



引物设计

生工生物市场部

引物设计专员

吴琼

2016年4月7日

引物设计背景介绍

引物设计在线提交流程

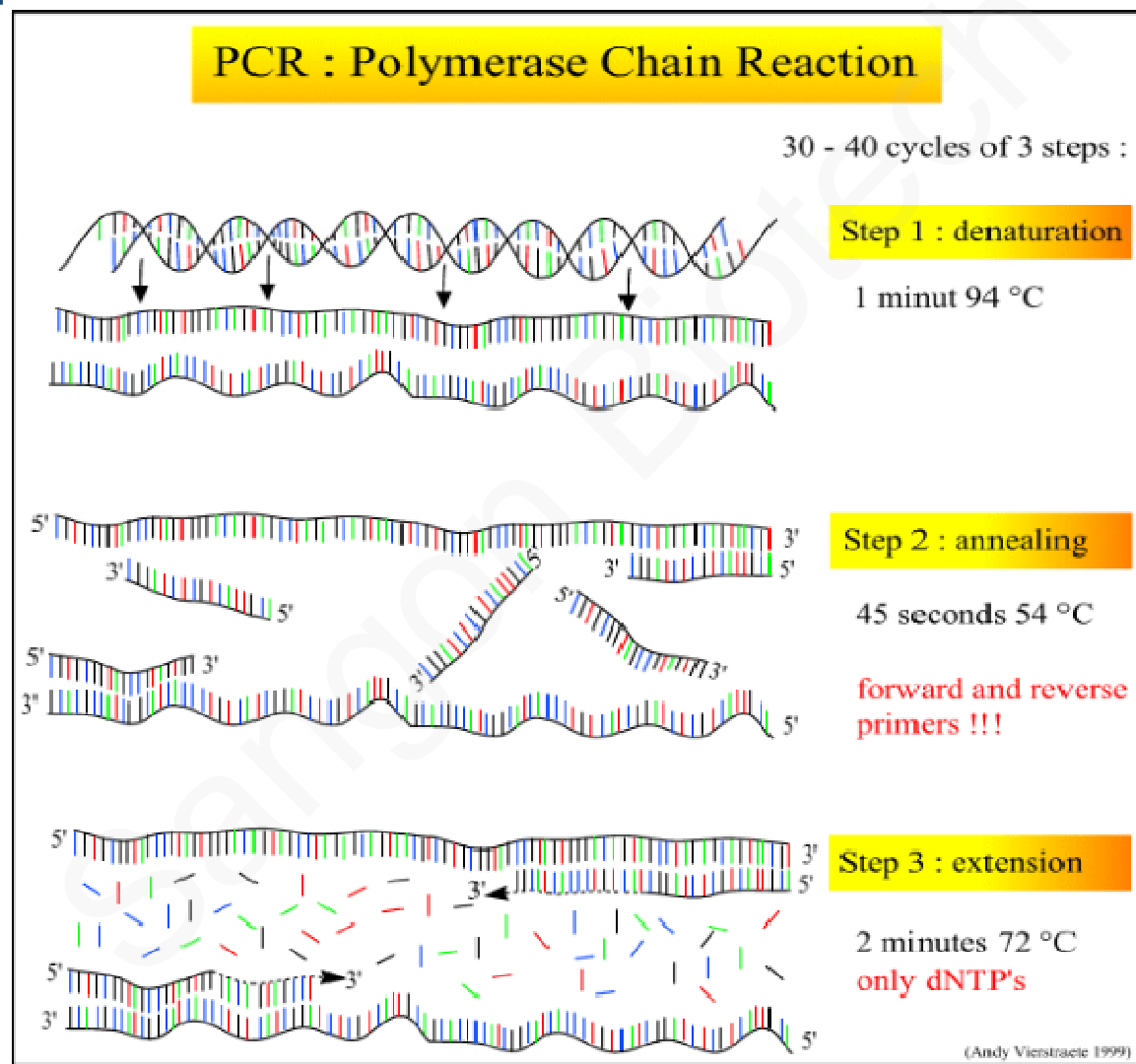
基因序列查找

引物特异性比对



引物设计背景介绍

PCR原理



引物设计注意事项

1. 引物长度

引物长度一般为15-30bp，常用的为18-27bp。

- 太短—低的特异性，结果可导致非特异性扩增。
- 太长—在退火时，可能导致与模板结合效率低，发夹结构的发生。

引物设计注意事项

2. 引物T_m值

上下游引物的T_m值 (Melting Temperature) 是寡核苷酸的解链温度，即在一盐浓度条件下，50 %寡核苷酸双链解链的温度。合适的T_m值：55 °C - 60 °C 。

- 上下游引物T_m值不匹配，可能导致扩增失败。
- 上下游引物退火温度最好<1°C

引物设计注意事项

3. 引物GC含量

在40%~60%之间。 GC含量 (composition) 过高或过低都不利于引发反应。

- GC含量太低导致引物T_m值较低，使用较低的退火温度不利于提高PCR的特异性。
- GC含量太高也易于引发非特异扩增。

引物设计注意事项

4. 引物自身及引物之间不应存在互补序列

- 引物**自身**不应存在互补序列，否则引物自身会折叠成**发夹结构**。这种二级结构会因空间位阻而影响引物与模板的复性结合。
- **两引物之间**也不应具有互补性，尤其应避免3' 端的互补重叠以防止**引物二聚体**的形成。



