

LncRNA 3'及 5'RACE 实验

合同号: PG1-1601004

1. 将提供的序列在 NCBI 上进行比对，比对结果如下：

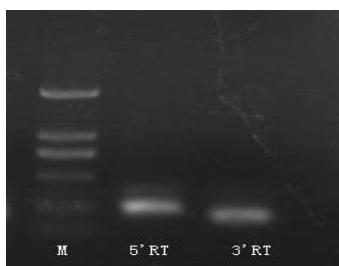
[illegible]

2. 我们采用能够比对得上的保守区域进行 5'及 3'两端引物的设计, 所用设计引物的序列标注红色如下:

TAGCAGTGCAGGTCATAGGAAAGCGAGTATGTGTCATAGAGGGTTGCCTTCACGTTACGGCAGCCAATAAAGTCCT
GAGGGTTTCAGCTTGTACAGGTCGAAGGTTACCCGGGCCACATCGATCTTCTTAGTTGGCTTCTGGGAAAGGGCTAGCTG
GGCTCTCTTCTGCGCTGCAAGACAAGCCCGTGAAGCTCACCTCTGGAACATCTCCCGAGCTCTCGACATTCACCCCTGC
CCCGGGAAGGCCGTACCTGTCTGATGGGGGCTTCCACTTCTGCCCTTCAGCAGGACCATGAACATGGTATCTTTTGC
CAGGGCTTGGAAGTATCTTCTGTCTCCAGTATGTGCCATCTTCTCCAGCACCAGGGAGAAGGGCTTGTCTTAAAGTTT
CAAGATGTCCTGGACCTGGAAGA

AGttccagggatctgtcacctctgcaggcctcgtggacactgcgatcatatgggtgtaaaaaCTATACACCAGAGAGCCGGGACTAAAGGTGT
GTGCCACCAAAGTGGTTAAGACGAGTACTTTATAAATGCCTACACATCCGTAGGCACAGCTGATCAGGAAGCTGATTCTAA
GGCAGCAACACGAAGCTAGAGCTGTACAGCCTTTCTGTCTGTAGTCACCTGTCCGTGGGGACTACTGGCTCTCACGA
GATGCATTATTTAGAGCTAAGCCAATCCCTGGAAGAGGCGACATTCCTTCATCGAGTACTTTCAGTGTGTGCCACGCTAGG
GGCTGACAATACATCAGAAAGCAAGACGGCCTTAGCCACACCTGGAGGAGCTGGGCTGGAACAGAAGCAAATGCAC
GAATGAGCAATGGGagccaggagtggatgatcacccctctctcaccggagcctcagaggctgacgcacaaacatcaccagtctaagcaggc
ctgggccacacagtgaaggctcttacctcaaagaaaaaaaAAAGTTTGTATTATTATTCGTTGTTTCATGTTGTTTCTTCTTTGTTTC
CTAAT

- 2.1 以红色部分作为原始序列进行引物的设计,以提取的 RNA 逆转录后的 cDNA 为模板进行 RT-PCR 扩增,扩增结果经琼脂糖凝胶电泳检测如下:



注：采用 DL2000 的 Marker



2.2 RT-PCR 产物送测序，将测序结果进行比对，比对结果如下：

3'比对结果：

Download GenBank Graphics				
PREDICTED: Mus musculus uncharacterized LOC105242739 (LOC105242739), ncRNA				
Sequence ID: reflXR_861982.1 Length: 751 Number of Matches: 1				
Range 1: 512 to 674 GenBank Graphics ▼ Next Match ▲ Previous Match				
Score	Expect	Identities	Gaps	Strand
294 bits(159)	3e-76	162/163(99%)	1/163(0%)	Plus/Plus
Query 11	GCTAGGGGCTGAC-ATACATCAGAAAGCAAGACGGCCTTAGCCCACACCTGGAGGAGCTG	89		
Sbjct 512	GCTAGGGGCTGACAATACATCAGAAAGCAAGACGGCCTTAGCCCACACCTGGAGGAGCTG	571		
Query 70	GGCTGGAACAGAAAGCAAATGCACGAATGAGCAATGGGAGCCAGGAGTGGTGTATGTACAC	129		
Sbjct 572	GGCTGGAACAGAAAGCAAATGCACGAATGAGCAATGGGAGCCAGGAGTGGTGTATGTACAC	631		
Query 130	CCCTCTCTCTCACCCTGGAGCCTCCAGAGGCTGACGCACAAACA	172		
Sbjct 632	CCCTCTCTCTCACCCTGGAGCCTCCAGAGGCTGACGCACAAACA	674		

5'比对结果：

PREDICTED: Mus musculus uncharacterized LOC105242739 (LOC105242739), ncRNA				
Sequence ID: reflXR_861982.1 Length: 751 Number of Matches: 1				
Range 1: 286 to 509 GenBank Graphics ▼ Next Match ▲ Previous Match				
Score	Expect	Identities	Gaps	Strand
390 bits(211)	5e-105	220/224(98%)	1/224(0%)	Plus/Plus
Query 15	ACTGGTT-CGACGAGTACTTTATAAATGCCTACACATCCGTAGGCACAGCTGATCAGGAA	73		
Sbjct 286	ACTGGTTAAGACGAGTACTTTATAAATGCCTACACATCCGTAGGCACAGCTGATCAGGAA	345		
Query 74	GCTGATTCTAAGGCAGCAACACGAAGCTAGAGCTGTACAGCCTTTCTGTCTGTAGTCAC	133		
Sbjct 346	GCTGATTCTAAGGCAGCAACACGAAGCTAGAGCTGTACAGCCTTTCTGTCTGTAGTCAC	405		
Query 134	CTGTCCGGTGGGACTCACTGGCTCTCACGAGATGCATTATTTAGAGCTAAGCCAATCCC	193		
Sbjct 406	CTGTCCGGTGGGACTCACTGGCTCTCACGAGATGCATTATTTAGAGCTAAGCCAATCCC	465		
Query 194	TGGAAGAGGCGACATTCCTTCATCGAGTACTTTAAATGTGTGCC	237		
Sbjct 466	TGGAAGAGGCGACATTCCTTCATCGAGTACTTTCACTGTGTGCC	509		

2.3 RT-PCR 结果比对正确，下一步安排进行 5'及 3'race 实验扩增尝试