Primer Design

G18

马越，邓迪，郭柏宏，刘璐萍

OUTLINE

1 背景介绍

2 设计与检索

3 验证

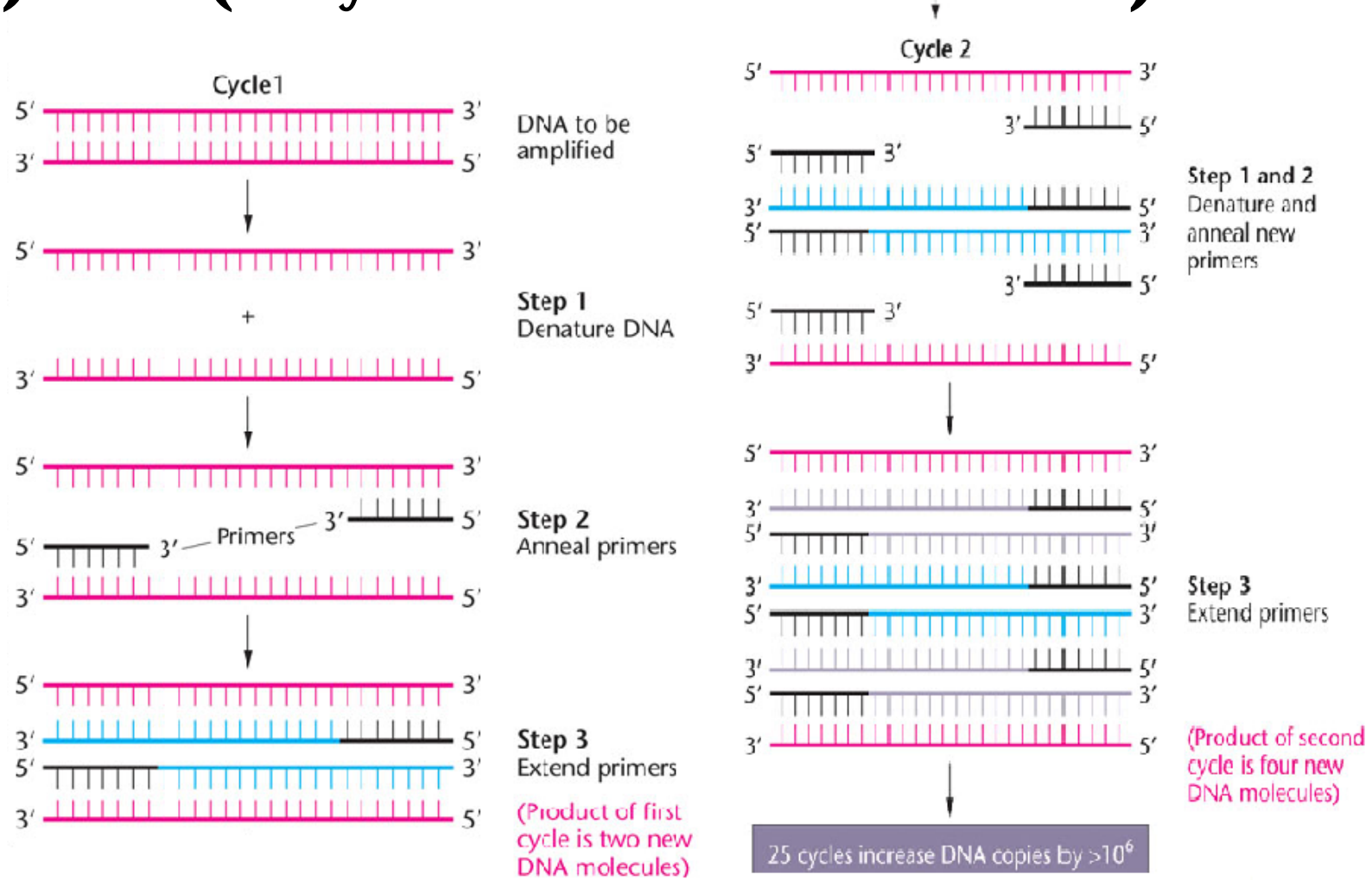
OUTLINE

1 背景介绍

2 设计与检索

3 验证

(1) PCR (Polymerase Chain Reaction)

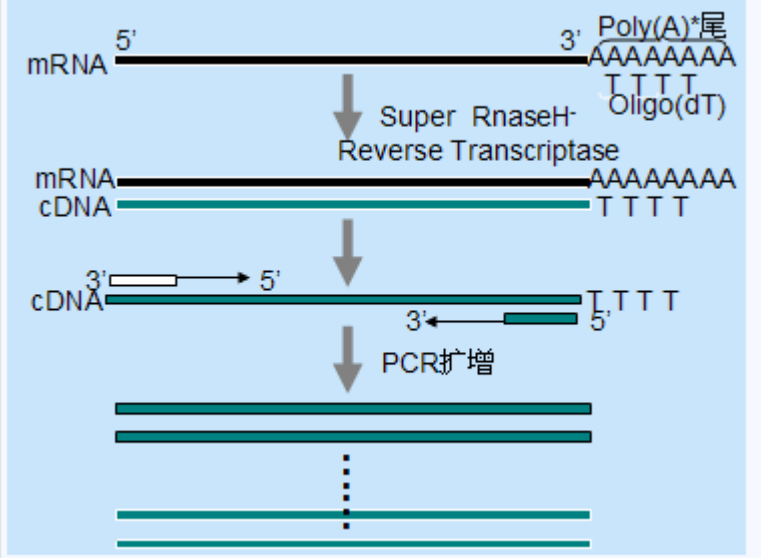


(2) PCR Varieties

- 反转录PCR (RT-PCR)
- 荧光定量PCR (RTFQ PCR)
- 反向PCR (Inverse PCR)
- 不对称PCR (Asymmetric PCR)
- 多重PCR (multiplex PCR)
- 巢式PCR (queshipcr)
- 递减PCR (touchdown PCR)

(2.1) 反转录PCR (RT-PCR)

先将mRNA反转录成cDNA，然后再以cDNA为模板，对mRNA进行扩增。

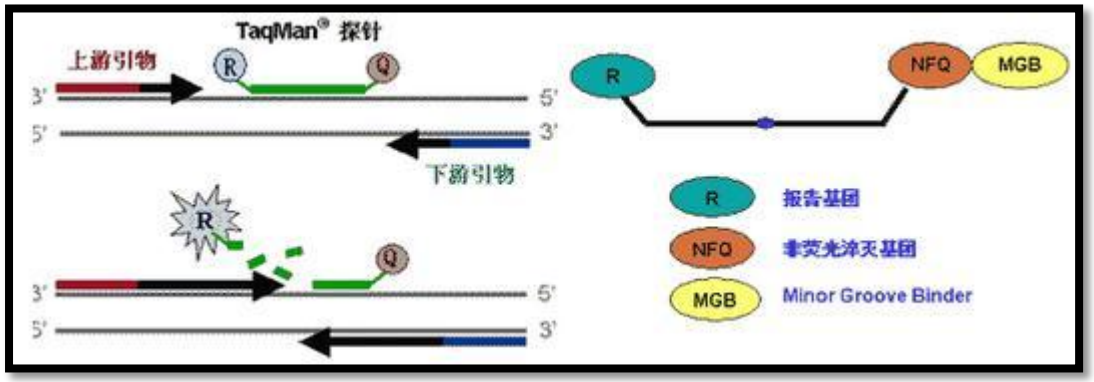


mRNA的引物选择：

1. 随机六聚体引物——不特异
2. Oligo (dT)——对mRNA特异
3. 特异性引物——最特异的引发方法

(2.2) 荧光定量PCR (RTFQ PCR)

通过荧光染料或荧光标记的特异性的探针，利用荧光信号积累实时监测整个PCR进程，通过标准曲线对未知模板进行定量分析的方法。



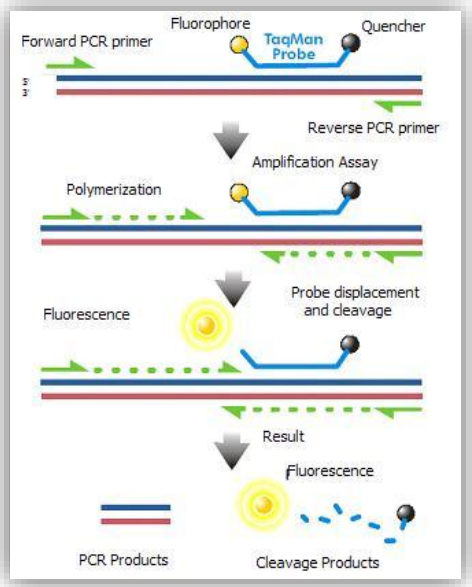
荧光定量PCR引物设计需注意：

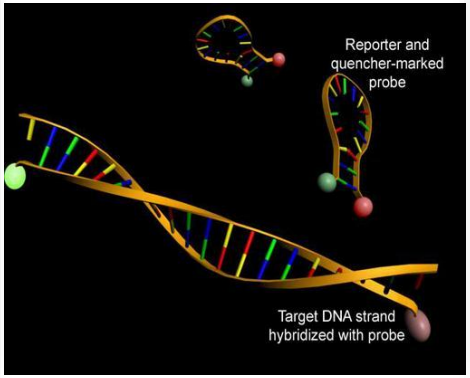
1. 扩增的片段不能太大，最好在100bp-250bp之间；

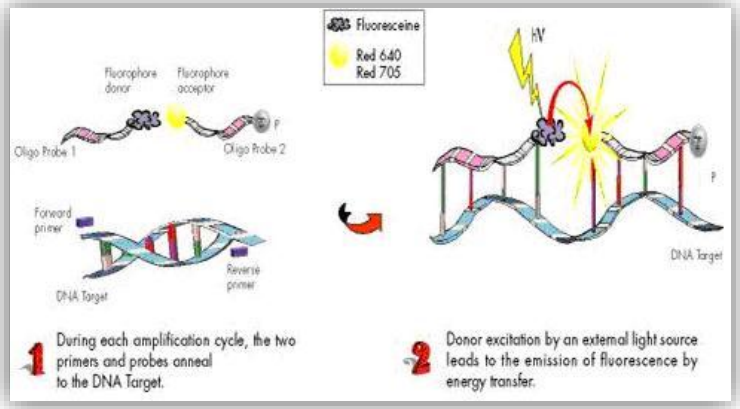
2. 引物的特异性强。

(2.2) 荧光定量PCR (RTFQ PCR)

技术	引物	探针
Taqman 技术	相同	直线型的寡核苷酸
Beacon 技术		环状的寡核苷酸
FRET 技术		两条直线型寡核苷酸







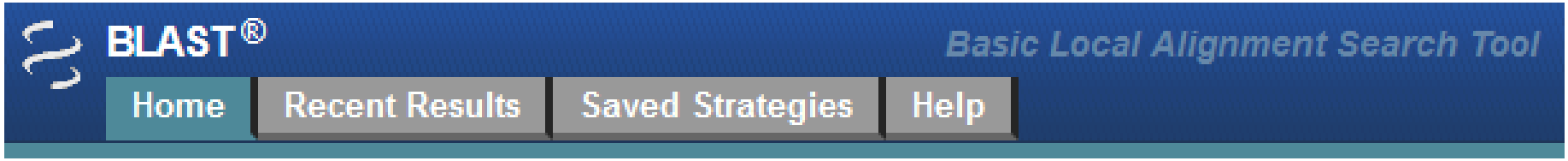
(3) Influencing Factor

Influencing Factor	Optimization
长度	常用的是18-27 bp，但不应大于38bp
碱基组成	GC含量一般为40-60%，上下游引物的GC含量不能相差太大，四种碱基要均匀分布
解链温度 Tm值	引物的Tm值为55~65℃，尽量保证上下游引物Tm值一致，二者差异最好不要超过5℃
3’端	与模板严格配对，尽量为G或C,但不能为3个以上连续碱基
5’端	可以引进修饰位点或标记物
自由能 △G	3’端ΔG值较低（绝对值不超过9），而5’端和中间ΔG值相对较高的引物

(4) 引物设计相关软件或网站

software or website	characteristic
Primer-BLAST	在线设计引物,验证设计好的引物
BioWeb	讲解详细，适合初学者
Primer Premier 5.0	引物设计的专业软件，功能全面
ProbeBase	在线的寡聚核苷酸探针和引物检索的数据库