引物设计在线提交流程

生工免费引物设计流程：

1.进入生工官网首 (<http://www.sangon.com/>)

2.登录 (新客户请先注册)



The screenshot displays the Sangon Biotech website interface. On the left, a promotional banner features the text "体验精品尊享品牌" (Experience Premium Brand) and "BBIAB近万抗体助您科研梦" (BBIAB's nearly 10,000 antibodies help your research dream). Below this, it states "近万种抗体现货促销" (Near 10,000 types of antibodies on sale), "无需等待漫长货期，即买即发" (No need to wait for a long delivery period, buy and ship immediately), and "活动时间：2015年4月1日-2015年6月30日" (Activity time: April 1, 2015 - June 30, 2015). On the right, the "会员登录" (Member Login) section is visible, including a link for "新会员注册" (New Member Registration). The login form includes a note: "所有用户名已更新为手机号/生工ID登陆, 什么是生工ID?" (All usernames have been updated to mobile number/Sangon ID login, what is Sangon ID?). There are two login options: "普通登录" (General Login) and "手机动态密码登录" (Mobile Dynamic Password Login). The form fields are labeled "手机号/生工ID:" and "密码:". A "忘记密码?" (Forgot Password?) link is also present. A large orange "登录" (Login) button is at the bottom of the form.

3.在线填写申请单，简易流程如下：进入首页导航栏“技术服务>在线提交引物设计订单”



订购中心

免费引物设计

DNA 合成

我的订单

我的购物车

产品订单

合成订单

测序订单

引物设计列表

积分优惠券

查看积分记录

查看优惠券

积分商城

积分订单

账户中心

账户信息

试用装申请

地址管理

发票信息

负责人管理

售后服务

免费引物设计

服务说明

我们为在生工生物网站注册的用户提供免费的引物设计服务。我们在设计时尽可能选择理论上最佳的结果，但不保证引物的应用效果，请您务必自行检测引物的可行性，并确认是否符合您所做实验的要求。

请仔细以下表单，如需帮助请点击帮助按钮  或联系人工在线客服 [点击留言](#)

1. 您所要的引物是用于以下哪种实验的？

☐ 普通PCR ☐ 半定量RT-PCR ☐ 荧光定量 Real-Time PCR ☐ 其他（请注明）

2. 您的目的基因序列属于以下哪种类型？

☐ genomic DNA ☐ mRNA/cDNA

3. 您的目的基因属于哪个物种？

☐ Human ☐ rat ☐ mouse ☐ 其他（请注明）

4. 基因名称（只能填写一个）

5. 请您填写基因序列（只能填写一个）



6. 备注

注：1. 若是microRNA，请注明逆转录使用的是茎环法还是加尾法，如果是加尾法请提供下游引物Tm值（Tm值具体咨询试剂盒公司）
2. 若需要扩增序列全长，请在备注中注明 3. 若需要扩增特定位置的碱基或序列，请注明其在序列中的bp范围 4. 若需要设计探针，请在备注中注明其类型，目前提供Taqman及MGB探针的设计

- 4. 2个工作日内，请进入“我的会员中心”，左侧“我的订单>引物设计列表”进行查看。

订购中心

免费引物设计

DNA 合成

我的订单

我的购物车

产品订单

合成订单

测序订单

引物设计列表

积分优惠券

查看积分记录

查看优惠券

积分商城

积分订单

账户中心

账户信息

试用理由

订单编号

订单编号	PCR类型	序列类型	物种信息	基因名称	基因序列	状态	创建日期	操作
201603290109	荧光定量 Real-Time PCR	mRNA/cDNA	rat	fructose-1,6-bisphosphatase 1	查看序列	查看结果	2016-03-29	
201603290036	荧光定量 Real-Time PCR	mRNA/cDNA	Human	fructose-bisphosphatase 1	查看序列	查看结果	2016-03-29	
201603290027	荧光定量 Real-Time PCR	mRNA/cDNA	rat	pyruvate carboxylase	查看序列	查看结果	2016-03-29	
201603280100	荧光定量 Real-Time PCR	mRNA/cDNA	rat	Glucose kinase	查看序列	查看结果	2016-03-28	
201602290020	荧光定量 Real-Time PCR	mRNA/cDNA	mouse	CD36	查看序列	查看结果	2016-02-29	
201601290079	荧光定量 Real-Time PCR	mRNA/cDNA	Human	CDH1	查看序列	查看结果	2016-01-29	



