

# microRNA引物设计

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所用软件：

**1、Premier Primer**

**2、Oligo**

## 引物设计的原则

- 1、引物与模板的序列要紧密互补
- 2、引物不能在模板的非目的位点引发

## DNA聚合反应

- 3、引物与引物之间应避免形成稳定的二聚体或发夹结构

# 具体考虑的因素

- 1、引物的长度一般为15-30 bp，常用的是18-27 bp，但不应大于38，因为过长会导致其延伸温度大于74℃，不适于Taq DNA聚合酶进行反应
- 2、引物序列在模板内应当没有相似性较高，尤其是3'端相似性较高的序列，否则容易导致错配。引物3'端出现3个以上的连续碱基，如GGG或CCC，也会使错误引发机率增加
- 3、引物3'端的末位碱基对Taq酶的DNA合成效率有较大的影响，末位碱基为A的错配效率明显高于其他3个碱基，因此应当避免在3'端使用碱基A

4、引物序列的GC含量一般为40-60%，过高或过低都不利于引发反应，且上下游引物的GC含量不能相差太大

5、引物所对应模板位置序列的T<sub>m</sub>值在72℃左右可使复性条件最佳

6、 $\Delta G$ 值是DNA双链形成所需的自由能，该值反映了双链结构内部碱基对的相对稳定性。应当选用3'端 $\Delta G$ 值较低（绝对值不超过9），而5'端和中间 $\Delta G$ 值相对较高的引物。引物的3'端的 $\Delta G$ 值过高，容易在错配位点形成双链结构并引发DNA聚合反应

7、引物存在二聚体或发夹结构会影响PCR反应的进行，但5'端序列对PCR影响不太大，常用来引进修饰位点或标记物

8、引物二聚体及发夹结构的能值过高（超过4.5kcal/mol）易导致产生引物二聚体带，并且降低引物有效浓度而使PCR反应不能正常进行

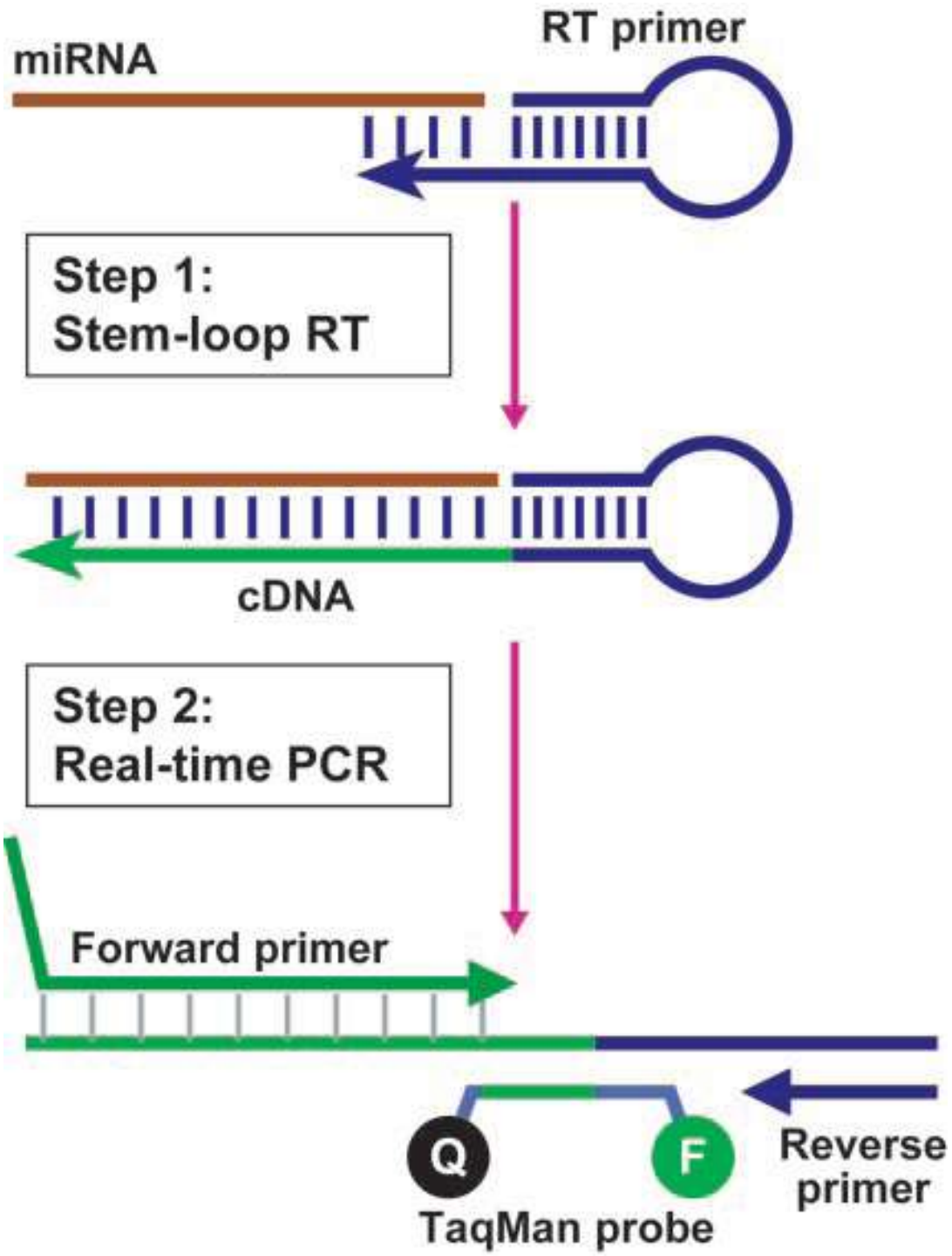
9、对引物的修饰一般是在5'端增加酶切位点，应根据下一步实验中要插入PCR产物的载体的相应序列而确定

# miRNA引物设计

(1) 以成熟序列设计

(2) 以前体序列设计

以成熟序列设计





# Premier Primer

Primer Premier

File Function Help


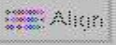
- New ▶ DNA Sequence
- Open ▶ Protein Sequence
- Preferences Ctrl+R
- Degenerate bases
- Quit
- 1 C:\PROGRA~1\PRIMER~1\data\DemoTNF.ALN

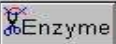
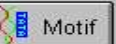
Taskbar icons: Start button, Internet Explorer, File Explorer, Recycle Bin, Microsoft Word, Adobe Reader, and a custom icon.

System tray: Network, Volume, and other background services.


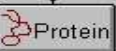
System clock: 22:41 2012/10/30



GeneTank - NewSequence

Function:  Primer  Align

 Enzyme  Motif

Translation: Active Sequence:

Translations:   DNA  Protein

5' Seq No Header 3 10 Find Find Next S A dsDNA  

```
1  AGGTTGGTGA TAATTGTTGA CAGAAGATAG AGAGCACTAA TGATGATATG CTAATTTCAI
61 TCAGCAAAAAG CACTCACTT CATTGTGCT CTCTATGCTT CTGTATCAC CTTGCCCCTC
```

Pos: 00121

Primer Premier

Primer: S A Search Results Edit Primers

Direct Select:

3' TCCAACCCTATTAAACAACTGTCTT 5'  
 5' AGGTTGGTGATAATTGTTGACAGAAGATAGAGAGCACTAATGATGATATGCTAATTCATTTCAGCAAAGCATCTCACTTCA 3'

