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NUCLEOTIDE SEQUENCES CODING FOR THE BOVINE BETA 3-ADRENERGIC RECEPTOR, AND APPLICATIONS THEREOF

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(56) Prior Art Documents
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(57) Claim

1. An isolated and purified nucleotide sequence, which comprises the cDNA coding for the bovine β_3-adrenergic receptor, comprising the nucleotide sequence SEQ ID No. 1.

9. A nucleotide probe, which hybridizes with nucleotide sequences according to any one of Claims 1 to 10 or the complementary sequence under conditions of hybridization such that it does not hybridize with the genes or messenger RNA coding for the β_1- and β_2-adrenergic receptors, hybridizes with the nucleic acid according to Claim 1 or the complementary sequence under conditions of hybridization such that it does not hybridize with the genes or messenger RNA of the β1 or β2 adrenergic receptors, wherein said probe is selected from the group of nucleotide probes consisting of:

the nucleic acid sequence which corresponds to nucleotides 332-403 of the sequence ID No. 1,

the nucleic acid sequence which corresponds to

.../2

nucleotides 572-640 of the sequence ID No. 1,

the nucleic acid sequence which corresponds to nucleotides 983-1048 of the sequence ID No. 1, and

the nucleic acid sequence which corresponds to nucleotides1070-1147 of the sequence ID No. 1.

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(54) Titre: SEQUENCES NUCLEOTIDIQUES CODANT POUR LE RECEPTEUR β3-ADRENERGIQUE (RAβ3) BOVIN ET LEURS APPLICATIONS

(57) Abstract

Nucleotide sequences coding for the bovine β3-adrenergic receptor (RAβ3), use of said sequences as probes and for the expression of peptides and/or fragments thereof having a bovine RAβ3 activity, vector useful for said expression as well as cellular hosts containing said vectors. Method for screening a substance having an agonist or antagonist action with respect to peptides having a β3-adrenergic receptor activity, particularly selective with respect to the bovine receptor.

(57) Abrégé

Séquences nucléotidiques codant pour le récepteur β3-adrénergique (RAβ3) bovin, utilisation desdites séquences comme sondes et pour l'expression de peptides et/ou de fragments de ceux-ci ayant une activité de RAβ3 bovin, vecteur utile pour ladite expression ainsi que les hôtes cellulaires contenant ledit vecteur. Procédé de criblage d'une substance, à action agoniste ou antagoniste vis-à-vis des peptides ayant une action de récepteur β3-adrénergiques, particulièrement sélective vis-à-vis du récepteur bovin.

NUCLEOTIDE SEQUENCES CODING FOR THE BOVINE β_3-ADRENERGIC RECEPTOR (ARβ_3) AND THEIR APPLICATIONS

The present invention relates to nucleotide sequences coding for the bovine β_3-adrenergic receptor (ARβ_3), to the use of the said sequences as probes and for the expression of peptides and/or of fragments of the latter having bovine ARβ_3 activity, to the vector which is useful for the said expression and also to host cells containing the said vector.

The present invention also relates to a method for the screening of substances possessing an agonist or antagonist action with respect to peptides of bovine origin having β_3-adrenergic receptor activity.

Catecholamines such as adrenaline and noradrenaline, synthetic agonists of these catecholamines which mimic their biological functions and antagonists which block these biological functions are known to exert their effects by binding to specific recognition sites (membrane receptors) located on the cell membranes.

Two main classes of adrenergic receptors have been defined, α-adrenergic receptors and β-adrenergic receptors.

In the set of these two classes, five subtypes of catecholamine receptors are now distinguished (α_1-, α_2-, β_1-, β_2- and β_3-AR). Their genes have recently been isolated and identified (S. COTECCHIA et al., 1988, Proc. Natl. Acad. Sci. USA, 85, 7159-7163; B.K. KOBILKA et al., 1987, Science, 238, 650-656; T. FRIELLE et al., 1987, Proc. Natl. Acad. Sci. USA 84, 7920-7924; L.J. EMORINE et al., 1987, Proc. Natl. Acad. Sci., USA, 84, 6995-6999; L.J. EMORINE et al., 1989, Science, 245, 1118-1121). Analysis of these genes has enabled them to be recognized as belonging to a family of integral membrane receptors displaying certain homologies (R.A.F. DIXON et al., 1988, Annual Reports in Medicinal Chemistry, 221-233; L.J. EMORINE et al., 1988, Proc. NATO Adv. Res. Workshop), in particular in respect of 7 transmembrane regions which are coupled to

regulatory proteins, known as G proteins, capable of binding guanosine triphosphate (GTP) molecules.

These membrane receptors, when they have bound the appropriate ligand (agonist or antagonist), undergo a change in conformation, which induces an intracellular signal which modifies the behaviour of the target cell.

In the case of β-adrenergic receptors, when they bind to catecholamine agonists, they catalyse the activation of a class of G proteins, which in turn stimulates adenylate cyclase activity, while ARβ antagonists act in competition with the agonists for binding to the receptor and prevent activation of adenylate cyclase.

When adenylate cyclase is activated, it catalyses the production of an intracellular mediator or second messenger, in particular cyclic AMP.

The inventors have recently demonstrated new β-adrenergic receptors in man, designated Hu-ARβ_3, and in the mouse (International Application WO 92/12,246), designated Mu-ARβ_3, and characterized by properties different from those of the β_1 and β_2 receptors, in particular in that they behave differently with respect to substances which are, respectively, β_1- and β_2-receptor antagonists and agonists (International Application WO 90/08,775).