- 4. 2个工作日内，请进入“我的会员中心”，左侧“我的订单>引物设计列表”进行查看。

订购中心

免费引物设计

DNA 合成

我的订单

我的购物车

产品订单

合成订单

测序订单

引物设计列表

积分优惠券

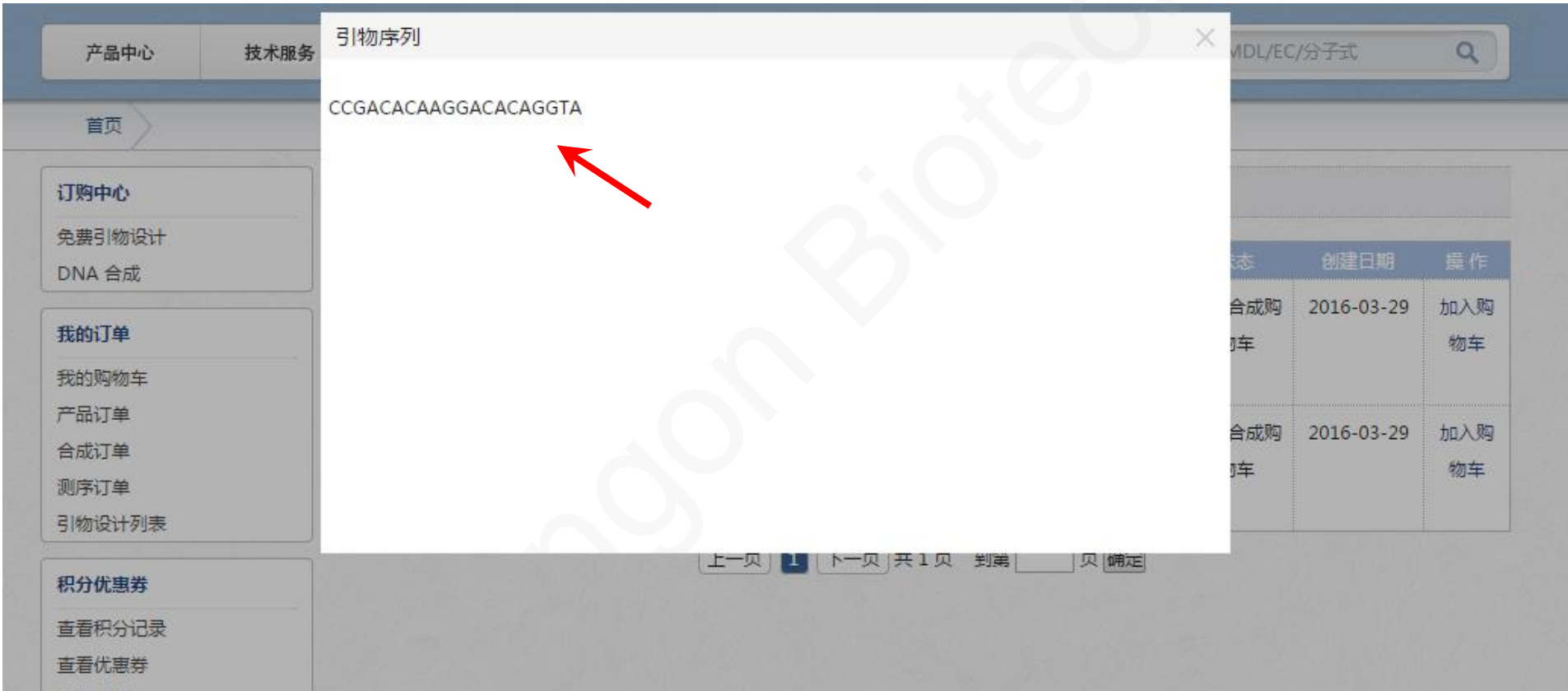
去查看公告

全部 ▼ 订单编号: 201603290109 基因名称: 查询

订单编号	基因名称	方向	Len	tm	prodSize	序列	状态	创建日期	操作
201603290109	fructose-1,6-bisphosphatase 1	REVERSE	20	59.59	169	查看序列	未转合成购物车	2016-03-29	加入购物车
201603290109	fructose-1,6-bisphosphatase 1	FORWARD	20	60.37	169	查看序列	未转合成购物车	2016-03-29	加入购物车

上一页 1 下一页 共 1 页 到第 页 确定

- 4. 2个工作日内，请进入“我的会员中心”，左侧“我的订单>引物设计列表”进行查看。



The screenshot shows the Sangon Biotech website interface. On the left, there is a navigation menu with sections: '订购中心' (Order Center) containing '免费引物设计' (Free Primer Design) and 'DNA 合成' (DNA Synthesis); '我的订单' (My Order) containing '我的购物车' (My Cart), '产品订单' (Product Order), '合成订单' (Synthesis Order), '测序订单' (Sequencing Order), and '引物设计列表' (Primer Design List); and '积分优惠券' (Points and Coupons) containing '查看积分记录' (View Points Record) and '查看优惠券' (View Coupons). The main content area is titled '引物序列' (Primer Sequence) and displays the sequence 'CCGACACAAGGACACAGGTA'. A red arrow points to this sequence. Below the sequence, there is a pagination bar showing '上一页', '1', '下一页', '共 1 页', '到第', '页', and '确定'. On the right side, there is a search bar with 'MDL/EC/分子式' and a magnifying glass icon, and a table with columns '状态', '创建日期', and '操作'. The table contains two rows of data, both showing '合成购' (Synthesis Purchase) and '加入购物车' (Add to Cart) actions, with a creation date of '2016-03-29'.