R L V I I V D R R - R A L M M I C - F H S A K A S H F I

	Rating	Seq No	Length	Tm [°C]	GC%	Δ G [kcal/mol]	Activity [μg/OD]	Degeneracy	Ta Opt [°C]
Sense	91	1	25	60.0	36.0	-42.8	30.3	1	--
Anti-sense	91	25	25	60.0	36.0	-42.8	32.4	1	--
Product	35	--	25	63.9	36.0	--	--	--	37.7

	Hairpin	Dimer	False Priming	Cross Dimer	No Hairpins Found
Sense	None	Found	None	Found	
Anti-sense	None	Found	None		

DNA → Protein

TATG CTAATTCAT  
 TCAC CTTGCCCCC

Pos: 00121

Primer: Search Results Edit Primers

Direct Select:

```

3' TCCAACCACIATTAACAACIGTCTT 5'
|||||
5' AGGTTGGTIGATAATIGTTGACAGAAGATAGAG
      10      20      30
R L V I I V D R R - R A
    
```

	Rating	Seq No	Length	Tm [°C]
Sense	91	1	25	60.3
Anti-sense	91	25	25	60.3
Product	35	-	25	63.3

	Hairpin	Dimer	False Priming
Sense	None	Found	None
Anti-sense	None	Found	None

### Search Criteria

**Search For:**

PCR Primers     Sequencing Primers     Hybridization Probes

**Search Type:**

Sense Primer                       Compatible with Sense Primer  
 Anti-sense Primer                   Compatible with Anti-sense Primer  
 Both                                       Pairs

**Search Ranges:**

**Sense Primer:**  
 to

**Anti-sense Primer:**  
 to

**PCR Product Size:**  
 bp to  bp

**Primer Length:**  
 bp ?  bp

**Search Mode:**

Automatic     Manual

Primer:

Direct Select:

```

3' TCCAACCACCTATTAACAACACTGTCTT 5'
|||||
5' AGGTTGGTGATAATTGTTGACAGAAGATAGAGAGCA
    10      20      30
R L V I I V D R R - R A I
    
```

	Rating	Seq No	Length	Tm [°C]
Sense	91	1	25	60.0
Anti-sense	91	25	25	60.0
Product	35	--	25	63.9

	Hairpin	Dimer	False Priming	Cr Di			
Sense	<input type="button" value="None"/>	<input type="button" value="Found"/>	<input type="button" value="None"/>	<input button"="" type="button" value="None"/>	<input type="button" value="Found"/>	<input type="button" value="None"/>	

### Search Progress

Search Complete.

Primer Search Results:

	Remaining/Rejected	
	Sense:	Anti-sense:
Total Possible	505	505
<input checked="" type="checkbox"/> Tm	1	1
<input checked="" type="checkbox"/> GC%	43	43
<input checked="" type="checkbox"/> Degeneracy	0	0
<input checked="" type="checkbox"/> 3' End Stability	20	0
<input checked="" type="checkbox"/> GC Clamp	79	79
<input checked="" type="checkbox"/> Redundancy	318	336
<input checked="" type="checkbox"/> Repeats/Runs	14	14
<input checked="" type="checkbox"/> Dimer/Hairpin	0	1
<input checked="" type="checkbox"/> False Priming	1	1
<input checked="" type="checkbox"/> Optimal Primers	29	30

Stringency:

- Very High
- High
- Moderate
- Low
- Very Low
- Manual

Primer Pairs:

Pairs Found  
100

Primer: S A Search Results Edit Primers

Direct Select:

3' TCCAACCACTATTAACAACCTGTCTT 5'  
 5' AGGTGGTGATAATTGTTGACAGAAGATAGAGAGCACTAATGATGATATGCTAATTTTCATTTCAGCAAAGCATCTCACTTCA 3'

R L V I I V D R R - R A L M M I C - F H S A K A S H F I

	Rating	Seq No	Length	Tm [°C]	GC%	Δ G [kcal/mol]	Activity [μg/OD]	Degeneracy	Ta Opt [°C]
Sense	91	1	25	60.0	36.0	-42.8	30.3	1	--
Anti-sense	91	25	25	60.0	36.0	-42.8	32.4	1	--
Product	35	--	25	63.9	36.0	--	--	--	37.7

	Hairpin	Dimer	False Priming	Cross Dimer
Sense	None	Found	None	Found
Anti-sense	None	Found	None	

No Hairpins Found All

Pos: 00121

Search Results

Sense  Anti-sense  Pairs

100 pairs found.

#	Rating	Tm [°C]	Product Size	Ta Opt [°C]	Mark
1	89	47.5 47.8	33	38.4	<input type="checkbox"/>
2	89	43.3 47.5	42	38.2	<input type="checkbox"/>
3	88	43.3 44.6	52	39.4	<input type="checkbox"/>
4	88	42.4 47.1	78	41.8	<input type="checkbox"/>
5	87	43.3 49.1	40	37.3	<input type="checkbox"/>

Primer Premier

Primer:

Direct Select:

5' GTGGTGATAATTGTGA 3'  
 3' TCCCAACCACATATAACAACCTGCTTCTATCTCTCGTGATTACTACTATACGATTAAAGTAAGTCGTTTTCGTAGAGTGAAGT 5'

R L V I I V D R R - R A L M M I C - F H S A K A S H F I

	Rating	Seq No	Length	Tm [°C]	GC%	Δ G [kcal/mol]	Activity [μg/OD]	Degeneracy	Ta Opt [°C]
Sense	91	3	18	42.4	33.3	-29.7	31.3	1	--
Anti-sense	100	80	18	47.1	38.9	-32.3	32.5	1	--
Product	88	--	78	77.3	34.6	--	--	--	41.8

	Hairpin	Dimer	False Priming	Cross Dimer
Sense	None	Found	None	None
Anti-sense	None	None	None	

Most Stable Dimer:  
 ΔG = -5.4 [kcal/mol]  
 5' GTGGTGATAATTGTGA 3'  
 3' AGTTGTTAATAGTGGTIG 5'

Search Results

Sense  Anti-sense  Pairs

100 pairs found.

#	Rating	Tm [°C]	Product Size	Ta Opt [°C]	Mark
1	89	47.5 47.8	33	38.4	<input type="checkbox"/>
2	89	47.5	42	38.2	<input type="checkbox"/>
3	88	43.3 44.6	52	39.4	<input type="checkbox"/>
4	88	42.4 47.1	78	41.8	<input type="checkbox"/>
5	87	43.3 49.1	40	37.3	<input type="checkbox"/>

Pos: 00121



Primer Premier

Primer:  Search  Results  Edit Primers

Direct Select:

3' TCGTTTTCGTAGAGTGA 5'  
 5' ATAATGCTAATTTCAATTCAGCAAAGCATCTCACTTCAATTTGTGCTCTCTATGCTTCTGTGATCACCTTCGCCCTC 3'

I C - F H S A K A S H F I C A L Y A S V I T F A L

	Rating	Seq No	Length	Tm [°C]	GC%	Δ G [kcal/mol]	Activity [μg/OD]	Degeneracy	Ta Opt [°C]
Sense	91	3	18	42.4	33.3	-29.7	31.3	1	--
Anti-sense	100	80	18	47.1	38.9	-32.3	32.5	1	--
Product	88	--	78	77.3	34.6	--	--	--	41.8

	Hairpin	Dimer	False Priming	Cross Dimer
Sense	None	Found	None	None
Anti-sense	None	None	None	

Most Stable Dimer:  
 ΔG = -5.4 [kcal/mol]  
 5' GTTGGTGATAATTGTTGA 3'  
 3' AGTTGTTAATAGTGGTIG 5'

