

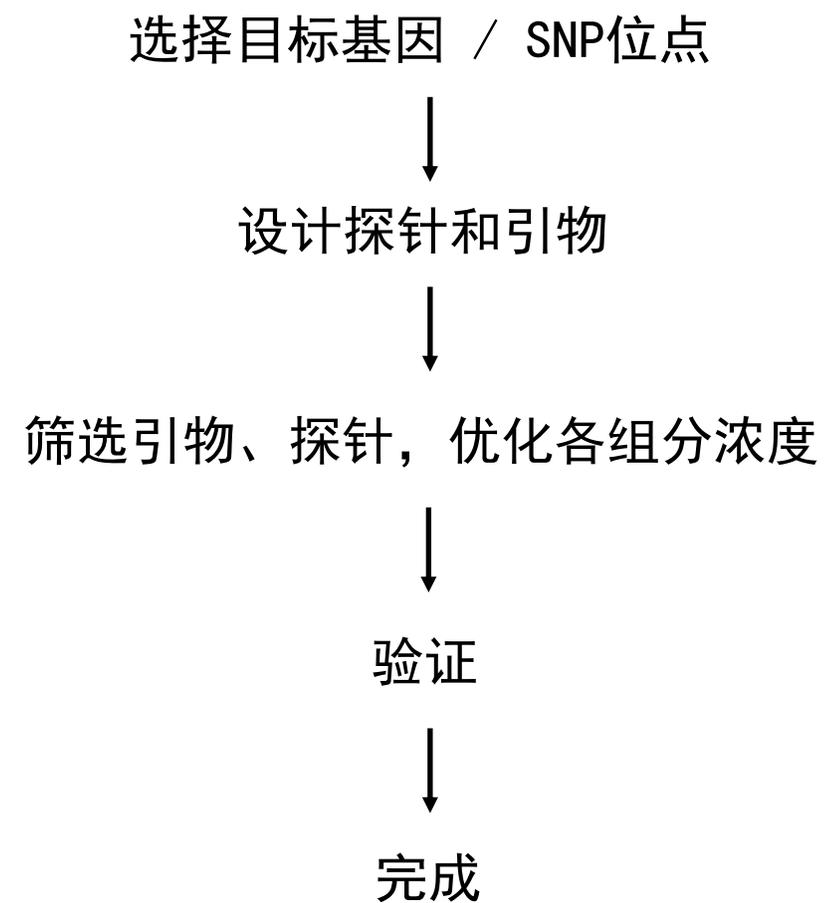


ThermoFisher
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引物探针的设计流程

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引物探针的设计流程



选择目的基因/SNP位点

PubMed Home - Microsoft Internet Explorer

Address <http://www.ncbi.nlm.nih.gov/pubmed>

NCBI PubMed www.pubmed.gov

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All Databases PubMed Nucleotide Protein Genome Structure OMIM PMC Journals

Search Nucleotide for Enterovirus Go Clear

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Internet

序列查找

The screenshot shows a Microsoft Internet Explorer browser window displaying the NCBI Entrez Nucleotide search results for the query 'Enterovirus'. The address bar shows the URL 'http://www.ncbi.nlm.nih.gov/sites/entrez'. The search bar contains 'Enterovirus' and the results are displayed in a list format. The top navigation bar includes 'All Databases', 'PubMed', 'Nucleotide', 'Protein', 'Genome', 'Structure', 'PMC', 'Taxonomy', and 'Books'. The search results section shows 'Found 14530 nucleotide sequences. Nucleotide [14439] EST [91]'. The display options are set to 'Summary', 'Show 20', and 'Sort by'. The results list includes items 1 through 6, each with a checkbox, a link to the sequence, and a brief description. A 'Top Organisms [Tree]' sidebar is visible on the right, listing various organisms and their sequence counts.

NCBI Nucleotide

Search Nucleotide for Enterovirus Go Clear Save Search

Limits Preview/Index History Clipboard Details

History has expired.

