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Nucleotide sequence of the gamma chain gene of rat fibrinogen: conserved intronic sequences

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The complete nucleotide sequence of the rat γ fibrinogen gene, including immediate 5' and 3' flanking regions has been determined. The gene is composed of a total of ten exons which by a process of alternative splicing produce two polypeptides called γ and γ' consisting of 412 and 420 amino acids respectively (1). The immediate 5' region of the gene possesses 3 areas of sequence homology common to the 5' flanking regions of the fibrinogen α and β chains (2). Sequence comparison to that of the human γ fibrinogen gene shows that the third intron is highly conserved between the two genes (3). This intron is one of two introns highly conserved over one billion years of evolution (4).

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TCGATAATTA TTGAGTGTTA ACTGTCACTC TACTAGCTGC TGTAGTGAAA CACCGCATTT CCATCATTAG GTTAACGGTT ATATAATGCA AGGTTCTTAT   -1388

AAGTCCACAG AGCATACAGA GCTGGGTCTG GGTTGTAAAA TAAAGCTGTG CTAGAGGGAA CCAGACTCCA GAAACAGGAC TTCCGGGGTC TGTTTGTTGT   -1288

TTGTTTTTGA TTTGTTTGTT TTGACAGCCA GACTTTGCCG TAATTCCTAT GAATGTTGGC AAATAAAACC AAGAAGAAGA AAAAAGAAAA AGAAAAGTAG   -1188

ATAGTATAGG AAGACAGAGC AAACAGCAGA GAGGAAGTAG CTAAGGGCTA GGAACTAAGC CGGTGATTGG TGACACTGCT GGAGCTATTC TGTAAGGATA   -1088

CTGGCAAATG GCTTGCAGCA GGAAGTGACT GAAGTGGAGG GCAGTCCCGT CAGCAAGGGA AGTGGTTACA TATTTGGTTT GATGAGTTCA CTTAGTTAAA    -988

ATGGTTTCTG ACTACAGAGC ATAAACATTT ATTTGTTTTA ATGTCAAAGG AGACACGATC AAAACTTCTT GTATCAAATT GTTAGCAATT CTTTCAAATT    -888

AGTGCATTTC TCCCAATCCC GTAGTTCTTC AGTTTATCAG GAAGCTTGGG TCACAAGAGG AAGAAAGTGA CTGCACACAT CTGTCACAGT GCTACAGCTG    -788

CTTGTCATCA CCAAAGAGAG GTGACAGTGC CTTTTTCTCC ACGACAGCAA TTAAACTTCT CAGTATCTCT CTGTGTCCCT TCTCCTTGAG AGAGGCTTGT    -688

AAGGGAGTAT GTGAGAAGTG GGTTTCTCTG AGTCCAGGTT TTTCCTCCAG GAACTAGATG GGGCTTTGGG ACAAGCCAGT GAACTGGCAC CTTCATTTTG    -588

GCGGCAGATG GGAGGTAGCC TATCTTCCAG TCATTAAACA AGTTGAGCCC AAAACTCAAT GAGAACTGAC CTAAGAAAAA GGAGAGCAAG GAGGAGGGGC    -488

AGGAACCGGT TTCCAACATC ATCGATCCTT GGGAAATCAG ACCTGAGGAG TGAAAGGAGG AAAAGTTCCC TTCTTCCACC CCTTGGCTAA GACAGGTCTT    -388

ACAGTAATAG CGTGTGGCTA CAGAAGGCCA GAACTCAGGC TGGGTTGGTT GCAGGAGGTC AGAACCCTGT GTTTGTTCTT CTGGAGGCGG TGTACTGGAA    -288

GTAATTGCAA CACGCAGCCT CTGCAGTCCT CGCGCAGCCC CCACTGTTTA GCTGGCTTTC AGCAGATAGA TAGCTTTGGG TCTGAGGAGA CAGGTTGGGC    -188

TGAGCCCAAA AGGAGGAGCT AAAACCTGTG TGCAAATCTG GGAATCCCTC GGAGCACAGG TGAGAGCCAG AATGAGCAGA AAGAGCCCAC ATTTCTGGTA     -88

AAAGAGACCC GTGACCAGTT CCAGCCACTC TTAGTCCCGC CCAGACTGGG AATTCATATA AAGCCCCAAG GAGAGCCCAA GAGGTCACAG TGCTGGCTGT      12

                                   Met Asn Trp Ser Leu Gln Leu Arg Ser Phe Ile Leu Cys Trp Ala Leu Leu Leu
AAGGGGCTGG GAGCACCGGT CACCCAGACA CT ATG AAT TGG TCC TTG CAA CTC CGG AGT TTC ATT CTA TGC TGG GCG CTT TTA CTA      98