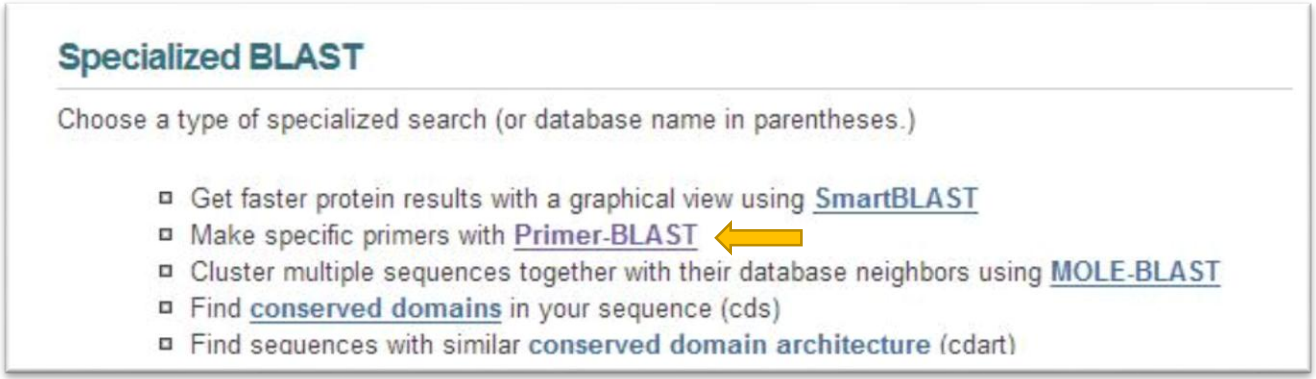
(4.1) 引物设计网站Primer-BLAST



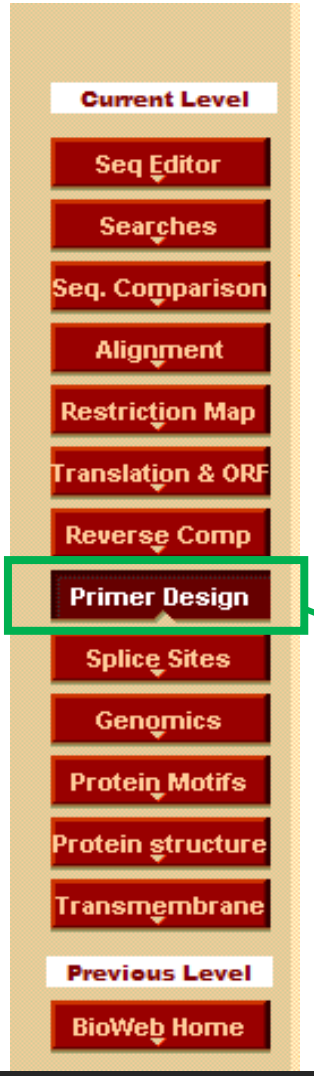
Primer-BLAST

1.在线设计引物——整合了**Primer3**软件

2.验证设计好的引物——**NCBI**的**Blast**进行引物特异性验证



(4.2) 引物设计网站BioWeb

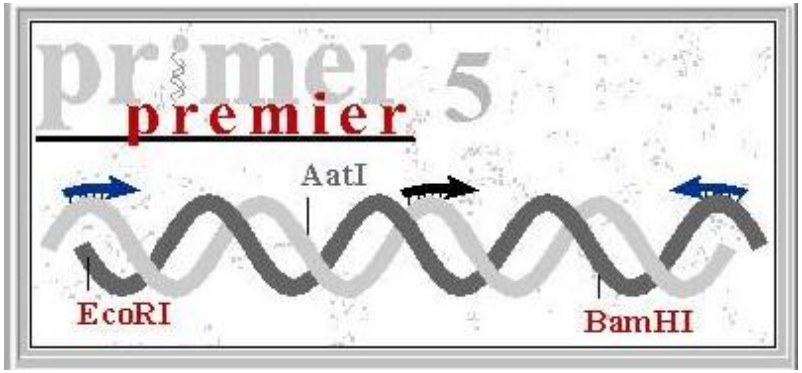


- 讲解详细，适合初学者学习
  - ✓ 基本的定义
  - ✓ 基本的原理和图示解释
  - ✓ 相关的例子和练习
  - ✓ 相关软件的链接**Biology WorkBench**
  - ✓ 除了引物设计之外其它的功能

1. What is a primer?
2. Analysis of primer sequences
3. Some thoughts on designing primers
4. Designing degenerate oligo- nucleotides

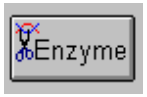
(4.3) 引物设计软件Primer Premier 5

Primer 5



Primer 5 四大主要功能：

引物设计 

限制性内切酶位点分析 

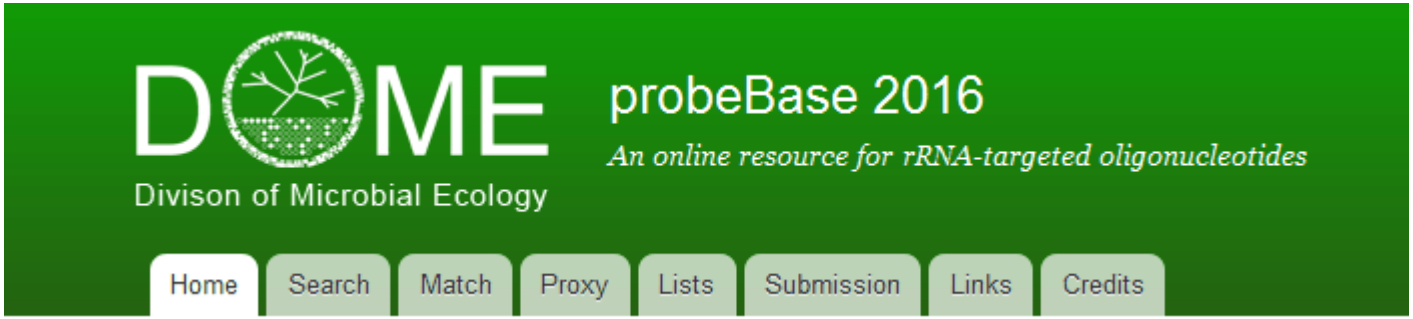
DNA基元查找 

同源性分析 

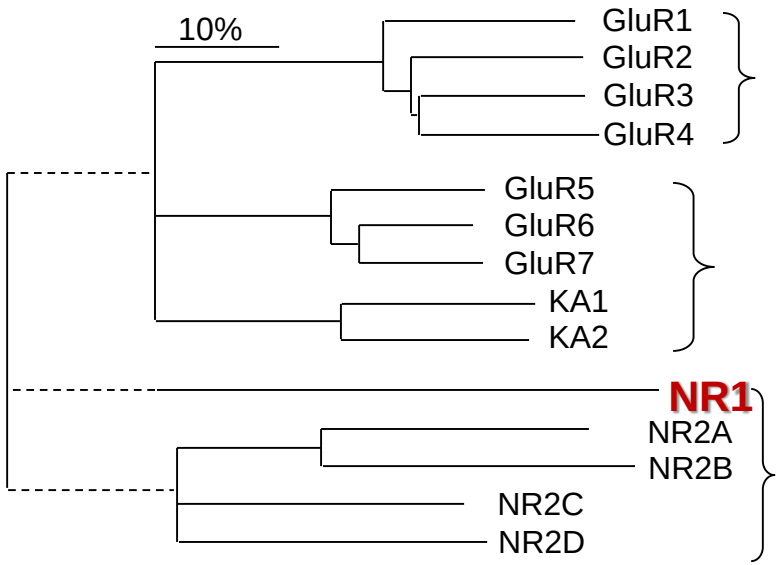
(4.4) 探针、引物检索网站ProbeBase

ProbeBase数据库是一个以rRNA为靶标，进行在线的寡聚核苷酸探针和引物检索的数据库。

由维也纳大学微生物与生态科学学院维护（Department of Microbiology and Ecosystem Science at the University of Vienna）。



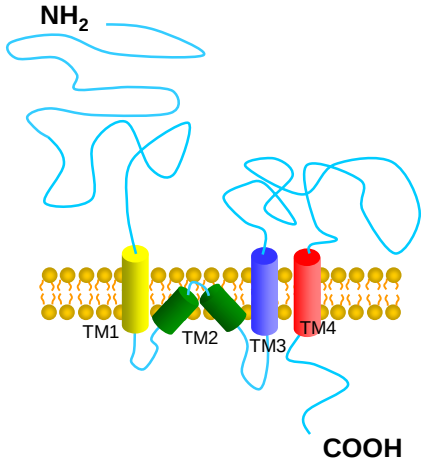
1 Background

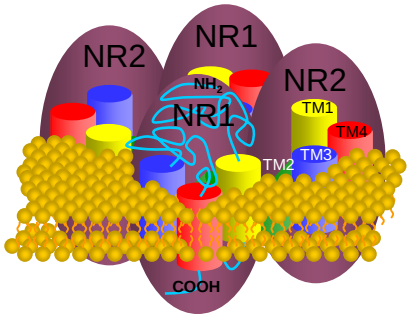


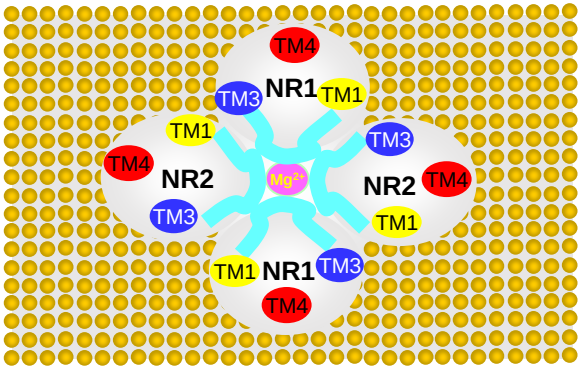
AMPA:
α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid

kainate

NMDA: *N*-methyl-D-aspartate
Coincidence detector because of voltage dependent Mg^{2+} block





