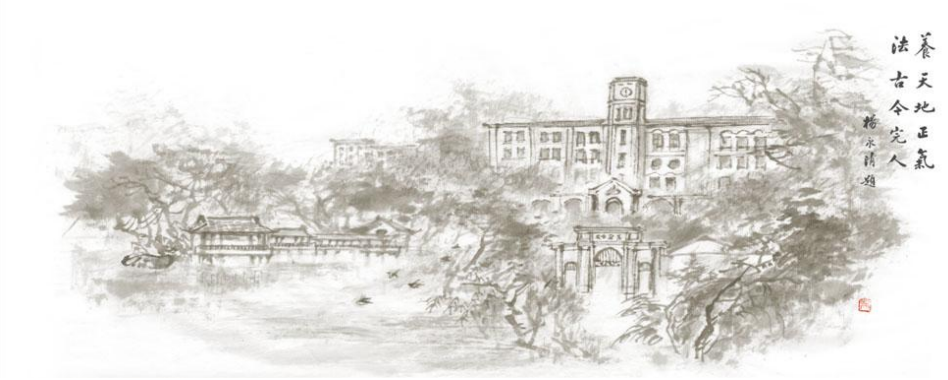

生物信息学
引物设计

张高川

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QQ: 2257916241

基因表达的分子生物学检测方法？
【RT-PCR => 引物设计】

如何设计特异性引物？
【Primer-Blast】

NCBI Resources How To Sign in to NCBI

NCBI
National Center for Biotechnology Information

All Databases | Search

NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation

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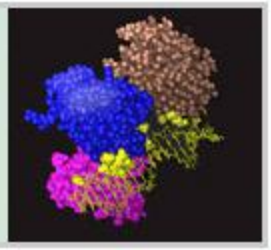
About the NCBI | Mission | Organization | Research | NCBI News

Get Started

- Tools: Analyze data using NCBI software
- Downloads: Get NCBI data or software
- How Tos: Learn how to accomplish specific tasks at NCBI
- Submissions: Submit data to GenBank or other NCBI databases

3D Structures

Explore three-dimensional structures of proteins, DNA, and RNA molecules. Examine sequence-structure relationships, active sites, molecular interactions, biological activities of bound chemicals, and associated biosystems.



II 1 2 3 4 5 6 7 8

Popular Resources

PubMed
Bookshelf
PubMed Central
PubMed Health
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem

NCBI Announcements

NCBI webinar A Submitter's Guide to GenBank, Part 2 on January 7th
Dec 31, 2014
On January 7th, NCBI will present the

GenBank release 205.0 is now available via FTP
Dec 16, 2014
Release 205.0 (12/12/2014) has

specific roles, they often work together in the cell as protein machines. REGULATORY NETWORKS UNC DOEGenomesToLife.org

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast, delta-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

Specialized BLAST

Choose a type of specialized search (or database name in parentheses.)

- 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)
- Search sequences that have gene expression profiles (GEO)
- Search immunoglobulins and T cell receptor sequences (IgBLAST)
- Screen sequence for vector contamination (vecscreen)
- Align two (or more) sequences using BLAST (bl2seq)
- Search protein or nucleotide targets in PubChem BioAssay
- Search SRA by experiment
- Constraint Based Protein Multiple Alignment Tool
- Needleman-Wunsch Global Sequence Alignment Tool
- Search RefSeqGene
- Search trace archives

Tip of the Day

How to save custom search pages.

So you have made a few BLAST searches and after adjusting the database, organism limits and maybe a few Algorithm Parameters you arrive at what you think is a good search strategy.

More tips...

Primer-BLAST *A tool for finding specific primers*

Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page | Save search parameters | Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range

From To

Forward primer **Reverse primer** Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	70	1000
# of primers to return	10	

	Min	Opt	Max	Max T_m difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span No preference

Exon junction match

Exon at 5' side	Exon at 3' side
7	4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

Min	Max
1000	1000000

Primer-BLAST *A tool for finding specific primers*

BI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Or, upload FASTA file 选择文件 未选择文件

Range

	From	To
Forward primer		
Reverse primer		

Clear

在这里输入模板序列

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	70	1000

# of primers to return: 10

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match

Exon at 5' side	Exon at 3' side
7	4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

Min	Max
1000	1000000

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Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range

From To

Forward primer

Reverse primer

Clear

Or, upload FASTA file 选择文件 未选择文件

在这里设定上下游引物搜索区间

Primer Parameters

Use my own forward primer (5'->3' on plus strand)

Use my own reverse primer (5'->3' on minus strand) Clear

PCR product size Min 70 Max 1000

# of primers to return 10

Primer melting temperatures (Tm) Min 57.0 Opt 60.0 Max 63.0 Max Tm difference 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span No preference

Exon junction match Exon at 5' side 7 Exon at 3' side 4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range Min 1000 Max 1000000

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Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range: From / To

Forward primer

Reverse primer

Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

在这里输入你的引物序列

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	70	1000
# of primers to return	10	

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match: Exon at 5' side 7; Exon at 3' side 4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min 1000; Max 1000000

Primer-BLAST *A tool for finding specific primers*

Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page | Save search parameters | Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range: From / To

Forward primer

Reverse primer

Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

PCR product size: Min 70, Max 1000

在这里输入产物大小

# of primers to return: 10

Primer melting temperatures (Tm): Min 57.0, Opt 60.0, Max 63.0, Max Tm difference 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match: Exon at 5' side 7, Exon at 3' side 4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

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Intron length range: Min 1000, Max 1000000

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Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range: From / To

Forward primer

Reverse primer Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

PCR product size: Min 70 Max 1000

# of primers to return: 10

Primer melting temperatures (Tm): Min 57.0 Opt 60.0 Max 63.0 Max Tm difference 3

在这里输入筛选出来的引物数量

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match: Exon at 5' side 7 Exon at 3' side 4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min 1000 Max 1000000

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Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range: From / To

Forward primer

Reverse primer

Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

PCR product size: Min 70, Max 1000

# of primers to return: 10

Primer melting temperatures (Tm): Min 57.0, Opt 60.0, Max 63.0, Max Tm difference 3