Leu Ser Pro Thr Gly Leu Ala
CTT TCT CCA ACG GGC CTG GCA GTAA GTGCTCTTCA GAAGCCATAT TGTTTGAGAG AGAGAGAGAG AGAGAGAGAG AGAGAGAGAG AGAGAGAGAG     193

                                                Gln Tyr Thr Ala Thr Arg Asp Asn Cys Cys Ile Leu Asp Glu Arg
AGAGAGAGAA CCACTGGCAA ATATATTTTT GTTTTTCTCC CACAG CAG TAC ACT GCT ACT AGA GAT AAC TGT TGC ATC CTA GAT GAG AGA     283

Phe
TTC GTAAGT AGTTTCTGTG TCACCCTCTT TCCCGGAAGT AGAGAAAGGT GGGAAACAGG GCGGTACTTT CATGTATCAG TTAAGCTTTT GGGA          376

                                                                                Gly Ser Tyr Cys Pro Thr
AACGGTTTAC AATGTAAGGG TAGTAACTAA AACTTATCTC CTCAGGTGTT TAATTATCTG CTTACTGACT TTGCAG GGT AGT TAC TGC CCA ACC     470

Thr Cys Gly Ile Ser Asp Phe Leu Asn Ser Tyr Gln Thr Asp Val Asp Thr Asp Leu Gln Thr Leu Glu Asn Ile Leu Gln
ACT TGT GGC ATC TCA GAC TTC CTG AAT TCT TAC CAA ACC GAC GTG GAC ACT GAC CTC CAG ACT CTG GAA AAC ATC TTA CAA     551

Arg Ala Glu Asn Arg Thr Thr Glu Ala Lys Glu Leu Ile Lys Ala Ile Gln Val Tyr Tyr Asn Pro Asp Gln Pro Pro Lys
CGA GCT GAA AAC AGA ACC ACA GAA GCC AAG GAA CTG ATT AAA GCC ATC CAG GTT TAC TAC AAC CCG GAC CAA CCC CCA AAG     632

Pro
CCG G GTGA GGAAACCCAC TGTGGAGTTG TTACCTTTCC TGCTTTCGGT TGTGCTATTT GCTTAAGTGA CTTTTCTGTC CCTGGCCCCT GTGACAG      727

Gly Met Ile Glu Gly Ala Thr Gln Lys Ser Lys Lys Met Val Glu Glu Ile Leu Lys Tyr Glu Ala Leu Leu Leu Thr His
 GT ATG ATA GAG GGT GCC ACT CAG AAG TCT AAG AAG ATG GTA GAA GAA ATC TTG AAG TAC GAA GCA CTG TTA CTA ACC CAT     807
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Glu Ser Ser Ile Arg
GAG TCG AGT ATC CG  G TATGTGTCTG GGTTTAATGT ACTCCCAAGG CTCTGGAGAT TTTACTTAAA ATTCCACATT CAAAGAAAGT TGTCTTGGAA     902