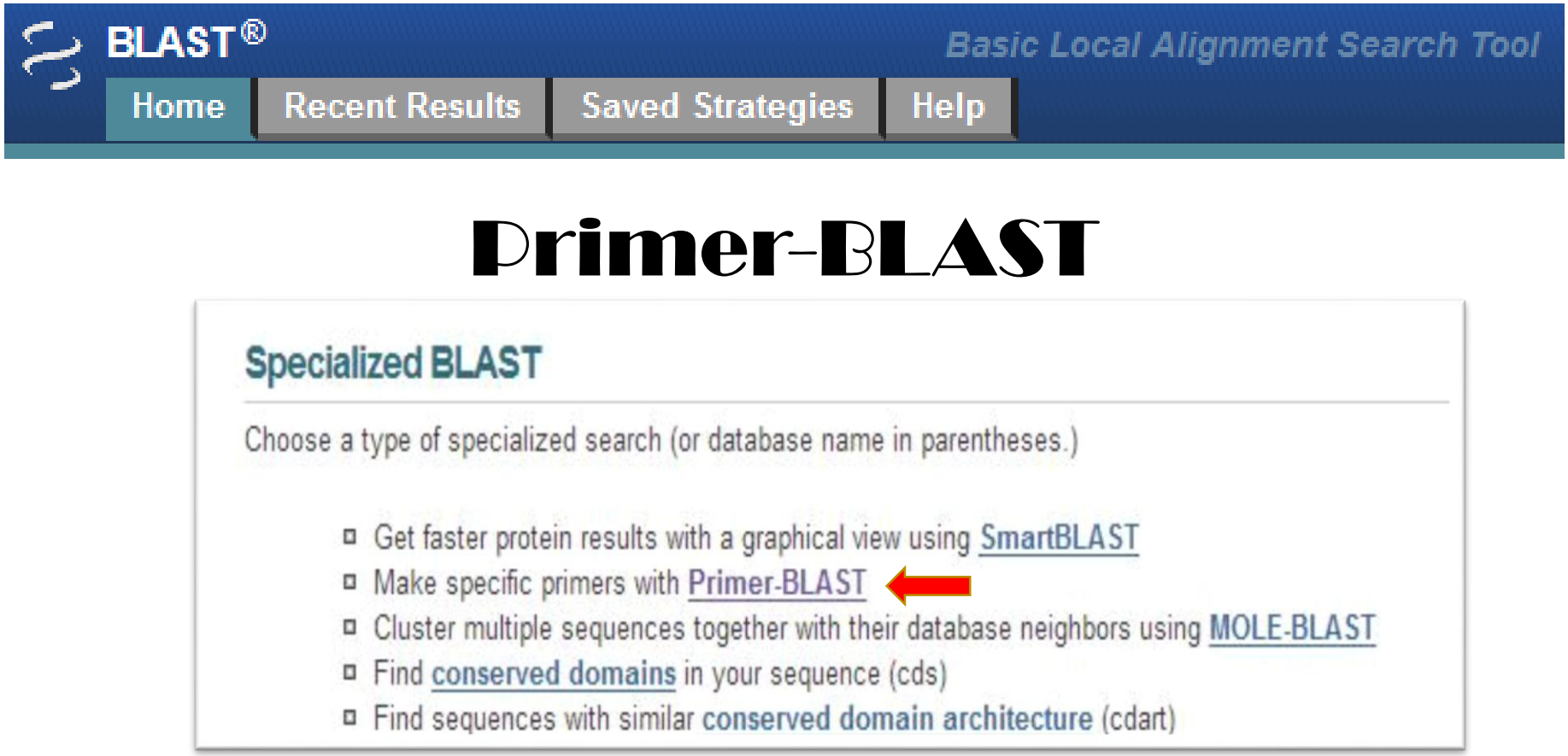


——引自朱曦Bio2000

OUTLINE

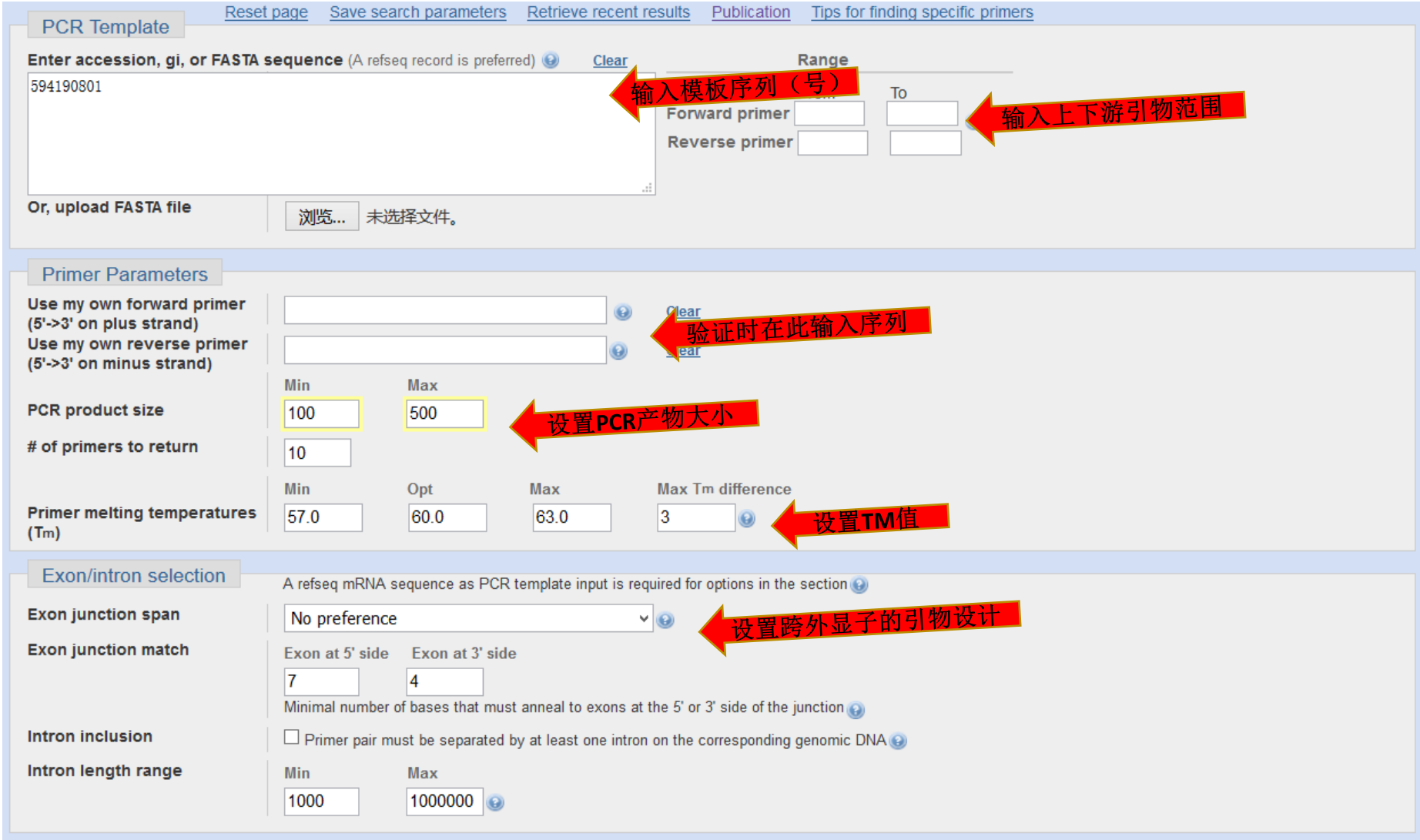
背景介绍 1 | 设计与检索 2 | 验证 3

- **Primer-BLAST**
- **BioWeb and Biology WorkBench**
- **primer premier 5.0**
- **ProbeBase**


BLAST®
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
Primer-BLAST
Specialized BLAST
Choose a type of specialized search (or database name in parentheses.)
Get faster protein results with a graphical view using SmartBLAST
Make specific primers with Primer-BLAST
Cluster multiple sequences together with their database neighbors using MOLE-BLAST
Find conserved domains in your sequence (cds)
Find sequences with similar conserved domain architecture (cdart)

2 Design & Search

Primer-BLAST



2 Design & Search

Primer-BLAST

Primer Pair Specificity Checking Parameters

Specificity check: ☑ Enable search for primer pairs specific to the intended PCR template

Search mode: Automatic

Database: Refseq mRNA — 设置检索数据库

Exclusion: ☑ Exclude predicted Refseq transcripts (accession with XM, XR prefix) ☑ Exclude uncultured/environmental sample sequences

Organism: Homo sapiens — 选择物种

Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.

Add more organisms

Entrez query (optional)

Primer specificity stringency

Primer must have at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps at the 3' end.

Ignore targets that have 6 or more mismatches to the primer.

Max target size: 4000

Splice variant handling: ☐ Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)

Primer Parameters

高级设置里的参数

	Min	Opt	Max	
PCR Product Tm				产物TM值
Primer Size	15	20	25	引物大小
Primer GC content (%)	20.0		80.0	引物GC比
GC clamp	0			设置GC夹
Max Poly-X	5			最大单核苷酸聚合体
Max 3' Stability	9			
Max GC in primer 3' end	5			

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTGCAACCTGACTTCCACCT	Plus	20	3840	3859	59.89	55.00	4.00	0.00
Reverse primer	AAGGTTGGGTGAGTGACACC	Minus	20	4151	4132	59.82	55.00	5.00	3.00
Product length	312								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTGCTCTCTGCAACCTGACT	Plus	20	3833	3852	59.68	55.00	4.00	1.00
Reverse primer	CCCTAGACATCTGGGAGCCA	Minus	20	4008	3989	60.40	60.00	6.00	3.00
Product length	176								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCAACCTGACTTCCACCTCT	Plus	20	3842	3861	59.60	55.00	2.00	0.00
Reverse primer	TGGACACTGGGAGAGCTAGT	Minus	20	4178	4159	59.59	55.00	4.00	2.00
Product length	337								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACAATCCTCCCACGACCCTT	Plus	20	3884	3903	60.84	55.00	2.00	0.00
Reverse primer	TCTTGGAGTCTGGAGCCCTA	Minus	20	4023	4004	59.29	55.00	3.00	2.00
Product length	140								

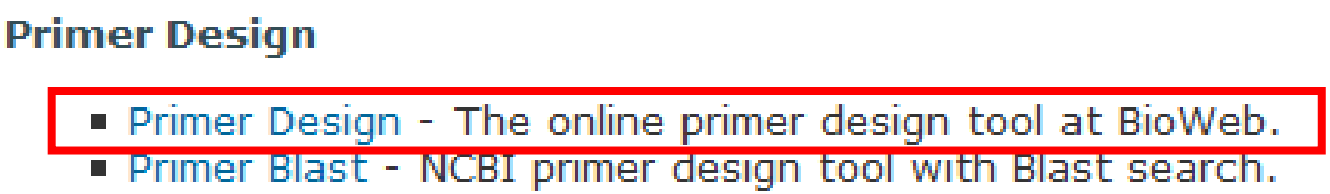
Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTGCACAATCCTCCCACGA	Plus	19	3880	3898	59.70	57.89	4.00	1.00
Reverse primer	CAAGCCAGCGTCTGAGGAAG	Minus	20	4080	4061	61.02	60.00	4.00	1.00
Product length	201								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCAGATCCCTCGGTCAGCA	Plus	20	3396	3415	61.04	60.00	4.00	0.00
Reverse primer	GGATTGTGCAGCAGGAGAGAA	Minus	21	3890	3870	60.34	52.38	5.00	0.00
Product length	495								

