

# Mir 前体克隆步骤

# 1、首先进入 mirbase 网站 <u>http://www.mirbase.org/</u>

miRBase	miRBase
Home Search Browse Help D	pwnload Blog Submit
Latest miRBase blog posts	
miRBase 19 released miRBase 19 is now available, brought to y time, we have added more than the usual miRBase 19 is coming We're scrambling to release miRBase 19 ir including the first entries for 25 new specie More []	By <u>sam</u> (August 1, 2012) ou from the Benasque RNA meeting in the sunny Pyrenees, and with a slightly larger time gap than usual. In that extended number of new sequences — 3171 new hairpins and 3625 novel mature products, bringing the totals to 21264 [] By <u>sam</u> (July 31, 2012) in the next few days from the Benasque RNA meeting in middle of the sunny Pyrenees. We have over 3000 new sequences, es (mostly plants). We've also put some effort into cleaning up some old entries, deleting over 130 misannotated sequences.
miRBase: the microf	NA database
2、search 需克隆的 mir (	以 has-mir-143 为例)
如下图找到工具栏的	"search"按钮,单击进入:
miRBase	miRBase
Home Search Browse Help D	ownload Blog Submit
Latest miRBase blog posts	
miRBase 19 released miRBase 19 is now available, brought to yu time, we have added more than the usual miRBase 19 is coming We're scrambling to release miRBase 19 in including the first entries for 25 new specie More []	By sam (August 1, 2012) bu from the Benasque RNA meeting in the sunny Pyrenees, and with a slightly larger time gap than usual. In that extended number of new sequences — 3171 new hairpins and 3625 novel mature products, bringing the totals to 21264 [] By sam (July 31, 2012) i the next few days from the Benasque RNA meeting in middle of the sunny Pyrenees. We have over 3000 new sequences, es (mostly plants). We've also put some effort into cleaning up some old entries, deleting over 130 misannotated sequences.
miRBase: the microf	NA database
在接下来弹出的对话框中	输入 hsa-mir-143,单击"提交查询内容"
Search miRBase	vord

Enter a miRNA accession, name or keyword:

hsa-mir-143

提交查询内容 重置 Example

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 <sup>3</sup> 汉恒生物科技▼

Solution www.hanbio.net



#### 弹出以下画面:

	1 1 1 2			
Stem-loop see	Juence nsa-mir-143			
Accession	M10000459			
Symbol	HGNC:MIR143			
Description	Homo sapiens miR-143 stem-loop			
Gene family	MIPF0000094; <u>mir-143</u>			
	This text is a summary paragraph taken from the Wikipedia entry entitled Mir-143, miRBa	se and <u>Rfam</u> are facilitating community annotation of microRNA families ar		
Community	In molecular biology mir-143 microRNA is a short RNA molecule. MicroRNAs function to regulate the expression lev			
annotation	Chem Wildende ander Wildende Chemistration and Chemistration and Chemistration and Chemistration and Chemistration			
	Show wikipedia enuy view @ wikipedia edu wikipedia enuy			
	-ge c ceug c ag g g u - ag			
Stem-loop	Stem-loop cruc ur arga ar gracue augueacada unaga cu ar u			
	cga u uuga aaa g a u g gg			
	Get sequence			
11 <u>1</u> 0000	12540 reads, 59 experiments			
Deep sequencing		read		
	GCGCAGCGCCCUGUCUCCCAGCCUGAGGUGCAGUGCGAGUCUCUGGGAGUCUGGGAGUCUGAGAUGAA	SCACUGUAGCAAGGAGAGAGAGAGUUGUUCUGCAGC		
	This miRNA sequence was predicted based on homology to a v	verified miRNA from mouse [1] Michael et al. subser		
Comments	of the miRNA in precancerous and neoplastic colorectal tissue	[2]. miR-143 cloned in [3] has a 1 nt 3' extension (A		
Genome	Coordinates Overlanning trans	crints		
context	chr5:         148808481-148808586 [+]         intergenic	стр. <del>с</del>		
单击 Stem-loop	,中的 Get sequence 按钮即可获得所需的 hsa	-mir-143 前体序列。		
	gcag gc ucuc c ccugag ugcag	g u - ag gugcu caucuc gg uc u		
Stem-loop		нін нин ії н		
Stem loop	cgucug agagig ggacuc auguo cga u uuga alaa g	acga guagag cu ag u a u g gg		
	Get sequence			
	<del>7</del>			
	chr5: 148808481-148808586 [-	+1		
单击 Genome c	context 中			
Genome	Coordinates	Overlapping transcripts		
context	<u>chr5: 148808481-148808586 [+]</u>	intergenic		
	,			
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在弹出的画面中单击 Let Export data