在这里设定引物的**Tm**值，以及两条引物**Tm**值的最大差异

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match: Exon at 5' side 7, Exon at 3' side 4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min 1000, Max 1000000

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Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range: From / To

Forward primer

Reverse primer

Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

PCR product size: Min 70, Max 1000

# of primers to return: 10

Primer melting temperatures (Tm): Min 57.0, Opt 60.0, Max 63.0, Max Tm diffe 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match: Exon at 5' side 7, Exon at 3' side 4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min 1000, Max 1000000

在这里设定引物是否跨越不同的外显子，以及跨越多少个碱基

Primer-BLAST *A tool for finding specific primers*

BI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range: From / To

Forward primer

Reverse primer

Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

PCR product size: Min 70, Max 1000

# of primers to return: 10

Primer melting temperatures (Tm): Min 57.0, Opt 60.0, Max 63.0, Max Tm difference 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for opti

Exon junction span: No preference

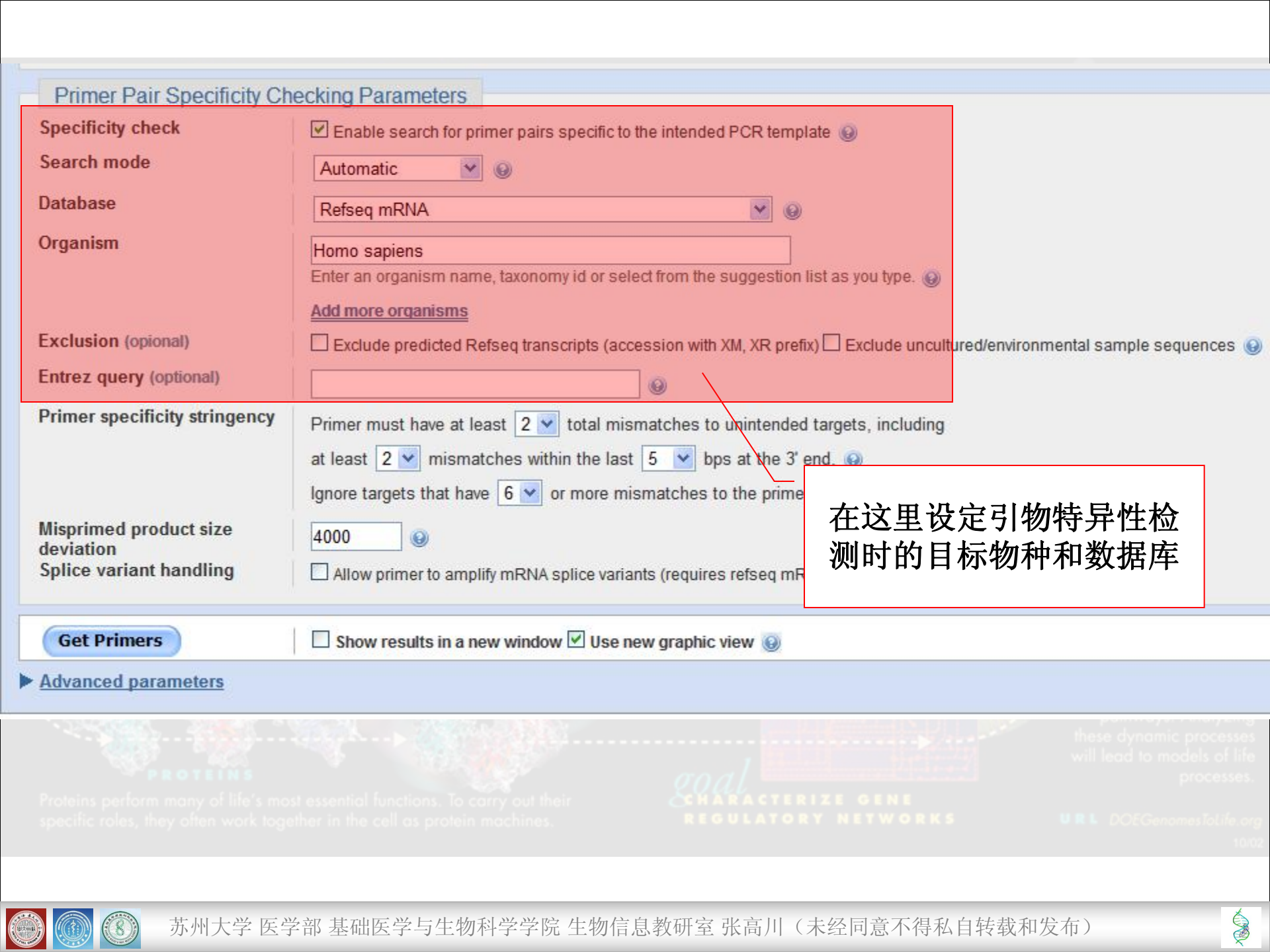
Exon junction match: Exon at 5' side 7, Exon at 3' side 4

