacttg tgcaagtgcc
caaatgagtt tactggtgat cgttgccaaa actacgtaat ggccagcttc taca
gtacgtccac tccctttctg tctctgcctg agtaggagca tgctcagtcg atgctgcttt
cttgttgcta catctcccct cagagtcacc tagagcctag aaccttctac caggtctaat
attgactgcc tctgcctgtc gcatgagaac attaacaagg ccgctgtgtt acttctctgt
ttgtgactag tcggctcaga gttactaaat aggtgtgtga ggccccagag gtttctgaaa
atggtcccgc agaaggtgat gcaaatcgat agtcaacatg ccaagagtga aataataata



Name: Sequence1
Long name:
Description:
Length: 1163
Type: DNA
checksum: 5082
Author:
Creation date: 24/10/201

Translation options

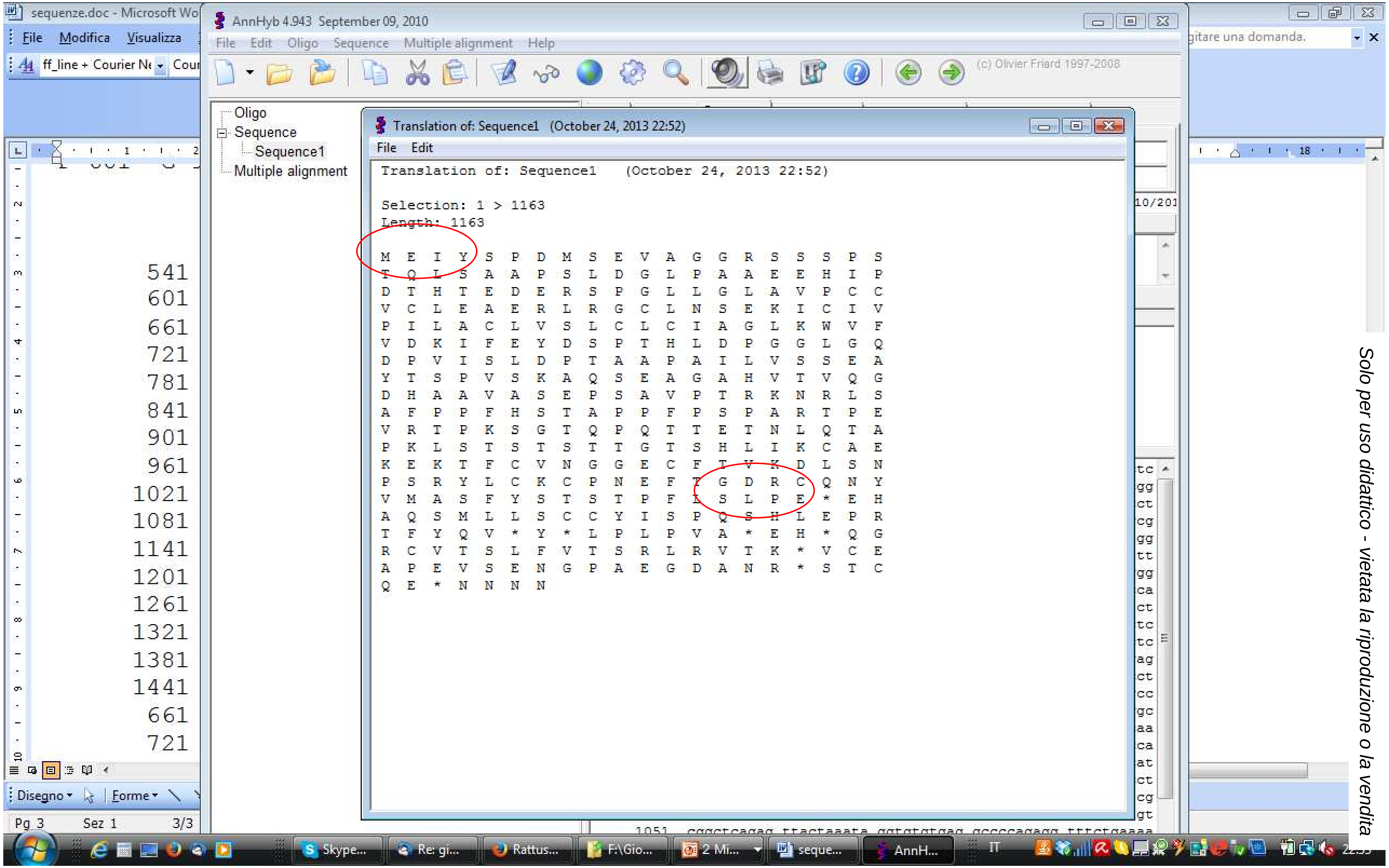
Amino acid code
● 1 character
○ 3 characters

☑ frame 1
☐ frame 2
☐ frame 3
☐ frame 4
☐ frame 5
☐ frame 6

☐ Include nucleotide sequence

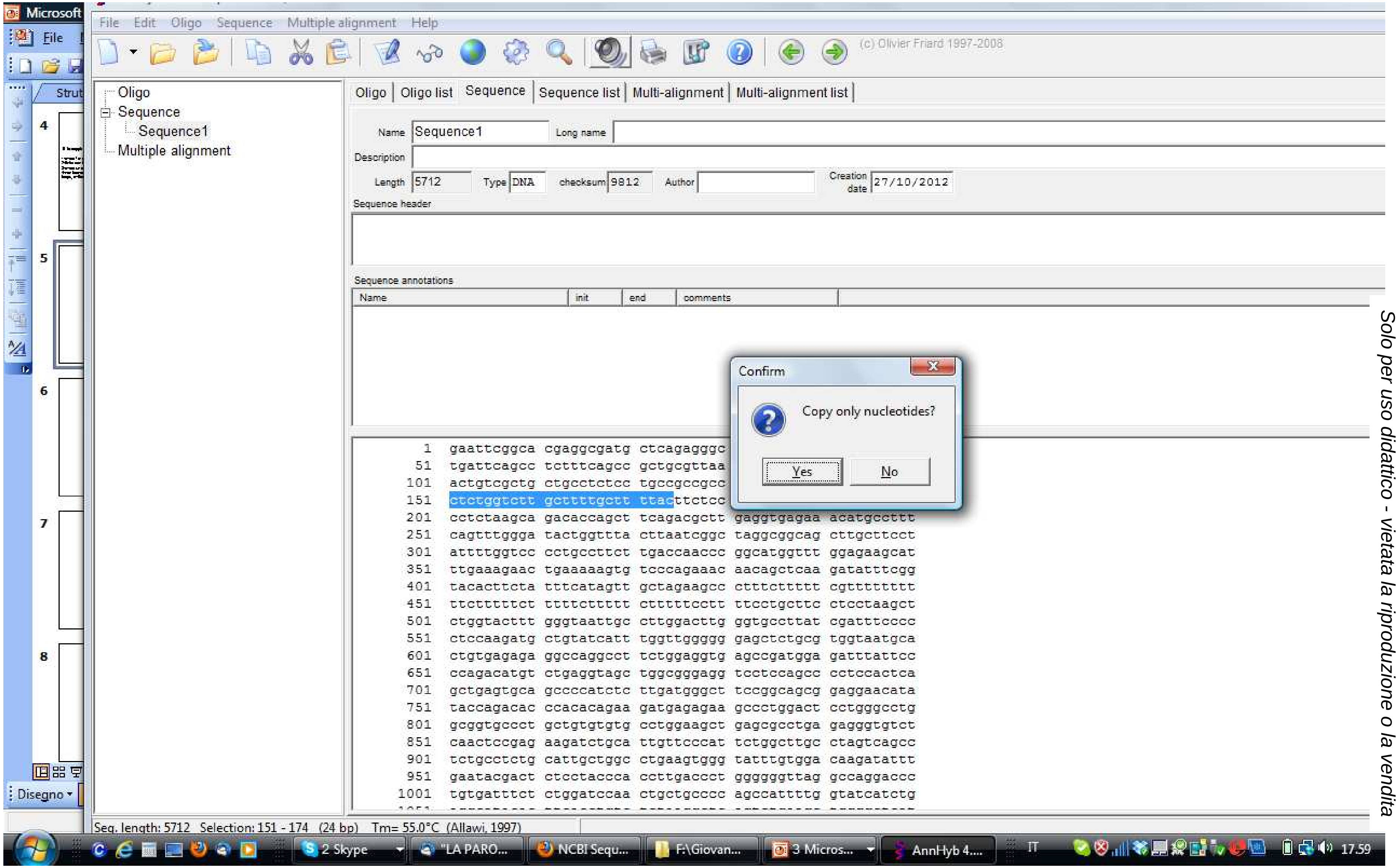
```
541
601
661
721
781
841
901
961
1021
1081
1141
1201
1261
1321
1381
1441
661
721
```

```
             tccccaga catgtctgag gtagctggcg ggaggtcctc
             tcagctga gtgcagcccc atctcttgat gggcttccgg
             atatacca gacacccaca cagaagatga gagaagccct
             ctggcggt gccctgctgt gtgtgcctgg aagctgagcg
             tctcaact ccgagaagat ctgcattgtt cccattctgg
             gcctctgc ctctgcattg ctggcctgaa gtgggtattt
             tttgaata 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  gtgcccaaat gagtttactg gtgatcgttg ccaaaactac gtaatggcca
 851  gcttctacag tacgtccact ccctttctgt ctctgcctga gtaggagcat
 901  gctcagtcga tgctgctttc ttgttgctac atctcccctc agagtcacct
 951  agagcctaga accttctacc aggtctaata ttgactgcct ctgcctgtcg
1001  catgagaaca ttaacaaggc cgctgtgtta cttctctgtt tgtgactagt
```



```
Translation of: Sequence1   (October 24, 2013 22:52)

Selection: 1 > 1163
Length: 1163

M  E  I  Y  S  P  D  M  S  E  V  A  G  G  R  S  S  S  P  S
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
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  C  Q  N  Y
V  M  A  S  F  Y  S  T  S  T  P  F  I  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  P  L  P  V  A  *  E  H  *  Q  G
R  C  V  T  S  L  F  V  T  S  R  L  R  V  T  K  *  V  C  E
A  P  E  V  S  E  N  G  P  A  E  G  D  A  N  R  *  S  T  C
Q  E  *  N  N  N  N
```

