

RACE 实验进展报告

客户合同号: PG1-1511003

传真: 027-88189683

1. 实验材料

1.1 主要实验仪器

| 名称 | 厂家 | 型号 |
|---------------|-----------|---------|
| 电泳仪 | 北京六一仪器厂 | DYY-6C |
| 定量 PCR 反应扩增仪 | ABI | 7900HT |
| NanoDrop 2000 | Thermo | ND-2000 |
| 超微量分光光度计 | | |
| 离心机 | Eppendorf | 5424 R |

1.2 主要实验试剂

| 名称 | 厂家 | 货号 |
|--------------------|------------|----------|
| TRIzol | Invitrogen | 15596018 |
| cDNA 第一链合成试剂盒 | Fermentas | #K1622 |
| SMARTerRACE 5' /3' | Clontech | 634858 |
| T4 DNA 连接酶 | Fermentas | |

2. 实验步骤

2.1 RNA 提取

- 1. 利用 TRIzol 提取 RNA。具体步骤可以参考试剂盒说明书。
- 2. NanoDrop 检测其浓度和纯度。

2.2 RNA 反转录及 cDNA 验证

- 1. 取 1ug 总 RNA 反转录成 cDNA, 步骤和体系按照说明书进行。
- 2. 以 cDNA 为模板,用 Real-Time PCR 内参基因 B-actin 引物做 RT-qPCR 扩增,验证 cDNA 的质量。

2.3 RT-PCR产物测序

- 1. RT-PCR 并且凝胶电泳验证
- 2. RT-PCR产物纯化后测序



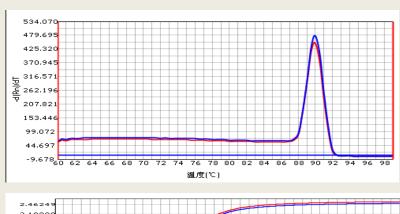
2.4 RACE 实验

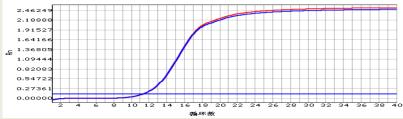
- 1. 利用 SMARTerRACE 5'/3'试剂盒做 RACE 实验。具体步骤参考说明书。
- 2. 测序
- 3. 实验结果及分析
- 3.1 RNA 浓度和纯度报告

纯度=260nm/280nm=0.550/0.296=1.85 浓度=8.8ug/u1 (未稀释)

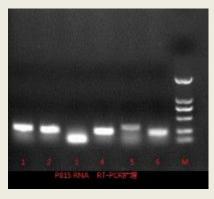
3.2 RT-qPCR 检测 cDNA 质量 (B-actin 基因):

Ct=11.3





3.3 RT-PCR 引物、凝胶电泳图片以及测序结果: RT-PCR 扩增电泳图:



AK149641 基因有 1-4 共四对 RT 引物; AK007111 基因有 5-6 共两对 RT 引物:

RT-F1: TAGAGCCTTATAGGTTTGTCT RT-R1: ACTGAACAGAGCCCTAGTGTA

扩增基因片段大小: 273bp



RT-F2: TTGCTATGGTCTTCTGCTCAC RT-R2: ATATGCAGGCAAACACTTATA

扩增基因片段大小: 261bp

RT-F3: ACTGAGCCTTGCTGACAGTTC RT-R3: TGCCTGCTGATTCAGATGTAG

扩增基因片段大小: 114bp

RT-F4: GCACATCCCAGAACAGCAAAG RT-R4: GCTCAGTTAAATGATTCCCTAT

扩增基因片段大小: 251bp

RT-F5: GATAATGCCGTGGGATGTGGA RT-R5: CCGTTGCCTGCTTGGCTTACT

扩增基因片段大小: 322bp

RT-F6: GGAGTTGGTGGCAGGAGTTCA RT-R6: TTGGTGCATATTCAGAGTTTCAGG

扩增基因片段大小: 239bp

RT-PCR-1 测序结果 (测序引物 F1):