ABC ⟶ Tools ⟶ Primer design ⟶ Bioweb



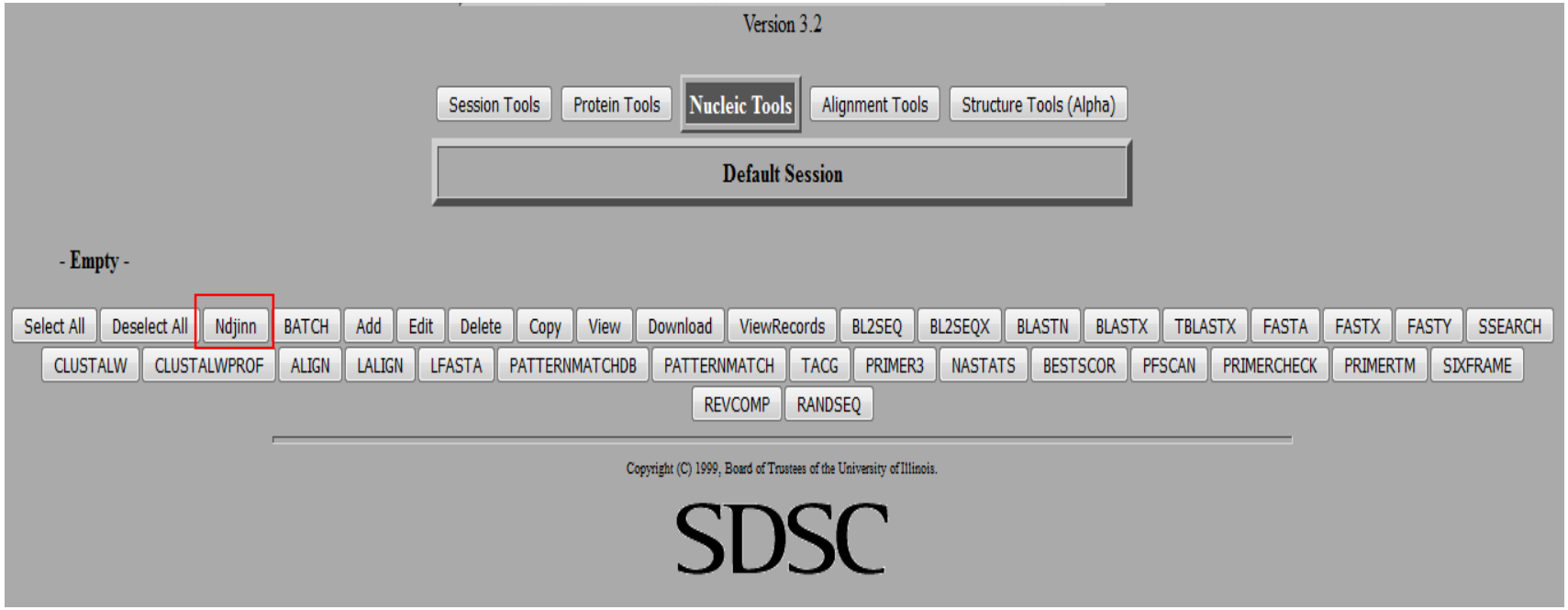
BioWeb

SDSC
SAN DIEGO SUPERCOMPUTER CENTER

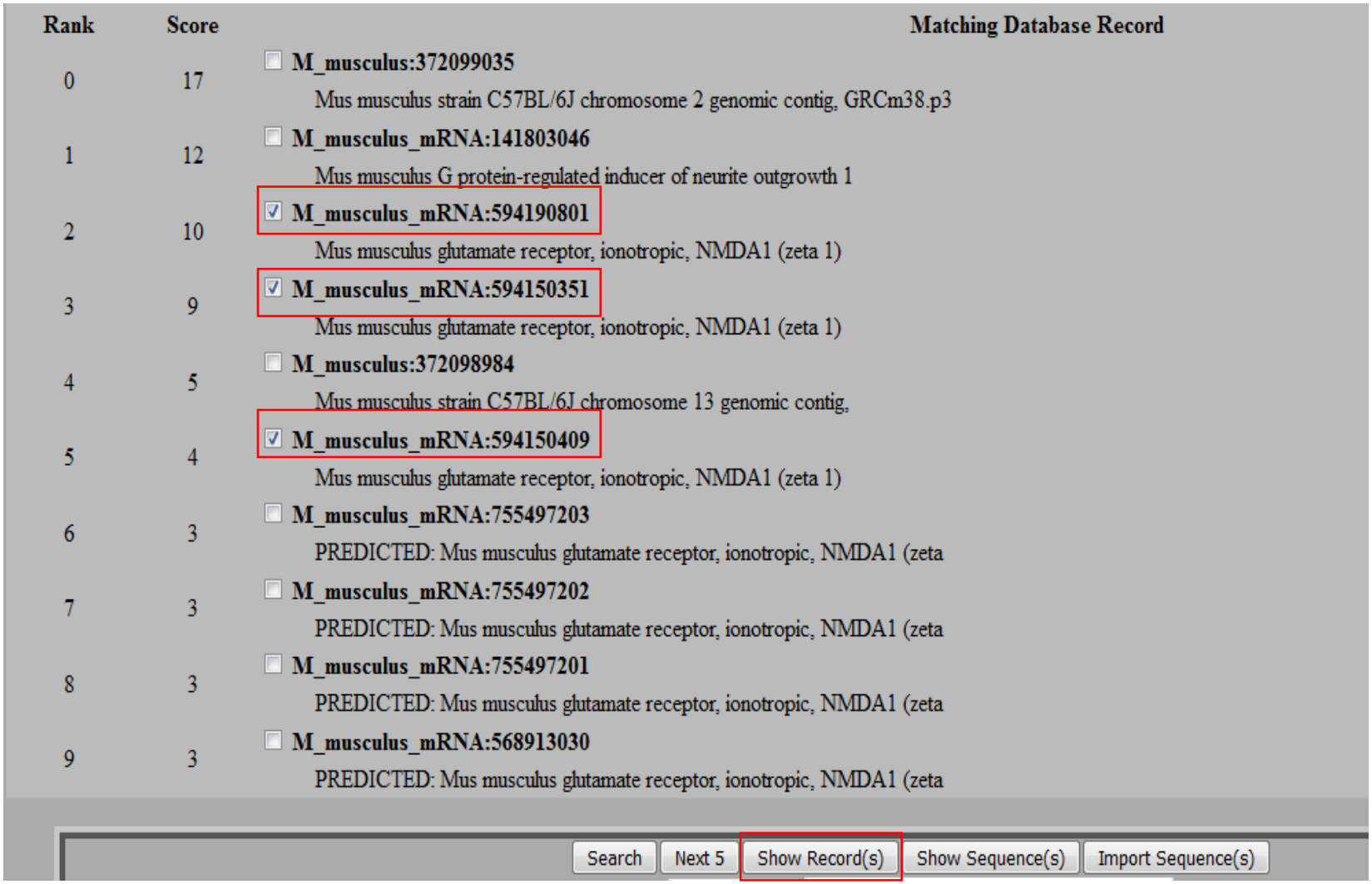
Biology WorkBench

(1) Nucleic tools

- 选择Ndjinn，输入基因名，进行检索

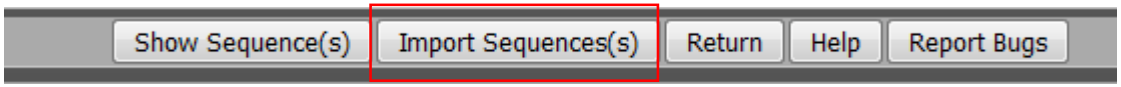


(1) Nucleic tools



(1) Nucleic tools

Locus	NM_008169 4326 bp mRNA linear ROD 17-MAY-2014
Definition	**Mus musculus glutamate receptor, ionotropic, NMDA1 (zeta 1) (Grin1), transcript variant 1, mRNA.**
Accession	NM_008169 VERSION NM_008169.3 GI:594190801
Keywords	RefSeq.
Source	Mus musculus (house mouse)
Organism	**Mus musculus** • Eukaryota • Metazoa • Chordata

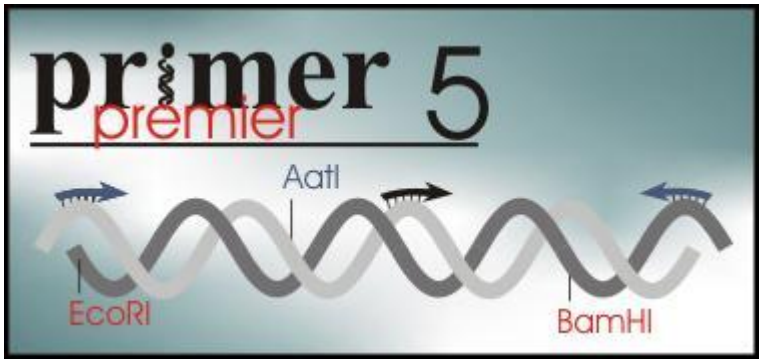


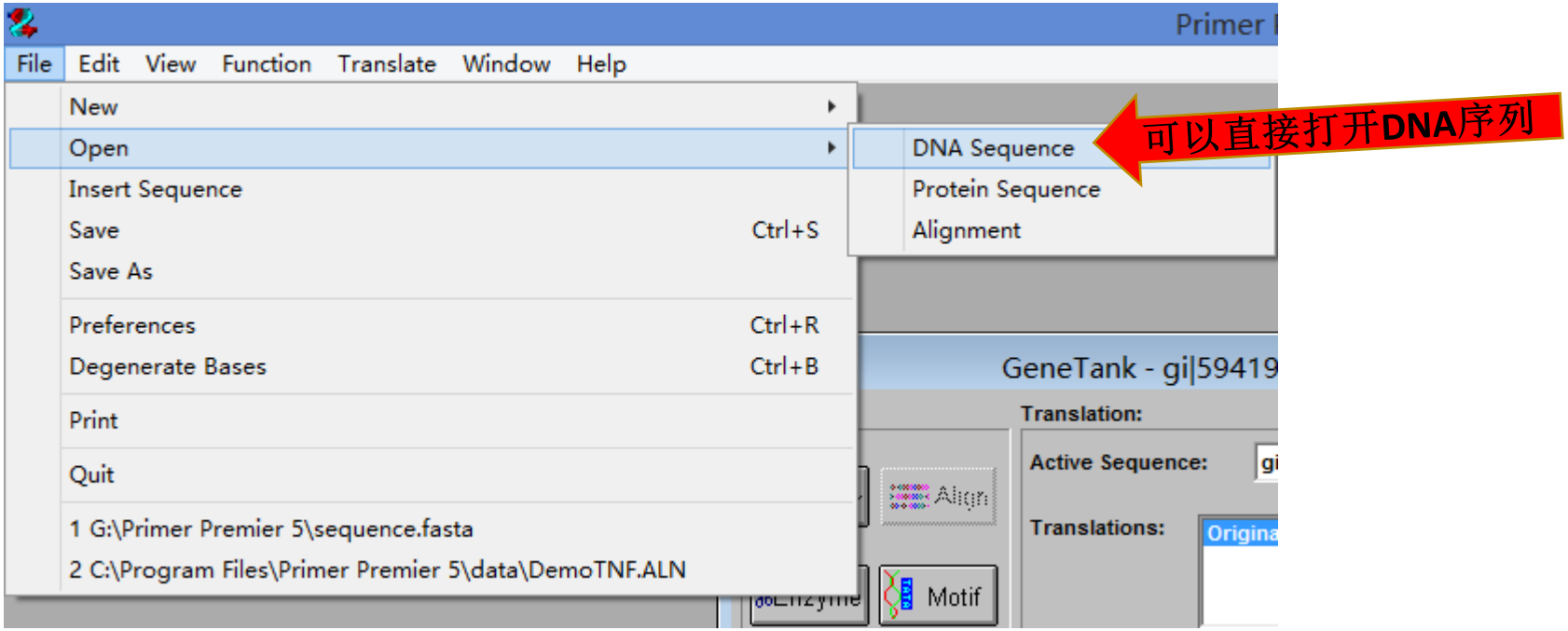
(2) Primer design

GC Clamp Size 设置GC夹	0	Number of 3' bases required to be either G or C (Default is 0 bases)
Optimal Primer Size 最适引物大小	20	Primer3 will attempt to select primers near this size. (Default is 20 bases; Maximum = 35)
Minimum Primer Size 最小引物大小	18	Minimum acceptable primer length. (Default is 18 bases; Maximum = 35)
Maximum Primer Size 最大引物大小	27	Maximum acceptable primer length. (Default is 27 bases; Maximum = 35)
Optimal Melting Temperature (°C) 最适TM值		Primer3 will attempt to select primers with TM's near this value. (Default is 60.0°C)
Minimum Melting Temperature (°C) 最小TM值		Minimum acceptable melting temperature. (Default is 57.0°C)
Maximum Melting Temperature (°C) 最大TM值	63	Maximum acceptable melting temperature. (Default is 63.0°C)
Maximum Melting Temperature Difference (°C) 最大TM值差	100	Maximum acceptable difference in melting temperatures between paired primers. (Default is 100.0°C)
Minimum GC Content (Percent) 最小TM值		Minimum GC content allowed in any primer. (Default is 20.0%)
Maximum GC Content (Percent) 最小TM值		Maximum GC content allowed in any primer. (Default is 80.0%)
Salt Concentration (mM)	50	Millimolar concentration of salt in the amplification reaction. (Default is 50.0mM)
DNA Concentration (nM)	50	Nanomolar concentration of DNA in the amplification reaction. (Default is 50.0nM)
Maximum Number Unknown Bases	0	Maximum number of unknown bases allowable in any primer. (Default is 0 bases)
Accept ambiguous bases? (0 = No; 1 = Yes)	0	accept IUB / IUPAC codes for ambiguous bases (i.e. by changing all unrecognized bases to N). If you wish to include an ambiguous base in an oligo, you must set maximum number of unknown bases allowable in any primer (the parameter above) to a non-0 value. (Default is 0 - Don't accept ambiguous bases)
Maximum Similarity Score for Complementarity	8	Maximum allowable similarity score for self-complementarity and primer pair complementarity. (Default is 8.00)

(2) Primer design

		start	len	tm	gc%	any	3'	seq	
1	LEFT PRIMER	596	20	60.01	55.00	2.00	0.00	AACGACCACTTCACTCCCAC	☐
	RIGHT PRIMER	777	20	59.98	50.00	6.00	1.00	TTGTAGACGCGCATCATCTC	☐
	PRODUCT SIZE: 182, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 0.00								
2	LEFT PRIMER	590	20	60.01	55.00	2.00	0.00	ACTCCCAACGACCACTTCAC	☐
	RIGHT PRIMER	775	20	59.98	50.00	6.00	0.00	GTAGACGCGCATCATCTCAA	☐
	PRODUCT SIZE: 186, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00								
3	LEFT PRIMER	590	20	60.01	55.00	2.00	0.00	ACTCCCAACGACCACTTCAC	☐
	RIGHT PRIMER	777	20	59.98	50.00	6.00	1.00	TTGTAGACGCGCATCATCTC	☐
	PRODUCT SIZE: 188, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 0.00								
4	LEFT PRIMER	460	20	59.98	55.00	6.00	2.00	CGGCTCTTGGAAGATACAGC	☐
	RIGHT PRIMER	615	20	60.01	55.00	2.00	0.00	GTGGGAGTGAAGTGGTCGTT	☐
	PRODUCT SIZE: 156, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00								