Come disegnare primers con Annhyb



Name Sequence1 Long name

Description

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

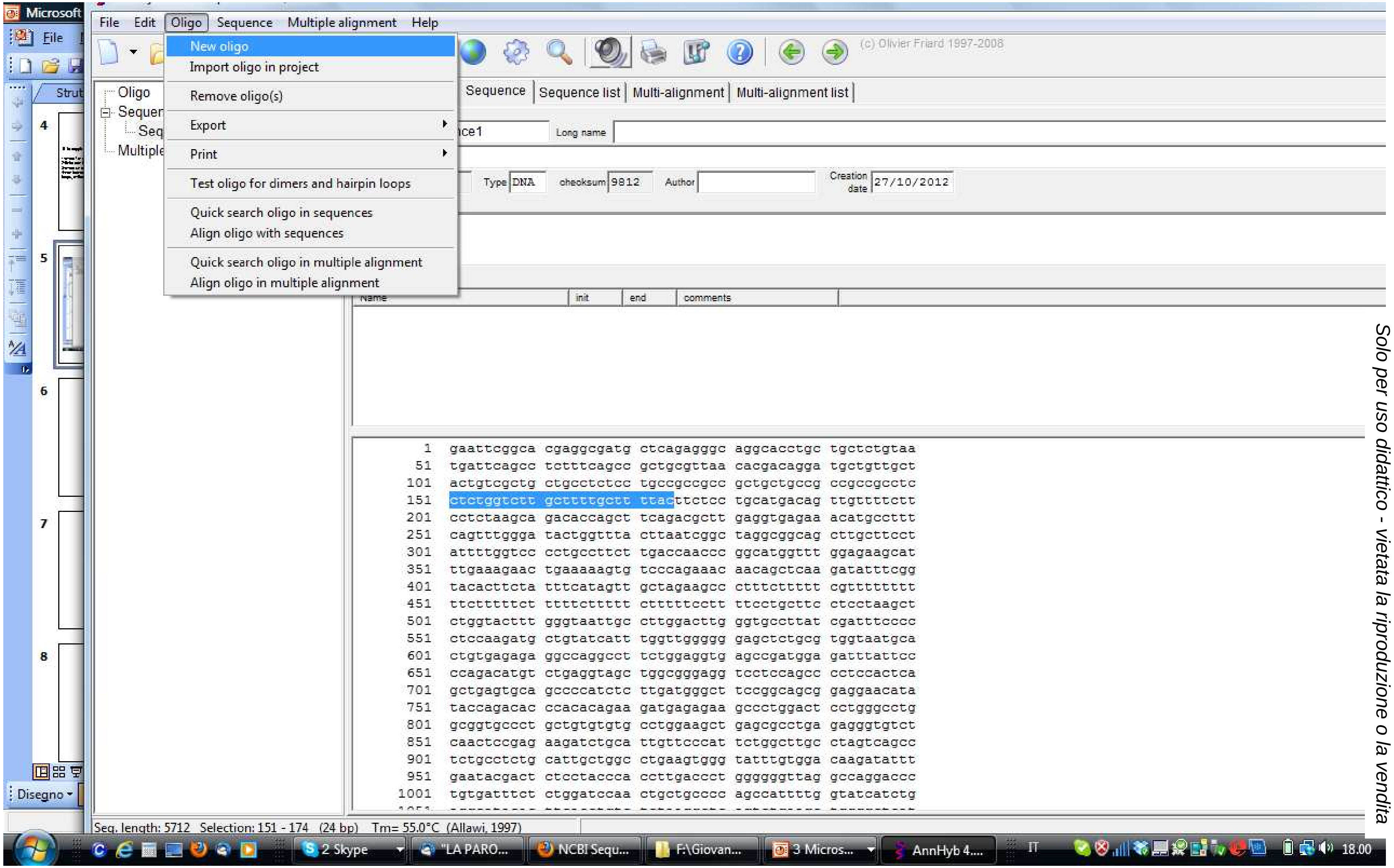
Confirm

Copy only nucleotides?

Yes No

```
   1  gaattcggca cgaggcgatg ctcagagggc
  51  tgattcagcc tctttcagcc gctgcgttaa
 101  actgtcgctg ctgcctctcc tgccgccgcc
 151  ctctggtctt gcttttgctt ttacttctcc
 201  cctctaagca gacaccagct tcagacgctt gaggtgagaa acatgccttt
 251  cagtttggga tactggttta cttaatcggc taggcggcag cttgcttcct
 301  attttggtcc cctgccttct tgaccaaccc ggcatggttt ggagaagcat
 351  ttgaaagaac tgaaaaagtg tcccagaaac aacagctcaa gatatttcgg
 401  tacacttcta tttcatagtt gctagaagcc ctttcttttt cgtttttttt
 451  ttcttttttct ttttcttttt ctttttcctt ttcctgcttc ctcctaagct
 501  ctggtacttt gggtaattgc cttggacttg ggtgccttat cgatttcccc
 551  ctccaagatg ctgtatcatt tggttggggg gagctctgcg tggtaatgca
 601  ctgtgagaga ggccaggcct tctggaggtg agccgatgga gatttattcc
 651  ccagacatgt ctgaggtagc tggcgggagg tcctccagcc cctccactca
 701  gctgagtgca gccccatctc ttgatgggct tccggcagcg gaggaacata
 751  taccagacac ccacacagaa gatgagagaa gccctggact cctgggcctg
 801  gcggtgccct gctgtgtgtg cctggaagct gagcgcctga gagggtgtct
 851  caactccgag aagatctgca ttgttcccat tctggcttgc ctagtcagcc
 901  tctgcctctg cattgctggc ctgaagtggg tatttgtgga caagatattt
 951  gaatacgact ctcctaccca ccttgaccct ggggggttag gccaggaccc
1001  tgtgatttct ctggatccaa ctgctgcccc agccattttg gtatcatctg
```

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

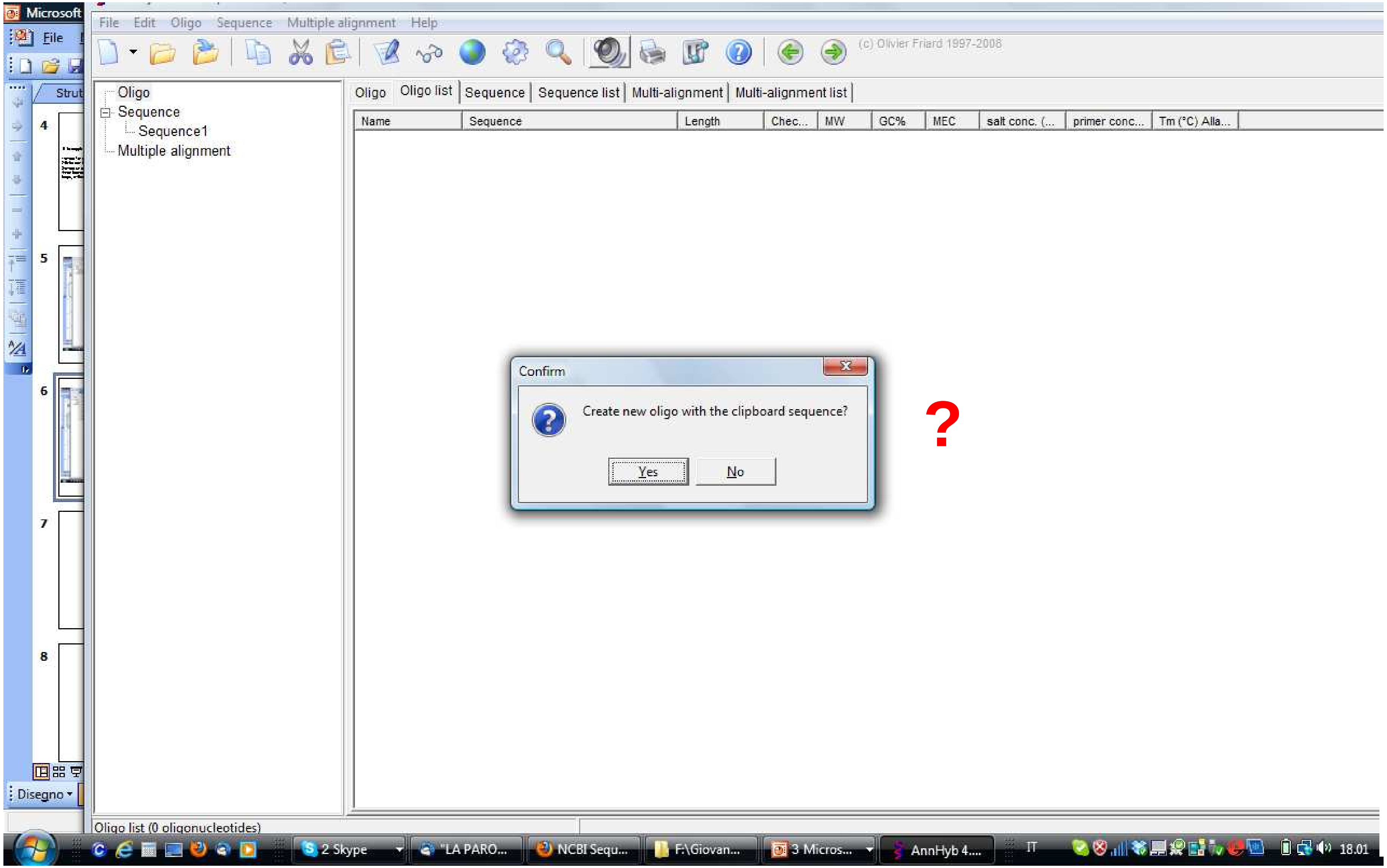


Type DNA checksum 9812 Author

Creation date 27/10/2012

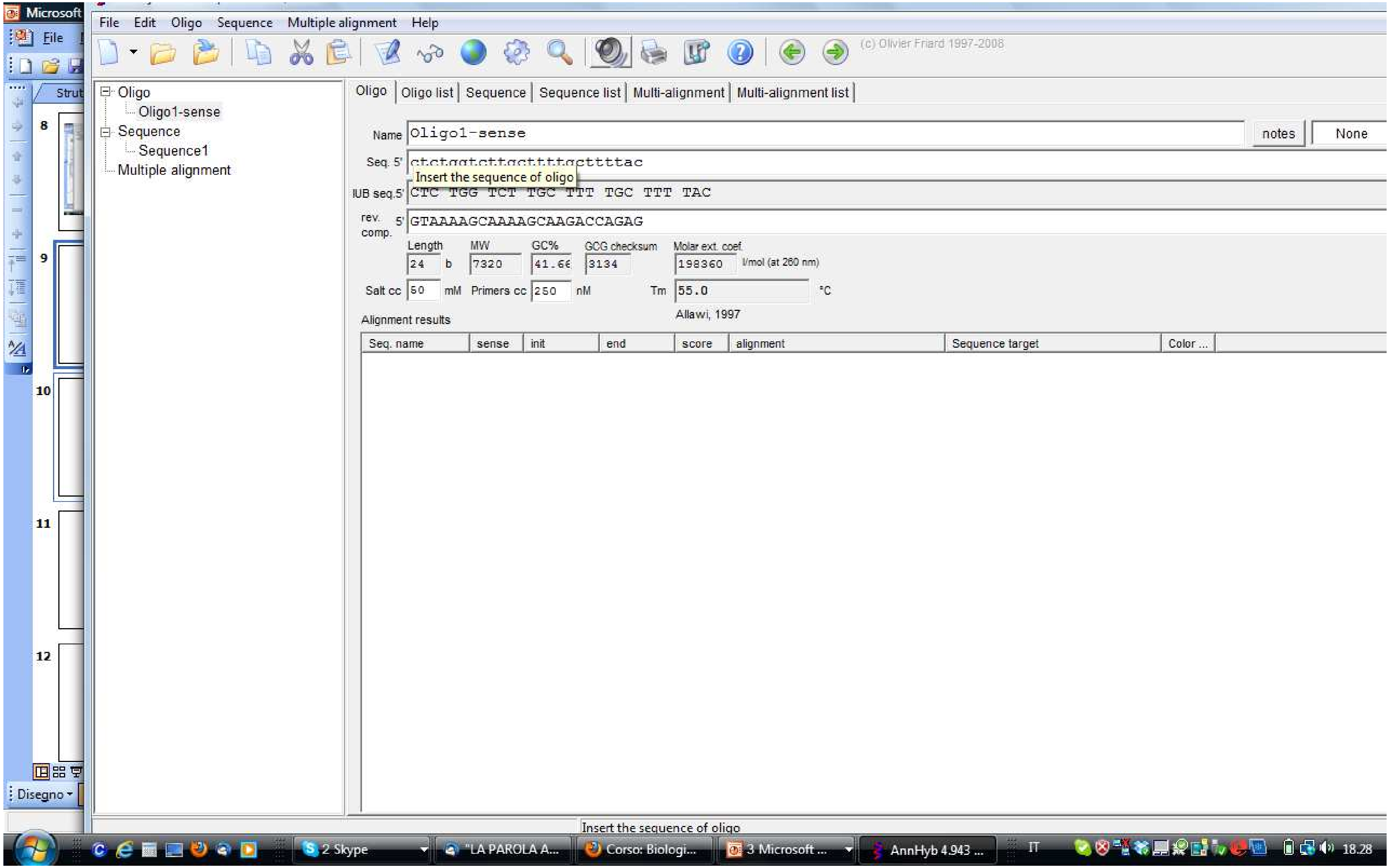
```
   1  gaattcggca cgaggcgatg ctcagagggc aggcacctgc tgctctgtaa
  51  tgattcagcc tctttcagcc gctgcgttaa cacgacagga tgctgttgct
 101  actgtcgctg ctgcctctcc tgccgccgcc gctgctgccg ccgccgcctc
 151  ctctggtctt gcttttgctt ttacttctcc tgcatgacag ttgttttctt
 201  cctctaagca gacaccagct tcagacgctt gaggtgagaa acatgccttt
 251  cagtttggga tactggttta cttaatcggc taggcggcag cttgcttcct
 301  attttggtcc cctgccttct tgaccaaccc ggcatggttt ggagaagcat
 351  ttgaaagaac tgaaaaagtg tcccagaaac aacagctcaa gatatttcgg
 401  tacacttcta tttcatagtt gctagaagcc ctttcttttt cgtttttttt
 451  ttcttttctt ttttcttttt ctttttcctt ttcctgcttc ctcctaagct
 501  ctggtacttt gggtaattgc cttggacttg ggtgccttat cgatttcccc
 551  ctccaagatg ctgtatcatt tggttggggg gagctctgcg tggtaatgca
 601  ctgtgagaga ggccaggcct tctggaggtg agccgatgga gatttattcc
 651  ccagacatgt ctgaggtagc tggcgggagg tcctccagcc cctccactca
 701  gctgagtgca gccccatctc ttgatgggct tccggcagcg gaggaacata
 751  taccagacac ccacacagaa gatgagagaa gccctggact cctgggcctg
 801  gcggtgccct gctgtgtgtg cctggaagct gagcgcctga gagggtgtct
 851  caactccgag aagatctgca ttgttcccat tctggcttgc ctagtcagcc
 901  tctgcctctg cattgctggc ctgaagtggg tatttgtgga caagatattt
 951  gaatacgact ctcctaccca ccttgaccct gggggttag gccaggaccc
1001  tgtgatttct ctggatccaa ctgctgcccc agccattttg gtatcatctg
```