Found 14530 nucleotide sequences. Nucleotide [14439] EST [91]

Display Summary Show 20 Sort by Send to

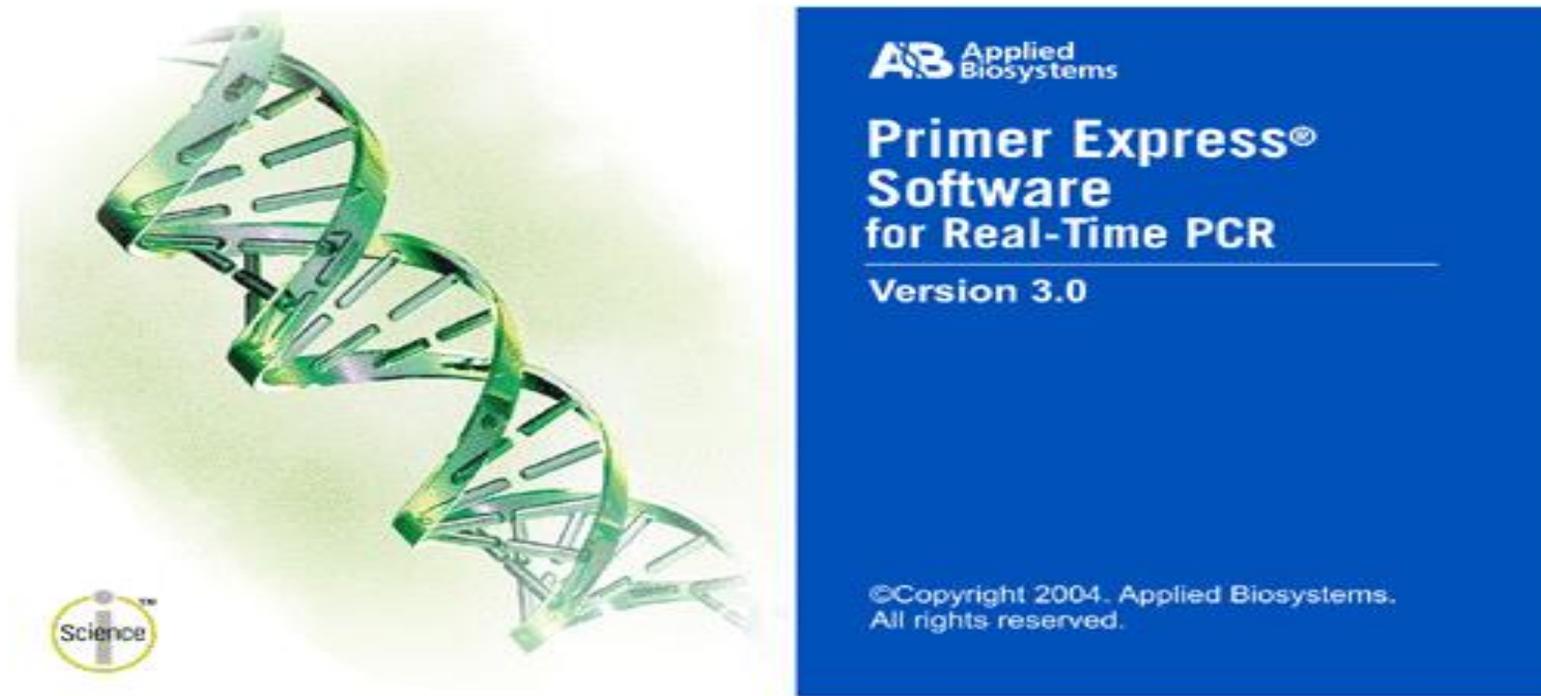
All: 14439 Bacteria: 0 RefSeq: 25 mRNA: 853