TTTAGTTTTC ATTTAGAGTA TAAAGTCCCC CAGTGGGGAT AAGAGAATTT AGTACAAAAT GAGTTGTACT AGCTTGTAAT TTTTAAATTA CATTGGAGAA    1002

AGGTTATTAT AGATATCTTT GAATGCCATT CTGGCTCTAT TGACTTGAAT GTCCCCATCC CCTTACAATA TAAAACCAAA TACATGGGAA CTGAAGTGTG    1102

GAAATGCTCT TCACAAATAT GTTACCAAAT CCACACTAAT AAATCAGAGA ACAGACAGAA GGAGGATCTT GGCTGCTATA GCAATTCATG TCCAAGGTGA    1202

AGGACAAAGG AAACGTTTTG AGCTCCTTGA CCAGAGTACA TGGACCTGAA ATTATTTCTC GTCCCCGTCC TCACGTCTAG CTGCCCAGAG ACTCTGGCAT    1302

GGAAGCGCAG GGATAGTTTT AACTCCTTTT CCTGTTTCCC CCAAACTAAA CGCACAGCAC CGTCGTCGGA AACTGAGCTA GATGGCCACT GGGGGAAGTG    1402

GCCTCCAACA CACTTCTCCT CCTCCTGTGA TCAGAGGGTA GGGCTTTAGT GCTGCTTATT AGTTTATCTA TTCCATTGCT TTTGCTCTAT CACTAGAGGG    1502

TAAGAAAACT GTGTGAGAAA ATGACTAAGA TAGGGAAATA AGGCGGTCCA CCATTGTGTC CCTCTGCTTT ACTTCAAAGA CAGACCCAGT GGTCTGGGCA    1602

TTGTGCATAC TCTCTGTTCA TGTCTGAATA TTTTTGAGCA ACCATGCAAA GGATGACCAA GGCAGAATTC TCACTTTCCC CACCTCTTTT CAAGAGCTAT    1702

AGGACCAGAA AAGGAAAGTA TCTGCCCTTT CATGTCTGGG AATGCCCCAG GGACTTTTCC CATGTAAGTT ATAGAACAAA CAGAAATTCC CAAATGGCAC    1802

ACAGAGCTC. ......... ......... ......... ......... ......... ......... ......... ......... .........                1902

......... ......... ......... ......... ......... .........C CCACAATTAG CCTGAGCCTC CTATGGATAG GGCAGGAGTC              2002

AGCAAAGGCC ACCTGTAGTG ACCTTGGTTA TTGACAGGGA CTTCTCCATT CCTGTTCAAG TCGAGACCAC CTGCCGACCC ACCCAATTCT CCTCCACAAG    2102

GCTTCAATTT AATTTTACAA TAGCAATTTT ACAATTTCCC AAGACAGAAG TAATTTATCA CAAAGGAGTT CTCCAGAAAA TAAAGCAGCC TTTCTAACAG    2202

                                                                                Tyr Leu Gln Asp Ile Tyr Thr
GGAAGATTAA AAAACCCTGT CTTCCGAAAA GGACCGCTTA GCCCCCTCTT CGCCACCGCT CTGTTTCTAG G TAT TTA CAG GAC ATC TAT ACT        2294

Ser Asn Lys Gln Lys Ile Thr Asn Leu Lys Gln Lys Val Ala Gln Leu Glu Ala Gln Cys Gln Glu Pro Cys Lys Asp Ser
TCA AAT AAG CAG AAG ATC ACG AAC CTG AAA CAG AAG GTG GCC CAG CTT GAA GCT CTG TGC CAG GAG CCT TGC AAG GAC TCT      2375

Val Arg Ile Tyr Asp Ile Thr Gly Lys
GTG CGA ATC TAT GAC ATA ACC GGA AAA G GTACA CACGAACTTA ATCCTGGGAT CAGGGTCATG AAGCAAGCAG CCAAAATAAA AGCAGTGTGCA    2469

CCTCGCACTG TACAGCAAGC TTGGAAAGTA TCTGACCTTA AAGCTAAGGG TTCCAACCAC CCAAGGCTCC TTGGGATTAG TCAGTAATTG GGAAGGGATG    2569

ACAATAGGCA GACTAGAGAG GCTGCTGATG CATTAACACC GCCTGGGGTT GGAGCCACTA CCCACTGCTG TGGTTTTACT TGCCCTTTCT TCTCCAG       2666

Asp Cys Gln Asp Thr Ala Asp Lys Gly Ala Lys Glu Ser Gly Leu Tyr Ser Ile Arg Pro Leu Lys Ala Thr Gln Gln Phe
 AT TGT CAG GAT ACC GCC AAC AAG GGT GCC AAA GAA AGT GGA CTT TAC TCC ATT CGG CCT TTG AAA GCC ACG GAG CAG TTC      2746

Leu Val Tyr Cys Glu Thr Asp Gly Pro Gly Asn Gly Trp Thr Glu Phe Lys Lys
TTA GTG TAC TGT GAA ACC GAC GGA CCT GGA AAT GGC TGG ACC GAA TTC AAG AAG GTAA GGGCCTCTGA CTGCATATGT ATTAAAAAAA    2834