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



Create new oligo with the clipboard sequence?

Oligo list (0 oligonucleotides)



Name Oligo1-sense

Seq. 5' ctctggtcttgcttttgcttttac

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

rev. comp. 5' GTAAAAGCAAAAGCAAGACCAGAG

Length 24 b

MW 7320

GC% 41.66

GCG checksum 3134

Molar ext. coef. 198360 l/mol (at 260 nm)

Salt cc 50 mM

Primers cc 250 nM

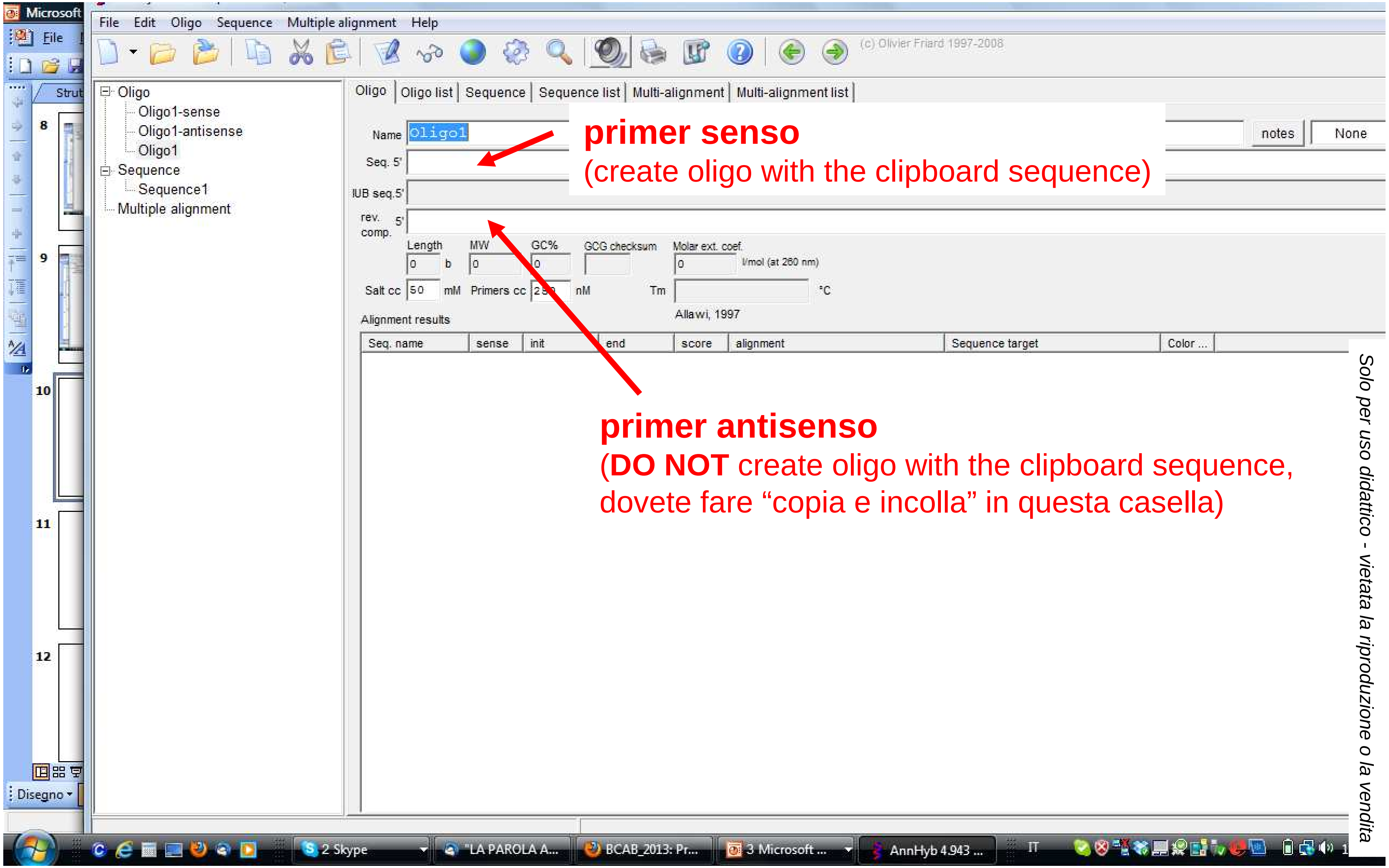
Tm 55.0 °C

Allawi, 1997

Alignment results

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

Insert the sequence of oligo



Name: Oligo1

Length: 0 b

MW: 0

GC%: 0

Molar ext. coef.: 0 l/mol (at 260 nm)

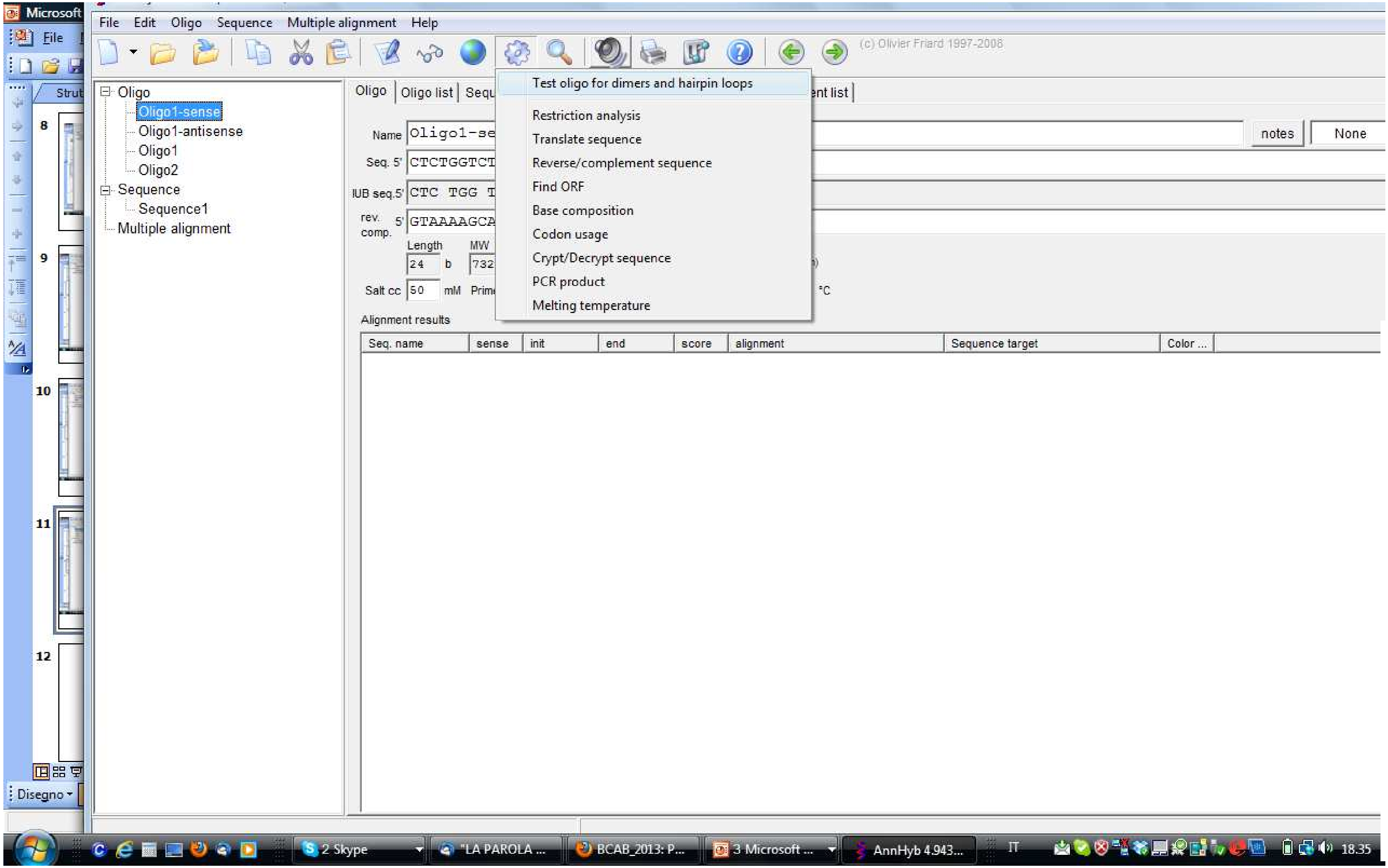
Salt cc: 50 mM

Primers cc: 250 nM

Allawi, 1997

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)