状态	创建日期	操作
合成购	2016-03-29	加入购物车
合成购	2016-03-29	加入购物车



基因序列查找

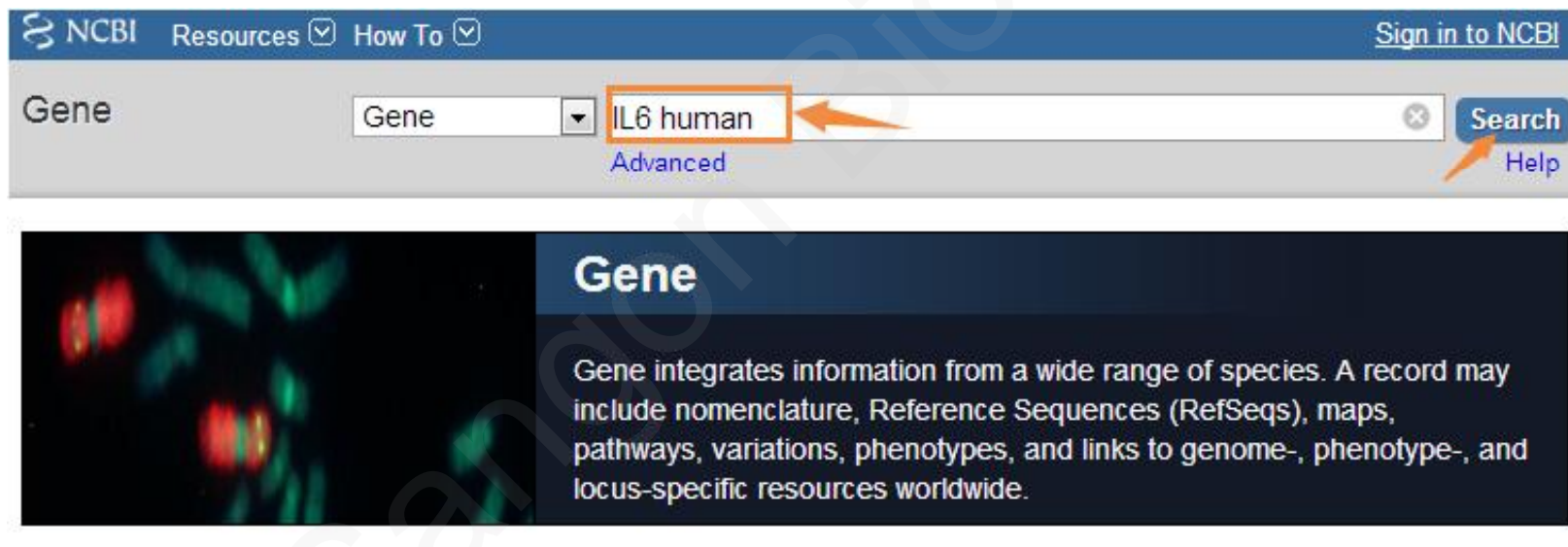
1.Genomic , mRNA序列查找

2.microRNA序列查找



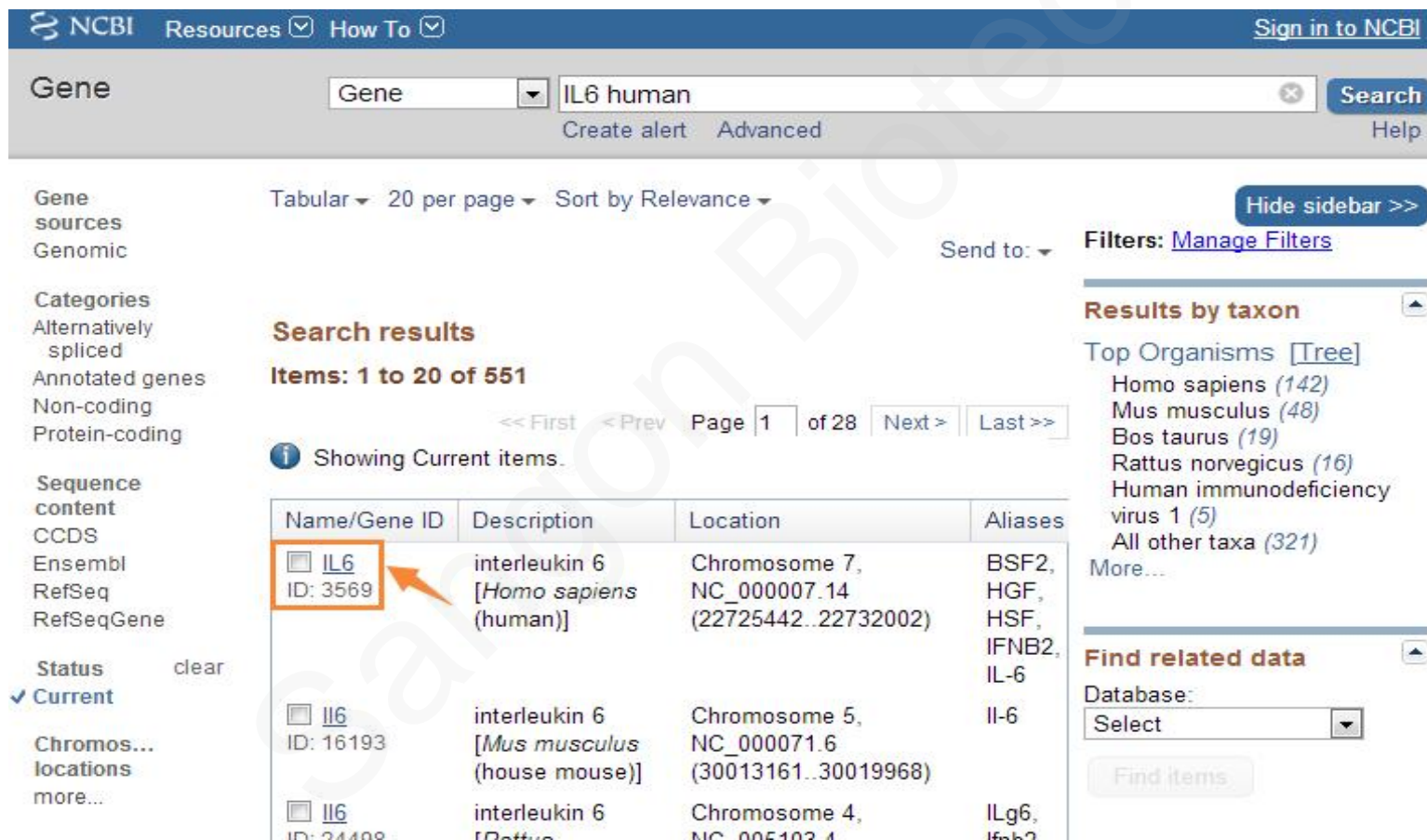
mRNA基因查找

- 1、打开**NCBI**主页 (<http://www.ncbi.nlm.nih.gov/gene/>) , 在搜索框输入 “IL6 human” , 点击 “Search” 。



mRNA基因查找

2、单击“Search”后得到以下页面：



The screenshot shows the NCBI Gene search results for 'IL6 human'. The search bar at the top contains 'IL6 human' and a 'Search' button. Below the search bar, there are links for 'Create alert' and 'Advanced'. The left sidebar contains various filters and categories, including 'Gene sources', 'Categories', 'Sequence content', and 'Status'. The main content area displays 'Search results' for 'IL6 human' with 551 items. The first item is highlighted with a red box and an orange arrow pointing to it. The table below shows the search results.

Name/Gene ID	Description	Location	Aliases
IL6 ID: 3569	interleukin 6 [<i>Homo sapiens</i> (human)]	Chromosome 7, NC_000007.14 (22725442..22732002)	BSF2, HGF, HSF, IFNB2, IL-6
Il6 ID: 16193	interleukin 6 [<i>Mus musculus</i> (house mouse)]	Chromosome 5, NC_000071.6 (30013161..30019968)	Il-6
Il6 ID: 24409	interleukin 6 [<i>Rattus norvegicus</i> (Norway rat)]	Chromosome 4, NC_005102.4	ILg6, Il-6

On the right side of the page, there are sections for 'Results by taxon' and 'Find related data'. The 'Results by taxon' section lists top organisms: Homo sapiens (142), Mus musculus (48), Bos taurus (19), Rattus norvegicus (16), Human immunodeficiency virus 1 (5), and All other taxa (321). The 'Find related data' section includes a 'Database' dropdown menu and a 'Find items' button.

mRNA基因查找

在新弹出的页面中，点击右侧**NCBI Reference Sequences**，快速定位至**序列页面**

IL6 interleukin 6 [*Homo sapiens* (human)]

Gene ID: 3569, updated on 3-Apr-2016

Summary

Official Symbol IL6 provided by HGNC