primer 5
premier
AatI
EcoRI
BamHI


File
Edit
View
Function
Translate
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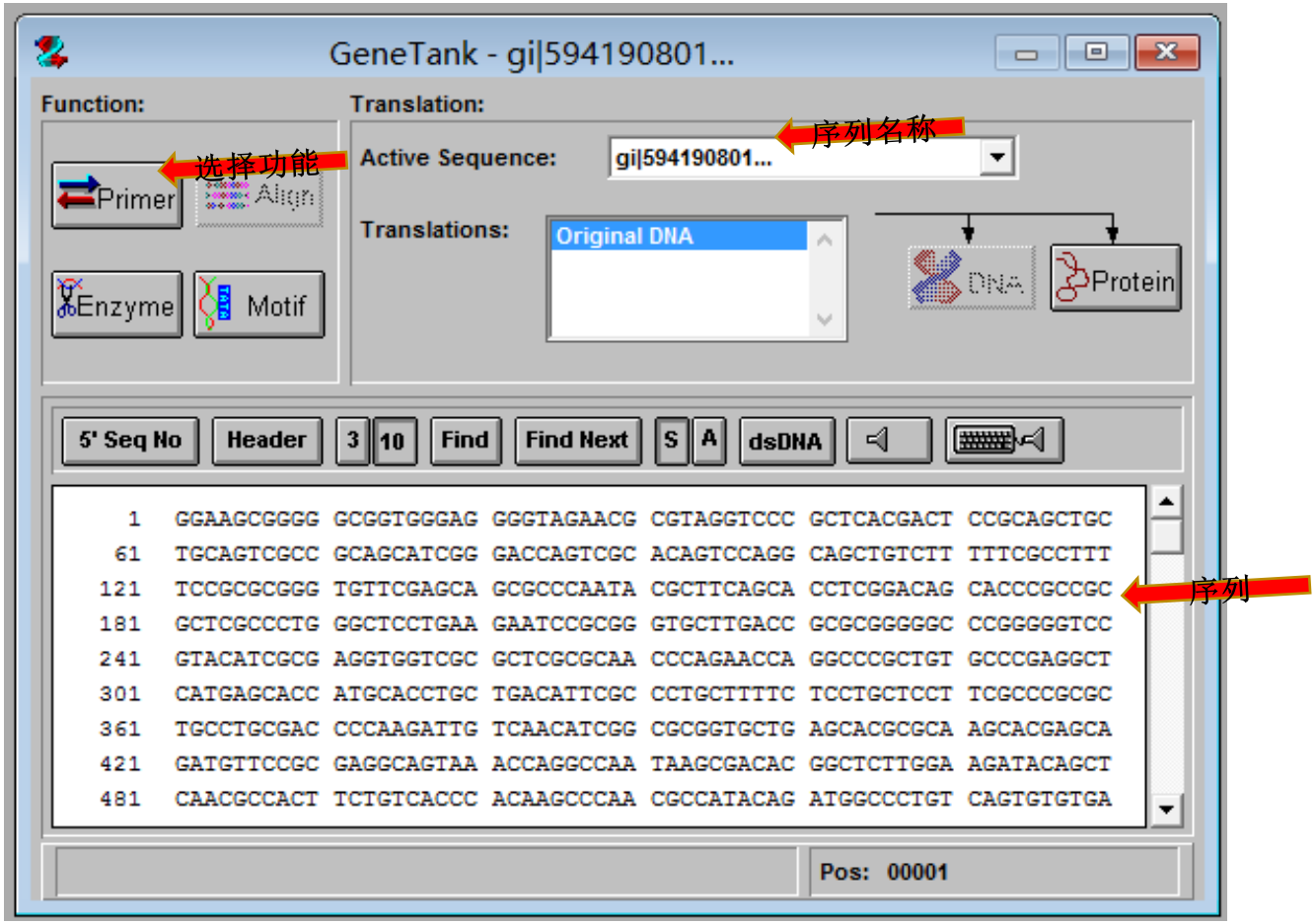
Help
New
Open
Insert Sequence
Save Ctrl+S
Save As
Preferences Ctrl+R
Degenerate Bases Ctrl+B
Print
Quit
1 G:\Primer Premier 5\sequence.fasta
2 C:\Program Files\Primer Premier 5\data\DemoTNF.ALN
DNA Sequence
Protein Sequence
Alignment
可以直接打开DNA序列
GeneTank - gi|59419
Translation:
Active Sequence:
Translations:
Align
Motif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选择功能
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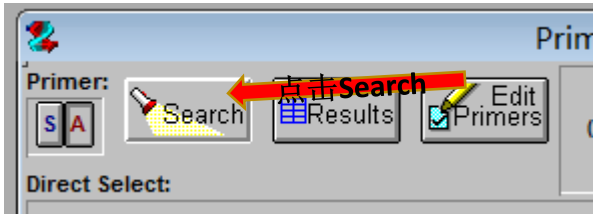
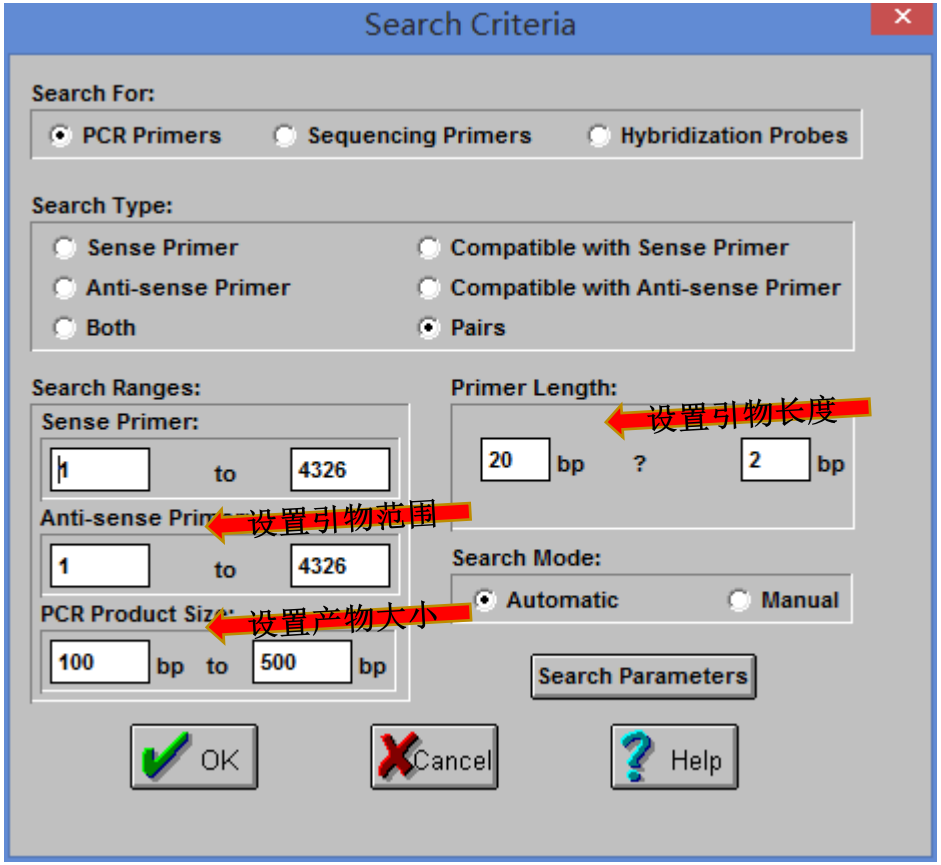
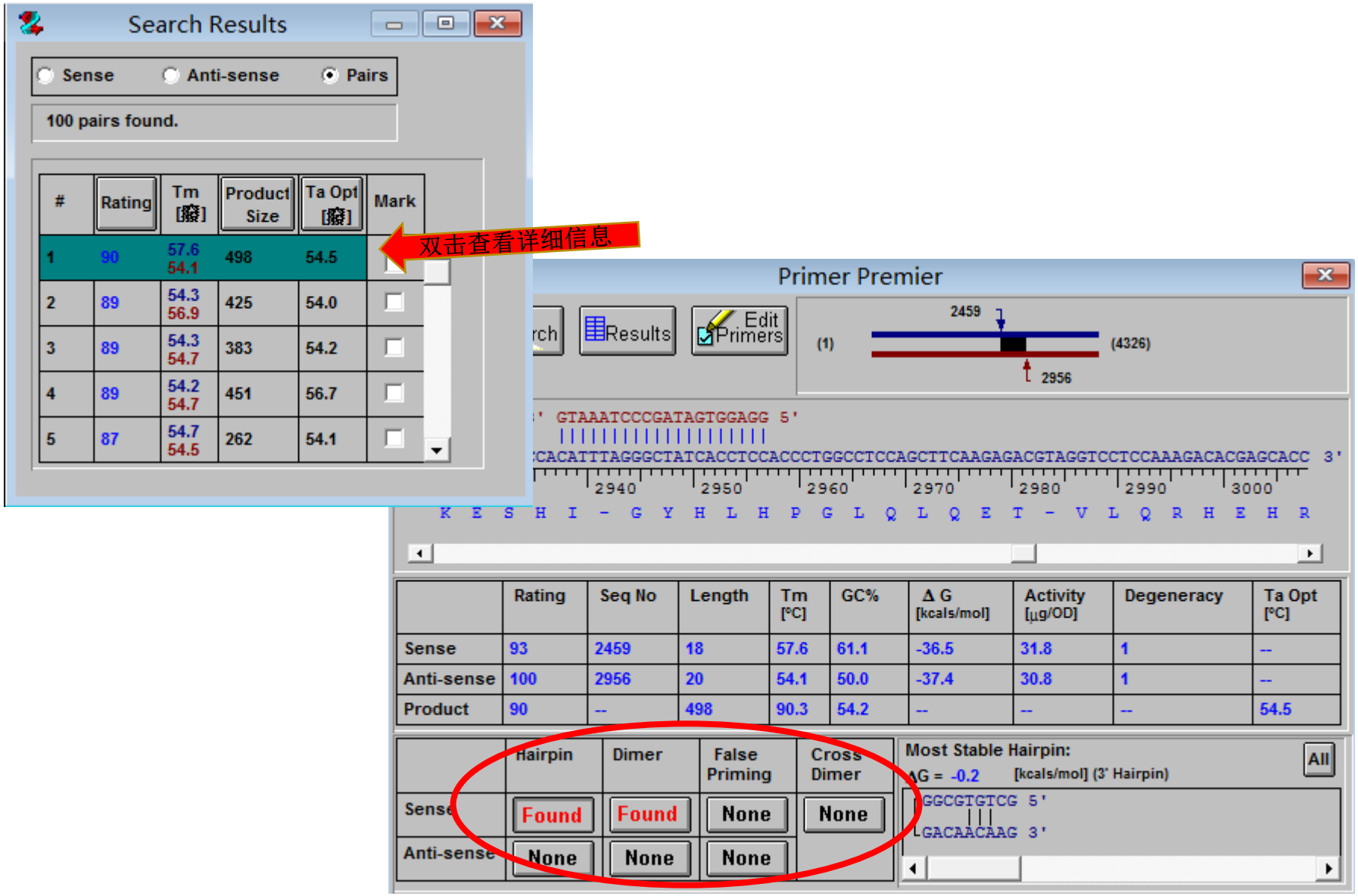
Primer
Align
Enzyme
Motif
Active Sequence:
gi|594190801...
Translations:
Original DNA
DNA
Protein
5' Seq No
Header
3
10
Find
Find Next
S
A
dsDNA
1 GGAAGCGGGG GCGGTGGGAG GGGTAGAACG CGTAGGTCCC GCTCACGACT CCGCAGCTGC
61 TGCAGTCGCC GCAGCATCGG GACCAGTCGC ACAGTCCAGG CAGCTGTCTT TTTCGCCTTT
121 TCCGCGCGGG TGTTCGAGCA GCGCCCAATA CGCTTCAGCA CCTCGGACAG CACCCGCCGC
181 GCTCGCCCTG GGCTCCTGAA GAATCCGCGG GTGCTTGACC GCGCGGGGGC CCGGGGGTCC
241 GTACATCGCG AGGTGGTCGC GCTCGCGCAA CCCAGAACCA GGCCCGCTGT GCCCGAGGCT
301 CATGAGCACC ATGCACCTGC TGACATTCGC CCTGCTTTTC TCCTGCTCCT TCGCCCGCGC
361 TGCCTGCGAC CCCAAGATTG TCAACATCGG CGCGGTGCTG AGCACGCGCA AGCACGAGCA
421 GATGTTCCGC GAGGCAGTAA ACCAGGCCAA TAAGCGACAC GGCTCTTGGA AGATACAGCT
481 CAACGCCACT TCTGTCACCC ACAAGCCCAA CGCCATACAG ATGGCCCTGT CAGTGTGTGA
序列
Pos: 00001


Prim
Primer:
Search
Results
Edit Primers
Direct Select:
点击Search


Search Criteria
Search For:
PCR Primers
Sequencing Primers
Hybridization Probes
Search Type:
Sense Primer
Anti-sense Primer
Both
Compatible with Sense Primer
Compatible with Anti-sense Primer
Pairs
Search Ranges:
Sense Primer:
1
to
4326
Anti-sense Primer:
1
to
4326
PCR Product Size:
100
bp
to
500
bp
Primer Length:
20
bp
?
2
bp
Search Mode:
Automatic
Manual
Search Parameters
OK
Cancel
Help
设置引物长度
设置引物范围
设置产物大小