Name Oligo1-se

Seq. 5' CTCTGGTCT

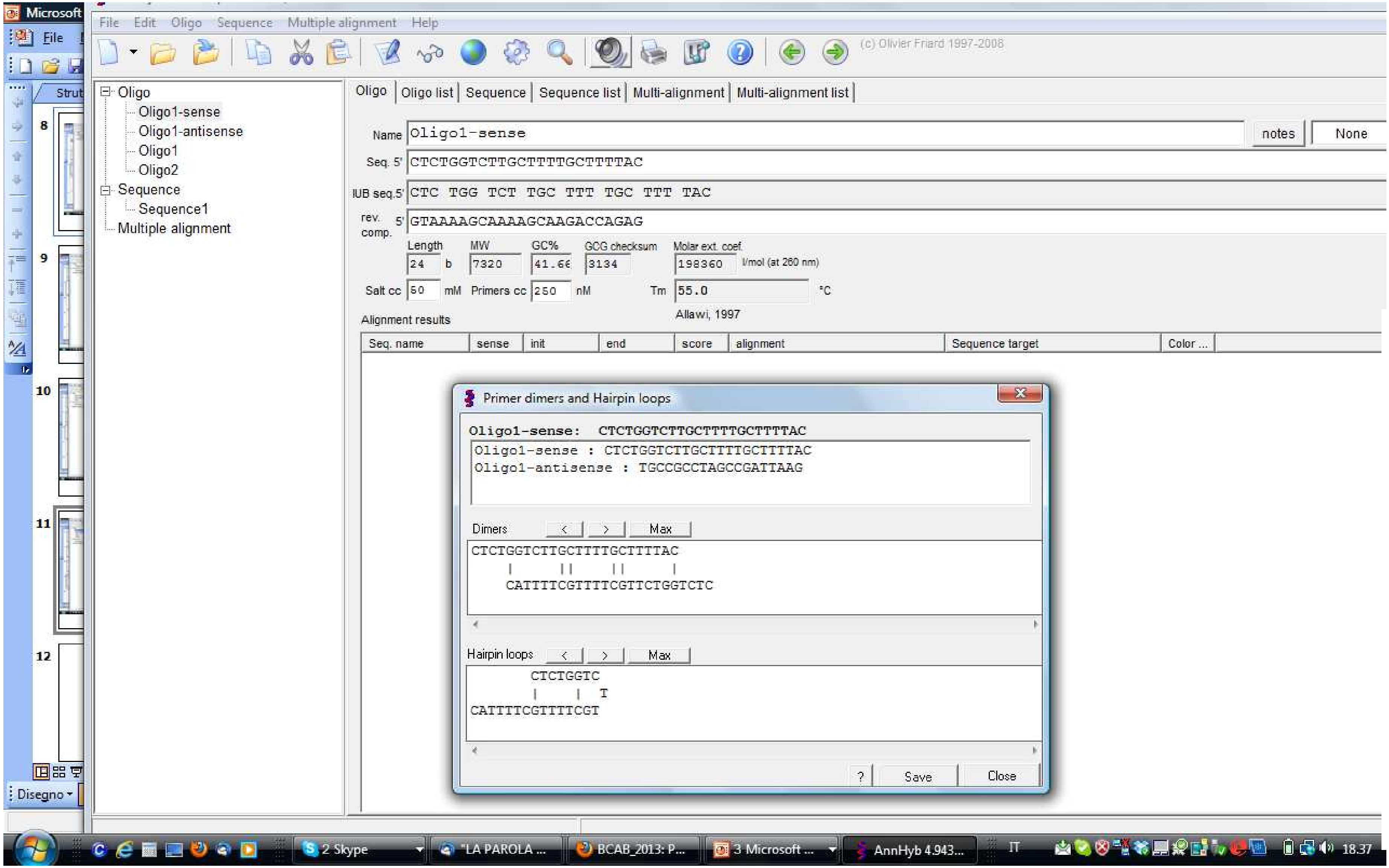
IUB seq.5' CTC TGG T

rev. comp. 5' GTAAAAGCA

Length 24 b

MW 732

Salt cc 50 mM



Name: Oligo1-sense
None

Seq. 5' CTCTGGTCTTGCTTTTGCTTTTAC

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

rev. comp. 5' GTAAAAGCAAAAGCAAGACCAGAG

Length: 24 b

MW: 7320

GC%: 41.66

GCG checksum: 3134

Molar ext. coef: 198360 l/mol (at 260 nm)

Salt cc: 50 mM

Primers cc: 250 nM

Tm: 55.0 °C

Allawi, 1997

Alignment results

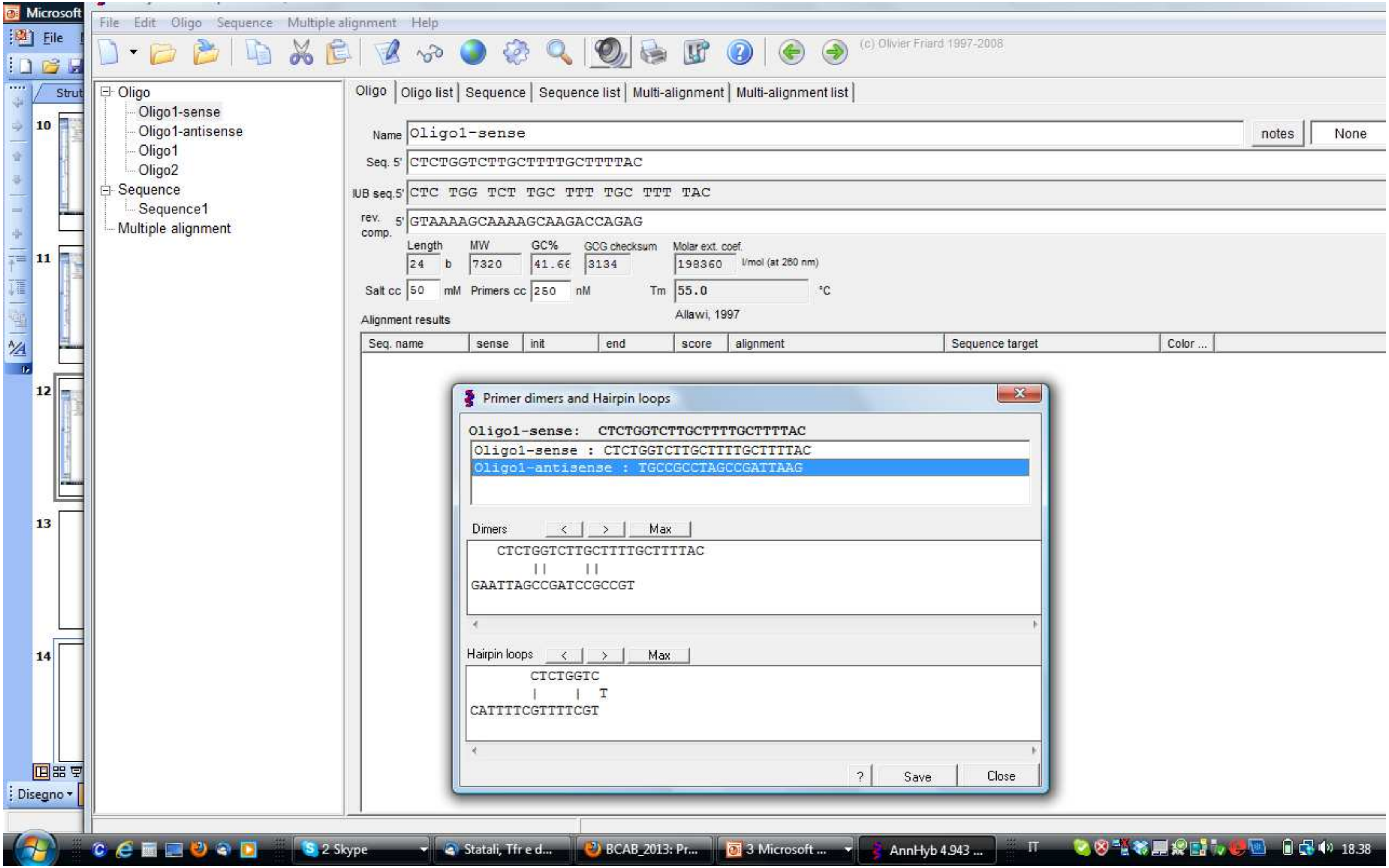
```
Oligo1-sense:  CTCTGGTCTTGCTTTTGCTTTTAC
Oligo1-sense : CTCTGGTCTTGCTTTTGCTTTTAC
Oligo1-antisense : TGCCGCCTAGCCGATTAAG
```

Dimers

```
CTCTGGTCTTGCTTTTGCTTTTAC
     |      ||     ||       |
    CATTTTCGTTTTCGTTCTGGTCTC
```

Hairpin loops

```
        CTCTGGTC
        |    |  T
CATTTTCGTTTTCGT
```



Name: Oligo1-sense

Seq. 5': CTCTGGTCTTGCTTTTGCTTTTAC

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

rev. comp. 5': GTAAAAGCAAAAGCAAGACCAGAG

Length	MW	GC%	GCG checksum	Molar ext. coef.
24 b	7320	41.66	3134	198360 l/mol (at 260 nm)

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

Allawi, 1997

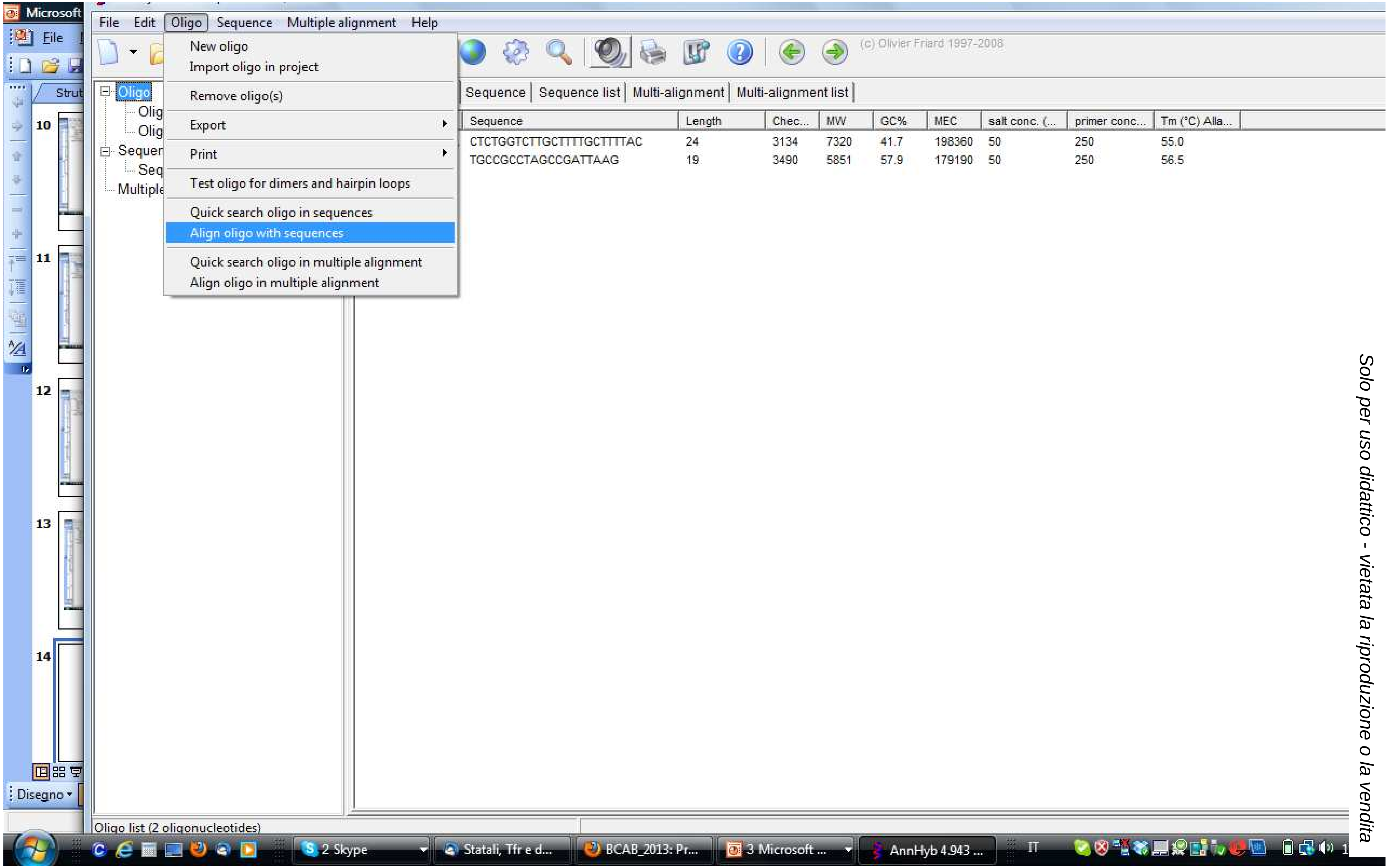
```
Oligo1-sense:   CTCTGGTCTTGCTTTTGCTTTTAC
Oligo1-sense : CTCTGGTCTTGCTTTTGCTTTTAC
Oligo1-antisense : TGCCGCCTAGCCGATTAAG
```