TGGCCCACGT TGCTCCAGAT GGTTCTCTAA GCATAGAAGA GACGTCGGCC TCTTTGAGAT GAGGACTGGT CTACCTTAAC CCACAGAAGA GACGGAAGCA    2934

TGAGATCGCC TAGGTAATGA GGAACAGGCA GTGTTTGAAT CCAGCAGCCA GGCTTTGTGG TGCTCTGAGG CAACACCATG CTGACTGTCA CAGAAGCCAA    3034

GTCCGTTTAG TGAGGTGACA TGCGAAGCCT CTTTCCTCAG ATCAAGAGCT AAGGCTGGCC CTTAACAATA ACCCACAGGC CTCTCTGGAG GATATAATTG    3134

GGCAGCTCAC AACAAAGCCT TTTTCGATCC GCCTGCCTGG TACCACTGCA AAACTGGCCT TTTTAACCTT GTAAGCTTAT GAGTTCTGAA GACCTCCATG    3234

GGTCTTTTAA AAGTGCACAA CTAAAACAAA AAACAAAATT AGCCAGCAAC GACAAAAATG TCCATACCAT TATAGTAGGA ATAAGCATGT AGCAGGTGTG    3334

TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGAT CCTCAGAGCA CCAGCATGCC ATTTGACAGT TGGGGAGATA GAGGCAGCTT GGAAGAGCA     3433

CAGTTAGCTG GTAATTGGCC AAACCGTATC CTCCACACAT TGAATGTTGT TTCTTTATAA GCACCAATCA TCTTTCCTCT GCTCTTGCCC ATACTGTTAT    3533

          Arg Leu Asp Gly Ser Val Asp Phe Leu Lys Asn Trp Ile Gln Tyr Lys Glu Gly Phe Gly His Leu Ser Pro Thr
GCCAACTAG AGG CTT GAT GGC AGT GTG GAT TTC TTG AAG AAC TGG ATT CAG TAT AAA GAA GGA TTT GGA CAC CTG TCA CCT ACT    3617

Gly Thr Thr Glu Phe Trp Leu Gly Asn Glu Lys Asn His Leu Ile Ser Met Gln Ser Thr Ile Pro Tyr Ala Leu Arg Ile
GGC ACC ACA GAG TTT TGG CTG GGC AAT GAG AAG AAT CAT CTG ATA AGC ATG CAG TCC ACC ATC CCA TAC GCA CTG AGA ATA     3698

Gln Leu Lys Asp Trp Ser Gly Arg Thr Ser
CAG CTC AAA GAC TGG AGT GGC AGG ACC AG  GTA TGGAAGGGAC TTTAACATTT TGTCTTGGGC TGGGGACATA GCTCAGTTGG TAGAGTACTT    3790