Search Results
Sense
Anti-sense
Pairs
100 pairs found.
Rating Tm [℃] Product Size Ta Opt [℃] Mark
1 90 57.6 54.1 498 54.5
2 89 54.3 56.9 425 54.0
3 89 54.3 54.7 383 54.2
4 89 54.2 54.7 451 56.7
5 87 54.7 54.5 262 54.1
双击查看详细信息
Primer Premier
Results
Edit Primers
2459
(1)
(4326)
2956
GTAAATCCCGATAGTGGAGG 5'
CACATTTAGGGCTATCACCTCCACCCTGGCCTCCAGCTTCAAGAGACGTAGGTCCTCCAAAGACACGAGCACC 3'
2940 2950 2960 2970 2980 2990 3000
K E S H I - G Y H L H P G L Q L Q E T - V L Q R H E H R
Rating Seq No Length Tm [℃] GC% Δ G [kcals/mol] Activity [μg/OD] Degeneracy Ta Opt [℃]
Sense 93 2459 18 57.6 61.1 -36.5 31.8 1 --
Anti-sense 100 2956 20 54.1 50.0 -37.4 30.8 1 --
Product 90 -- 498 90.3 54.2 -- -- -- 54.5
Hairpin Dimer False Priming Cross Dimer
Sense Found Found None None
Anti-sense None None None
Most Stable Hairpin:
All
ΔG = -0.2 [kcals/mol] (3' Hairpin)
GGCGTGTCG 5'
GACAACAAG 3'

DOME Divison of Microbial Ecology

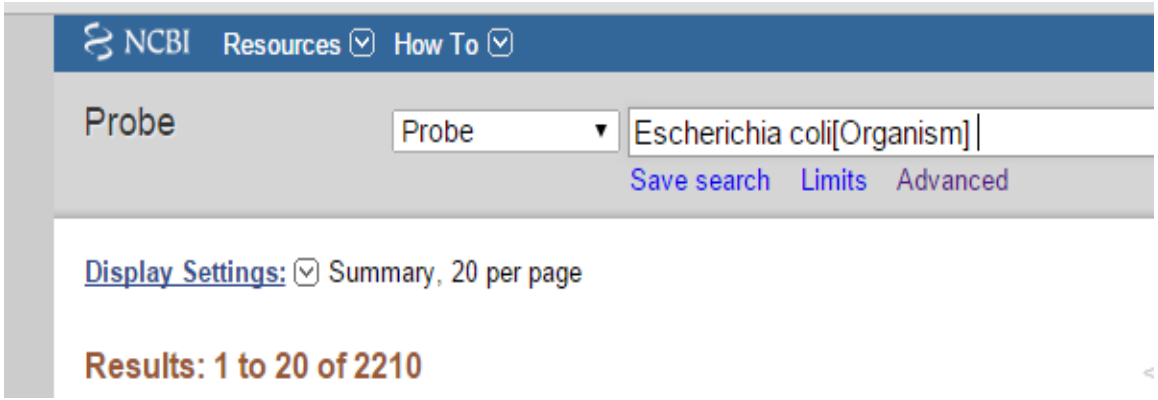
probeBase 2016

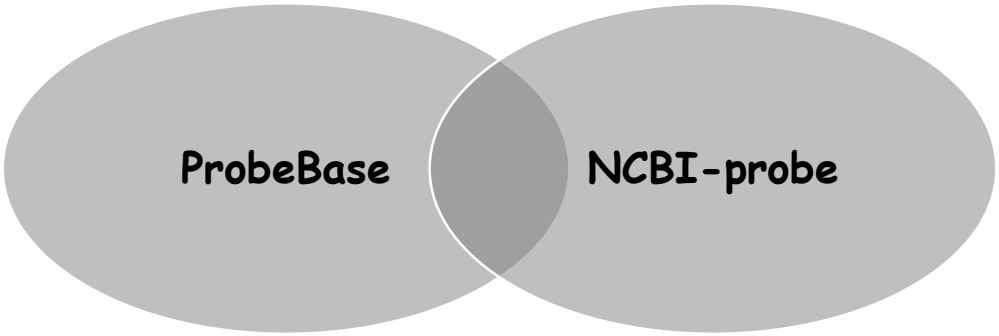
An online resource for rRNA-targeted oligonucleotides

(1)Search

A. 根据**organism**搜索

- 对比NCBI-probe数据库中的检索结果，各有交叉





(1)Search

B. 根据primer/probe names搜索

Uni1390R	To be used as primer!
Accession no.	pB-3760
Taxonomy	cellular organisms
Specificity	Bacteria, Archaea, Eukarya
Category	primer
Full name (Alm et al. 1996)	S-*-Univ-1390-a-A-18
Position	1390-1407
Direction	reverse primer
Sequence	5'- GACGGGCGGTGTGTACAA -3'
G+C content [%]	61
Length [nt]	18
Check specificity/coverage	silva RDP
Evaluate primer pair	Select 2nd primer
Tm	
Formamide [%]	not determined
Reference(s)	Zheng D., Alm E. W., Stahl D. A. and Raskin L. (1996). Characterization of universal small-subunit rRNA hybridization probes for quantitative molecular microbial ecology studies. Appl. Environ. Microbiol. 62: 4504-4513. Pubmed

选取E.coli的一个探针

探针名: S-*-EBAC-1790-a-A-18

- 对比NCBI-probe数据库中的检索结果

序列信息一致，但所引用的参考文献不同，且probebase的注释信息更丰富

Synopsis

Field Name	Values
Name	S-*-Univ-1390-a-A-18
Type	microarray element
Application	gene expression
Source organism	
Source sequence	
Target organism	cellular organisms

Sequences

>Probe|10169021|ASSAY_SEQUENCE assay sequence (18b)
GACGGGCGGTGTGTACAA

References

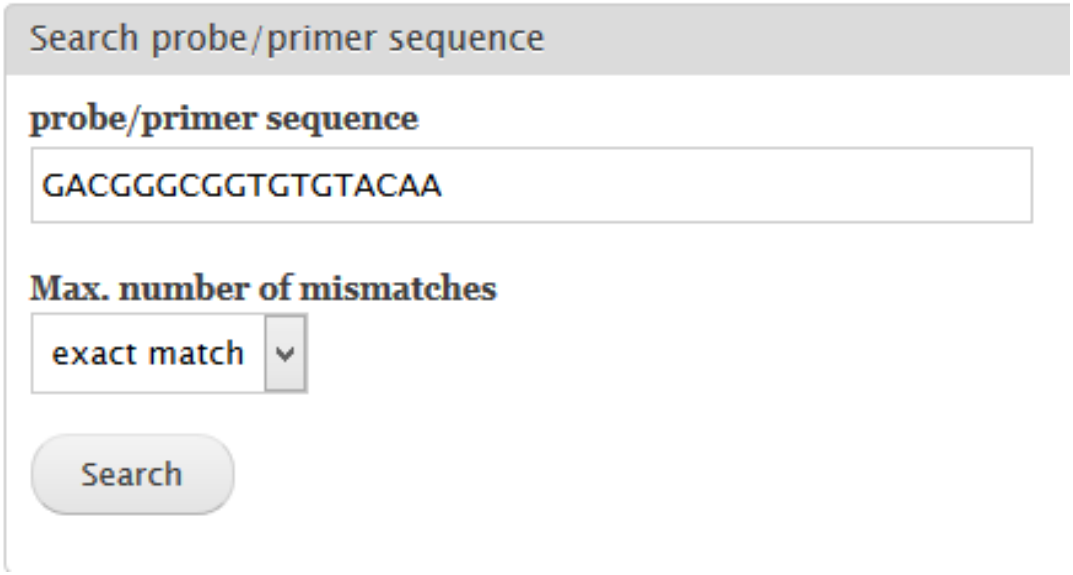
1. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations.
Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA.
Appl Environ Microbiol. 1990 Jun;56(6):1919-25.
PMID: 2200342
Other Probes in this publication

(1)Search

C. 根据sequence搜索

- 检索探针

  按照5’→3’的顺序输入序列 (无其他符号或空格)



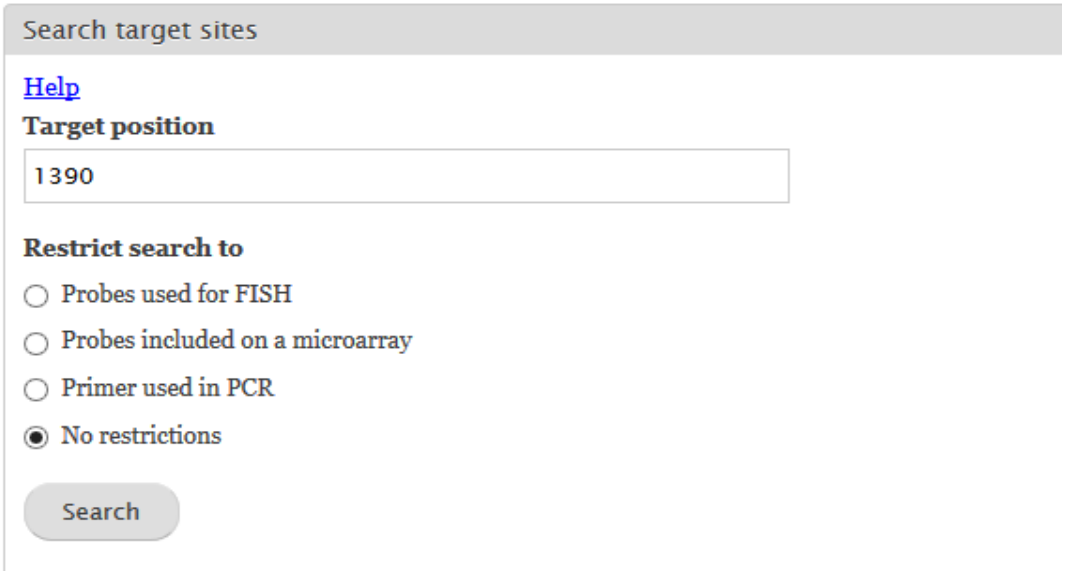
(1)Search

D. 根据target sites搜索

- 根据靶标位点的碱基号进行检索

  已知上述探针的靶标位点是1390-1407

Position	1390-1407
Direction	reverse primer
Sequence	5'- GACGGGCGGTGTGTACAA -3'



(2) Match

- Match中的功能主要是寻找与已知rRNA序列相配对的探针
- 要求rRNA序列的FASTA格式，以E.coli 16S ribosomal RNA为例。

E.coli 16S ribosomal RNA

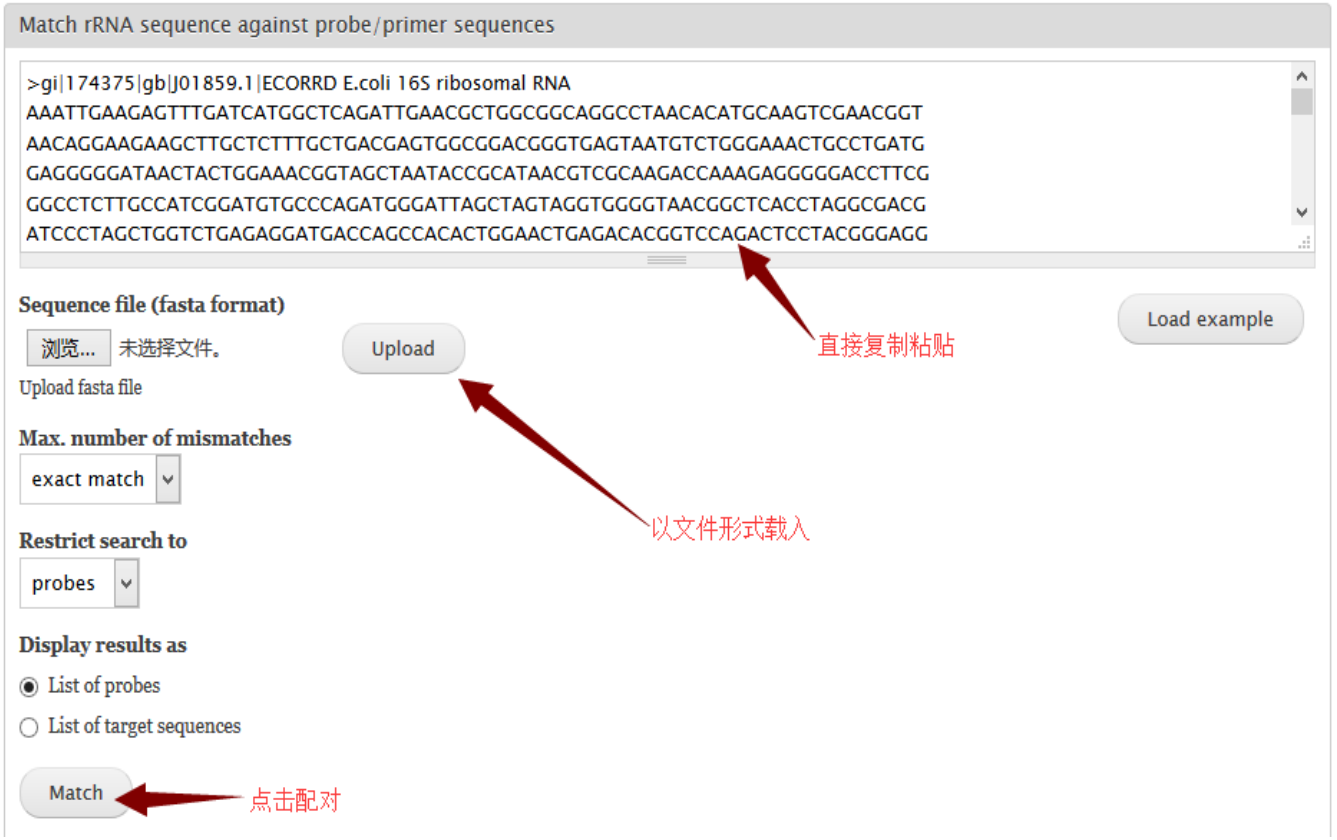
GenBank: J01859.1

GenBank Graphics

>gi|174375|gb|J01859.1|ECORRD E.coli 16S ribosomal RNA

AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGT
AACAGGAAGAAGCTTGCTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATG
GAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCG
GGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACG
ATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTT
CGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCG
CAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAAT
TACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAC
TGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGT
AGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCC
TTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACT
CAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCT
TACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGC
TGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCT
TTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGA
CGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGA
CCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATG
AAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTT
TGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTT
A