Items 1 - 20 of 14439 Page 1 of 722 Next

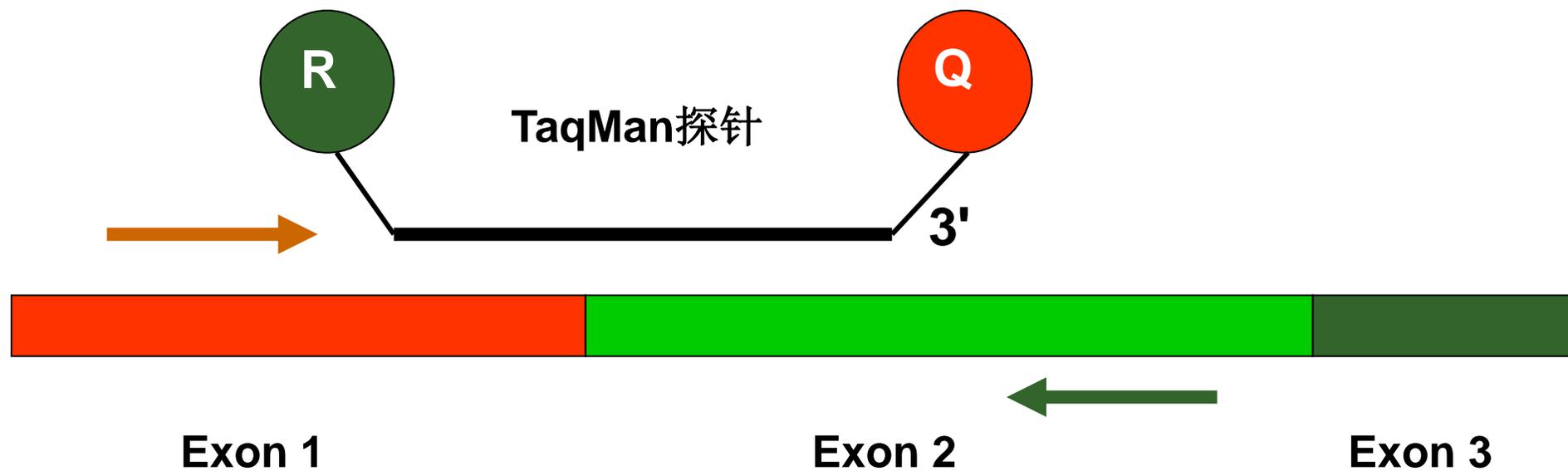
- 1: [NC_001430](#) Reports
Human enterovirus D, complete genome
gi|9626436|ref|NC_001430.1|[9626436]
- 2: [NC_001428](#) Reports
Human enterovirus C, complete genome
gi|9626433|ref|NC_001428.1|[9626433]
- 3: [AC024817](#) Reports
Caenorhabditis elegans cosmid Y54G2A, complete sequence
gi|7140379|gb|AC024817.1|[7140379]
- 4: [NM_201429](#) Reports
Homo sapiens reticulon 3 (RTN3), transcript variant 3, mRNA
gi|41393603|ref|NM_201429.1|[41393603]
- 5: [AM939639](#) Reports
Human enterovirus 71 EP/449/2000 partial 3D pol gene for polymerase 3D, genomic RNA
gi|186929183|emb|AM939639.1|[186929183]
- 6: [AM939638](#) Reports
Human enterovirus 71 EP/2063/2001 partial 3D pol gene for polymerase 3D, genomic RNA
gi|186929181|emb|AM939638.1|[186929181]

Top Organisms [Tree]