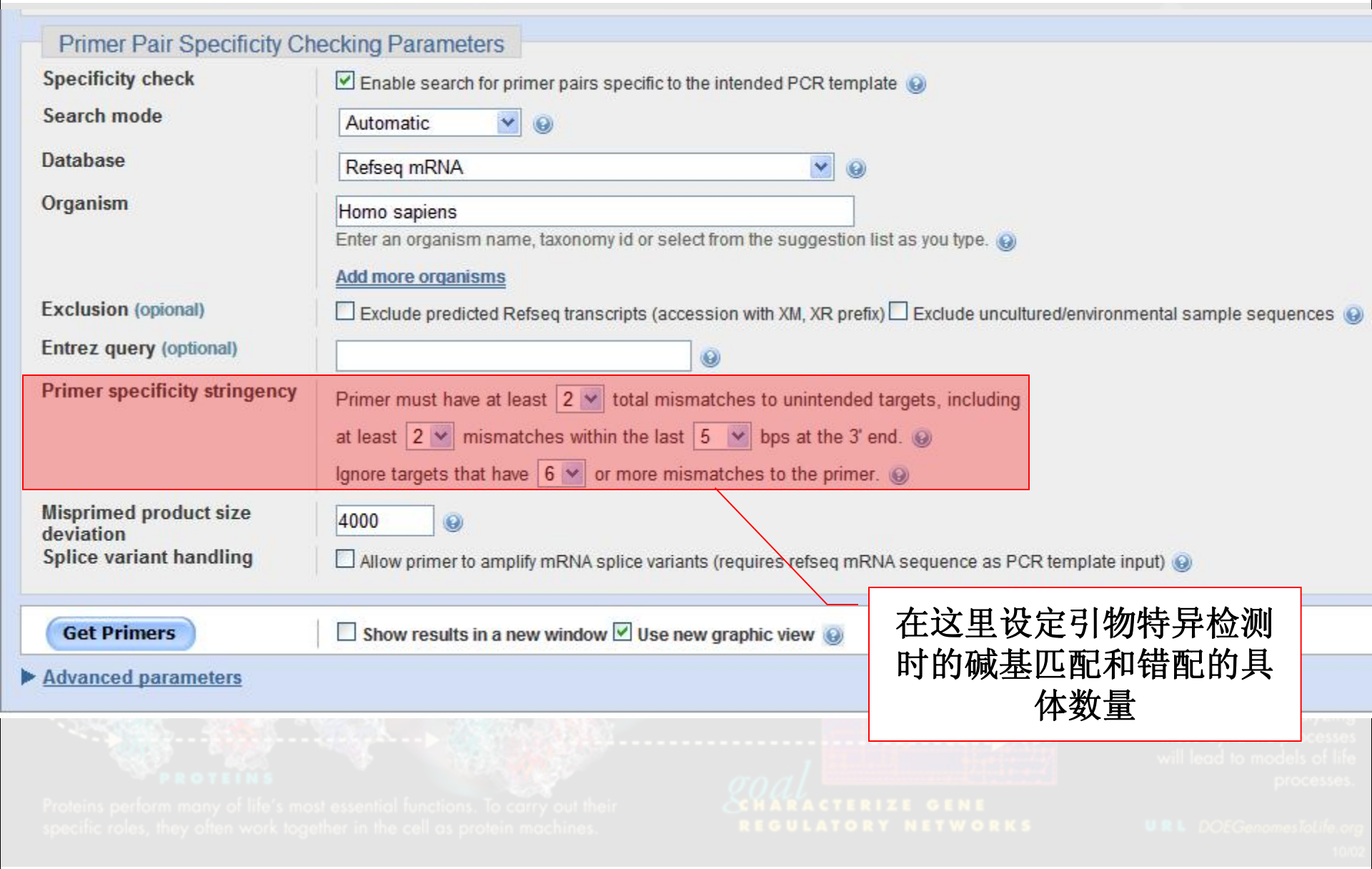
Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

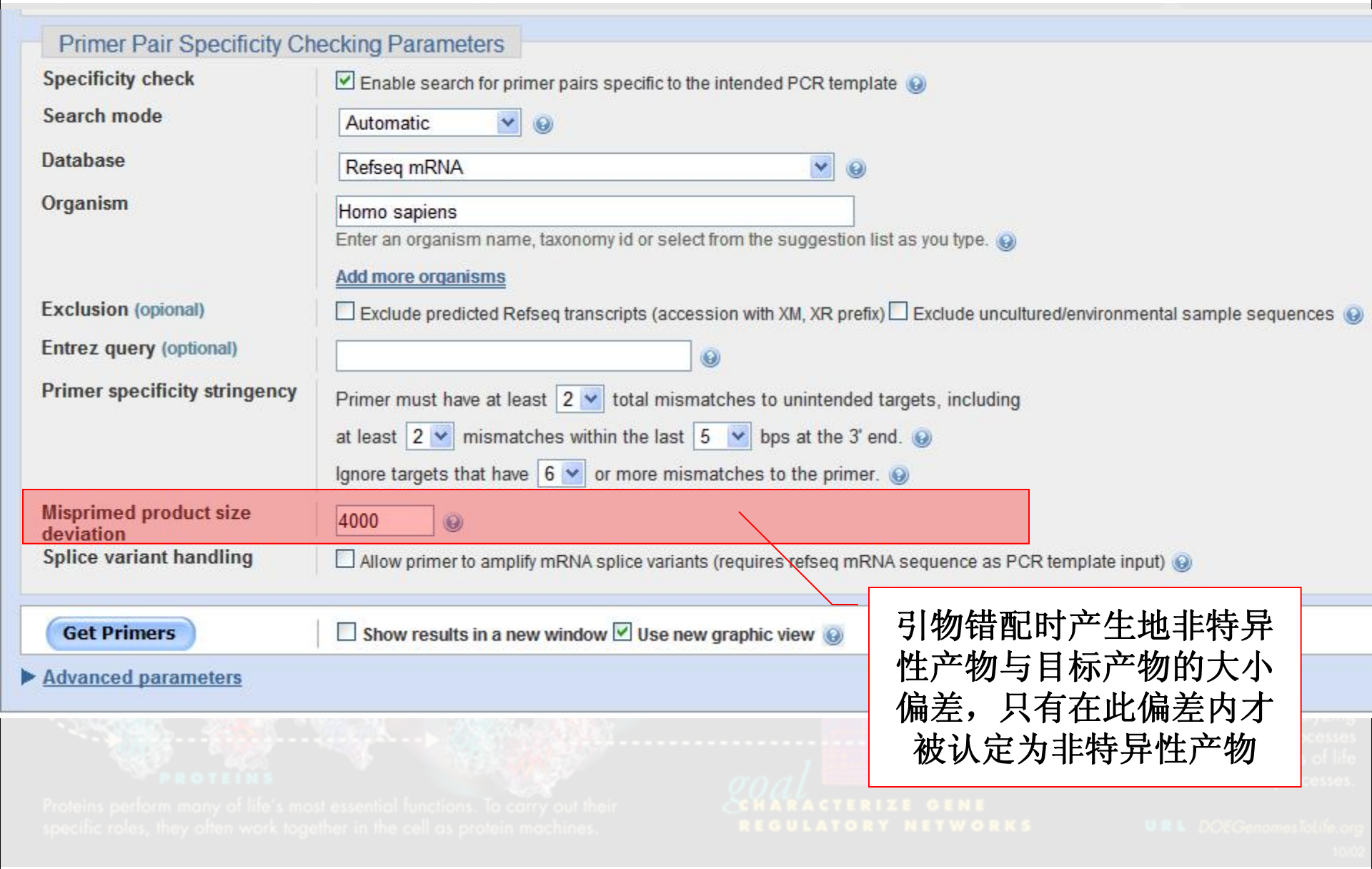
Intron inclusion: ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min 1000, Max 1000000