| 69 to 304 | <u>Graphics</u> | | | ▼ Next Match 🔺 Pre |
|-------------|---|---|---|----------------------------|
| (221) | Expect 7e-119 | Identities 232/237(98%) | Gaps 2/237(0%) | Strand Plus/Plus |
| 71111111111 | 111111111111111111 | 1 111111111111111111111111111 | | |
| 11111111111 | 1111111111111111 | | | |
| | | 111111111111111111111111111111111111111 | | |
| 11111111111 | | 11111111111111111111111 | | |
| 8 8 | (221) AGTGTGACTG AGTGTGACTG AATTGAGGTA: B AATTGAGGTA: TTCTGCTCAC: | AGTGTGACTGTCGCCATTTCATGGG | Expect Identities (221) 7e-119 232/237(98%) AGTGTGACTGTCGCCATTTCATGGG-AGCCCAGAATGGTCCTGGCCAGGC | Expect Identities Gaps |

RT-PCR-2 测序结果 (测序引物 F2):

| Range 1: | 217 to 437 | <u>Graphics</u> | | | 🔻 Next Match 🔺 Pre |
|---------------------|-------------|------------------|----------------------------|------------------|-----------------------|
| Score 387 bits(2 | 200) | Expect 3e-112 | Identities 218/222(98%) | Gaps 2/222(0% | Strand) Plus/Plus |
| 307 DICS(2 | 209) | 36-112 | 210/222(9070) | 2)222(070 |) Plus/Plus |
| Query 8 | ACAGTCTGACC | TATTTCCTTCTTG- | -ACATTCAGAAAGACTTCTT | CTACTGGAACATTCCA | 66 |
| Sbjet 217 | ACAGTCTGACC | TATT-CCTTTTG | AÁCÁTTCÁGÁÁÁÁGÁCTTCTT | ĊŤÁĊŤĠĠÁÁĊÁŤŤĊĊÁ | 275 |
| Query 67 | AAGATCTCTAC | ACTAGGGCTCTGTT | CAGTTTTTGGCTTCCTTAT | GTGACATGGGGCCTAG | 126 |
| Sbjet 276 | AAGATCTCTAC | ACTAGGGCTCTGT | icágtítítíggetteettát | ĠŤĠĂĊĂŤĠĠĠĠĊĊŤĂĠ | 335 |
| Query 127 | GTCAACATTTG | GGGGTCTAAATAT? | TACTTTTCTAAGCATTGTT | ATAATTTAAATTCCTT | 186 |
| Sbjet 336 | GTCAACATTTG | GGGGTCTAAATAT | TACTTTTCTAAGCATTGTT | | 395 |
| Query 187 | TAAGTTATTCT | 'ATTTTCTGTGTATA | AAGTGTTTGCCAGCATAT : | 228 | |
| Sbjct 396 | TAAGTTATTCT | 'ATTTTCTGTGTATA | AAGTGTTTGCCTGCATAT | 437 | |

RT-PCR-3 测序结果(测序引物 F3):



| Range | e 1: 3 | 294 to | 358 <u>Gra</u> p | <u>ohics</u> | | | | | Next Match |
|-------|--------|--------|------------------|--------------|--------------|-------------|--------------|-----|------------|
| Score | е | | Exp | ect | Identities | | Gaps | | Strand |
| 110 b | oits(5 | i9) | 2e-2 | 29 | 63/65(97% | 6) | 0/65(0%) | | Plus/Plus |
| Query | 20 | | 111111111111 | | ATTGGAAGAGCT | 11111111111 | | 79 | |
| Sbjet | 294 | AATGGT | GTCATGAAGG(| CCAACATGA | GTTGGAAGAGCT | GAGAGTTCTAC | ATCTGAATCAGC | 353 | |
| Query | 80 | AGGCA | 84 | | | | | | |
| Sbjct | 354 | ÁĠĠĊÁ | 358 | | | | | | |