Pos: 00121

Search Results

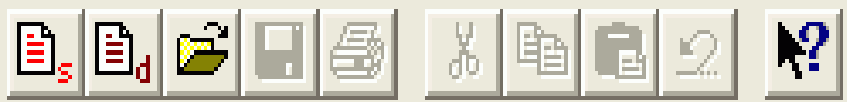
Sense  Anti-sense  Pairs

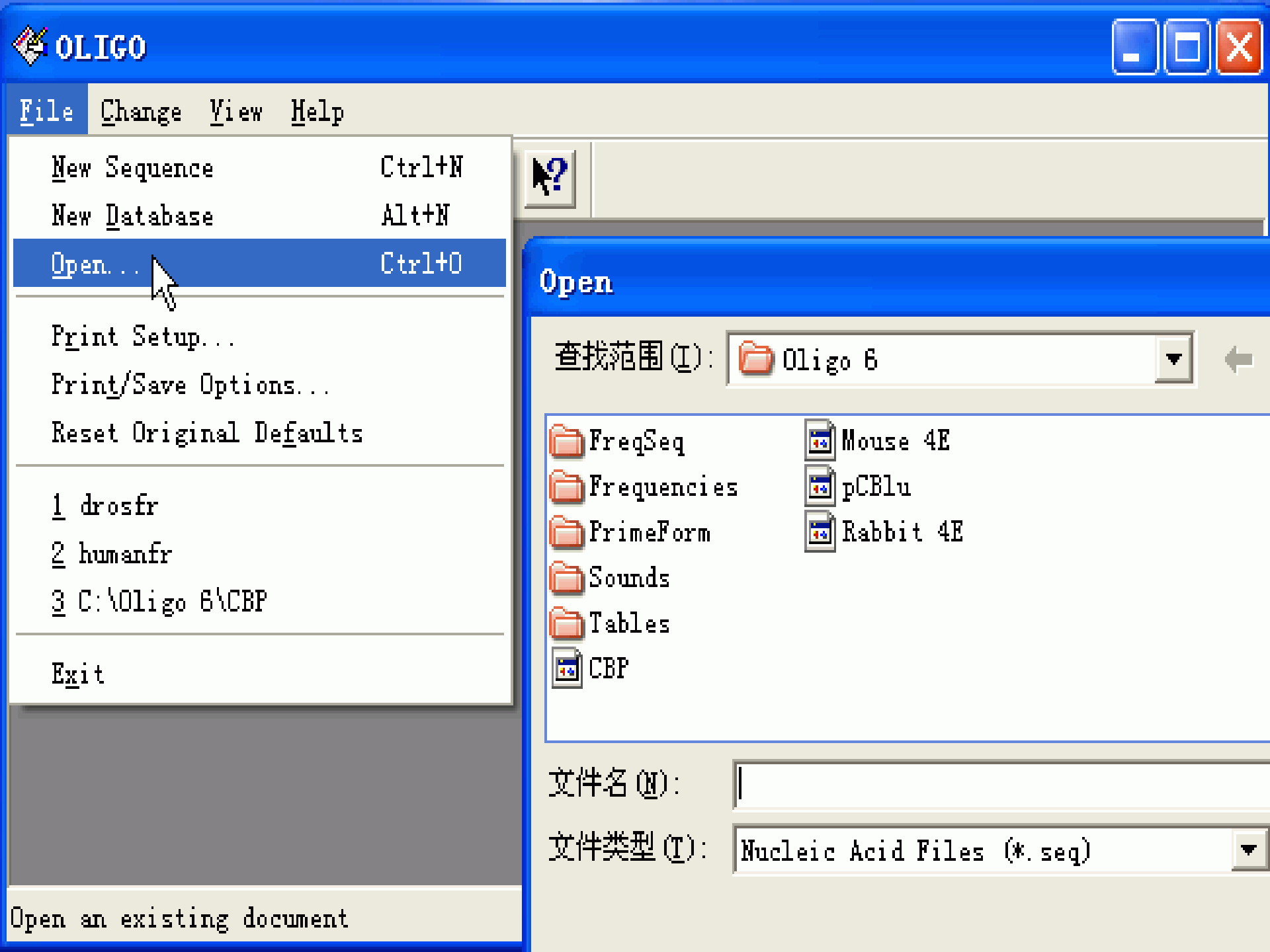
100 pairs found.

#	Rating	Tm [°C]	Product Size	Ta Opt [°C]	Mark
1	89	47.5 47.8	33	38.4	<input type="checkbox"/>
2	89	43.3 47.5	42	38.2	<input type="checkbox"/>
3	88	43.3 44.6	52	39.4	<input type="checkbox"/>
4	88	42.4 47.1	78	41.8	<input type="checkbox"/>
5	87	43.3 49.1	40	37.3	<input type="checkbox"/>

**OLOGO 6.0**

File Change View Help





- New Sequence Ctrl+N
- New Database Alt+N
- Open... Ctrl+O**
- Print Setup...
- Print/Save Options...
- Reset Original Defaults
- 1 drosfr
- 2 humanfr
- 3 C:\Oligo 6\CBP
- Exit



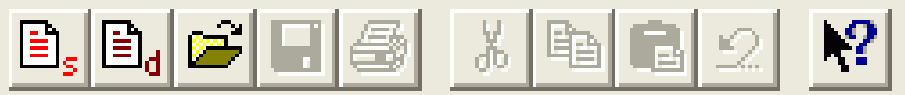
Open

查找范围 (I):

- FreqSeq
- Frequencies
- PrimeForm
- Sounds
- Tables
- CBP
- Mouse 4E
- pCBlu
- Rabbit 4E

文件名 (N):

文件类型 (T):



Open



查找范围 (I):



- FreqSeq
- Frequencies
- PrimeForm
- Sounds
- Tables
- CBP
- Mouse 4E
- pCBlu
- Rabbit 4E

文件名 (N):

打开 (O)

文件类型 (T):

取消

Open as:

Preview



## Open



查找范围 (I): FreqSeq



- drosfr
- humanfr
- mousefr
- ratfr
- wheatfr
- yeastfr

文件名 (N): 