在这里设定引物对是否跨越内含子，以及内含子长度区间


Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq mRNA
Organism
Homo sapiens
Enter an organism name, taxonomy id or select from the suggestion list as you type.
Add more organisms
Exclusion (opional)
Exclude predicted Refseq transcripts (accession with XM, XR prefix)
Exclude uncultured/environmental sample sequences
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 prime
Misprimed product size deviation
4000
Splice variant handling
Allow primer to amplify mRNA splice variants (requires refseq mR
在这里设定引物特异性检测时的目标物种和数据库
Get Primers
Show results in a new window
Use new graphic view
Advanced parameters


Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq mRNA
Organism
Homo sapiens
Enter an organism name, taxonomy id or select from the suggestion list as you type.
Add more organisms
Exclusion (opional)
Exclude predicted Refseq transcripts (accession with XM, XR prefix)
Exclude uncultured/environmental sample sequences
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.
Misprimed product size deviation
4000
Splice variant handling
Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)
Get Primers
Show results in a new window
Use new graphic view
Advanced parameters
在这里设定引物特异检测时的碱基匹配和错配的具体数量


Primer Pair Specificity Checking Parameters
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Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq mRNA
Organism
Homo sapiens
Enter an organism name, taxonomy id or select from the suggestion list as you type.
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Ignore targets that have 6 or more mismatches to the primer.
Misprimed product size deviation
4000
Splice variant handling
Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)
Get Primers
Show results in a new window
Use new graphic view
Advanced parameters
引物错配时产生地非特异性产物与目标产物的大小偏差，只有在此偏差内才被认定为非特异性产物


Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq mRNA
Organism
Homo sapiens
Enter an organism name, taxonomy id or select from the suggestion list as you type.
Add more organisms
Exclusion (opional)
Exclude predicted Refseq transcripts (accession with XM, XR prefix)
Exclude uncultured/environmental sample sequences
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.
Misprimed product size deviation
4000
Splice variant handling
Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)
Get Primers
Show results in a new window
Use new graphic view
Advanced parameters
是否允许引物扩增mRNA变异体

Primer Pair Specificity Checking Parameters

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

Search mode Automatic

Database Refseq mRNA

Organism Homo sapiens

Enter an organism name, taxonomy id or select from the suggestion list as you type.

Add more organisms

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

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.

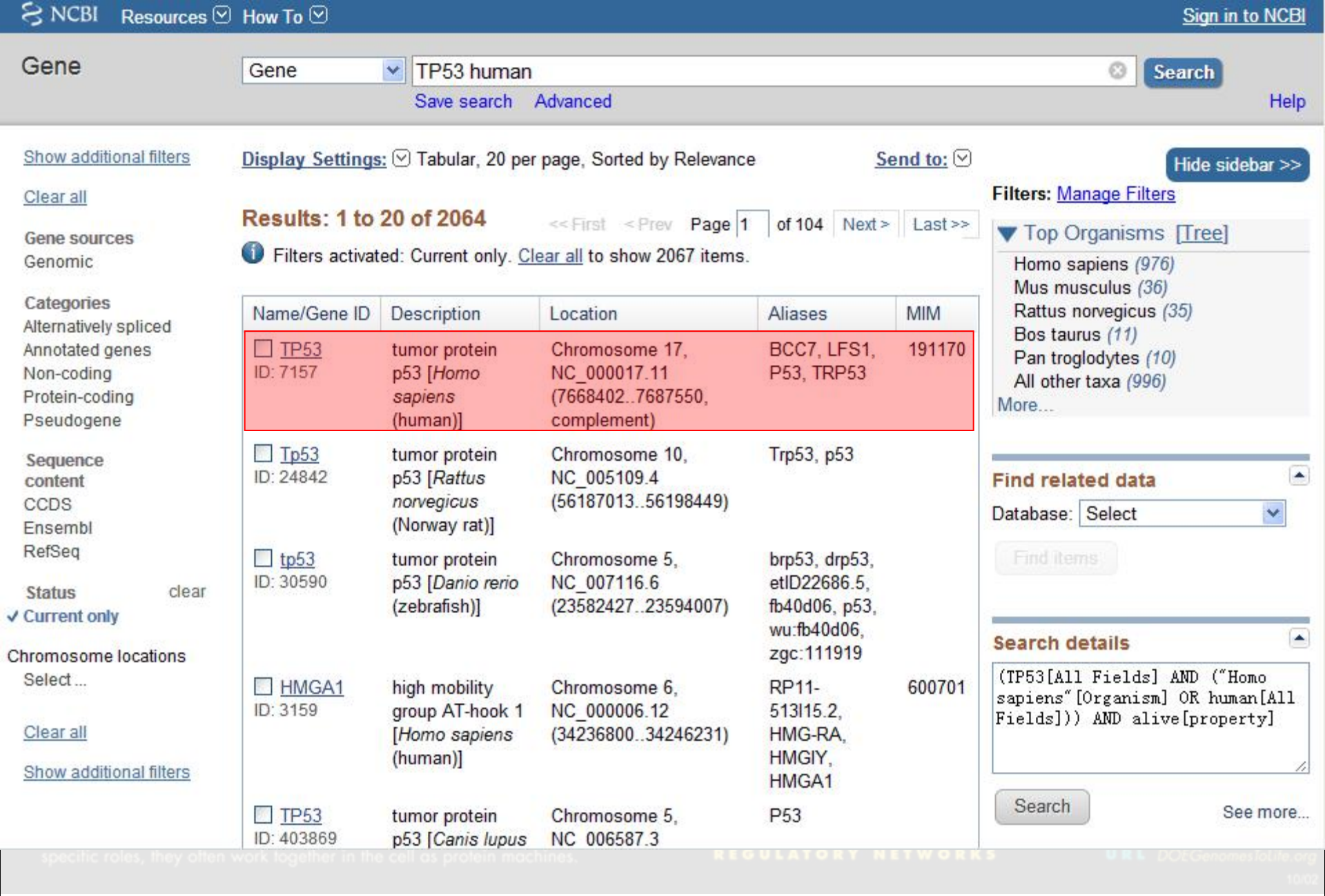
Ignore targets that have 6 or more mismatches to the primer.