GCCTAGCATG CATGAAGTCC TGAGTTCAAT ATCCAGTGCT ACATAAACCA AGGAATGGCG GTGGTAACTA TCTATAGTTC TAGCACCCAG GAAGTAGAAG    3890

CAGTAAGGTT GGGAGATCAA GGTCCATCTC CAGTTACATA GTGAGCTCGA GGCTAGCATG ACTCACATTA AGCTGTCTCC AAAAAAAAAA AAAATTTGTC    3990

TTAGCTGTCT GCTACATTTA TTAGACATCA ATATATTATC AGCTAAATGG ACCGTCAGCT GGCAATGGCC TAACAGCAAA CAACCTGACA AGGGCAAAGA    4090

GCATGACCAG GCTCTTTCCA AGAAGAACTT GGACAGTCCA AAGAGAGTGA GAAAGCTAGG CTGTCTCCTC TAGGCGGGAT GTGGATCGTG GGGCATTCAC    4190

ATGTCCAATT TACACACCTT GGACGCATTC GGTGGTTGCT ATAGGGTGTT CAATATGAAC CAGAAATTGA GCATGAAATT AAATTCAGTC AAATTCAAGC    4290

ATTCATGTTT CCCACTCATA CAATGATTGT TCACTCCGTC AAATCACAGA CTGTCTGAAA ATTAACTGAC TCCTGTGGGG ATCTGCAGCC CTGCCAGGCT    4390

GAGCGATGCT CTGGGGTTTG GGTTTTAACC ACCTCATGTA AGTGAAAAAA TTGCAGAGCC TCTAATTAGA CATGAAATAT TGAAACATGC TCCATTTCCA    4490

AGTGCATTTT TTAAACTTTA TTTTAATCCC CGAAACTATC CCAGCAGCAA GCAACATTTT CCTATGTGAG GCATGCTCCA CTTCCCAAGG ATGAGATATG    4590

GGGAAAGAGA TTAACTATTT TCTAGGAATT CTCTATTTAG TCACAAAAGA CCATTTTTCT CTCTACTCTG CAAAACCATA GCTGCCTTTA CTAACTTTCC    4690

CAGAGGAGGT CTCTCTGACT CTTGAGGCTC TCATTTAATG CATCACATAG GGTTCGTGGC TTCTCGAACA TTCTAGAAAG TCTCTTTTCG AGGTAACATT    4790

GCTTTGGAAA AGAAAACCCT TTTGTACCCT CACACTTTAA GTGTTGGGGA TGGGTAAGGA AACCCGTCTC CTTTAAAGGA ACATGGGTTC ACTCATTGTA    4890

GATAATTACT CTGACCTATG TGTGTGCAGT GCTAACAAAG GCCTTAAATG AAAGCTGAAA TAATTACATT TTCCATCTGT TTCCATTTGA TCTCAGATTG    4990

TGTAACTAAA AAGCCAGGAG GTGGCTTTTA GTTTTTCTGA GATTTGCTGA AGGCTCTTTA GATCCATCCT GGTGCGGGAT GTGATCTGGA CTGGTGCACA    5090

                                                                                        Thr Ala Asp Tyr Ala
TGGTATTGCC TGGAGTTGCA GAAAACGTTG TCATTCCATT CCCCTCTTTG TGACTTGAGA TCCTCCACTC CACCCTCACA G C ACC GCG GAC TAT GCC   5187

Met Phe Arg Val Gly Pro Glu Ser Asp Lys Tyr Arg Leu Thr Tyr Ala Tyr Phe Ile Gly Gly Asp Ala Gly Asp Ala Phe
ATG TTC AGG GTG GGT CCT GAA TCC GAC AAA TAC CGC CTG ACC TAT GCC TAC TTC ATT GGT GGA GAT GCC GGG GAT GCC TTC     5268
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Asp Gly Tyr Asp Phe Gly Asp Asp Pro Ser Asp Lys Phe Phe Thr Ser His Asn Gly Met His Phe Ser Thr Trp Asp Asn
GAC GGC TAC GAT TTT GGT GAT GAT CCC AGT GAC AAG TTT TTC ACA TCC CAC AAC GGC ATG CAC TTC AGT ACC TGG GAC AAT      5349

Asp Asn Asp Lys Phe Glu Gly Asn Cys Ala Glu Gln Asp Gly Ser Gly Trp Trp Met Asn Lys Cys His Ala Gly His Leu
GAC AAC GAC AAG TTC GAA GGC AAC TGT GCT GAG CAG GAT GGA TCT GGC TGG TGG ATG AAC AAG TGT CAC GCT GGC CAC CTC      5430

Asn Gly Val Tyr Tyr Gln
AAT GGA GTT TAT TAC CAA G GTATTT TTTTAAGTTA TGAGTATATG TGTAACAGAT CTCTATGGAA GGTAATAAGC AGAAGCAGAG AGATGAAGCAT   5526