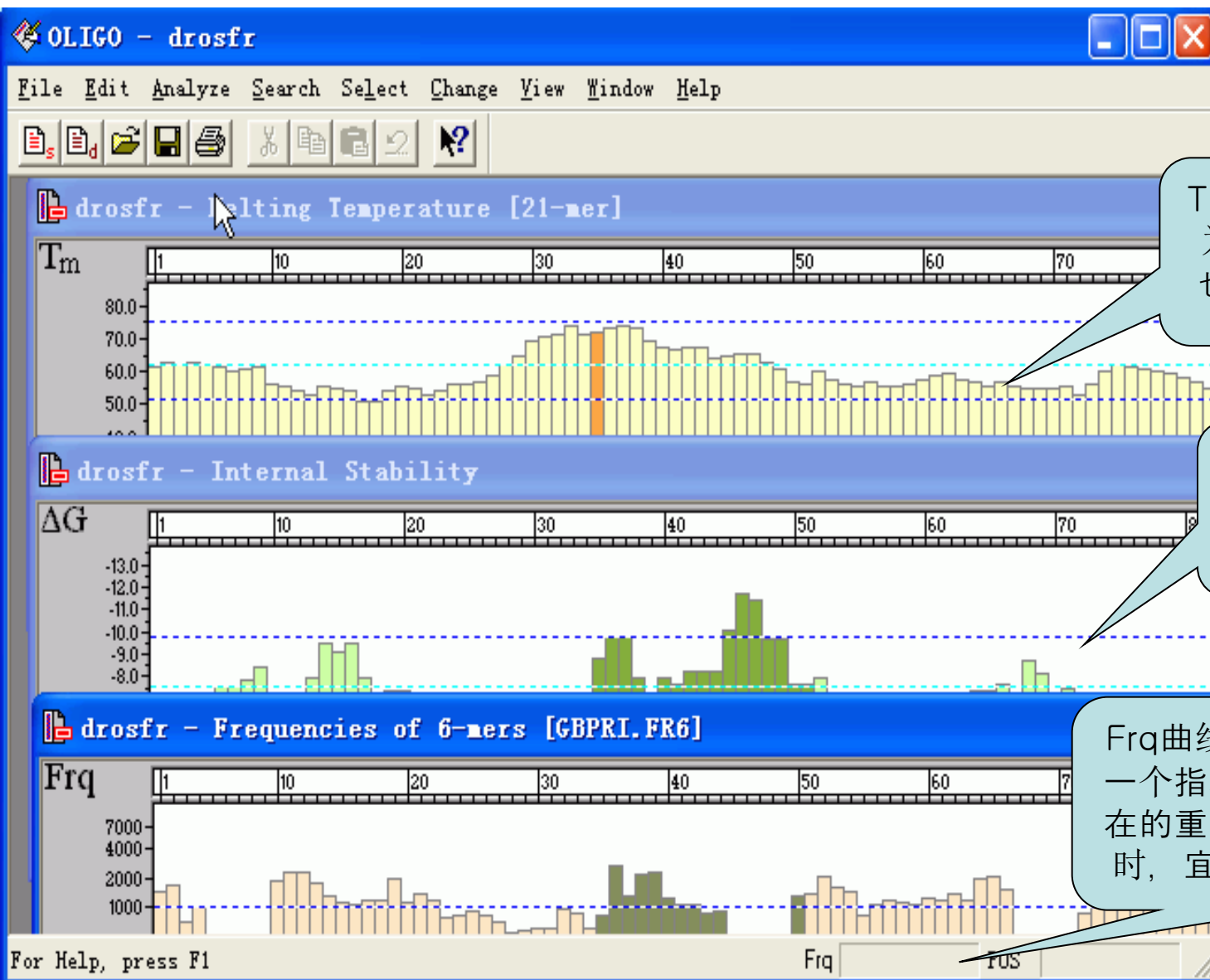
打开 (O)

文件类型 (T): 

取消

Open as: 

Preview



$T_m$ 值曲线以选取72°C附近为佳，5'到3'的下降形状也有利于引物引发聚合反应

$\Delta G$ 值反映了序列与模板的结合强度，最好引物的 $\Delta G$ 值在5'端和中间值比较高，而在3'端相对低

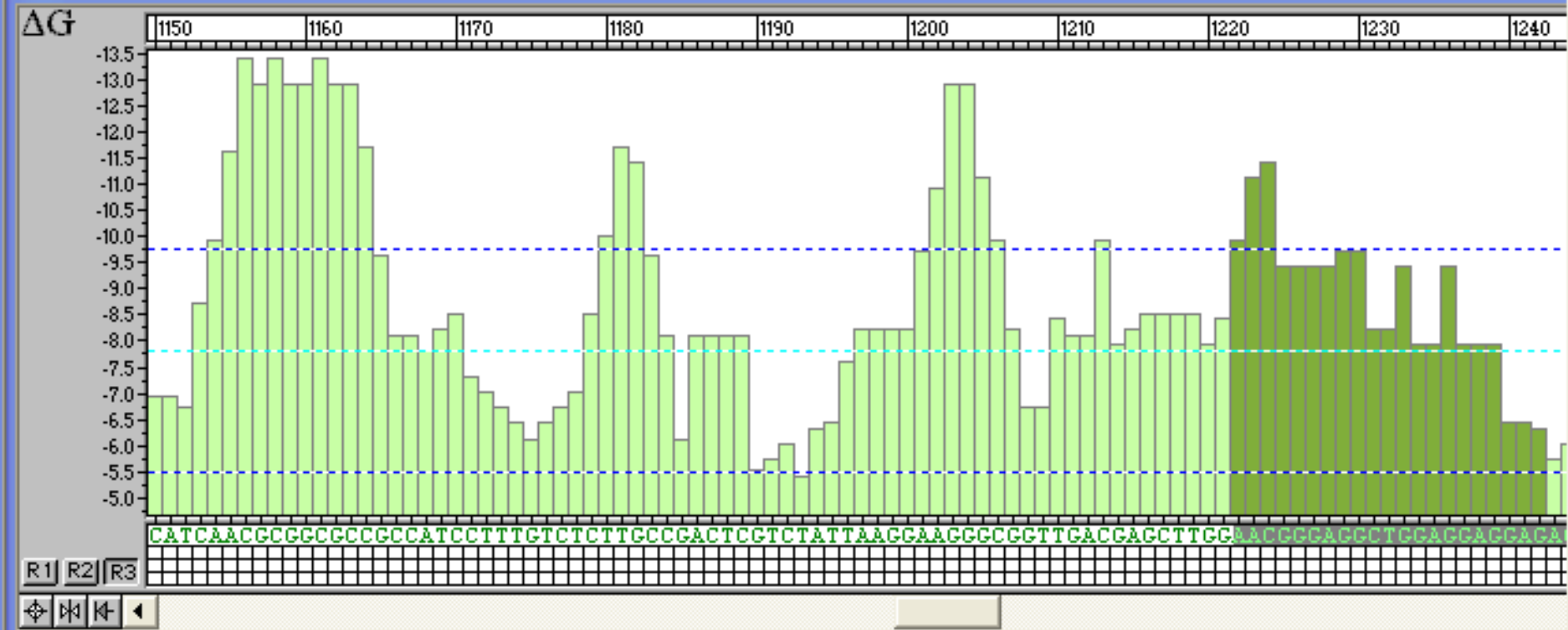
Frq曲线为“Oligo 6”新引进的一个指标，揭示了序列片段存在的重复机率大小。选取引物时，宜选用3'端Frq值相对较低的片段。



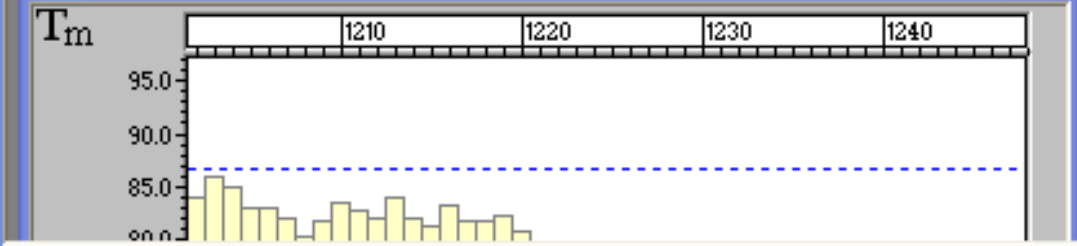
File Edit Analyze Search Select Change View Window Help



BX000496-LOC\_0s12g01350 - Internal Stability



BX000496-LOC\_0s12g01350 - Melting T...



BX000496-LOC\_0s12g01350 - Upper Pr...