Misprimed product size deviation 4000

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

Get Primers ☐ Show results in a new window ☑ Use new graphic view

▶ Advanced parameters


NCBI Resources How To
Sign in to NCBI
Gene
Gene
TP53 human
Search
Save search Advanced
Help
Show additional filters
Clear all
Gene sources
Genomic
Categories
Alternatively spliced
Annotated genes
Non-coding
Protein-coding
Pseudogene
Sequence content
CCDS
Ensembl
RefSeq
Status clear
Current only
Chromosome locations
Select ...
Clear all
Show additional filters
Display Settings: Tabular, 20 per page, Sorted by Relevance
Send to:
Hide sidebar >>
Filters: Manage Filters
Results: 1 to 20 of 2064
<< First < Prev Page 1 of 104 Next > Last >>
Filters activated: Current only. Clear all to show 2067 items.
Name/Gene ID
Description
Location
Aliases
MIM
TP53 ID: 7157
tumor protein p53 [Homo sapiens (human)]
Chromosome 17, NC_000017.11 (7668402..7687550, complement)
BCC7, LFS1, P53, TRP53
191170
Tp53 ID: 24842
tumor protein p53 [Rattus norvegicus (Norway rat)]
Chromosome 10, NC_005109.4 (56187013..56198449)
Trp53, p53
tp53 ID: 30590
tumor protein p53 [Danio rerio (zebrafish)]
Chromosome 5, NC_007116.6 (23582427..23594007)
brp53, drp53, etID22686.5, fb40d06, p53, wu:fb40d06, zgc:111919
HMGA1 ID: 3159
high mobility group AT-hook 1 [Homo sapiens (human)]
Chromosome 6, NC_000006.12 (34236800..34246231)
RP11-513I15.2, HMG-RA, HMGIY, HMGA1
600701
TP53 ID: 403869
tumor protein p53 [Canis lupus
Chromosome 5, NC_006587.3
P53
Top Organisms [Tree]
Homo sapiens (976)
Mus musculus (36)
Rattus norvegicus (35)
Bos taurus (11)
Pan troglodytes (10)
All other taxa (996)
More...
Find related data
Database: Select
Find items
Search details
(TP53[All Fields] AND ("Homo sapiens"[Organism] OR human[All Fields])) AND alive[property]
Search
See more...

这么多转录变异体，
接下来该怎么办？

```
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:9606"
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                     /map="17p13.1"
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                     /gene_synonym="BCC7; LFS1; P53; TRP53"
                     /note="tumor protein p53"
                     /db_xref="GeneID:7157"
                     /db_xref="HGNC:HGNC:11998"
                     /db_xref="MIM:191170"
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                     /gene_synonym="BCC7; LFS1; P53; TRP53"
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     misc_feature    95..97
                     /gene="TP53"
                     /gene_synonym="BCC7; LFS1; P53; TRP53"
                     /note="upstream in-frame stop codon"
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                     /gene_synonym="BCC7; LFS1; P53; TRP53"
                     /inference="alignment:Splign:1.39.8"
     CDS             203..1384
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                     /gene_synonym="BCC7; LFS1; P53; TRP53"
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                     protein 53; mutant tumor protein 53; cellular tumor
                     antigen p53; phosphoprotein p53; transformation-related
                     protein 53; p53 tumor suppressor; antigen NY-CO-13"
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                     /product="cellular tumor antigen p53 isoform a"
                     /protein_id="NP_000537.3"
                     /db_xref="GI:120407068"
```

UCSC Genome Browser
[UCSC Genome Browse

Related information

- Related Sequences
- Annotated Genomic
- BioSystems
- CCDS
- Components (Core)
- Components (EST)
- Full text in PMC
- Gene
- GeneView in dbSNP
- HomoloGene
- Map Viewer
- OMIM
- Probe
- Protein
- PubMed
- PubMed (RefSeq)
- PubMed (Weighted)
- SNP
- Taxonomy
- UniGene

>gi|371502114|ref|NM_000546.5| Homo sapiens tumor protein p53 (TP53), transcript variant 1, mRNA