Dimers

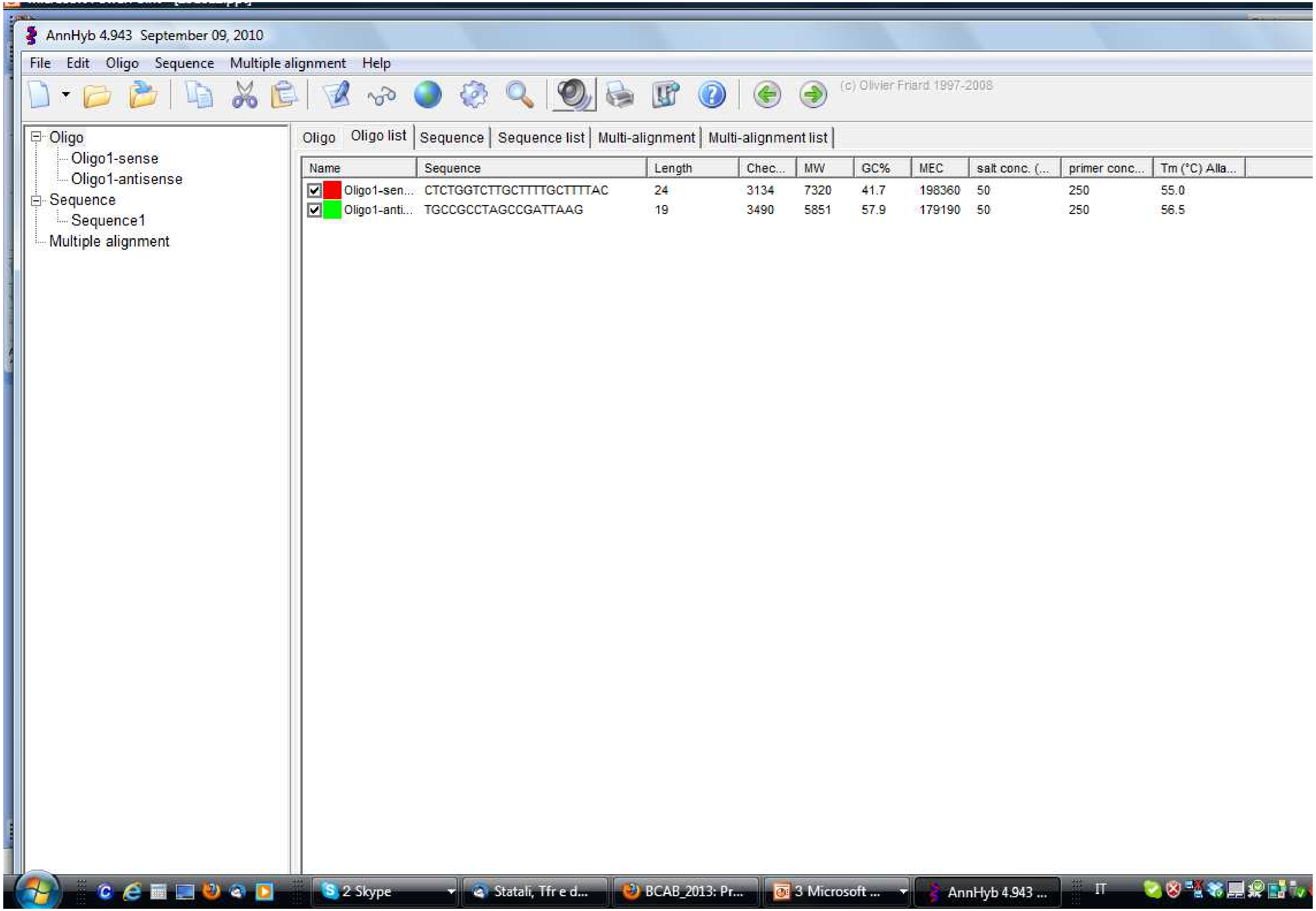
```
   CTCTGGTCTTGCTTTTGCTTTTAC
        ||    ||
GAATTAGCCGATCCGCCGT
```

Hairpin loops

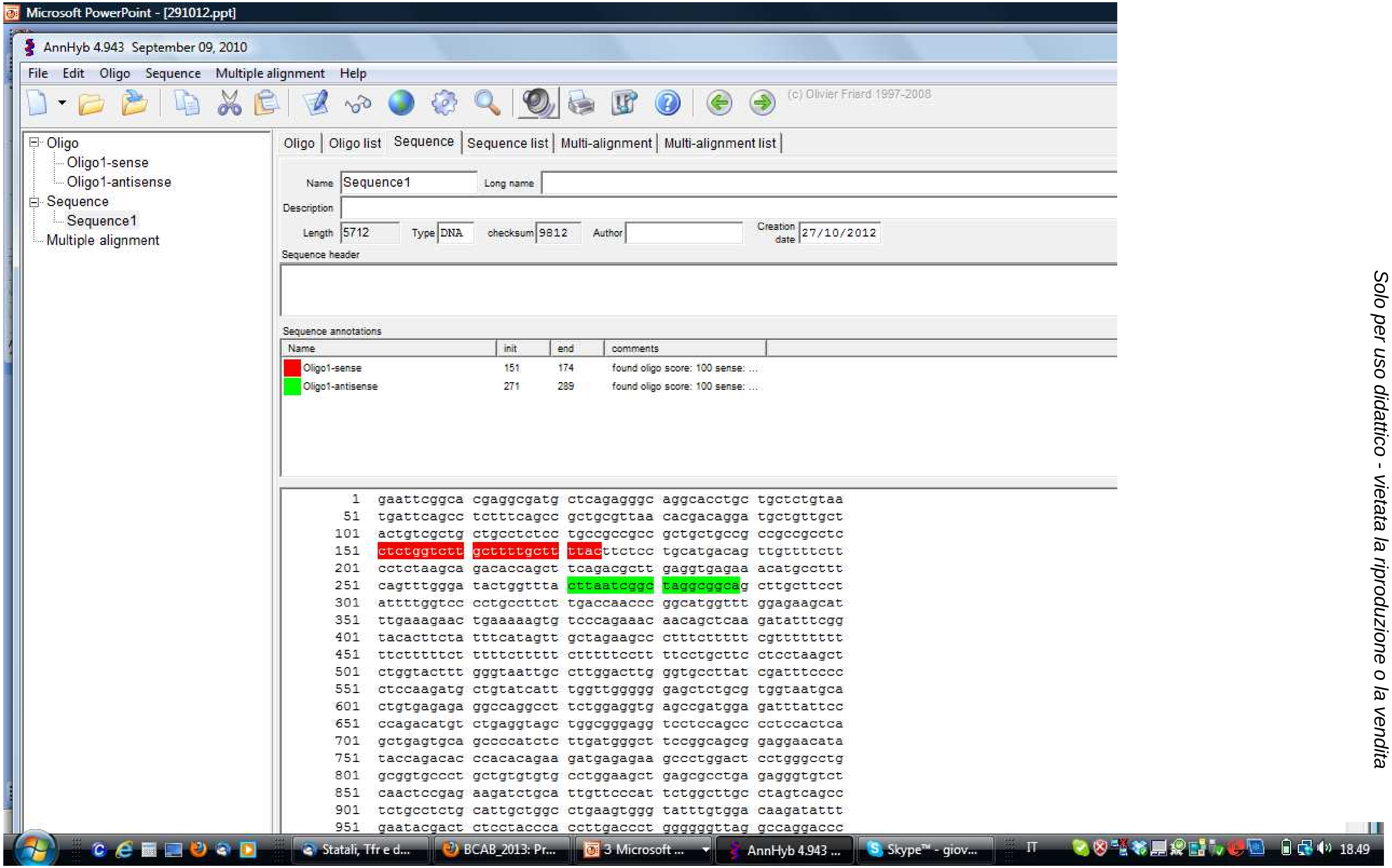
```
       CTCTGGTC
       |    |  T
CATTTTCGTTTTCGT
```



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



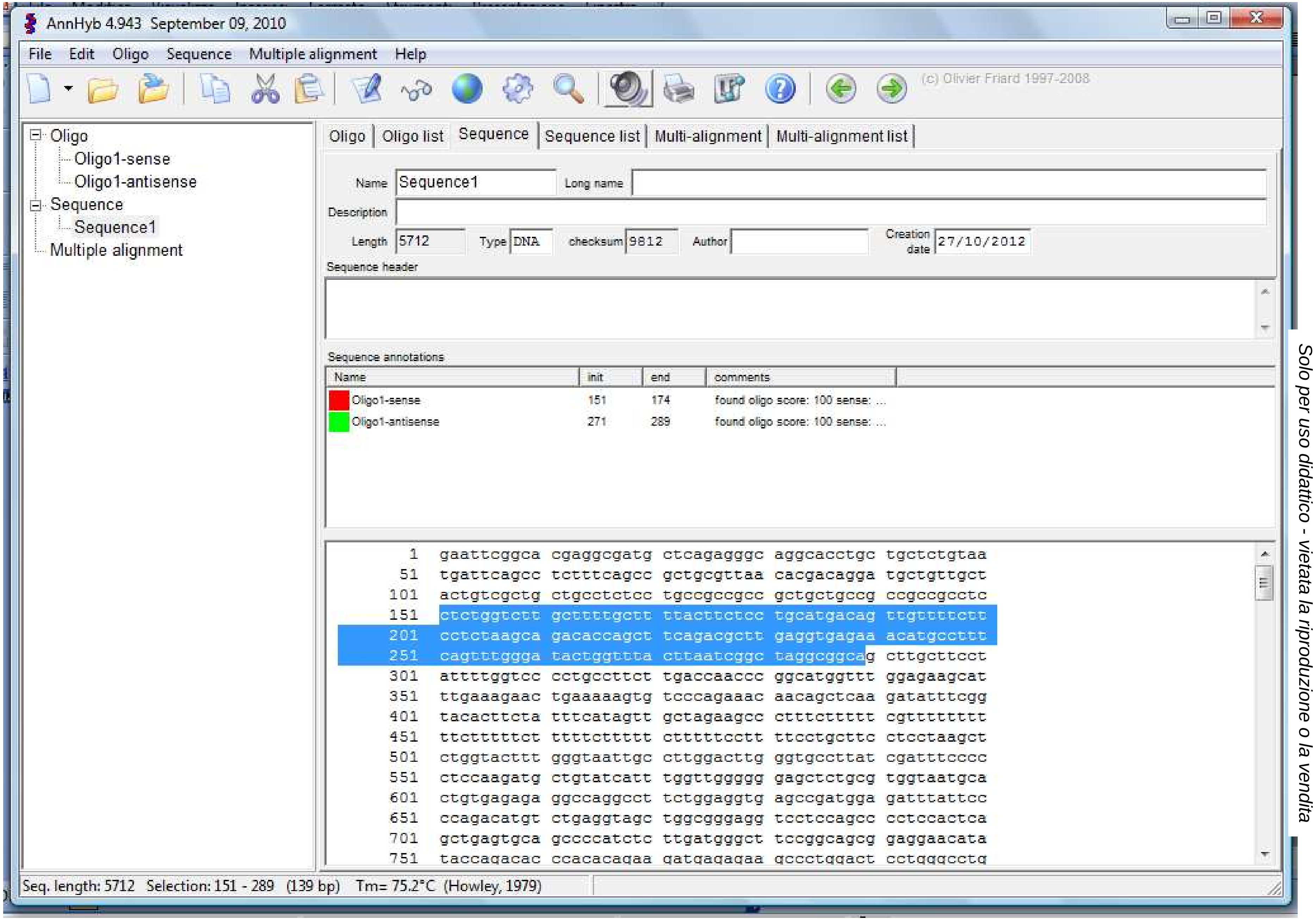
		Name	Sequence	Length	Chec...	MW	GC%	MEC	salt conc. (...	primer conc...	Tm (°C) Alla...
☑	🟥	Oligo1-sen...	CTCTGGTCTTGCTTTTGCTTTTAC	24	3134	7320	41.7	198360	50	250	55.0
☑	🟩	Oligo1-anti...	TGCCGCCTAGCCGATTAAG	19	3490	5851	57.9	179190	50	250	56.5