RT-PCR-4 测序结果 (测序引物 F4):

| Range 1: | 45 to 252 | <u>Graphics</u> | | | ▼ Next Match 7 |
|-----------|-------------|-------------------------|----------------------------|----------------|----------------|
| Score | | Expect | Identities | Gaps | Strand |
| 366 bits | (198) | 3e-106 | 205/208(99%) | 1/208(0%) | Plus/Plus |
| Query 16 | AGTGGGCAGGI | ACTGAATTATGTTG | GATGAAAAGATGTTCATGAAATAATG | TTAACTATGA 75 | |
| Sbjet 45 | ÁGTGGGCÁGGG | GACTGAATTATGTT | GATGAAAAGATGTTCATGAAATAATG | TTAACTATGA 104 | |
| Query 76 | ACTATGTTTTC | TACAGATGCACATI | TTGCTTTGTTTAATTGCTTTATAAGT | GTTTTATATT 135 | |
| Sbjet 105 | ACTATGTTT | TÁCÁGÁTGCÁCÁTT | TGCTTTGTTTÄÄTTGCTTTÄTÄÄGT | ĠŤŤŤŤÁŤÁŤŤ 164 | |
| Query 136 | GGGTCTTTGTT | TTATTCAATTGTTC | CTATTAGTATGAAAAGACACCTTGAC | CACAGCACCT 195 | |
| Sbjet 165 | GGGTCTTTGT1 | TTATTCAATTGTTC | TATTAGTATGAAAAGACACCTTGAC | CÁCÁGGÁCCT 224 | |
| Query 198 | GTTATATAGGG | GAATC-TTTAACTGA | AGCC 222 | | |
| Sbjet 225 | GTTÁTÁTÁGGG | GAÁTCATTTÁACTG <i>I</i> | ÁĠĊĊ 252 | | |

RT-PCR-5 测序结果(测序引物 F5):

| Range 1: | 78 to 278 | <u>Graphics</u> | | | ▼ Next Match 』 |
|-----------|---------------|------------------|-----------------------------|----------------|----------------|
| Score | | Expect | Identities | Gaps | Strand |
| 372 bits | (201) | 6e-108 | 201/201(100%) | 0/201(0%) | Plus/Plus |
| Query 20 | ACATGCTCAG | CAGATTCGCGGCAC | AGAAGGACGTCAAGTGCTGCCTAAATG | GCAAAGCTT 79 | |
| Sbjet 78 | ÁCÁTGCTCÁG | ĊĀĠĀŤŤĊĠĊĠĠĊĀĊ | ÁGÁÁGGÁCGTCÁÁGTGCTGCCTÁÁÁTG | ĠĠĊĀĀĀĠĊŤŤ 137 | |
| Query 80 | CAGAAGACAG. | AAAGGTCCAGCCTT | TGAGGACATAGGAAGAAAGGTTCATGI | TCCTCCGAA 139 | |
| Sbjet 138 | B CÁGÁÁGÁCÁG. | ÁÁÁGGTCCÁGCCTI | ŤĠŔĠĠŔĊŔŤŔĠĠŔŔĠŔŔŔĠĠŤŤĊŔŤĠĬ | TCCTCCGÁÁ 197 | |
| Query 140 | TTCGGTGAAA | AATATTGAGGAGCC | TTTATAGACGGGAGATAATGATTTTAC | TTTGGACTG 199 | |
| Sbjet 198 | B ŤŤĊĠĠŤĠĂĂĂ | á at attgaggagec | ŤŤŤÁŤÁĠÁĊĠĠĠĠĠĠŤÁÁŤĠÁŤŤŤÁĊ | TTTĠĠÁĊŤĠ 257 | |
| Query 200 | TAGGAAGTGT | CCTGGCTATG 2 | 20 | | |
| Sbjet 258 | B TAGGÁÁGTGT | CCCTGGCTATG 2 | 78 | | |

RT-PCR-6 测序结果 (测序引物 F6):