- 导入序列，点击配对


Match rRNA sequence against probe/primer sequences
>gi|174375|gb|J01859.1|ECORRD E.coli 16S ribosomal RNA
AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGT
AACAGGAAGAAGCTTGCTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATG
GAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCG
GGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACG
ATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
直接复制粘贴
Load example
Sequence file (fasta format)
浏览...
未选择文件。
Upload
Upload fasta file
以文件形式载入
Max. number of mismatches
exact match
Restrict search to
probes
Display results as
List of probes
List of target sequences
Match
点击配对

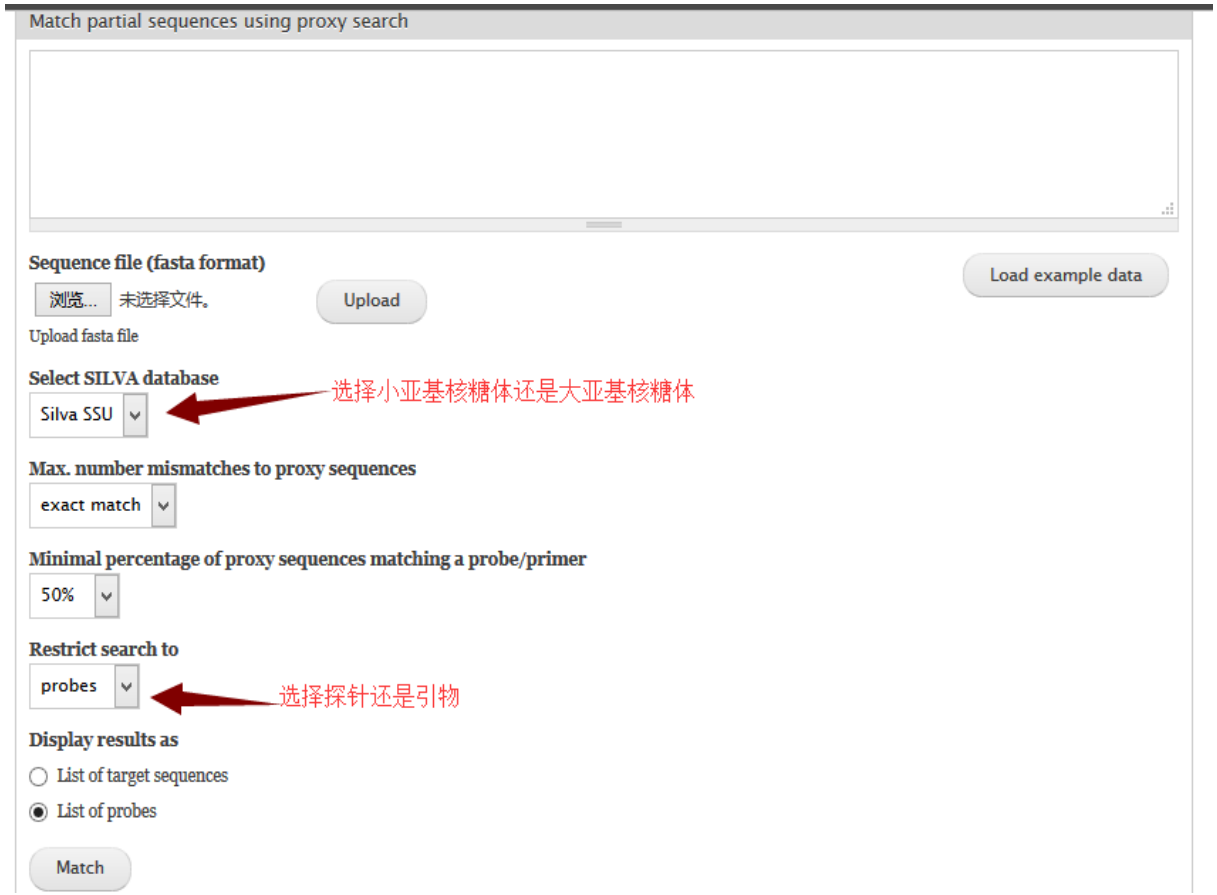
- 弹出配对结果

Probes found targeting query sequence(s)

	Probe name	Specificity	No. of target hits
[+]	7	Bacteria	1
[+]	Bact1492	most Bacteria	1
[+]	Enterbact D	some members of the Enterobacteriaceae	1
[+]	6	Bacteria	1
[+]	3	Universal	1
[+]	Eub927	most Bacteria	1
[+]	E11	Eubacteria	1
[+]	Enterbact B	some members of the Enterobacteriaceae and Vibrionaceae	1
[+]	5	Bacteria	1
[+]	ENT183	Enterobacteriaceae	1
[+]	EUB342	Most Bacteria	1
[+]	UNIV1389a	Bacteria, not Epsilonproteobacteria	1
[+]	Enter1432	enteric group (Enterobacteriales)	1
[+]	UNIV1390	all Organisms	1
[+]	Ecoli1b	Escherichia/Citrobacter/Salmonella/Shigella cluster	1
[+]	EUB338 (Bact338) (bacterial)	most Bacteria	1

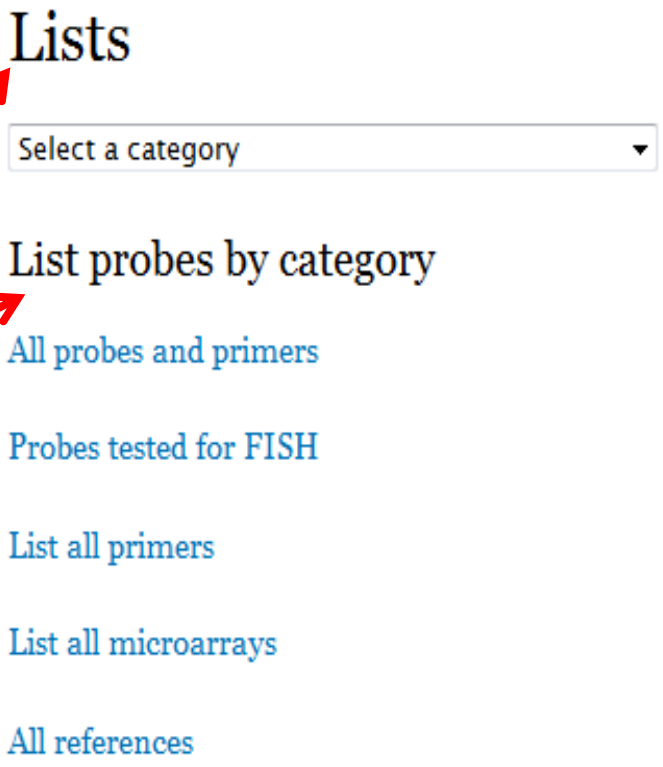
(3) Proxy

- Match功能的补充，proxy是对部分的序列，通过SILVA数据库进行检索
- 可以选择小亚基核糖体还是大亚基核糖体，可以选择是找探针还是引物



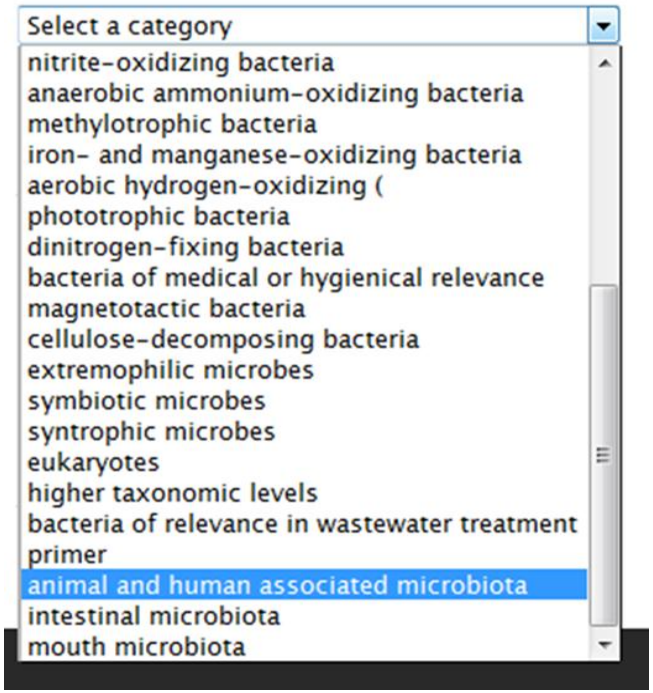
(4) Lists

- 分为两部分，一部分是下拉框（Select a category），一部分是list probes by category。



(4) Lists

A. Select a category

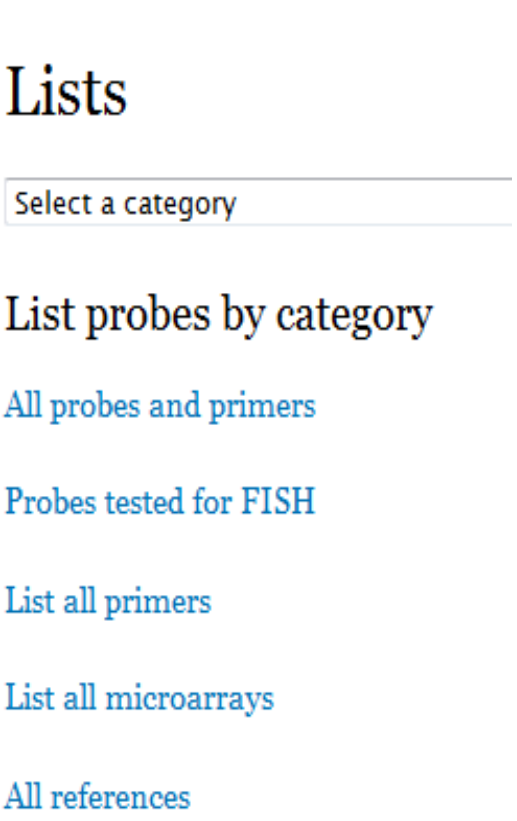


Probes targeting animal and human associated microbiota

Probe name	Specifity
MUC-1437	Akkermansia muciniphila
Acac194	Anaerostipes caccae
Aqua828	Aquabacterium spp.
ATO291	Atopobium cluster
Bacid1	B. acidifaciens Group 1
Bacid2	B. acidifaciens Group 2

(4) Lists

A. list probes by category



1 List of all probes and primers

2 List of probes tested for in situ hybridization

3 List of all primers

4 Complete list of DNA microarrays at probeBase

5 List of papers

Reference
Adamczyk J., Hesselsoe M., Iversen N., Horn M., Lehner A., Nielsen P. H., Schloter M., Roslev P. and Wagner M. (2003).The isotope array, a new tool that employs substrate-mediated labeling of rRNA for determination of microbial community structure and function. Appl. Environ. Microbiol. 69: 6875-6887.
Allen MA, Goh F, Burns BP, Neilan BA. Bacterial, archaeal and eukaryotic diversity of smooth and pustular microbial mat communities in the hypersaline lagoon of Shark Bay. Geobiology 7, 82-96 (2009).
Amann R. I., Binder B. J., Olson R. J., Chisholm S. W., Devereux R. and Stahl D. A. (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56: 1919-1925.
Amann R. I., Krumholz L. and Stahl D. A. (1990). Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. 172: 762-770.