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

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 tctttcagcc gctgcgttaa cacgacagga tgctgttgct
101  actgtcgctg ctgcctctcc tgccgccgcc gctgctgccg ccgccgcctc
151  ctctggtctt gcttttgctt ttacttctcc tgcatgacag ttgttttctt
201  cctctaagca gacaccagct tcagacgctt gaggtgagaa acatgccttt
251  cagtttggga tactggttta cttaatcggc taggcggcag cttgcttcct
301  attttggtcc cctgccttct tgaccaaccc ggcatggttt ggagaagcat
351  ttgaaagaac tgaaaaagtg tcccagaaac aacagctcaa gatatttcgg
401  tacacttcta tttcatagtt gctagaagcc ctttcttttt cgtttttttt
451  ttctttttct ttttcttttt ctttttcctt ttcctgcttc ctcctaagct
501  ctggtacttt gggtaattgc cttggacttg ggtgccttat cgatttcccc
551  ctccaagatg ctgtatcatt tggttggggg gagctctgcg tggtaatgca
601  ctgtgagaga ggccaggcct tctggaggtg agccgatgga gatttattcc
651  ccagacatgt ctgaggtagc tggcgggagg tcctccagcc cctccactca
701  gctgagtgca gccccatctc ttgatgggct tccggcagcg gaggaacata
751  taccagacac ccacacagaa gatgagagaa gccctggact cctgggcctg
801  gcggtgccct gctgtgtgtg cctggaagct gagcgcctga gagggtgtct
851  caactccgag aagatctgca ttgttcccat tctggcttgc ctagtcagcc
901  tctgcctctg cattgctggc ctgaagtggg tatttgtgga caagatattt
951  gaatacgact ctcctaccca ccttgaccct gggggttag gccaggaccc
```



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 ctcagagggc aggcacctgc tgctctgtaa
   51  tgattcagcc tctttcagcc gctgcgttaa cacgacagga tgctgttgct
  101  actgtcgctg ctgcctctcc tgccgccgcc gctgctgccg ccgccgcctc
  151  ctctggtctt gcttttgctt ttacttctcc tgcatgacag ttgttttctt
  201  cctctaagca gacaccagct tcagacgctt gaggtgagaa acatgccttt
  251  cagtttggga tactggttta cttaatcggc taggcggcag cttgcttcct
  301  attttggtcc cctgccttct tgaccaaccc ggcatggttt ggagaagcat
  351  ttgaaagaac tgaaaaagtg tcccagaaac aacagctcaa gatatttcgg
  401  tacacttcta tttcatagtt gctagaagcc ctttcttttt cgtttttttt
  451  ttcttttttct ttttcttttt ctttttcctt ttcctgcttc ctcctaagct
  501  ctggtacttt gggtaattgc cttggacttg ggtgccttat cgatttcccc
  551  ctccaagatg ctgtatcatt tggttggggg gagctctgcg tggtaatgca
  601  ctgtgagaga ggccaggcct tctggaggtg agccgatgga gatttattcc
  651  ccagacatgt ctgaggtagc tggcgggagg tcctccagcc cctccactca
  701  gctgagtgca gccccatctc ttgatgggct tccggcagcg gaggaacata
  751  taccagacac ccacacagaa gatgagagaa gccctggact cctgggcctg
```

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

PROGETTO DETTAGLIATO BIOTECNOLOGIE CELLULARI

1 - Studio delle sequenze presenti in banca dati riguardanti la NRG1-typeIII-beta 3
2 - Scelta dei primers per amplificare la NRG1-typeIII-beta 3 di ratto (tenendo conto del fatto che poi la cloneremo *in frame* in un vettore esprimente la GFP).
3 - RT-PCR
4 - Clonaggio di NRG1-typeIII-beta 3 in vettore pCR-bluntII-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-typeIII-beta3 in vettore virale adenoassociato pAAV-MCS
10 - Subclonaggio di NRG1-typeIII-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 antisenso

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1998 by The American Society for Biochemistry and Molecular Biology, Inc.

Vol. 273, No. 46, Issue of November 13, pp. 30643–30650, 1998
Printed in U.S.A.

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)

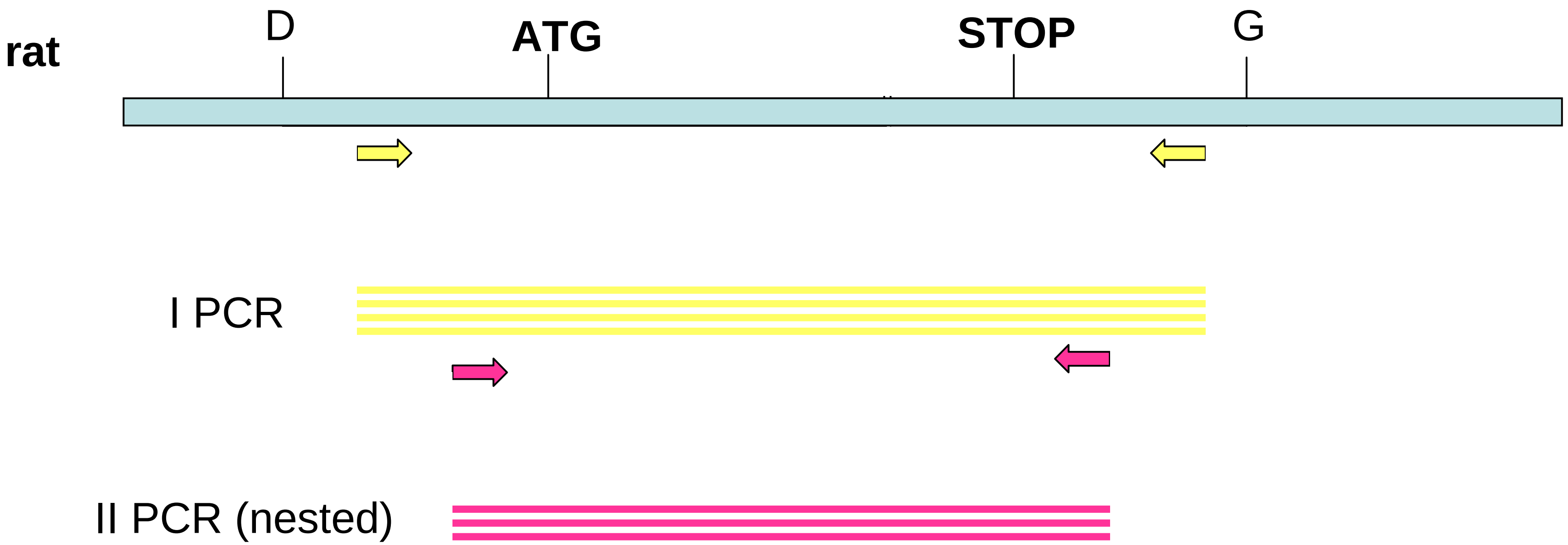
Allen Schroering and David J. Carey‡
From the Weis Center for Research, Penn State College of Medicine, Danville, Pennsylvania 17822-2613

TABLE I
Primers used for PCR amplification of human SMDF

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

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 *Eco*RI 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.



rat

D

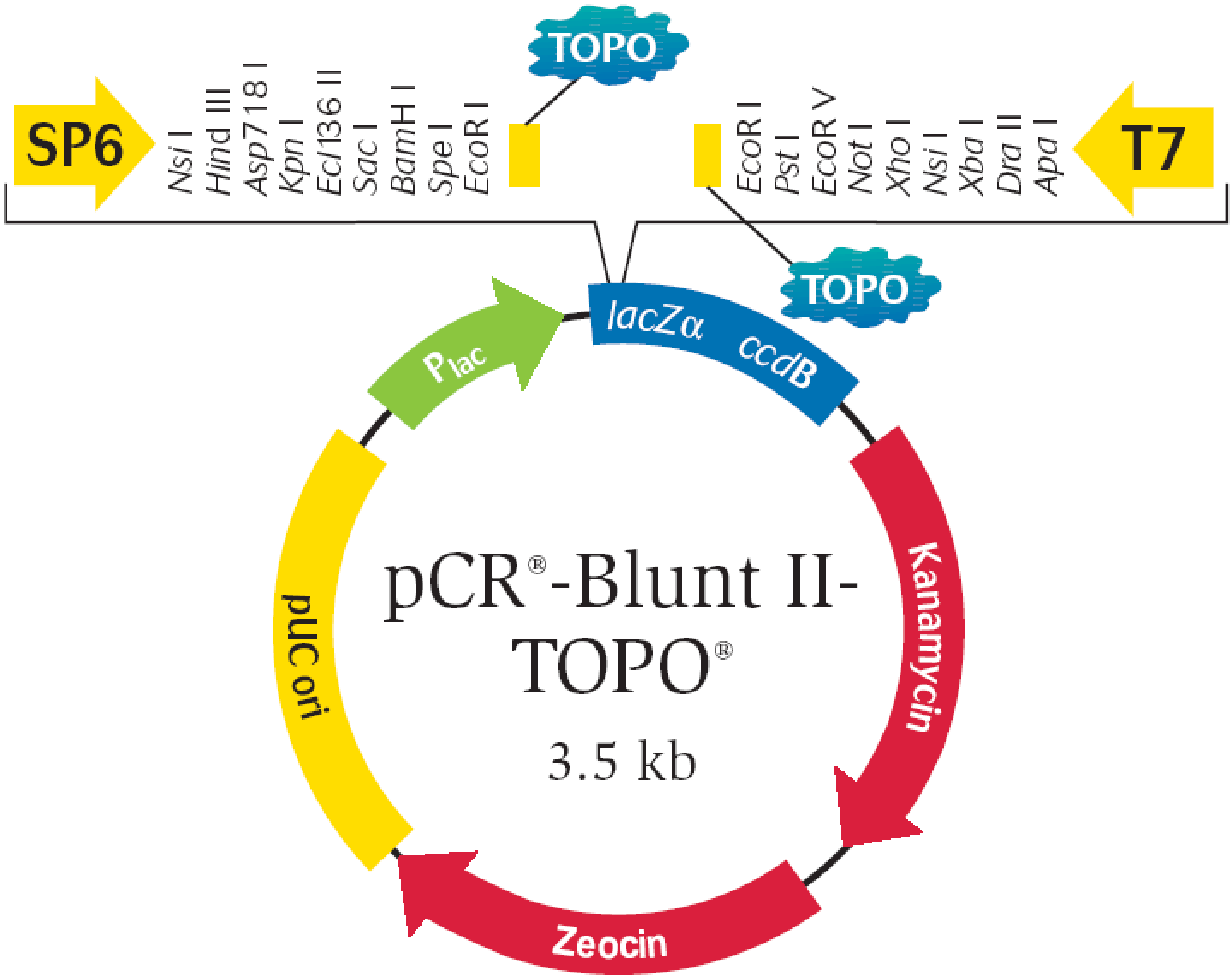
ATG

STOP

G

I PCR

II PCR (nested)