Official Full Name interleukin 6 provided by HGNC
Primary source [HGNC:HGNC:6018](#)
See related [Ensembl:ENSG00000136244](#); [HPRD:00970](#); [MIM:147620](#); [Vega:OTTHUMG00000023178](#)
Gene type protein coding
RefSeq status REVIEWED
Organism [Homo sapiens](#)
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as CDF; HGF; HSF; BSF2; IL-6; BSF-2; IFNB2; IFN-beta-2
Summary This gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, the encoded protein has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. The functioning of this gene is implicated in a wide variety of inflammation-associated disease states, including susceptibility to diabetes mellitus and systemic juvenile rheumatoid arthritis. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Dec 2015]
Orthologs [mouse](#) [all](#)

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[HIV-1 interactions](#)

[Pathways from BioSystems](#)

[Interactions](#)

[General gene information](#)

[Markers, Homology, Gene Ontology](#)

[General protein information](#)

[NCBI Reference Sequences \(RefSeq\)](#)

[Related sequences](#)

[Additional links](#)

[Locus-specific Databases](#)

Related information

[Order cDNA clones](#)

mRNA基因查找

- 3、若您的模板是基因组则点击“**Genomic**”下方的“**FASTA**”进入页面复制序列，若模板是**mRNA/cDNA**则点击“**NM_00600.3**”进入页面复制序列。

☐ [RefSeqs maintained independently of Annotated Genomes](#)

These reference sequences exist independently of genome builds. [Explain](#)

Genomic

NG_011640.1 RefSeqGene

Range	5001..9856
Download	GenBank FASTA Sequence Viewer (Graphics)

mRNA and Protein(s)

NM_00600.3 → [NP_000591.1](#) interleukin-6 precursor

[BioAssay](#)

[BioAssay by Target \(List\)](#)

[BioAssay by Target \(Summary\)](#)

[BioAssay, by Gene target](#)

[BioAssays, RNAi Target, Tested](#)

[BioProjects](#)

[BioSystems](#)

[CCDS](#)

[ClinVar](#)

[Conserved Domains](#)

[dbVar](#)