(5) Submission

➢Contact information:

First name, Last name, Email, Institute, University, City, Postal code, Country, Website

➢Probe information:

Short name, Probe name, Specificity, Taxonomy, Category, Target molecule, Position, Sequence, Competitor, Formamide, Remarks

➢Pubmed information:

PubmedID, Journal, Title, Authors, Year, Abstract

(6) Links

rRNA-related web resources

ARB A software environment for sequence data
SILVA Resource for quality checked and aligned ribosomal RNA sequence data.
The Ribosomal Database Project II (RDP) 16S rRNA database and online analysis tools
Greengenes 16S rRNA database and ARB compatible online-workbench
'The All-Species Living Tree' Project Curated rRNA datasets and trees including all sequenced type strains of hitherto classified species of Archaea and Bacteria.
probeCheck A central resource for evaluating probe and primer specificity
TestProbe SILVA Probe Match and Evaluation Tool
mathFISH A web tool for the computational evaluation of RNA-targeted FISH probes using Mathematical models
EzTaxon 16S rRNA gene sequence of type strains of validly published species
Sapelo Island Microbial Observatory (SIMO) 16S rRNA Database
rrndb rRNA Operon Number Database
CRW The Comparative RNA Web Site
The Ribosomal RNA Mutation Database
The RNA World Website Lists of links on RNA related topics

Taxonomy of Prokaryotes

Bacterial Nomenclature Up-to-date
List of Bacterial names with Standing in Nomenclature

Oligo


Oligo® .net
Primer Analysis Software
Since 1989
by
Molecular Biology Insights
Home
Tutorials
Downloads
Ordering
Contact Us

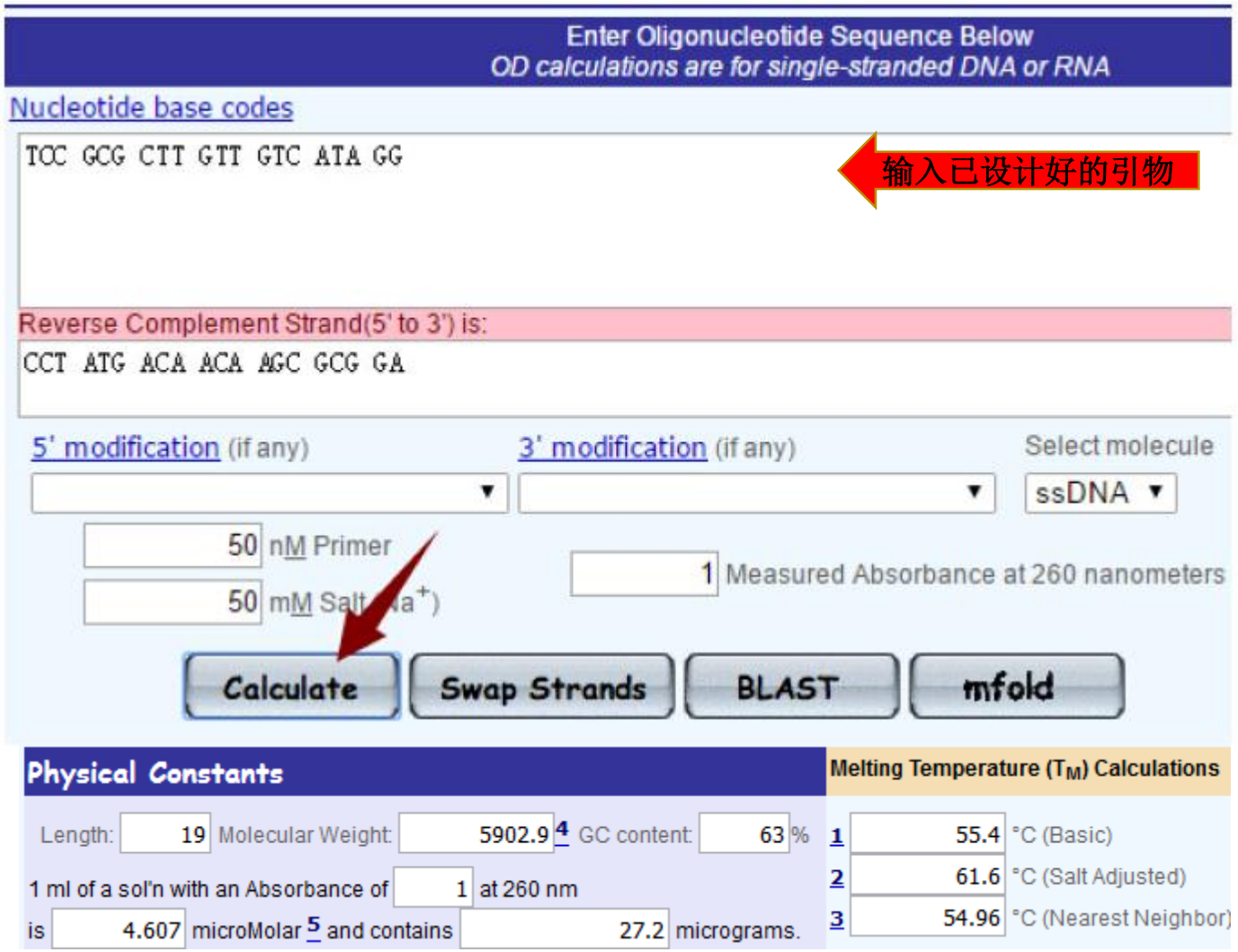
OUTLINE

1 背景介绍

2 设计与检索

3 验证

Oligonucleotide Properties Calculator



Oligonucleotide Properties Calculator

- 检验序列的Tm 值和GC 比是否符合要求
- 检验序列是否具有发卡结构，3’ 端互补重叠 (None可用)

Potential hairpin formation :

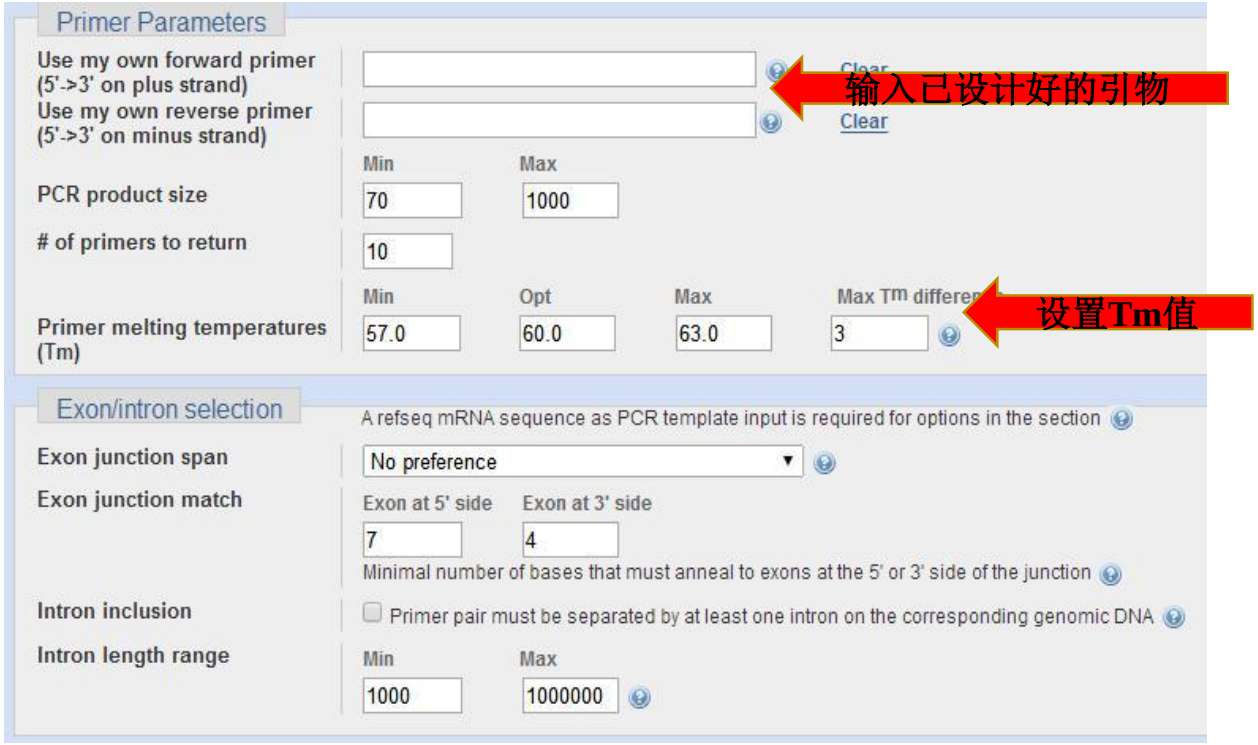
None !

3' Complementarity:
None !

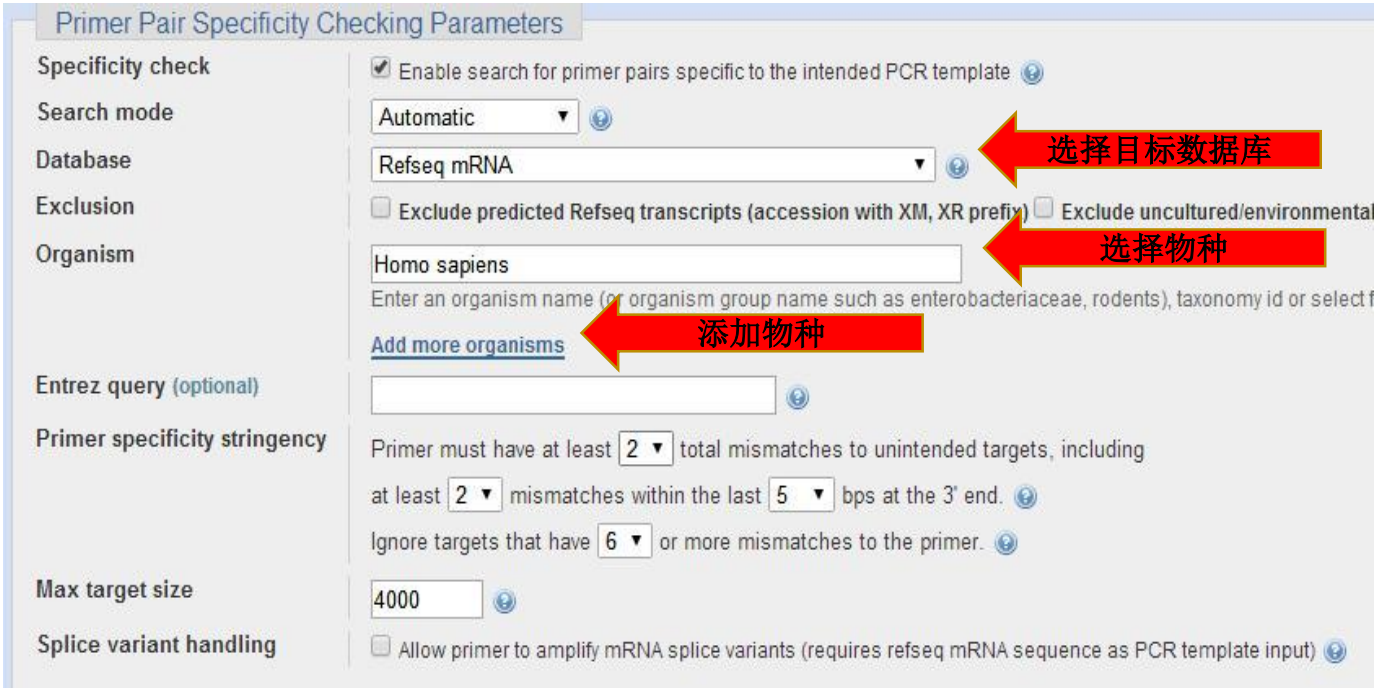
All potential self-annealing sites are marked in red (allowing 1 mis-match):

None !

Primer-BLAST



Primer-BLAST



根据相关参数，self complementarity和self 3‘ complementarity对应的数值越小越好，非特异性扩增和引物二聚体就越少。另外，检测出的模板越单一越好。

Thanks

G18

马越，邓迪，郭柏宏，刘璐萍