http://www.invitrogen.com/

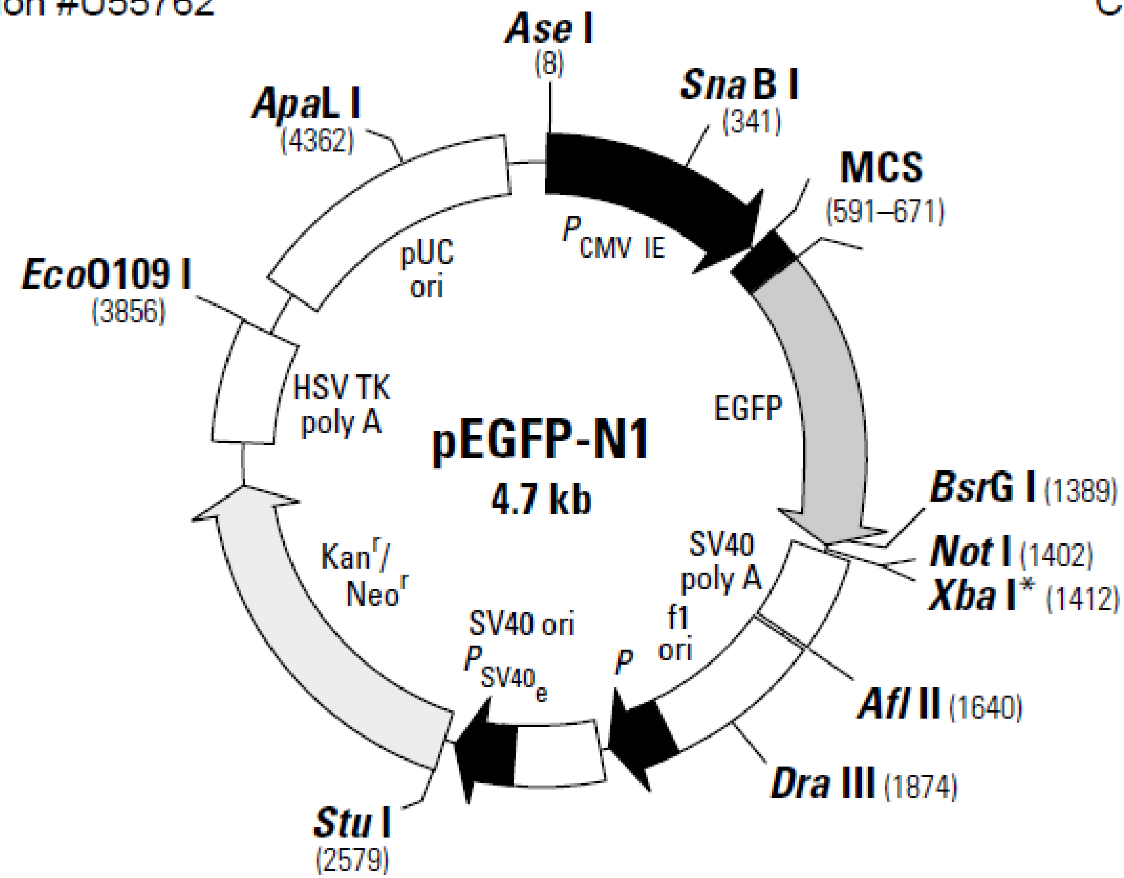
Vettore di clonaggio del prodotto di RT-PCR

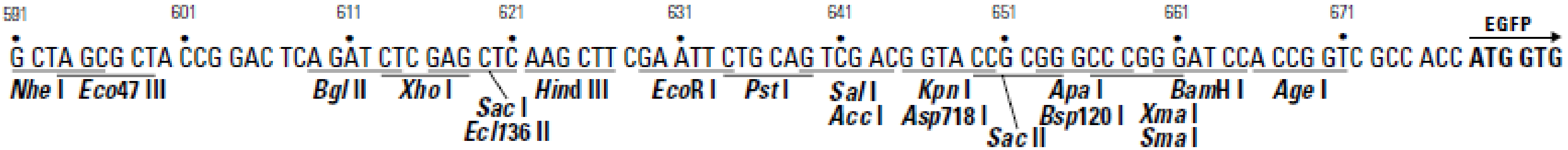
pEGFP-N1 Vector Information

PT3027-5

GenBank Accession #U55762

Catalog #6085-1





pEGFP-C3 Vector Information

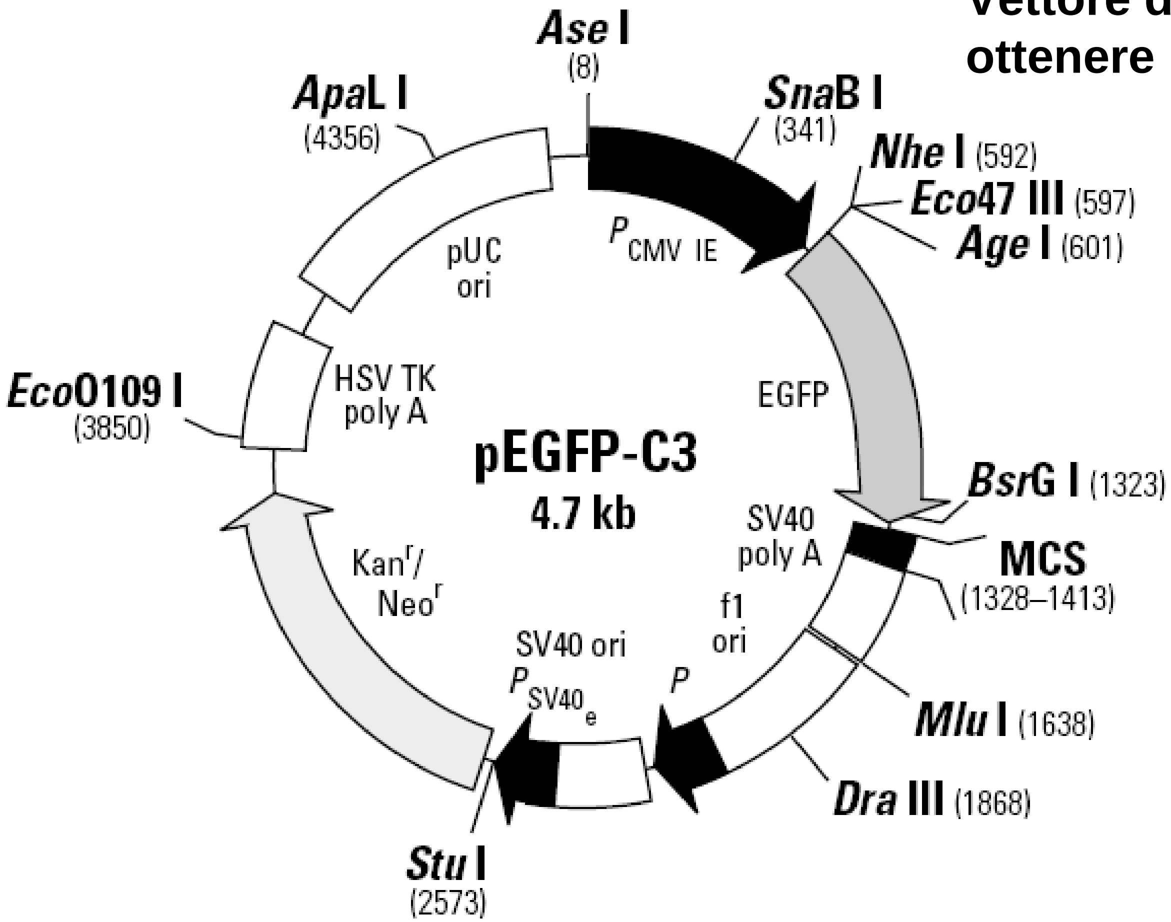
GenBank Accession #: U57607

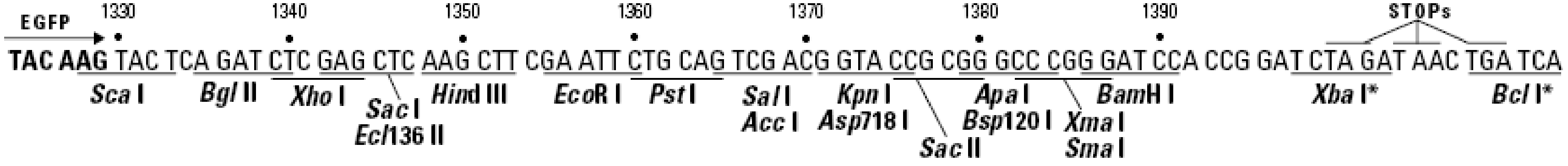
Clontech

PT3052-5

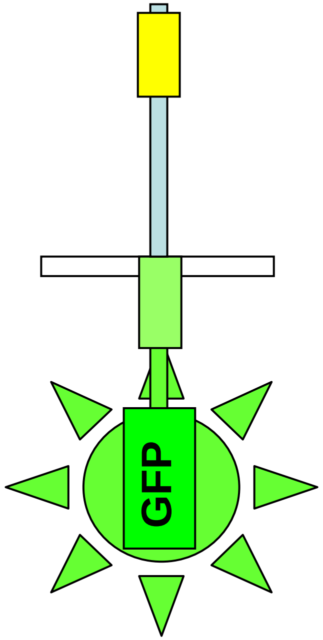
Catalog #6082-1

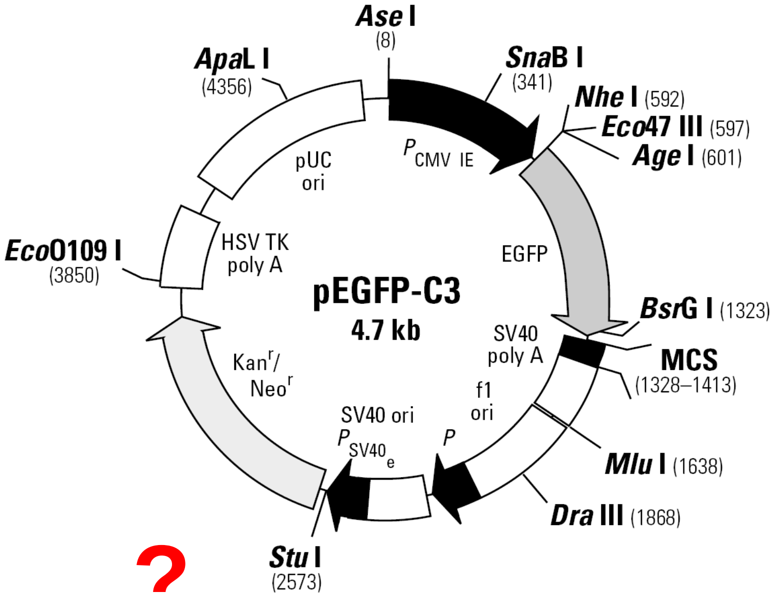
Vettore di espressione per ottenere proteina ibrida



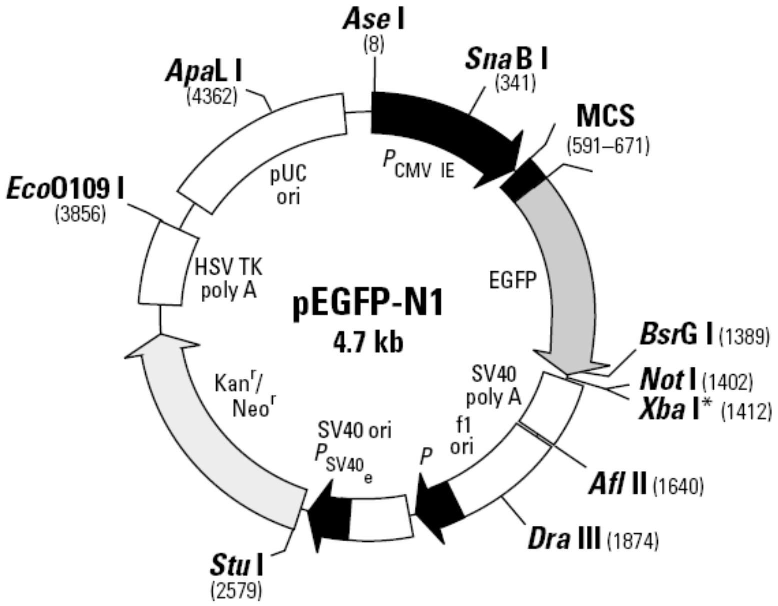


- Quale vettore devo usare per avere la GFP nella regione citoplasmatica?