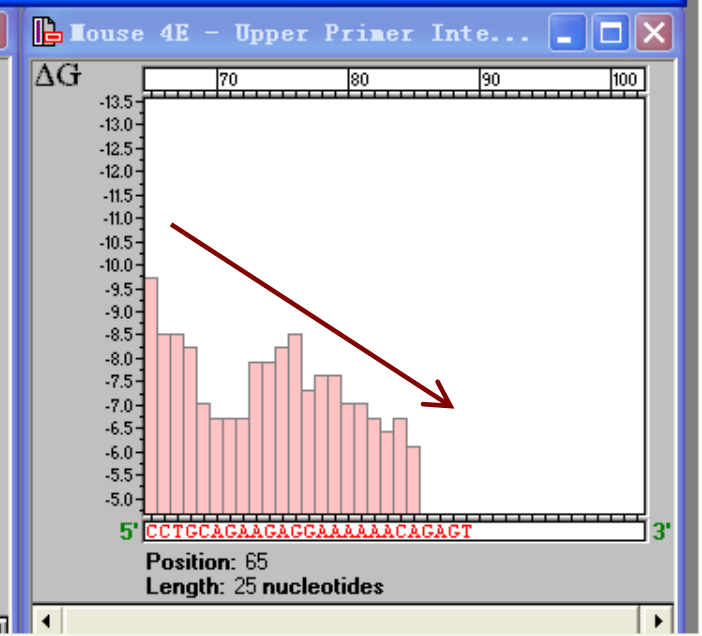
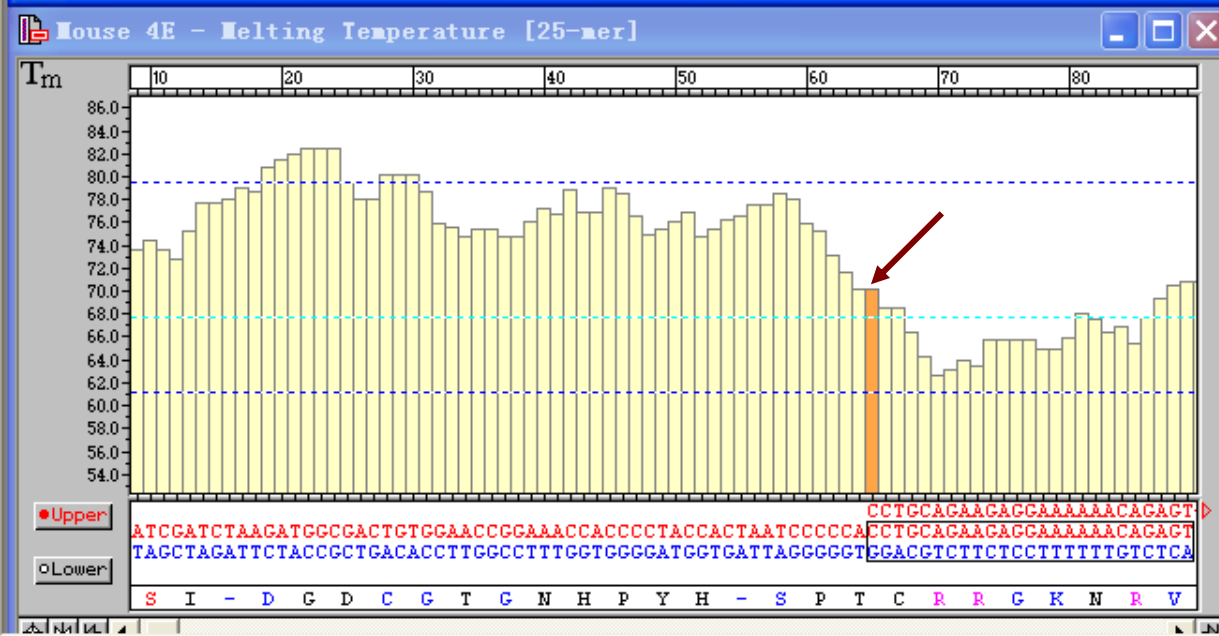
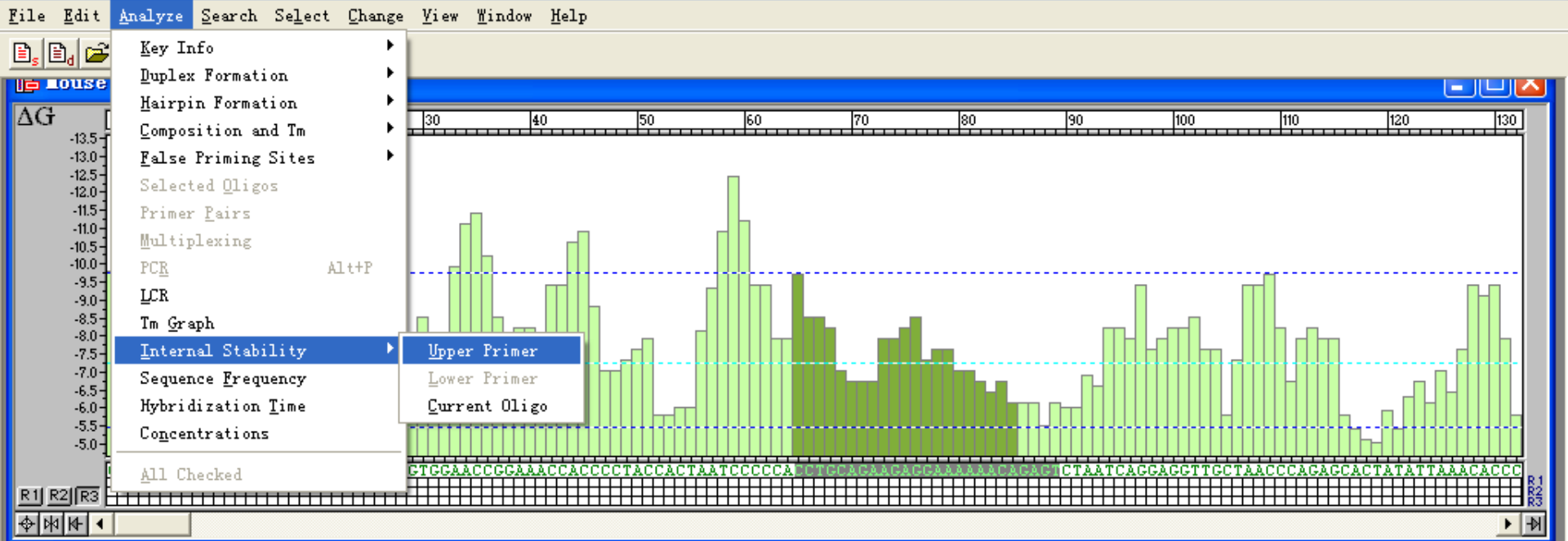
Display Hairpin with 2 or more bp Stems