[EST](#)

mRNA基因查找

4、进入IL6的mRNA页面，向下拖动页面，点击左侧的“CDS”，图中标红的序列即为所需mRNA编码区序列。

CDS

sig_peptide

ORIGIN

```

/note= upstream in frame stop codon
117..755
/gene="IL6"
/gene_synonym="BSF2; HGF; HSF; IFNB2; IL-6"
/note="hybridoma growth factor; interleukin BSF-2; B-cell
differentiation factor; CTL differentiation factor; CDF;
IFN-beta-2; interferon beta-2; B-cell stimulatory factor
2; interferon, beta 2"
/codon_start=1
/product="interleukin-6 precursor"
/protein_id="NP_000591.1"
/db_xref="GI:10834984"
/db_xref="CCDS:CCDS5375.1"
/db_xref="GeneID:3569"
/db_xref="HGNC:HGNC:6018"
/db_xref="HPRD:00970"
/db_xref="MIM:147620"
/translation="MNSFSTSAFGPVAFSLGLLLVLPAAFPAPVPPGEDSKDVAAPHR
QPLTSSERIDKQIRYILDGISA LRKETCNKSNMCESSKEALAENNLNLPKMAEKD GCF
QSGFNEETCLVKIITGLLEFEVYLEYLQNRFSSEEQARAVQMSTKVL IQFLQKKAKN
LDAITTPDPTTNASLLTKLQAQNQWLQDMTHLILRSFKEFLQSSLRALRQM"
117..203
/gene="IL6"
/gene_synonym="BSF2; HGF; HSF; IFNB2; IL-6"

1 aatattagag tctcaacccc caataaatat aggactggag atgtctgagg ctcatctctgc
61 cctcgagccc accgggaacg aaagagaagc tctatctccc ctccaggagc ccagctatga
121 actccttctc cacaagcgcc ttccgtccag ttgccttctc cctggggctg ctccctggtg
181 tgcctgctgc ctccctgccc ccagtacccc caggagaaga ttccaaagat gtacccgccc
241 cacacagaca gccactcacc tcttcagaac gaattgacaa acaaatctcg tacatcctcg
301 accgcctctc agccctgaga aaggagacat gtaacaagag taacatgtgt gaaagcagca
361 aagaggcact ggcagaaaaa aacctgaacc ttccaaagat ggctgaaaaa gatggatgct
421 tccaatctgg attcaatgag gagacttgcc tggtgaaaat catcactggt cttttggagt
481 ttgaggtata cctagagtac ctccagaaca gatttgagag tagtgaggaa caagccagag
541 ctgtgcagat gattacaaaa gtccctgatcc agttcctgca gaaaaaggca aagaatctag
601 atgcaataac caccctgac ccaaccacaa atgccagcct gctgacgaag ctgcaggcac
661 agaaccagtg gctgcaggac atgacaactc atctcattct ggcagcgttt aaggagtccc
721 tgcagtccag cctgagggct cttcgagcaa tgtagcatgg gcacctcaga ttgttgttgt
781 taatgggcat tcttcttctt ggtcagaaac ctgtccactg ggcacagaaac ttatgttgtt
841 ctctatggag aactaaaagt atgagcgta ggacactatt ttaattattt ttaatttatt

```

5、用鼠标选中标红序列，Ctrl+C 或者右键复制

```

/gene="IL6"
/gene_synonym="BSF-2; BSF2; CDF; HGF; HSF; IFN-beta-2;
IFNB2; IL-6"

ORIGIN
1  gtctcaatat tagagtctca accccaata aatataggac tggagatgtc tgaggctcat
61  tctgcctctg agcccaccgg gaacgaaga gaagctctat ctccctcca ggagcccagc
121 tatgaactcc ttctccaaa gcgcttctg tccagttgoc ttctcctgg ggtgctctct
181 ggtgttgctt gctgccttcc ctgcccagt acccccagga gaagattcca aagatgtagc
241 cgcaccacac agacagccac tcacctctc agaacgaatt gacaaacaaa ttcggtacat
301 cctcgacggc atctcagccc tgagaaagga gacatgtaac aagagtaaca tgtgtgaaag
361 cagcaaagag gcactggcag aaaacaacct gaaccttcca aagatggctg aaaaagatgg
421 atgcttccaa tctgattcca atgaggagac ttgcctggtg aaaatcatca ctggtctttt
481 ggagtttgag gtatacctag agtacctcca gaacagattt gagagtagtg aggaacaagc
541 cagagctgtg cagatgagta caaaagtctt gatccagttc ctgcagaaaa aggcaaagac
601 tctagatgca ataaccacc ctgacccaac cacaaatgcc agcctgctga cgaagctgca
661 ggcacagAAC cagtggctgc aggacatgac aactcatctc attctgcgca gcttgaagga
721 gttcctgcag tccagcctga gggctcttc gcaaatgtag
781 gttgttaatg ggcattcctt cttctggta gaaacctgtc
841 ttgttctcta tggagaacta aaagtatgag cgttaggaca
901 ttattaatat ttaaatatgt gaagctgagt taatttatgt
961 agaagtacca cttgaacat tttatgtatt agttttgaaa
1021 gcagtttgaa tatcctttgt ttcagagcca gatcatttct
1081 caaataaatg gctaacttat acatattttt aaagaaat at
1141 gtataaatgg tttttatacc aataaatggc attttaaaaa
//

```

复制(C)

复制为纯文本

用百度搜索"atgaactcc ttctccaaa gcgcttctg tccagttgoc ttctcctgg ggtgctctct"