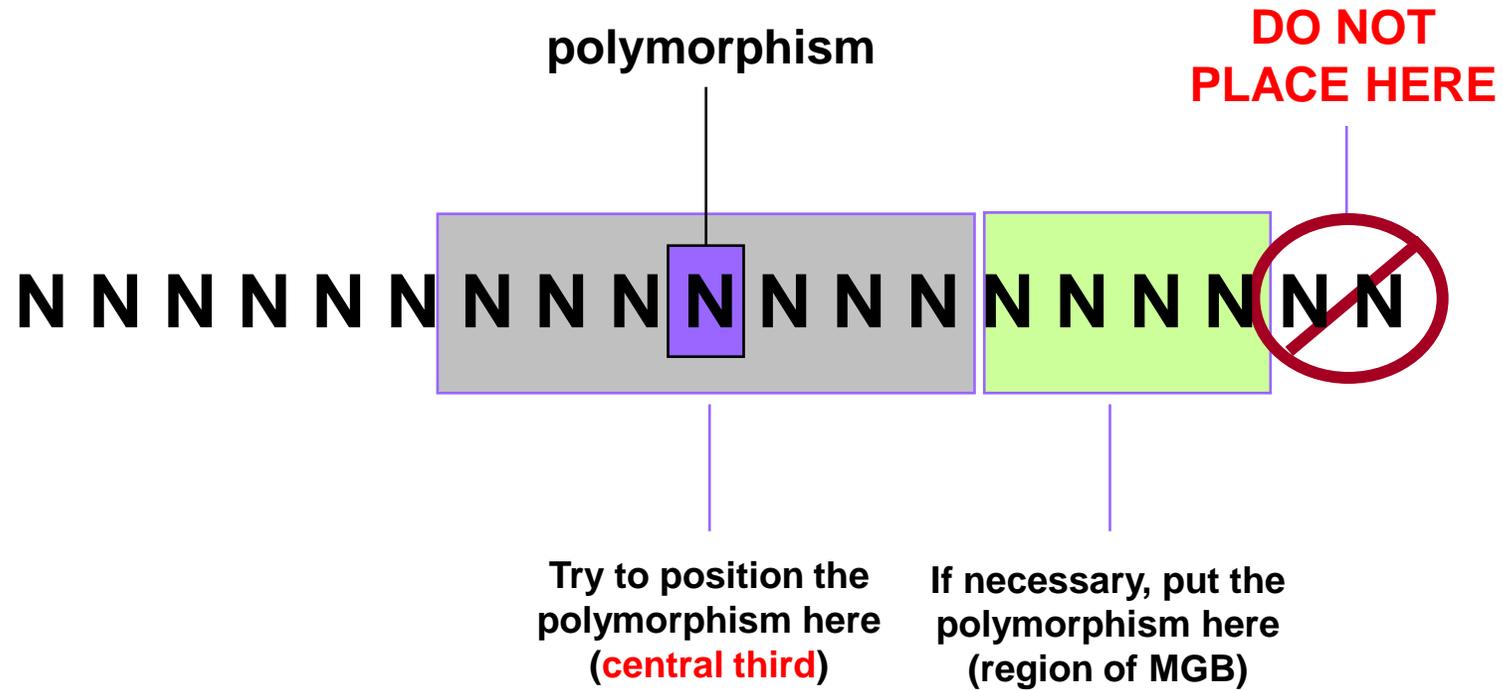
- Human enterovirus B (6738)
- Human enterovirus A (3241)
- Poliovirus (2179)
- Human enterovirus 71 (1952)
- Human echovirus 30 (1871)
- All other taxa (9811)
- More...