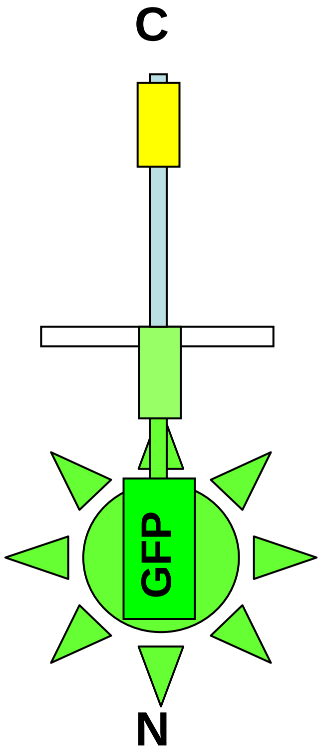


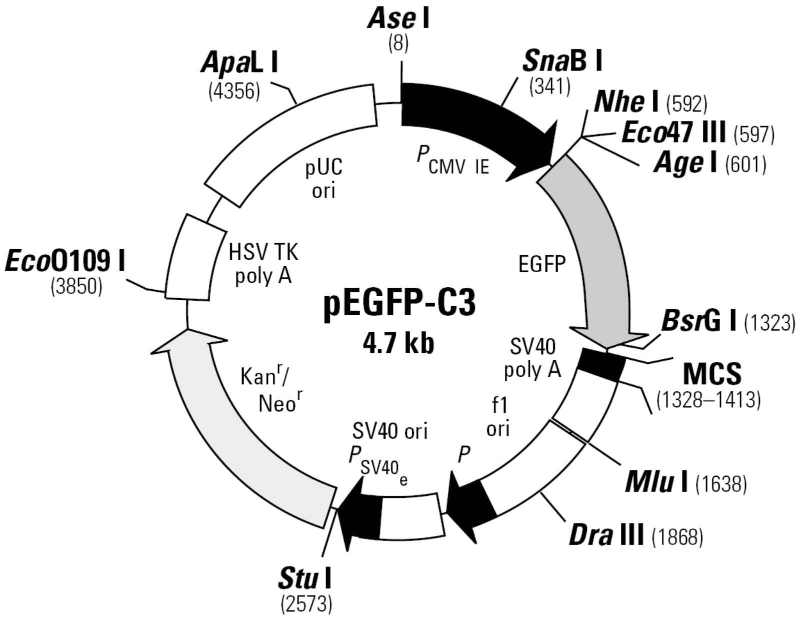


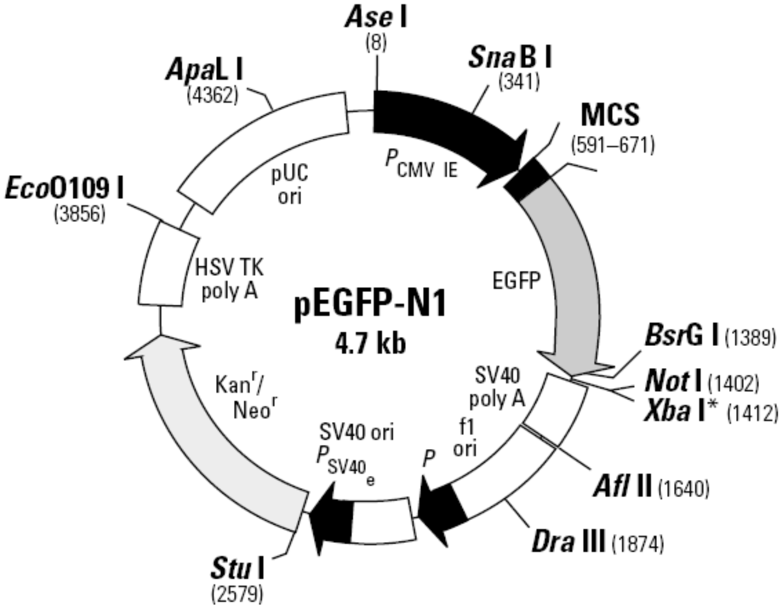
?



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



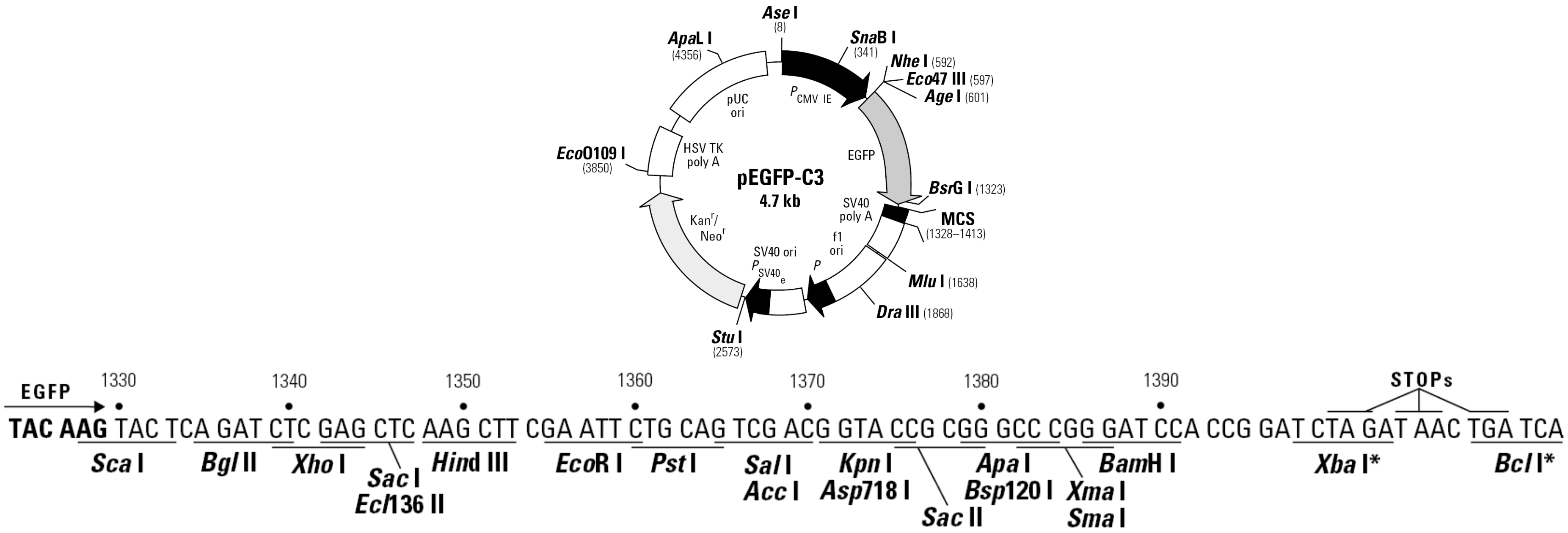




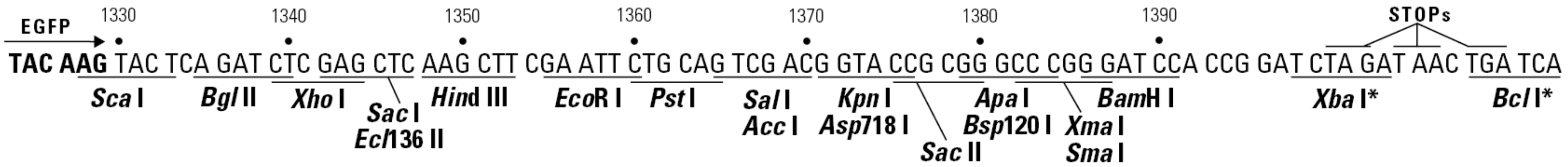
❉ per semplicità non faremo due cicli di PCR, ossia una PCR seguita da una “nested” PCR, bensì una sola reazione: scegliete dunque una sola coppia di primers per amplificare

❉ 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…



✳ 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 C**GA ATT C**TG 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 C**GA ATT C**NN 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 Tm 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 C**GA ATT C**NN 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

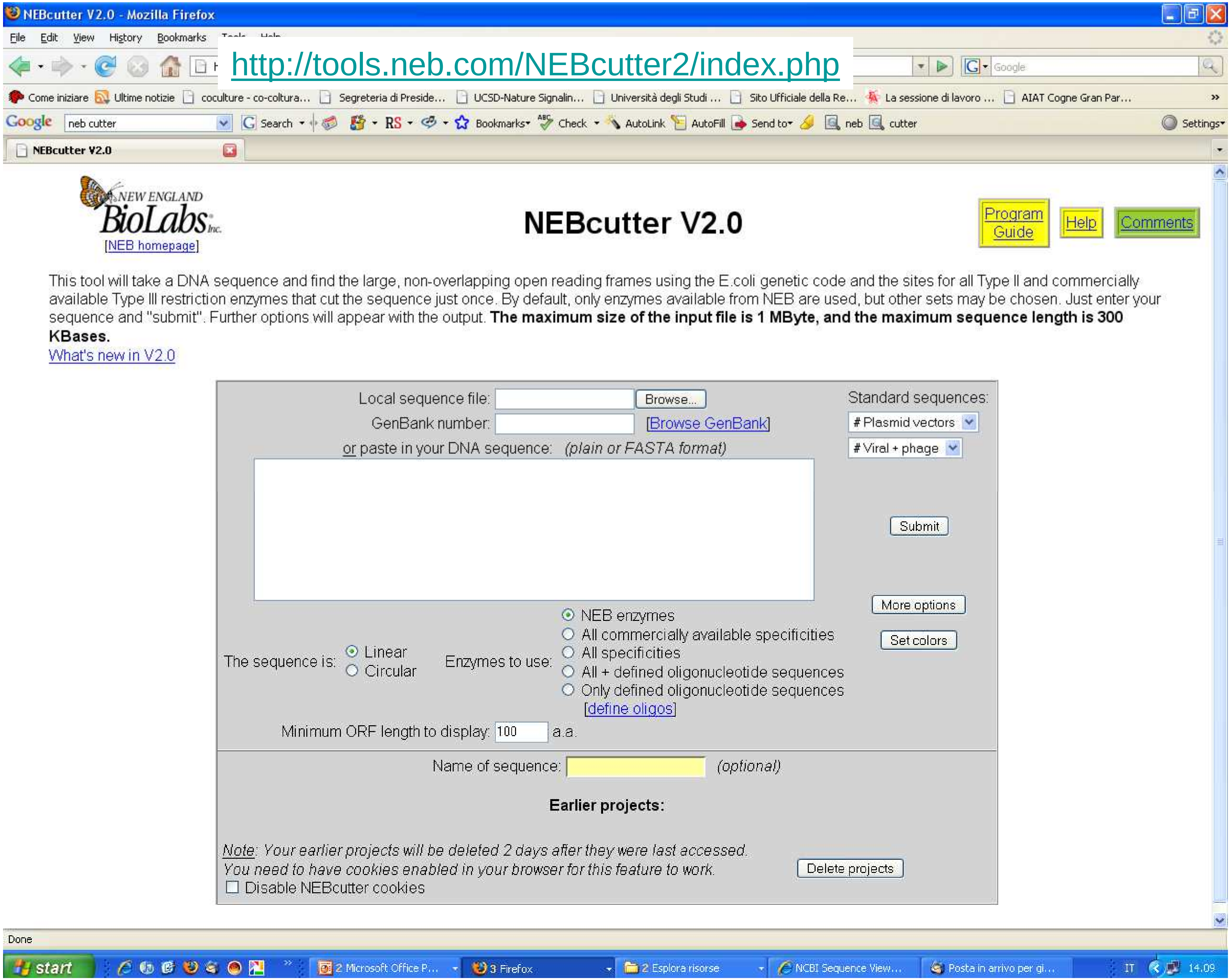
Per l’analisi di restrizione e l’identificazione degli ORF potete utilizzare il sito NEB cutter:

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/

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



NEBcutter V2.0

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.**

What's new in V2.0

Local sequence file:

GenBank number:

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: 100 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