发送文本到手机

审查元素

扫描二维码，发送文字到手机

6、粘贴到引物设计订单中的5.基因序列框中即可。

自行检测引物的可行性，并确认是否符合您所做实验的要求。

请仔细以下表单，如需帮助请点击帮助按钮  或联系人工在线客服 

1. 您所要的引物是用于以下哪种实验的？

☐ 普通PCR ☐ 半定量RT-PCR ☒ 荧光定量 Real-Time PCR ☐ 其他（请注明）


2. 您的目的基因序列属于以下哪种类型？

☐ genomic DNA ☒ mRNA/cDNA

3. 您的目的基因属于哪个物种？

☒ Human ☐ rat ☐ mouse ☐ 其他（请注明）

4. 基因名称（只能填写一个）

5. 请您填写基因序列（只能填写一个） 

```
atgaactcc ttctccacaa gcgccttcgg tccagttgcc ttctccctgg ggctgctcct
181 ggtgttgctt gctgccttcc ctgccccagt acccccagga gaagattcca aagatgtagc
241 cgccccacac agacagccac tcacctcttc agaacgaatt gacaaacaaa ttcggtacat
301 cctcgacggc atctcagccc tgagaaagga gacatgtaac aagagtaaca tgtgtgaaag
361 cagcaaagag gcactggcag aaaacaacct gaaccttcca aagatggctg aaaaagatgg
```

有多个NM号（转录变体）怎么办？

- 找到所有转录变体的共有序列/特异序列
- 联系技术支持

[NM_000600.4](#) → [NP_000591.1](#) interleukin-6 isoform 1 precursor

[See identical proteins and their annotated locations for NP_000591.1](#)

Status: REVIEWED

Description	Transcript Variant: This variant (1) represents the longer transcript and encodes the longer isoform (1).	
Source sequence(s)	AK301141 , BC015511 , CD013918 , HY076725	
Consensus CDS	CCDS5375.1	
UniProtKB/TrEMBL	B4DVM1	
UniProtKB/Swiss-Prot	P05231	
UniProtKB/TrEMBL	Q75MH2	
Related	ENSP00000258743 , ENST00000258743	

Conserved Domains (1) [summary](#)

pfam00489	IL6; Interleukin-6/G-CSF/MGF family
Location:57 → 210	

[NM_001318095.1](#) → [NP_001305024.1](#) interleukin-6 isoform 2

Status: REVIEWED

Description	Transcript Variant: This variant (2) lacks an exon in its 5' UTR and uses a downstream in-frame start codon, compared to variant 1. The encoded isoform (2) has a shorter N-terminus than isoform 1.	
Source sequence(s)	CD013918 , HY076725	
UniProtKB/TrEMBL	B5MC21	

Conserved Domains (1) [summary](#)

pfam00489	IL6; Interleukin-6/G-CSF/MGF family
Location:1 → 134	

microRNA基因查找

- 1、打开miRBase主页 (<http://www.mirbase.org/>) , 在搜索框输入“hsa-mir-21” , 点击 “Search” 。



The screenshot shows the miRBase website interface. At the top, there is a navigation bar with links: Home, Search, Browse, Help, Download, Blog, and Submit. A search box contains the text "hsa-mir-21" and a "Search" button. Below the navigation bar, there are three main sections:

- Latest miRBase blog posts**: This section contains two blog posts. The first is titled "High confidence miRNA set available for miRBase 21" by sam (July 3, 2014). The second is titled "miRBase 21 finally arrives" by sam (June 26, 2014).
- miRNA count: 28645 entries**: This section shows the total number of miRNA entries and the release date: "Release 21: June 2014".
- Search by miRNA name or keyword**: This section contains a search box and two buttons: "Go" and "Example".

At the bottom right, there is a link to "Tweets by @mirbase".

microRNA基因查找

2、单击“Search”后得到以下页面：



The screenshot shows the miRBase website interface. The header includes the miRBase logo, the text "miRBase", and a "MANCHESTER 1824" logo. Below the header is a navigation bar with links: Home, Search, Browse, Help, Download, Blog, Submit, and a search input field with the text "hsa-mir-21" and a "Search" button. The main content area displays the "Stem-loop sequence hsa-mir-21" and a table with the following information:

Accession	MI0000077
Symbol	HGNC:MIR21
Description	Homo sapiens miR-21 stem-loop
Gene family	MIPF0000060; mir-21

microRNA基因查找

- 3、向下拖动页面，拖至下图位置。选择以“5p”或者“3p”结尾的成熟miRNA序列，点击“Get sequence”。

Mature sequence hsa-miR-21-5p	
Accession	MIMAT0000076
Previous IDs	hsa-miR-21
Sequence	8 - uagcuuaucaagacugauguuga - 29 Get sequence
Mature sequence hsa-miR-21-3p	
Accession	MIMAT0004494
Previous IDs	hsa-miR-21*
Sequence	46 - caacaccagucgaugggcugu - 66 Get sequence
Deep sequencing	3950 reads, 53 experiments

microRNA基因查找

4、点击“Get sequence”获得如下页面，复制序列，把序列中“U”替换成“T”。

Mature sequence hsa-miR-21-5p

Accession	MIMAT0000076
Previous IDs	hsa-miR-21
Sequence	8 - uagcuuauca <u>gacug</u> auguuga - 29

[Get sequence](#)