GATGGGATTGGGGTTTTCCCCTCCCATGTGCTCAAGACTGGCGCTAAAAGTTTTGAGCTTCTCAAAAGTC
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GCCGCAGTCAGATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACATTTTCAGACCTATGGAAACTACTT
CCTGAAAACAACGTTCTGTCCCCCTTGCCGTCCCAAGCAATGGATGATTTGATGCTGTCCCCGGACGATA
TTGAACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCCAGAATGCCAGAGGCTGCTCCCCCCGT
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GTCCCTTCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCCA
AGTCTGTGACTTGCACGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGT
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CAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGCGATGGTCTGGCCC
CTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGGATGACAGAAACACTTTTCG
ACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAGGTTGGCTCTGACTGTACCACCATCCACTACAACTAC
ATGTGTAACAGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACT
CCAGTGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGACCGGCG
CACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCCAGGGAGCACTAAGCGA
GCACTGCCCAACAACACCAGCTCCTCTCCCCAGCCAAAGAAGAAACCACTGGATGGAGAATATTTCACCC
TTCAGATCCGTGGGCGTGAGCGCTTCGAGATGTTCCGAGAGCTGAATGAGGCCTTGGAACTCAAGGATGC
CCAGGCTGGGAAGGAGCCAGGGGGGAGCAGGGCTCACTCCAGCCACCTGAAGTCCAAAAAGGGTCAGTCT
ACCTCCCGCCATAAAAAACTCATGTTCAAGACAGAAGGGCCTGACTCAGACTGACATTCTCCACTTCTTG
TTCCCCACTGACAGCCTCCCACCCCCATCTCTCCCTCCCCTGCCATTTTGGGTTTTGGGTCTTTGAACCC
TTGCTTGCAATAGGTGTGCGTCAGAAGCACCCAGGACTTCCATTTGCTTTGTCCCGGGGCTCCACTGAAC
AAGTTGGCCTGCACTGGTGTTTTGTTGTGGGGAGGAGGATGGGGAGTAGGACATACCAGCTTAGATTTTA
AGGTTTTTACTGTGAGGGATGTTTGGGAGATGTAAGAAATGTTCTTGCAGTTAAGGGTTAGTTTACAATC
AGCCACATTCTAGGTAGGGGCCCACTTCACCGTACTAACCAGGGAAGCTGTCCCTCACTGTTGAATTTTC
TCTAACTTCAAGGCCCATATCTGTGAAATGCTGGCATTTGCACCTACCTCACAGAGTGCATTGTGAGGGT
TAATGAAATAATGTACATCTGGCCTTGAAACCACCTTTTATTACATGGGGTCTAGAACTTGACCCCCTTG
AGGGTGCTTGTTCCCTCTCCCTGTTGGTCGGTGGGTTGGTAGTTTCTACAGTTGGGCAGCTGGTTAGGTA
GAGGGAGTTGTCAAGTCTCTGCTGGCCCAGCCAAACCCTGTCTGACAACCTCTTGGTGAACCTTAGTACC
TAAAAGGAAATCTCACCCCATCCCACACCCTGGAGGATTTCATCTCTTGTATATGATGATCTGGATCCAC
CAAGACTTGTTTTATGCTCAGGGTCAATTTCTTTTTTCTTTTTTTTTTTTTTTTTCTTTTTCTTTGAGA
CTGGGTCTCGCTTTGTTGCCCAGGCTGGAGTGGAGTGGCGTGATCTTGGCTTACTGCAGCCTTTGCCTCC
CCGGCTCGAGCAGTCCTGCCTCAGCCTCCGGAGTAGCTGGGACCACAGGTTCATGCCACCATGGCCAGCC
AACTTTTGCATGTTTTGTAGAGATGGGGTCTCACAGTGTTGCCCAGGCTGGTCTCAAACTCCTGGGCTCA
GGCGATCCACCTGTCTCAGCCTCCCAGAGTGCTGGGATTACAATTGTGAGCCACCACGTCCAGCTGGAAG
GGTCAACATCTTTTACATTCTGCAAGCACATCTGCATTTTCACCCCACCCTTCCCCTCCTTCTCCCTTTT
TATATCCCATTTTTATATCGATCTCTTATTTTACAATAAAACTTTGCTGCCACCTGTGTGTCTGAGGGGT G

Primer-BLAST *A tool for finding specific primers*

BI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

```
>gi|371502114|ref|NM_000546.5| Homo sapiens tumor protein p53 (TP53), transcript
variant 1, mRNA
GATGGGATTGGGGTTTTCCCCTCCCATGTGCTCAAGACTGGCGCTAAAAGTTTTGAGCTTCTCAAAAGTC
TAGAGCCACCGTCCAGGGAGCAGGTAGCTGCTGGGCTCCGGGGACACTTTGCGTTCGGGCTGGGAGCGTG
CTTTCCACGACGGTGACACGCTTCCCTGGATTGGCAGCCAGACTGCCTTCCGGGTCACTGCCATGGAGGA
```

Range

	From	To
Forward primer	1	174
Reverse primer	1303	2591

Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	1000	2500

# of primers to return 5

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	50	60.0	70	2

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span Primer must span an exon-exon junction

Exon junction match

Exon at 5' side	Exon at 3' side
7	4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion ☑ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

Min	Max
1000	1000000

Note: Parameter values that differ from the default are highlighted in yellow

Primer Pair Specificity Checking Parameters

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

Search mode Automatic

Database Refseq mRNA

Organism Homo sapiens

Enter an organism name, taxonomy id or select from the suggestion list as you type.

Add more organisms

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

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.

Misprimed product size deviation 4000

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

Get Primers ☑ Show results in a new window ☑ Use new graphic view

▶ Advanced parameters

Note: Parameter values that differ from the default are highlighted in yellow

NCBI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST).

Input PCR template NM_000546.5 Homo sapiens tumor protein p53 (TP53), transcript variant 1, mRNA
Range 1 - 2591

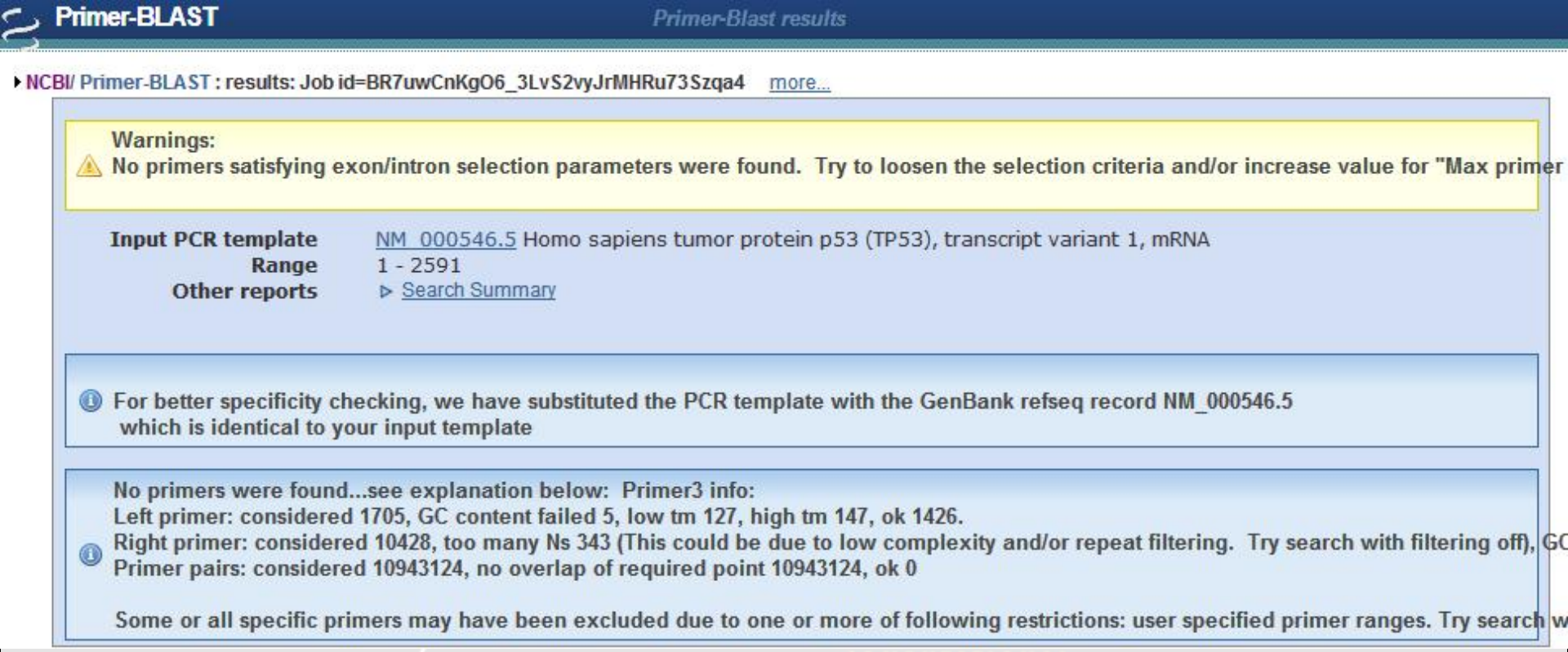
For better specificity checking, we have substituted the PCR template with the GenBank refseq record NM_000546.5 which is identical to your input template