| Range 1: | 55 to 252 | <u>Graphics</u> | | | ▼ Next Match , |
|--------------------|-------------|-----------------|----------------------------|----------------------|----------------------------|
| Score 337 bits(| 182) | Expect 2e-97 | Identities 193/198(97%) | Gaps 2/198(1%) | Strand Plus/Plus |
| Query 12 | ATAGTTGGAGA | -ATATAG-AACC | AGATCTCAGCAGGAGGGGTGA | AGCGGACCGTAATCGG 69 | |
| Sbjet 55 | ATAATTGGAGA | CATAGAGAAACC | AGATETEAACAGGAGGGGTGA | AGCGGACCGTAATCGG 114 | 4 |
| Query 70 | CAAGAGTGTTT | CCCAGGGCGAGG | TCACGTGTGTAATTCTTGCTA | GTCAGCCTTTTGGTTT 129 | 9 |
| Sbjet 115 | CAAGAGTGTTT | CCCAGGGCGAGG | TCACGTGTGTAATTCTTGCTA | GTCAGCCTTTTGGTTT 174 | 4 |
| Query 130 | CCCAAAGGGGG | GCTGTAAAAAGC | AATTTTAAAAAGGTCCCACTG | TCCTGGCTAGCCTGAA 189 | 9 |
| Sbjet 175 | ĊĊĊĂĂĂĠĠĠĠ | GCTGTAAAAAGC | AATTTTÄÄÄÄÄÄGGTCCCÄCTG | ŤĊĊŤĠĠĊŤÁĠĊĊŤĠÁÁ 234 | 4 |
| Query 190 | ACTCTGAATAT | GCACCAA 207 | , | | |
| Sbjet 235 | ÁCTCTGÁÁTÁT | ĠĊÁĊĊÁÁ 252 | | | |



3.4 目的基因已知序列:

AK149641:

5':gcttgactctaattggacctgttgggaaatttagagccttataggtttgtctttataagcatctgc aaagtgtgactgtcgccatttctgggaagcccagaatggtcctggccagggttaatcataaaattgagg tagtgattttattctcaactggtgggtttgacagtagctagttgctatggtcttctgctcacttcaaaa $\tt ctctgaagaaacacagtctgacctattcctttttgaacattcagaaagacttcttctactggaacattc$ tttgggggtctaaatatttacttttctaagcattgttataatttaaattcctttaagttattctattttgtagacagatgtgagccacccgtgggtgtcgggaacctaatctgggtccttctcagggggatcaattgc tccacagaggagatgcatattaggagagaaatgttagattaatgacaaaaccactcaaccaaattcttctgagtagctgtgctctttcccatgcatgctttttaattgcatacattctaacatgtgtgctcctaatctactta a accaata ta aa ta aa ta aa atctgttg cag attag atgctg aa tttgag ag accaa acgta ta ttcatattttccataaaaaattaaattttcatacagataaataggatactatcagaaaaataattgtaaag a a t ctt g ct a ttttta a ct g t g tat g tat c g g t g ttt t g c t g c a t g c a t g ta t a t g ta c c t c a t a c a t g c aacctaggaggcccaaagagggtgccaaataccctggaactgaagcggctcacagttgtaaggtatcacatgggttttgggatgttgaacctgtgtcctctgggtgagcagtcattgctttaaactgctgagtcacctctctagccccataaaagtcatttttaataaattcacactttggaatatatcttaaagaaatgttcttttaacttaatatcccaaaagtaaggtatgtaaagctgttaaagcagtattactcataacagaaaaggaattg gatttttttttttttttgagacaagtgtcacattatgtaccattgactgttctaaaactctctatgtaga ccaggctggccttgcactcacaaaggtgtgcctgcctctgcctcccaagttctgaaaataaaggcatgt $\tt gcctccatgatgcctggctggaaaactcattttcttaaagctttgtgttagactactatatatcatatt$ atggaatacataaccattaataattttctctctctctgtggtggcacttgcctttgatcccagcactct $ggaagagtt caa agc cag cct ggt cta cata {\color{red} gcacateccagaa cagcaa aggctacact gt gggacag}$ $\tt gcttaaagtgggcagggactgaattatgttgatgaaaagatgttcatgaaataatgttaactatgaact$ atgttttgtacagatgcacatttgctttgtttaattgctttataagtgttttatattcggtctttgttttatt ca att gtt ctatt ag tatgaa aa ga ca cctt ga cca cag ga cct gtt at at ag gg aa t catt ta a comment of the $\tt ctgagccttgctgacagttcagaggtttagtctgaacatggtgggaaaaatggtgtcatgaaggccaac$ 3' taatcattt

AK007111:

5': gaagaagggcacctttaaactacaacgaggataatgccgtgggatgtggaacaaggcagaagaaa atatgaacaggaacatgctcagcagattcgcggcacagaaggacgtcaagtgctgcctaaatggcaaag cttcagaagacagaaaggtccagcctttgaggacataggaagaaaggttcatgttcctccgaattcggt gaaaaatattgaggagcctttatagacgggagataatgattttactttggactgtaggaagtgccctg gctatgaaatgatgttagatgaagacagaagtgggtggaaacactgaggctgttacagtaagccaag



3.5 RACE 测序结果 (展示部分去掉克隆载体后的序列和 blast 之后的结果)

测序结果1如下:

gcacatcccagaacagcaaagtatgctccacaaaagcaagagaacctgagctcagatc ccaaccctcacaaggaaagccaggtgtggtggcttgtgtatagccaacctggctttcatc actgtggaggaggagatgcatttctgggctttgctgttctgggatgtgc

Blast之后如下:

| | | sequence from clo | | | |
|--------|------------------|---------------------|---------------------|---|--------------------|
| Sequen | ice ib. <u>e</u> | mb AL671173.12 Lo | | n chromosome 4, co ber of Matches: 1 | mplete sequence |
| Range | 1: 199 | 9240 to 199385 | GenBank Graphics | 5 | ▼ Next Match ▲ Pre |
| Score | • | Expect | Identities | Gaps | Strand |
| 254 b | its(137 |) 5e-64 | 144/147(98%) | 1/147(0%) | Plus/Plus |
| Query | 9 | CAGAACAGCAAAGTATGCT | CCACAAAAGCAAGAGAACC | TGAGCTCAGATCCCAACCCTCA | 68 |
| Sbjet | 199240 | CAG-ACAGCAAAGTATGCT | CCACAAAAGCAAGAGAACC | TGAGCTCAGATCCCAACCCTCA | 199298 |
| Query | 69 | CAAGGAAAGCCAGGTGTGG | TGGCTTGTGTATAGCCAAC | CTGGCTTTCATCACTGTGGAGG | 128 |
| Sbjet | 199299 | CAAGGAAAGCCAGGTGTGG | TGGCTTGTGTATAGCCAAC | CTGGCTTTCATCACTATGGAGG | 199358 |
| Query | 129 | AGAGAGATGCATTTCTGGG | CTTTGCTG 155 | | |
| Sbjet | 199359 | ÁGÁCÁGÁTGCÁTTTCTGGG | ĊŤŤŤĠĊŤĠ 199385 | | |

测序结果2如下:

Blast之后结果如下:

Mouse DNA sequence from clone RP23-123E1 on chromosome 4, complete sequence Sequence ID: emb|AL732520.14| Length: 167166 Number of Matches: 1

Range 1: 63463 to 63628 GenBank Graphics ▼ Next Match ▲ Prescree Expect Identities Gaps Strand 7307 bits(166) 4e-80 166/166(100%) 0/166(0%) Plus/Plus

| Range | e 1. o. | 5403 to 0 | 3020 Gene | Salik | Graphics | | - I | rext Match | A PIE |
|---------------|--------------|-----------|-----------------|-------|-----------------------|-------------------|-------|---------------------|-------|
| Scor 307 l | e bits(16 | 6) | Expect 4e-80 | | ntities /166(100%) | Gaps 0/166(0%) | | Strand Plus/Plus | |
| Query | 15 | ACAGCAAAG | GACTGCAAACCA | ACTCA | AGGCCAATTAAAA | GACGGAATTATT | 74 | | |
| Sbjet | 63463 | | | | AGGCCAATTAAAA | | 63522 | | |
| Query | 75 | | | | GAAGTTTGCCAAG | | 134 | | |
| Sbjet | 63523 | | | | SAAGTTTGCCAAG | | 63582 | | |
| Query | 135 | | | | PATCTCATTCATC | 180 | | | |
| Sbjet | 63583 | | | | PATCTCATTCATC | 63628 | | | |



测序结果3如下:

Blast之后结果如下:

Mus musculus BAC clone RP23-379J7 from 3, complete sequence Sequence ID: gb|AC126450.4| Length: 159914 Number of Matches: 1

| Range | e 1: 87 | 7665 to 8 | 7839 <u>G</u> e | enBank | Graphic | cs | | | W [| Next Match | ▲ Pre |
|-------|----------|-----------|-----------------|---------|------------|------------|-------------|-------|-------|------------|-------|
| Scor | | _ | Expect | | lentities | | Gaps | | | Strand | |
| 318 b | oits(17) | 2) | 2e-83 | 1 | 74/175(9 | 9%) | 0/175(| 0%) | | Plus/Plus | |
| Query | 12 | | | | CAGGTCCACA | | TCATTTGGCC | TTAA | 71 | | |
| Sbjet | 87665 | AACAGCAAA | GCTTGGAGG | TCACTGC | CAGGTCCACA | AGCTCACTCC | TCATTTGGCC | AATT | 87724 | | |
| Query | 72 | | | | | | CTGCATGGTTG | | 131 | | |
| Sbjet | 87725 | | | | | | CTGCATGGTT | | 87784 | | |
| Query | 132 | CTATCCCAA | AATGTCACC | GCAAGAG | TCCTATTATO | TGTCGTCCTT | CCTTTGCTGT | 186 | | | |
| Sbjet | 87785 | CTATCCCAA | AATGTCACC | GCAAGAG | TCCTATTATC | TGTCGTCCTT | CCTTTGCTGT | 87839 | 9 | | |

测序结果4如下:

ggagttggtggcaggagttcaagagggaactgacccagtcactcattggggacagaagt ccatggcagatggggaagggcctcatgtgacttgagacttaaaattcttgtcagttttgag agaaactaaagaaaccacatcttcccttcaggaaaagtttcaataatttgatagatgattg ggttgtttctgtcttgactgttgtaaatgatgctgttatgaatatttgtgtgcatgcttctg

Blast之后结果如下:

Mus musculus BAC clone RP24-81D14 from chromosome 8, complete sequence Sequence ID: gblAC128668.4 Length: 211075 Number of Matches: 1

| Score 424 b | e oits(229 | Expect) 5e-115 | Identities 238/242(98%) | Gaps 1/242(0%) | Strand Plus/Minus |
|----------------|---------------|----------------------|----------------------------|-------------------|----------------------|
| Query | 3 | | AGAGGGAACTGACCCAGTCACTC | ATTGGGGACAGAAGTCC | 61 |
| Sbjet | 153704 | | ágagggaactgacccagtcactc | ATTGGGGACAGAAGTCC | 153645 |
| Query | 62 | ATGGCAGATGGGGAAGGGCC | TCATGTGACTTGAGACTTAAAAT | TCTTGTCAGTTTTGAGA | 121 |
| Sbjet | 153644 | ATGGCAGATGGGGAAGGGCC | TCATGTGACTTGAGACTTAAAAT | TCTTGTCAGTTTTGAGA | 153585 |
| Query | 122 | | TTCCCTTCAGGAAAAGTTTCAAT | AATTTGATAGATGATTG | 181 |
| Sbjet | 153584 | GAAACTAAAGAAACCACATC | TTCCCTTCAGGAAAAGTTTCAAT | AATTTGATAGATGATTG | 153525 |
| Query | 182 | GGTTGTTTCTGTCTTGACTG | TTGTAAATGATGCTGTTATGAAT. | ATTTGTGTGCATGCTTC | 241 |
| Sbjet | 153524 | GGTTGTTTCTGTCTTGACTG | TTGTAAATGATGCTGTTATGAAT. | ATTTGTGTGCATGCTTC | 153465 |
| uery) | 242 | TG 243 | | | |
| Sbjet | 153464 | TG 153463 | | | |

注: 所有的测序原始结果(含有载体序列)如附件所示。我们已经比对了所有的测序结果,没有符合要求的结果。

另外,需要告知客户的是,AK007111 基因已知序列经过 blast 之后如下表所示。发现该基因和 Mus musculus 6 BAC RP23-330J17,完全重合。该基因的全长有可能从 Mus musculus 6 BAC RP23-330J17 基因上查找。而 RACE 实验也有可能得到的是该序列。



结论以及下步计划:

- 1、此次 RACE 实验,亚克隆之后没有得到理想结果。
- 2、我们会重新再做一次 RACE 实验,大概 2 周左右时间,如果还做不出来, 我们会建议客户取消此次实验。避免浪费客户时间。