TATAATAAGC ACAGAAAGTC TTTTATCTTA CAGCTTTTCA TTTGAAGTCA GAATTGGATA CACTCACTAT CCAACTGGCA TTACCTTAAA GAACAGAAAG   5626

AGTGCCTATG TGTCACCTCT AACTCCCCAC AAAGCATCAG TGCTTTGATA TGTGCTGAAA CTCATTATCT GACTTGGAAT TTGGTGTCTC CTTTAGACCT   5726

ACCATGTATA TGATAGCGTT ACCATGTCTG TGCTTGTGGA CACATTATCC AGGTTCAGCT GTCTATGAAC TATGTCAGGC ACAAGCAGTG AACAGTGGAT   5826

AGGTGTCCAG AGAAATGAAG TGAGAGGCAC AGAAGTACAG GAAGAGTGAA AAGTCATGGA AGCCTGGATG AAGGGTCTAG CCTGGGCTTG GAGATGCTCG   5926

TTTTAGCATG GGGGGAGCGA TGGTTTCTGT AAGGCTTAAG GCGTTTGGAG CACTGATTTC ACTGACCACC TAGCAGTTCT GTATGGAGAG ACTCCAGGGA   6026

TACCGAAGAT CTTTTAAGGC CCCATCTACT AATGAGAAAC TCCATGGTGA TGTAGAACAA CTTTCTACAG ACACCTTCTA TAGCCCGTAA TAGCATCTTC   6126

CATTAGCCAG ACAATTGTGC TGCCATCCAG TCAGACCACC TGATCAGGAA TCTCGGCTCT ACATATAATG GAATATAAGG ATTCCTTATA AAAGAAAGAC   6226

AGACAGAGGG AAACACAGAC AGACAGAGAG AAGTGCTCCA GCCCTGATAA AAATGGATAA ATTTTAAGTA CTGACATAAT CACCAAAACG ACTTATTGGC   6326

TCTTGGAATC TCTAGGGATG TTTTCAGTTA GGAGGAAATG CTGTAGACGT CACAGTAATT CTATTGGAAC TCTAATGAGC TGAGAAAATG TAGGGAAAAA   6426

                                   Gly Gly Thr Tyr Ser Lys Ser Ser Thr Pro Asn Gly Tyr Asp Asn Gly Ile Ile
AGACACACCC ATGCATCTCT GACTCCTTGC AG  GT GGC ACT TAC TCC AAG TCA TCT ACT CCT AAC GGT TAT GAC AAT GGC ATT ATT      6511

Trp Ala Thr Trp Lys Thr Arg Trp Tyr Ser Met Lys Glu Thr Thr Met Lys Ile Ile Pho Phe Asn Arg Leu Ser Ile Gly
TGG GCC ACG TGG AAA ACC CGC TGG TAT TCC ATG AAG GAA ACC ACC ATG AAG ATA ATT CCC TTC AAC AGA CTC TCC ATT GGA      6592
Asp Gly Gln Gln His His Met Gly Gly Ser Lys Gln
GAT GGG CAG CAA CAT CAC ATG GGA GGA TCC AAA CAG GTCAGC GTGGAGCATG AAGTGGATGT TGAATACCCG TAAATCCTCT GCCTAGACAT   6684

TTTAAATTAG ACACAAAGAA TCAACTATAA CTTCTATTAG CCTGTACCAA GTTCCAATAT TTTCCTCAAA TTTTCTTCTA TACCTCTATA TTCGAGTTAT   6784

TAATTTTGGT CTCTCTTAAA ATGATATTTA GCCATAAATG GACATTAAAC CCCACGTGAA CCATGTTTCT AAGTTACTTG AATCAAAGTT ATTAAAATTT   6884

GTTTGTTTGA ATGGTCAACA TTTTGTTTGA CCTTTCCCCT AAATATTAAA AGTAAAACTA CTGTATTTTA TTTTATGATC AGCTGTAATT ATTGTTTTTG   6984

TTGTTGTTGT TTCCTGAGTA TTTTTAGTAT GCACTAATAA AATAGGAGAA ATTTTAGAAC TTCACCTGTA TATTTTCCAT GTATTTTACC TCTACATCAT   7084

                                                                                Val Gly Asp End
TAGTATTTAA TTCTTCTTTT TAAATGAAAA GTTATATTTT TTAATATACC TTTTGTTTTA TTGTGTATTC ATAG GTT GGA GAC ATG TAA AGAACAT   7180

TTCCAAGGTG ATTTGCTCTT TAACGGACTT TATCCAAGCA GAGAGATATA TTTTTCCTAT GAGACCATGG AACCCACTTC CTTTACAGAG TTAATGGGAT   7280

CCATGATGCA AACTCCATTA GCAGTTTTAT GCTGGCGATA ATTTATCTAC ATGCATTTCA ATAAACATTT GTTTCCT                            7357
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Legend: Nucleotide sequence of the rat γ-fibrinogen gene. The sequence extends from -1488 bp 5' of the transcription initiation site to 12 bp 3' of the second potential polyadenylation site. The TATAAA and transcriptional initiation site are underlined while the two poly (A) addition sites are boxed.

Acknowledgements

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