Your PCR template is highly similar to the following sequence(s) from the search database. To increase the chance of finding specific primers, please review the list below and select all sequences (within the given sequence ranges) that are intended or allowed targets.

Select: All None Selected:1

Accession	Title	Identity	Alignment length	Seq. start	Seq. stop
☑ NM_001126112.2	Homo sapiens tumor protein p53 (TP53), transcript variant 2, mRNA	99.88%	2591	1	2588

Submit ☑ Show results in a new window



No primers were found...see explanation below: Primer3 info:

Left primer: considered 1705, GC content failed 5, low tm 127, high tm 147, ok 1426.

Right primer: considered 10428, too many Ns 343 (This could be due to low complexity and/or repeat filtering. Try search with filtering off), GC content failed 157, low tm 2181, high tm 53, long poly-x seq 20, ok 7674.

Primer pairs: considered 10943124, no overlap of required point 10943124, ok 0.
Some or all specific primers may have been excluded due to one or more of following restrictions: user specified primer ranges. Try search with less restrictions.

Primer-BLAST *A tool for finding specific primers*

CBI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). More... Tips for finding specific primers

Reset page Save search parameters Retrieve recent results

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

```
>gi|371502114|ref|NM_000546.5| Homo sapiens tumor protein p53 (TP53), transcript
variant 1, mRNA
GATGGGATTGGGGTTTTCCCCTCCCATGTGCTCAAGACTGGCGCTAAAAGTTTTGAGCTTCTCAAAAGTC
TAGAGCCACCGTCCAGGGAGCAGGTAGCTGCTGGGCTCCGGGGACACTTTGCGTTCGGGCTGGGAGCGTG
CTTTCCACGACGGTGACACGCTTCCCTGGATTGGCAGCCAGACTGCCTTCCGGGTCACTGCCATGGAGGA
```

Range

	From	To
Forward primer	1	500
Reverse primer	1000	2591

Clear

Or, upload FASTA file 选择文件 未选择文件

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	1000	2500

# of primers to return 5

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	50	60.0	70	2

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span Primer must span an exon-exon junction

Exon junction match

Exon at 5' side	Exon at 3' side
7	4

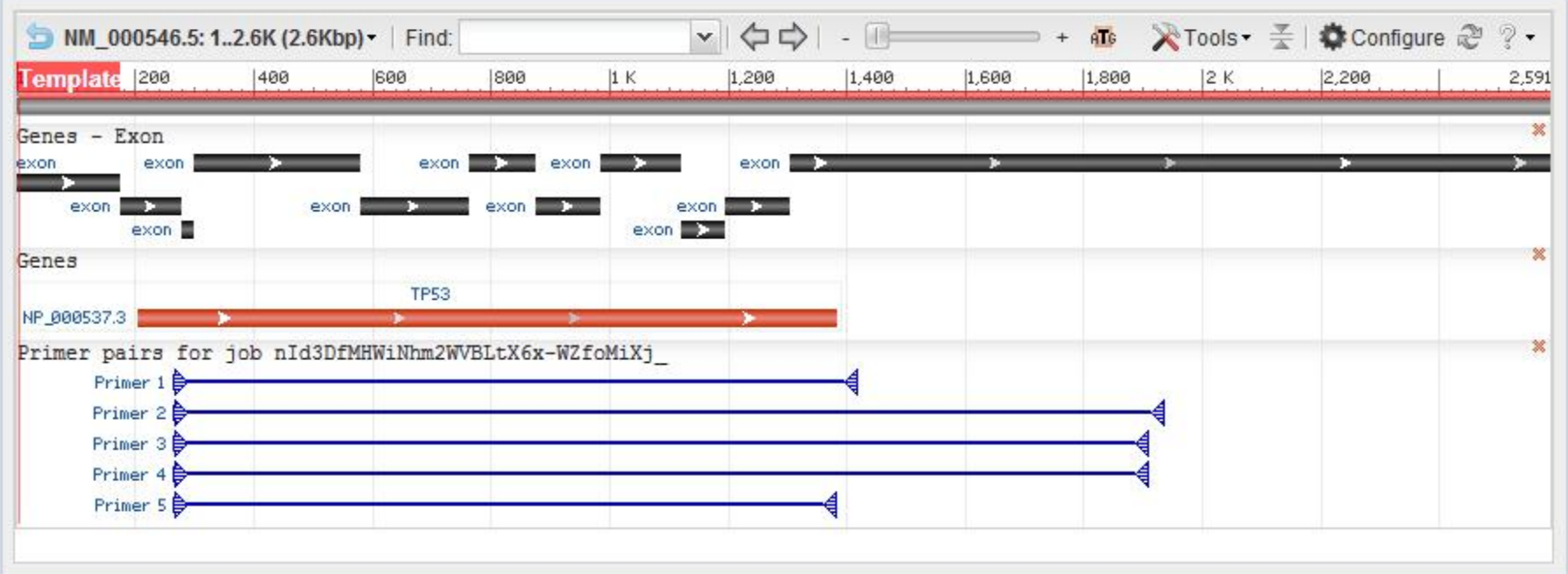
Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

NCBI/ Primer-BLAST : results: Job id=nId3DfMHWiNhm2WVBLtX6x-WZfoMiXj_ more...