>hsa-miR-21-5p MIMAT0000076
UAGCUUAUCAGACUGAUGUUGA

Mature sequence hsa-miR-21-3p

Accession	MIMAT0004494
Previous IDs	hsa-miR-21*
Sequence	46 - caacaccagucgaugggcugu - 66

[Get sequence](#)

UAGCUUAUCAGACUGAUGUUGA

查找和替换

查找(F) 替换(R) 定位(G)

查找内容(C): U

选项: 区分大小写

替换为(I): T

更多(M) >> 替换(R) 全部替换(A) 查找下一处(F) 取消

5、将变换之后的序列粘贴到序列框中，若使用**加尾法**进行miRNA的**逆转录**，需要在“备注”栏中填写**反向引物的Tm值**。

4. 基因名称（只能填写一个）

hsa-miR-21

5. 请您填写基因序列（只能填写一个）

TAGCTTATCAGACTGATGTTGA

6. 备注

miRNA逆转录为：加尾法，反向引物Tm值为58℃。

引物特异性比对

引物特异性比对

- 引物特异性检测工具：百度搜索**Primer blast**第一个就是。
- <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>

PCR Template [Reset page](#) [Save search parameters](#) [Retrieve recent results](#) [Publication](#) [Tips for finding specific primers](#)

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

Or, upload FASTA file

Range

Forward primer From To [Clear](#)

Reverse primer [Clear](#)

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 Max

of primers to return

Primer melting temperatures (T_m)

Min Opt Max Max T_m difference [?](#)

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section [?](#)

Exon junction span [?](#)

Exon junction match

Exon at 5' side Exon at 3' side

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

引物特异性比对

Primer Pair Specificity Checking Parameters

Specificity check

☒ Enable search for primer pairs specific to the intended PCR template

Search mode

Automatic

Database

Refseq mRNA

Exclusion

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

Organism

Homo sapiens

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)

Get Primers

☐ Show results in a new window ☒ Use new graphic view

Primer pair 2

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCTCCTCATCCTCTCCACA	Plus	20	1521	1540	60.03	60.00	2.00	0.00
Reverse primer	CTTTGGTCCGTCTCCTCCAC	Minus	20	1697	1678	60.04	60.00	3.00	0.00
Product length	177								

Products on intended target

>[NM_001122742.1](#) Homo sapiens estrogen receptor 1 (ESR1), transcript variant 4, mRNA

product length = 177
 Forward primer 1 CCTCCTCATCCTCTCCACA 20
 Template 1891 1910

Reverse primer 1 CTTTGGTCCGTCTCCTCCAC 20
 Template 2067 2048

>[NM_001122741.1](#) Homo sapiens estrogen receptor 1 (ESR1), transcript variant 3, mRNA

product length = 177
 Forward primer 1 CCTCCTCATCCTCTCCACA 20
 Template 1739 1758

Reverse primer 1 CTTTGGTCCGTCTCCTCCAC 20
 Template 1915 1896

>[NM_001122740.1](#) Homo sapiens estrogen receptor 1 (ESR1), transcript variant 2, mRNA

product length = 177
 Forward primer 1 CCTCCTCATCCTCTCCACA 20
 Template 1782 1801

Reverse primer 1 CTTTGGTCCGTCTCCTCCAC 20
 Template 1958 1939

>[NM_000125.3](#) Homo sapiens estrogen receptor 1 (ESR1), transcript variant 1, mRNA

product length = 177
 Forward primer 1 CCTCCTCATCCTCTCCACA 20
 Template 1755 1774

Reverse primer 1 CTTTGGTCCGTCTCCTCCAC 20



>[XM_011527014.1](#) PREDICTED: Homo sapiens SH3 and multiple ankyrin repeat domains 1 (SHANK1), transcript variant X3, mRNA

product length = 2982

Forward primer 1 CCTCCTCATCCTCTCCCACA 20

Template 6315C...A.GT....C 6334

Forward primer 1 CCTCCTCATCCTCTCCCACA 20

Template 9296T...C.....C 9277

>[XM_006723233.2](#) PREDICTED: Homo sapiens SH3 and multiple ankyrin repeat domains 1 (SHANK1), transcript variant X2, mRNA

product length = 2982

Forward primer 1 CCTCCTCATCCTCTCCCACA 20

Template 6342C...A.GT....C 6361

Forward primer 1 CCTCCTCATCCTCTCCCACA 20

Template 9323T...C.....C 9304

>[XM_011527013.1](#) PREDICTED: Homo sapiens SH3 and multiple ankyrin repeat domains 1 (SHANK1), transcript variant X1, mRNA

product length = 2982

Forward primer 1 CCTCCTCATCCTCTCCCACA 20

Template 6366C...A.GT....C 6385

Forward primer 1 CCTCCTCATCCTCTCCCACA 20

Template 9347T...C.....C 9328

引物特异性比对

特异性比对结果分析：

- 出现transcript variant结尾的基因是没有关系的，它指的是不同的转录变体，还是同一个基因；
- 出现的非目的条带和目的条带大小相差较大，相差1000bp以上可忽略不计，因为PCR扩增程序中延伸时间的限制，这种非目的条带扩增不出来；
- 有些非目的条带和引物之间有四五个碱基不能互补配对，一般是扩增不出来的，尤其是这种不能互补的碱基在引物的3'端时，扩增不出来的可能性更大。



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谢谢！