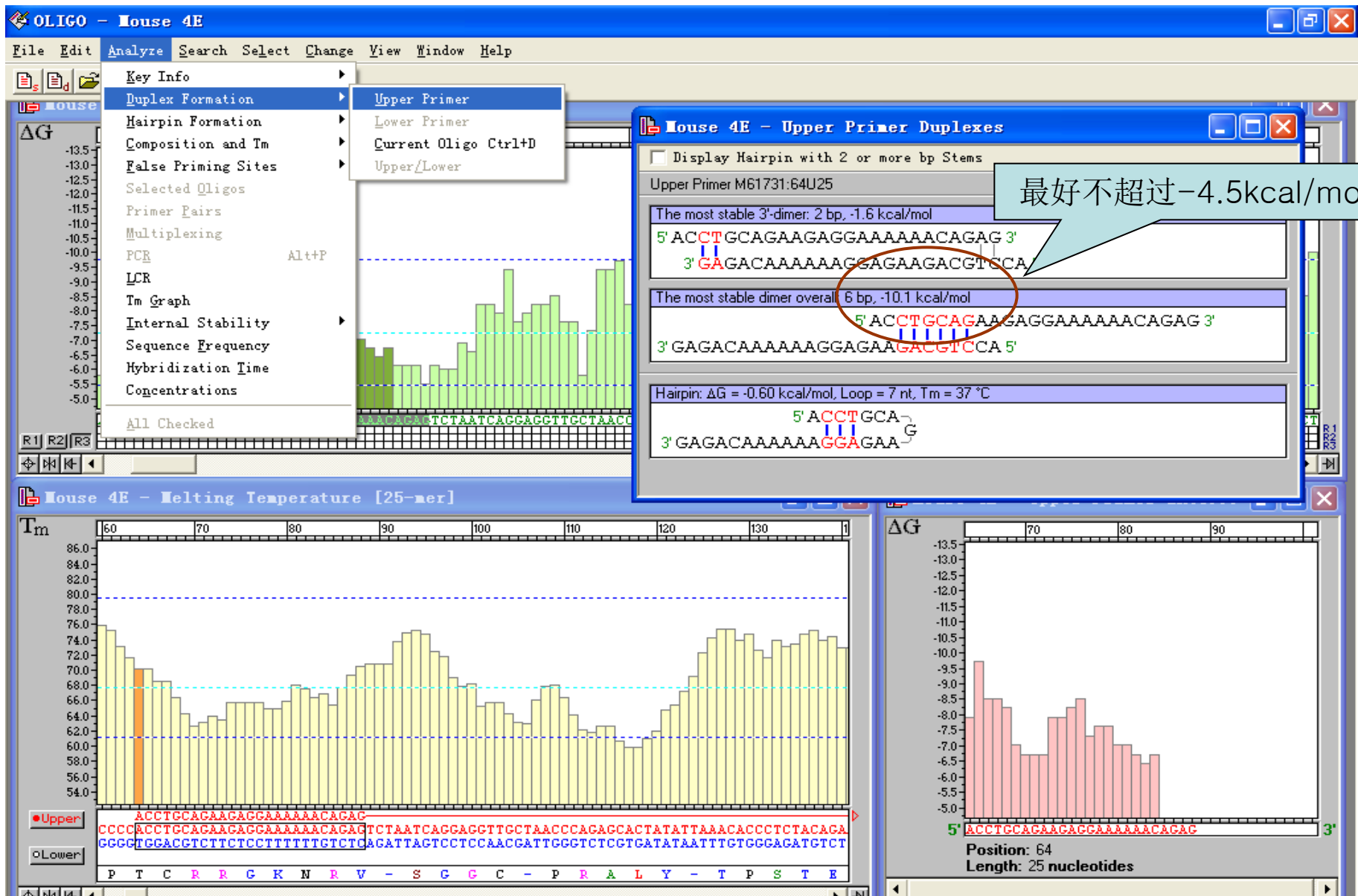
Upper Primer BX000496-LOC\_0s12g01350:1222U25

The most stable 3'-dimer: 2 bp, -1.5 kcal/mol

5' AACGGGAGGCTGGAGGAGGAGAGAT 3'

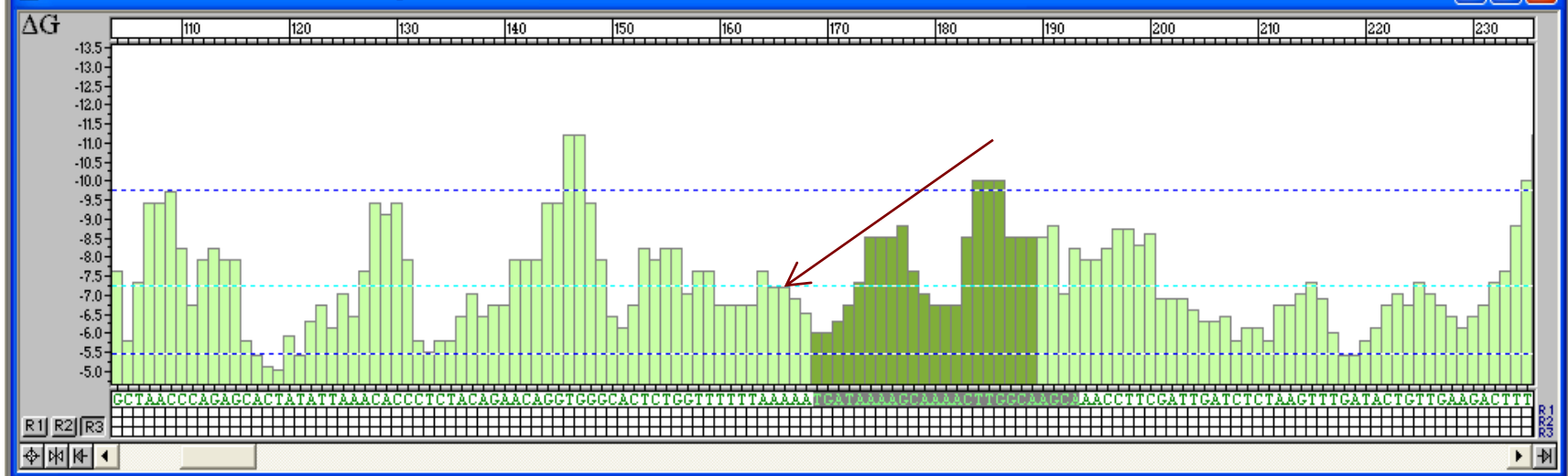




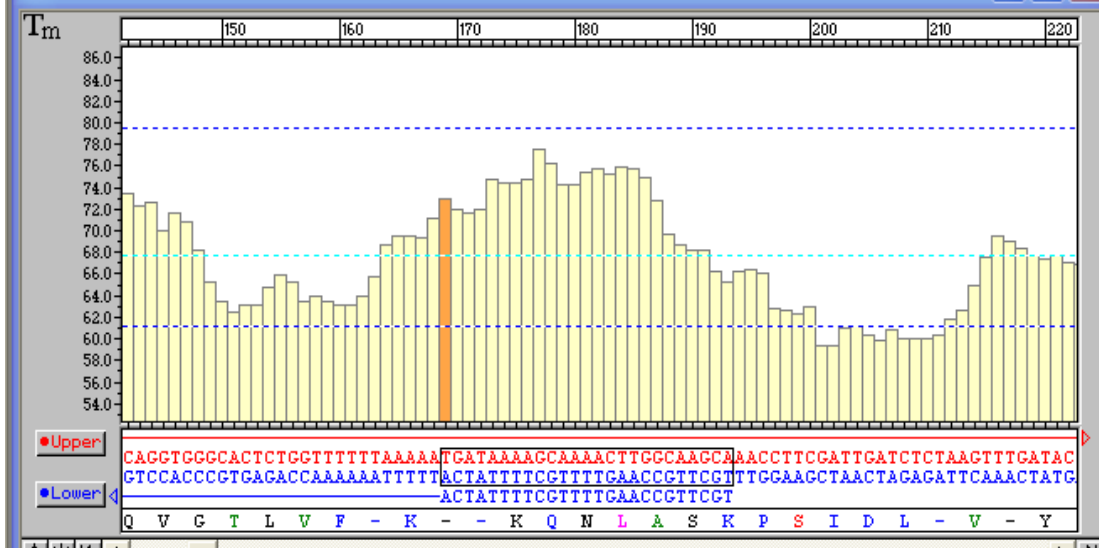




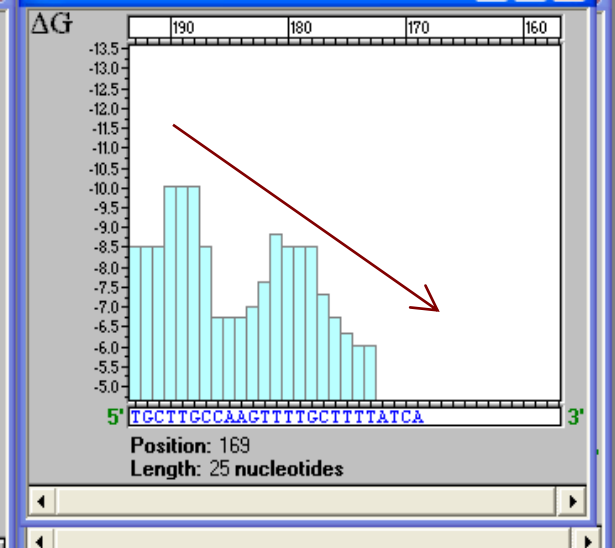
Mouse 4E - Internal Stability



Mouse 4E - Melting Temperature [25-mer]



Mouse 4E - Lower Primer Int...