Input PCR template	NM_000546.5 Homo sapiens tumor protein p53 (TP53), transcript variant 1, mRNA
Range	1 - 2591
Specificity of primers	Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports	▷ Search Summary

For better specificity checking, we have substituted the PCR template with the GenBank refseq record NM_000546.5 which is identical to your input template

Graphical view of primer pairs



Detailed primer reports

Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACCTATGGAAACTACTTCCTGAAAA	Plus	25	264	288	57.91	36.00	4.00	1.00
Reverse primer	GCTGTCAGTGGGGAACAAGA	Minus	20	1415	1396	59.89	55.00	5.00	0.00
Product length	1152								
Exon junction	276/277 (forward primer) on template NM_000546.5								
Total intron size	5687 (between pos. 7187395 and 7180703 on NT_010718.17)								

Products on intended target

>NM_001126112.2 Homo sapiens tumor protein p53 (TP53), transcript variant 2, mRNA

```
product length = 1152
Forward primer  1     ACCTATGGAAACTACTTCCTGAAAA  25
Template        261   .........................  285

Reverse primer  1      GCTGTCAGTGGGGAACAAGA  20
Template        1412   ....................  1393
```

>NM_000546.5 Homo sapiens tumor protein p53 (TP53), transcript variant 1, mRNA

```
product length = 1152
Forward primer  1     ACCTATGGAAACTACTTCCTGAAAA  25
Template        264   .........................  288

Reverse primer  1      GCTGTCAGTGGGGAACAAGA  20
Template        1415   ....................  1396
```

Products on allowed transcript variants

>NM_001126114.2 Homo sapiens tumor protein p53 (TP53), transcript variant 3, mRNA

```
product length = 1285
Forward primer  1     ACCTATGGAAACTACTTCCTGAAAA  25
Template        264   .........................  288

Reverse primer  1      GCTGTCAGTGGGGAACAAGA  20
Template        1548   ....................  1529
```

>NM_001126113.2 Homo sapiens tumor protein p53 (TP53), transcript variant 4, mRNA

```
product length = 1212
Forward primer  1     ACCTATGGAAACTACTTCCTGAAAA  25
Template        264   .........................  288

Reverse primer  1      GCTGTCAGTGGGGAACAAGA  20
Template        1475   ....................  1456
```

Primer pair 2

More PCR primer design softwares ?

GenScript Real-time PCR (TaqMan) Primer Design https://www.genscript.com/ssl-bin/app/primer

** This online tool helps you to design primers and probes for your Real-time PCR (TaqMan) experiments. You can customize the potential PCR amplicon's size range, Tm (melting temperature) for the primers and probes, as well as the organism. You can also decide how many Primer/Probe sets you want the tool to return to you. We recommend you using the GenBank Accession to input your target sequence. However, you can choose to input the sequences manually in raw format.

Number of Primer/Probe Sets to Return (Limit 20 Sets) 3

PCR Amplicon Size Range 50-150

Primer Tm Minimum 58 Optimum 59 Maximum 60 °C

Probe Tm Minimum 68 Optimum 69 Maximum 70 °C

Organism: Human

☐ Pick Primer/Probe Crossing Exon Junction

When *Pick Primer/Probe Crossing Exon Junction* is selected, the exon regions must be defined. If only raw sequence is provided, the sequence will be mapped on the genome (human, mouse or rat at present) sequences to locate the exon boundaries. Besides, there are other two ways to specify exon regions:

- The best way is to fill in *GenBank Accession* with the accession number (*e.g.* NM_145027 , please note the accession number is case-sensitive and should not contain version suffix like '.1' or '.2'). The sequence will be fetched from NCBI data center, and the exon regions are already defined in sequence annotations.
- Manually specify the exon junctions with ':' in the input sequence (e.g. 'ACGCGCG:CGTACG')

Target Nucleotide Sequences:

- GenBank Accession:
- or Paste in the DNA Sequence in raw format:

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【综合】

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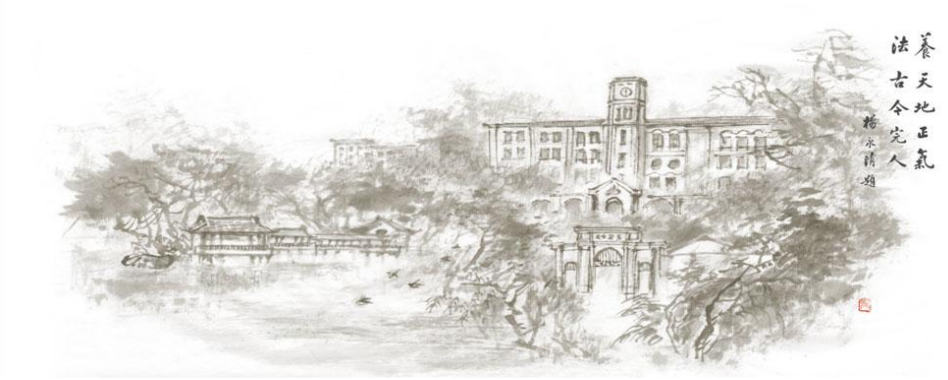
Genbank之GEO数据库及其在线分析工具

Genbank之EST和UniGene数据库，
以及在线DDD分析工具

基因功能注释【Gene Ontology, KEGG=>DAVID】

蛋白相互作用【STRING】

引物设计【Primer-Blast】



Thanks for your attention !