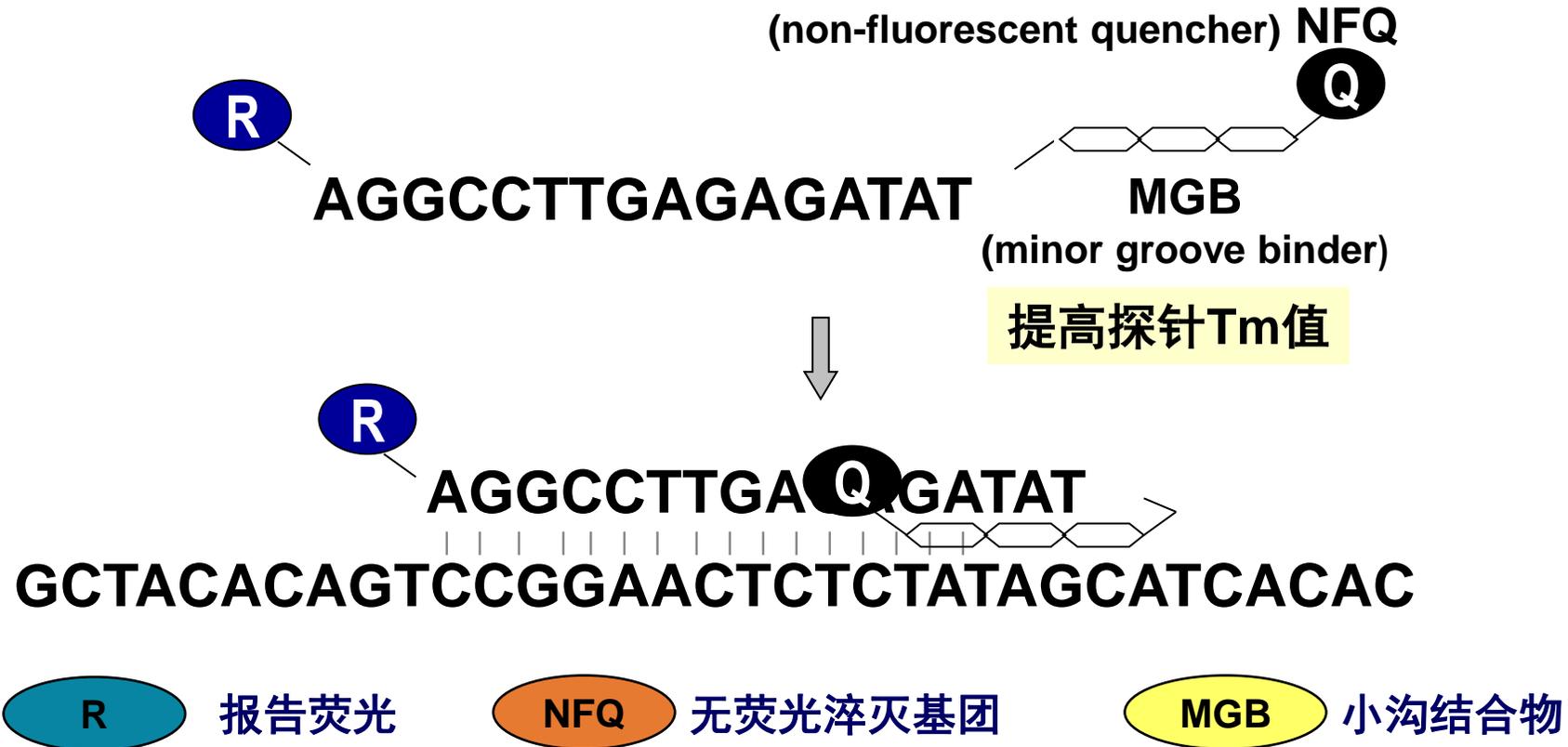


- 优先考虑探针位置
- 探针的5'端第一个碱基不能是G
- 基因表达探针T_m值：68-70°C；基因分型探针T_m值：65-67°C
- TaqMan探针长度13-30bp，TaqMan-MGB探针长度在13-25bp
- 避免同一碱基重复过多，特别是连续4个或更多的G
- C比G多的探针效果更好，如果C少于G，选择其互补链
- 多态位点应尽量位于探针中央，可以±3个碱基



基因表达、cDNA定量研究中，特别设计探针跨过两个外显子，以增强扩增特异性





荧光标记基团选择

荧光染料	最大发射峰(nm)	用途
6-FAM	~520	报告荧光
SYBR Green I	~520	报告荧光
JOE	~550	报告荧光
VIC	~550	报告荧光
TAMRA	~580	淬灭荧光
NED	~580	报告荧光
CY-3	~580	报告荧光
ROX	~610	参比荧光
TEXAS Red	~610	报告荧光
CY-5	~670	报告荧光

- 引物尽可能地接近探针，但是不可与探针重叠
- 引物长度9-40bp，最优20bp，GC含量保持在30-80%之间
- 避免同一碱基重复过多，特别是连续4个或更多的G
- 引物T_m值在58-60°C之间，上下游引物T_m不要超过2°C
- 引物3'端的5个碱基中，G和C加起来不要超过2个
- 引物3'端不要是碱基A，避免出现3个以上相同碱基
- 避免引物自身或与引物之间形成4个或4个以上连续配对
- 避免引物自身形成环状发卡结构
- 扩增片段的长度在50-150bp之间

- **NCBI**

BLAST引物扩增特异性

<http://blast.ncbi.nlm.nih.gov/Blast.cgi>