Key Info

- Duplex Formation
  - Upper Primer
  - Lower Primer
  - Current Oligo Ctrl+D
  - Upper/Lower
- Hairpin Formation
- Composition and Tm
- False Priming Sites
- Selected Oligos
- Primer Pairs
- Multiplexing
- PCR Alt+P
- LCR
- Tm Graph
- Internal Stability
- Sequence Frequency
- Hybridization Time
- Concentrations

All Checked



### Mouse 4E - Upper-Lower Duplexes

Display Hairpin with 2 or more bp Stems

Primers: M61731:64U25 M61731:169L25

The most stable 3'-dimer: 2 bp, -1.6 kcal/mol

```
5' ACCTGCAGAAGAGGAAAAACAGAG 3'
3' ACTATTTTCGTTTTGAACCGTTCGT 5'
```

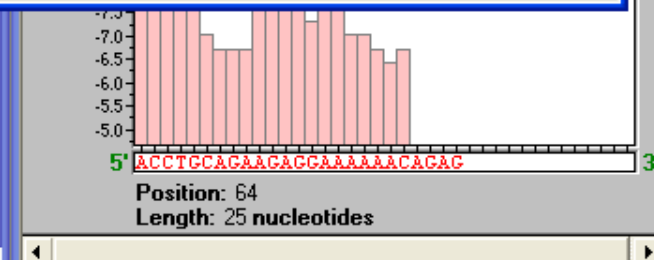
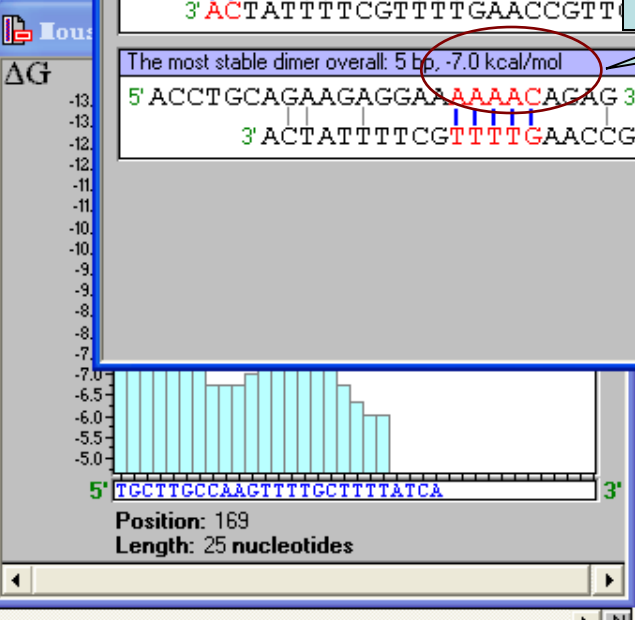
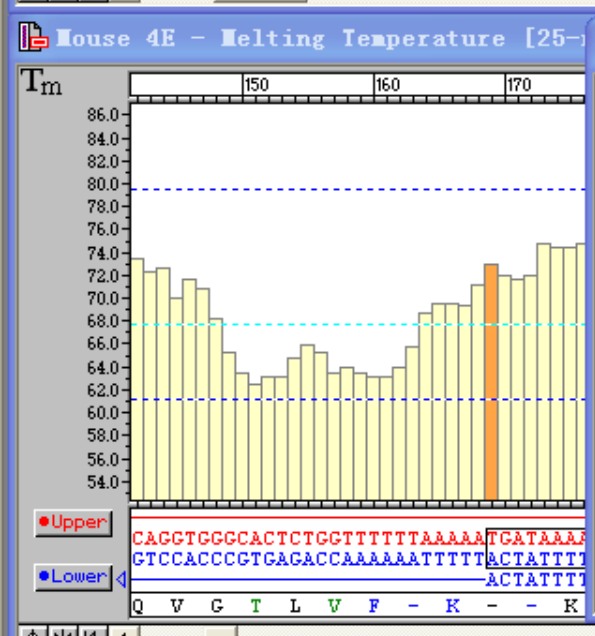
The most stable 3'-dimer: 2 bp, -1.9 kcal/mol

```
5' ACCTGCAGAAGAGGAAAAACAGAG
3' ACTATTTTCGTTTTGAACCGTT
```

The most stable dimer overall: 5 bp, -7.0 kcal/mol

```
5' ACCTGCAGAAGAGGAAAAACAGAG 3'
3' ACTATTTTCGTTTTGAACCGTTCGT 5'
```

最好不超过-5kcal/mol



OLIGO - drosfr

File Edit Analyze Search Select Change View Window Help

droshr - Primer Pairs

Tm	[1]	[2]	Sort	Se
80.	#	Pos		
70.	1	44		
60.	2	44		
50.	3	44		
40.	4	44		
	5	44		
	6	44		
	7	44		
	8	44		
	9	44		
	10	44		
	11	44		
	12	44		
	13	44		
	14	44		
	15	44		
	16	44		
	17	44	1615	1587 52.9 41.7

droshr - PCR

Optimal Annealing Temperature: 50.3° (Max: 70.5°)

	Position and Length	
Product	252	
Upper Primer	44	21
Lower Primer	276	20

Product Tm - Upper Primer Tm: 11.2  
Primers Tm difference: 8.5

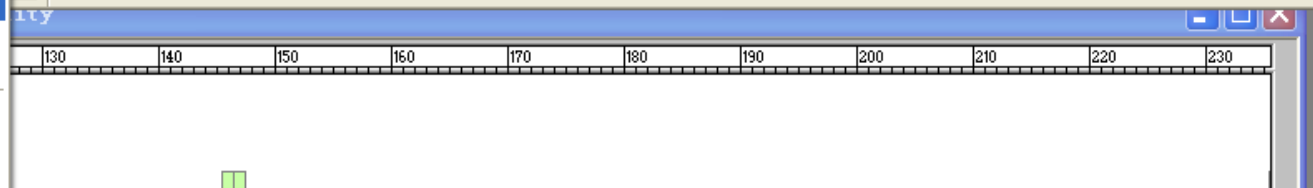
Concentration

For Help, press F1

“PCR”窗口，告知你扩增片断的位置，最适合的退火温度等等信息

上下游的Tm值的差值不能超过3，product与引物之间的差值不能超过23

- New Sequence Ctrl+N
- New Database Alt+N**
- Open... Ctrl+O
- Close Ctrl+W
- Save
- Revert
- Print... Ctrl+P
- Print Preview
- Print Setup...
- Print/Save Options...
- Reset Original Defaults
- 1 Mouse 4E
- 2 AF003255-LOC\_0s01g60280
- Exit