即可获得 mir 所在的基因组信息。

3、获得 mir 前体基因组序列并克隆

克隆的时候一般往上下游推。如下图:上下游各 300bp,一般长一点短一点都可以,总

长度 300bp 以上就差不多:

Location to export	CHIOHOSOHE.GRCh37.0.133017033.133101		
Output:	FASTA sequence 💌		
Select location:	6 * 133017695 * 133161157		
5' Flanking sequence (upstream):	300 × (M->		
3' Flanking sequence (downstream):	300 * (M->		
	Next >		

单击 Next > 即可获得 mir 前体基因组序列。

以此序列为模板, primer5 设计引物并加入酶切位点(设计引物时,最好将 mir 前体序列 置于整个 PCR 目的产物的中部附近,不要太靠近 PCR 目的产物的末端)。

4、设计好的引物还可以在 NCBI 验证其准确性

以 hsa-mir-143 为例,设计好的引物如下:

### Forward: GGTCAAGGTTTGGTCCTGGGTGCTC

#### Reverse: AGGGTGCTGGGGGAAATGCTAACGCC

首先进入 NCBI 网站主页

Welcome to NCBI	Popular Resources
The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.	PubMed
About the NCBI IMission IOrganization IResearch IRSS Feeds	Bookshelf
	PubMed Central
	PubMed Health
	BLAST
Get Started	Nucleotide
Tools: Analyze data using NCBI software	Genome
Downloads: Get NCBI data or software	SNP
How-To's: Learn how to accomplish specific tasks at NCBI	Gene
Submissions, Submit data to Genbank or other NCBI databases	Protein
	PubChem

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   <sup>2</sup>
   <sup>1</sup>
   </sup>
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## 单击 BLAST 按钮,进入 Basic BLAST 页面

Choose a	a BLAST pr	ogram to run.
nucleot	ide blast	Search a <b>nucleotide</b> database using a <b>nucleotide</b> query Algorithms: blastn, megablast, discontiguous megablast
prot	tein blast	Search <b>protein</b> database using a <b>protein</b> query Algorithms: blastp, psi-blast, phi-blast, delta-blast
	<u>blastx</u>	Search protein database using a translated nucleotide query
	<u>tblastn</u>	Search translated nucleotide database using a protein query
	<u>tblastx</u>	Search translated nucleotide database using a translated nucleotide query

单击 nucleotide blast 按钮,进入如下界面,并将上述设计好的引物输入方框内,如下图 选择 Somewhat similar sequences (blastn)

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Enter accession r	number(s), gi(s), or FASTA sequence(s) 😡	Clear	Query subrange 🧕
GGTCAAGGTTTGG AGGGTGCTGGGGA	TCCTGGGTGCTC AATGCTAACGCC	-	From To
Or, upload file Job Title	浏览 )	3	
Align two or r	nore sequences 😡		
Choose Sea	rch Set		
Database	Human genomic + transcript     O Mouse genomic + transcript	Others (nr etc.):	
Exclude Optional	Models (XM/XP) Uncultured/environmental sample sequences		
Entrez Query Optional	Enter an Entrez query to limit search 🕑		
Program Sel	ection		
Optimize for	<ul> <li>Highly similar sequences (megablast)</li> </ul>		
	More dissimilar sequences (discontiguous megablast)		
	Somewhat similar sequences (blastn)		
/			
RIAST	Search database Human G+T using Blastn (Optimize for some	what similar s	equences)

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单击 BLAST 按钮:如下图所示,第一个全绿色的是与上述引物完全匹配的基因组,

其余颜色均不完全匹配。



这时只要查询这个对应的基因组是否为 hsa-mir-143 前体序列即可。

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