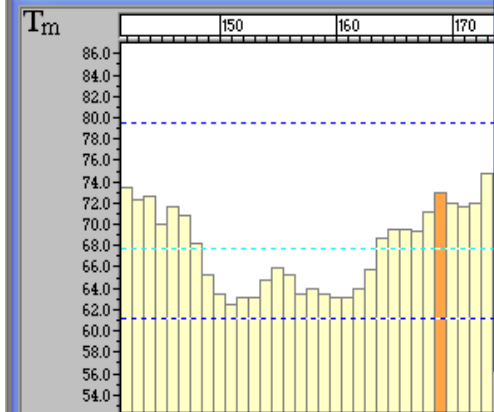


NewDatabase1 - Oligonucleotide Database

[1]	Sort	Sort	Sort	Sort	Sort	Sort	Sort
#	Date	ID Number	Sequence	Ref.	3' dim.ΔG	P.E.	Tm

R1 R2 R3  
 GCTAACCCAGAGCACTATATTAAACACCCTCT

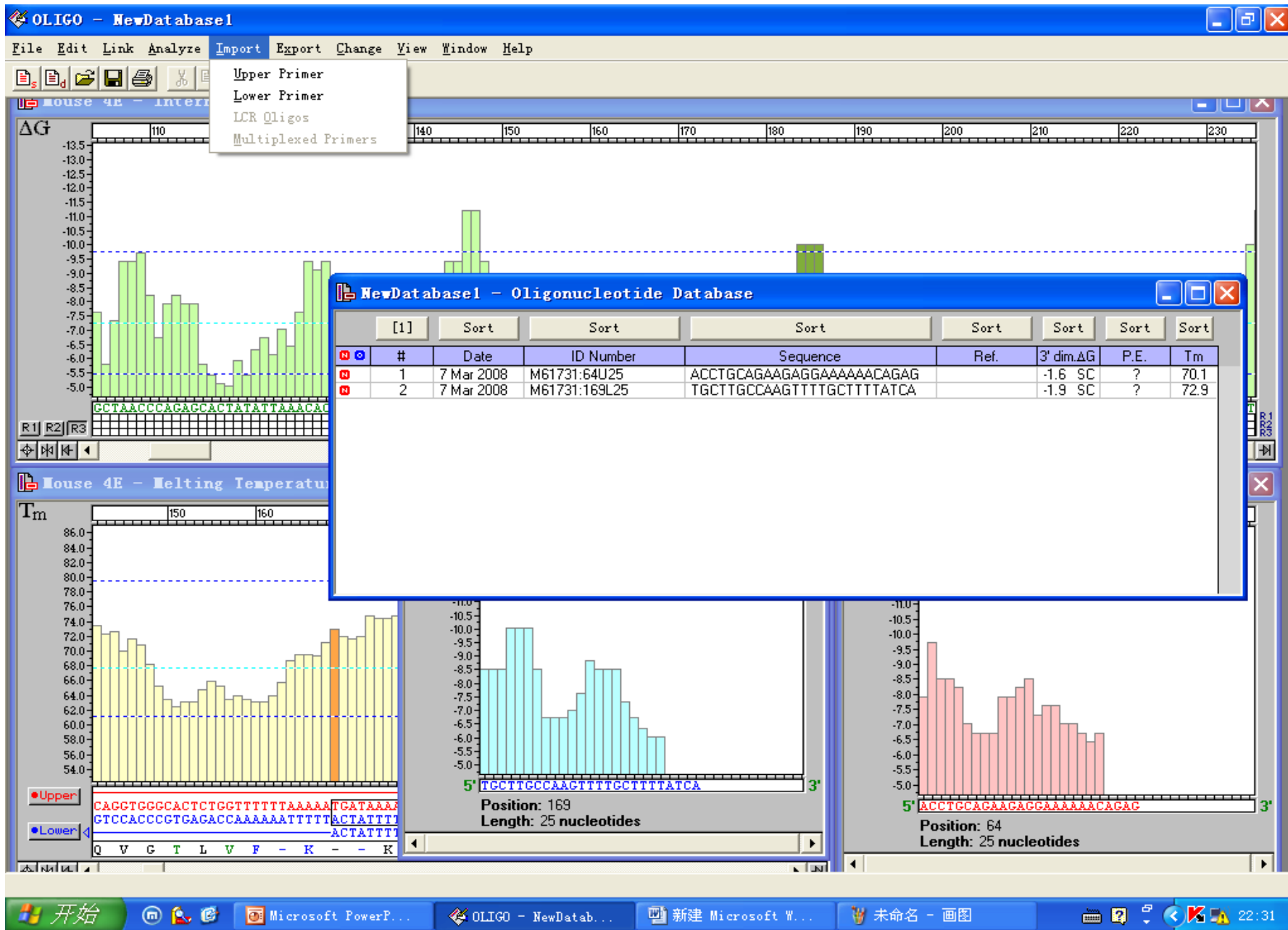
Mouse 4E - Melting Temperature [2]



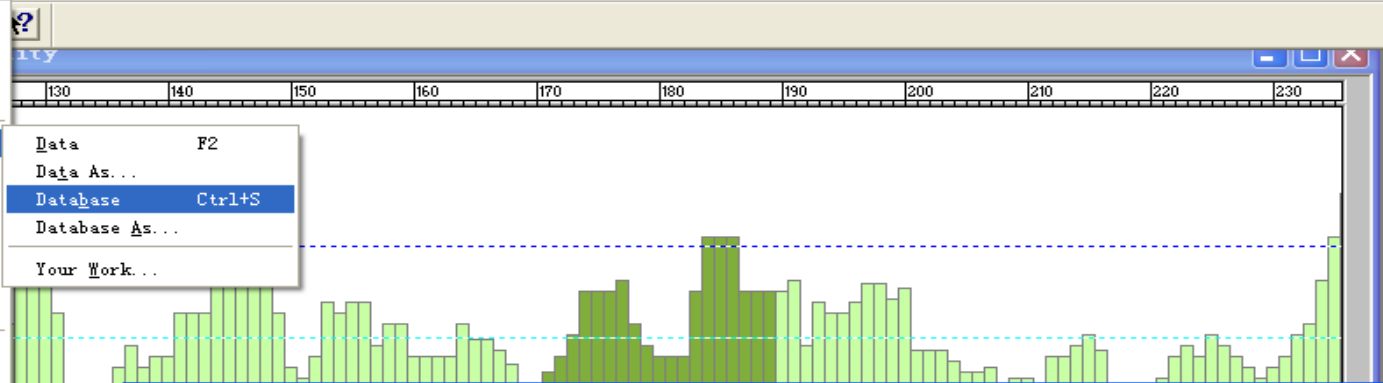
Upper  
 CAGGTGGGCACTCTGGTTTTTAAAAATGATAAA  
 Lower  
 CTCACCCCGTGACACCAAAAAATTTTACTATTTT  
 ACTATTTT  
 Q V G T L V F - K - - K

5' TCCTTCCCAAGTTTTGCTTTTATCA 3'  
 Position: 169  
 Length: 25 nucleotides

5' ACCTGCAGAAGAGCAAAAAACAGAC 3'  
 Position: 64  
 Length: 25 nucleotides



- New Sequence Ctrl+N
- New Database Alt+N
- Open... Ctrl+O
- Close Ctrl+W
- Save
- Revert
- Print... Ctrl+P
- Print Preview
- Print Setup...
- Print/Save Options...
- Reset Original Defaults
- 1 Mouse 4E
- 2 AP003255-LOC\_0s01g60280
- Exit



NewDatabase1 - Oligonucleotide Database

[1]	Sort	Sort	Sort	Sort	Sort	Sort	Sort
#	Date	ID Number	Sequence	Ref.	3' dim.ΔG	P.E.	Tm
1	7 Mar 2008	M61731:64U25	ACCTGCAGAAGAGGAAAAACAGAG		-1.6 SC	?	70.1
2	7 Mar 2008	M61731:169L25	TGCTTGCCAAGTTTTGCTTTTATCA		-1.9 SC	?	72.9