In particular, the Hu-ARβ_3 receptor consists, more especially, of a sequence of 408 amino acids, and is considered to contain seven hydrophobic transmembrane regions separated by intra- and extracellular hydrophilic loops, and the Mu-ARβ_3 receptor consists of a sequence of 400 amino acids and also contains 7 transmembrane regions.

The previous studies relating to Hu-ARβ_3 and Mu-ARβ_3 showed, in particular, that the β_3-adrenergic receptor participates in disorders such as diabetes and/or obesity, in as much as it is expressed in tissues which play an important part in metabolism (adipose tissues, skeletal muscles in particular).

Continuing his studies along these lines, one of the inventors sought to demonstrate such a β_3-adrenergic receptor in cattle (Bo-ARβ_3), so as to be able to have available a tool for regulating the amount of fats in these animals, in particular with the object of improving the quality of the meat.

The subject of the present invention is a nucleotide sequence, characterized in that it corresponds to the cDNA of the bovine gene coding for the bovine β_3-adrenergic receptor.

According to an advantageous embodiment of the said nucleotide sequence, it comprises the nucleotide sequence and the deduced amino acid sequence (SEQ ID No. 1) of the following formula (I):

```
CCCAGGCCAG GGAAATCGCT CCCACGCCCC GATGCCCCCG CCGCTGAGCA GGGTGAGCTG   60

GGAGACCCTT TCCCTCATTC CTTCCCGCCC CACGCGCGAC GCGGGG ATG GCT CCG     115
                                                   Met Ala Pro
                                                     1

TGG CCT CCT GGG AAC AGC TCT CTG ACC CCG TGG CCA GAT ATC CCC ACC     163
Trp Pro Pro Gly Asn Ser Ser Leu Thr Pro Trp Pro Asp Ile Pro Thr
      5                  10                  15

CTG GCA CCC AAT ACT GCC AAC GCG AGT GGG CTG CCA GGG GTG CCC TGG     211
Leu Ala Pro Asn Thr Ala Asn Ala Ser Gly Leu Pro Gly Val Pro Trp
 20                  25                  30                  35

GCG GTG GCG CTG GCG GGG GCG CTG TTG GCG CTA GCG GTG CTG GCC ACC     259
Ala Val Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Val Leu Ala Thr
                 40                  45                  50

GTG GGA GGC AAC CTG CTG GTA ATC GTG GCC ATC GCC CGG ACG CCG AGA     307
Val Gly Gly Asn Leu Leu Val Ile Val Ala Ile Ala Arg Thr Pro Arg
            55                  60                  65

CTC CAG ACC ATG ACC AAC GTG TTC GTG ACT TCG CTG GCC ACA GCC GAC     355
Leu Gln Thr Met Thr Asn Val Phe Val Thr Ser Leu Ala Thr Ala Asp
        70                  75                  80

CTG GTG GTG GGG CTC CTG GTC GTG CCC CCG GGG GCC ACG TTG GCG CTG     403
Leu Val Val Gly Leu Leu Val Val Pro Pro Gly Ala Thr Leu Ala Leu
     85                  90                  95

ACC GGC CAC TGG CCC CTG GGC GTC ACC GGT TGC GAG CTG TGG ACC TCA     451
Thr Gly His Trp Pro Leu Gly Val Thr Gly Cys Glu Leu Trp Thr Ser
100                 105                 110                 115

GTG GAC GTG CTG TGT GTG ACC GCC AGC ATC GAA ACC CTG TGC GCC CTG     499
Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Ala Leu
                120                 125                 130

GCG GTG GAC CGC TAC CTG GCC GTG ACC AAC CCG CTG CGC TAC GGC GCG     547
Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg Tyr Gly Ala
            135                 140                 145
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CTG GTC ACC AAA CGC CGC GCC CTA GCA GCC GTG GTC CTG GTG TGG GTG   595
Leu Val Thr Lys Arg Arg Ala Leu Ala Ala Val Val Leu Val Trp Val
        150                 155                 160

GTG TCC GCC GCG GTG TCG TTT GCG CCC ATC ATG AGC AAA TGG TGG CGC   643
Val Ser Ala Ala Val Ser Phe Ala Pro Ile Met Ser Lys Trp Trp Arg
    165                 170                 175

ATC GGG GCC GAT GCC GAG GCG CAG CGT TGC CAC TCC AAC CCG CGC TGC   691
Ile Gly Ala Asp Ala Glu Ala Gln Arg Cys His Ser Asn Pro Arg Cys
180                 185                 190                 195

TGC ACC TTC GCC TCC AAC ATG CCC TAC GCG CTG CTC TCC TCC TCG GTC   739
Cys Thr Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser Ser Ser Val
                200                 205                 210

TCG TTC TAT CTT CCG CTC CTG GTG ATG CTC TTC GTC TAC GCA CGA GTT   787
Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr Ala Arg Val
            215                 220                 225

TTC GTG GTG GCC ACG CGC CAG CTG CGC TTG CTG CGC CGG GAG CTG GGT   835
Phe Val Val Ala Thr Arg Gln Leu Arg Leu Leu Arg Arg Glu Leu Gly
        230                 235                 240

CGC TTC CCG CCA GAG GAG TCT CCG CCG GCT CCT TCT CGC TCC GGA TCC   883
Arg Phe Pro Pro Glu Glu Ser Pro Pro Ala Pro Ser Arg Ser Gly Ser
    245                 250                 255

CCT GGC CTG GCG GGG CCG TGC GCC TCG CCC GCG GGG GTG CCC TCC TAC   931
Pro Gly Leu Ala Gly Pro Cys Ala Ser Pro Ala Gly Val Pro Ser Tyr
260                 265                 270                 275

GGC CGG CGG CCG GCG CGC CTT CTG CCT CTG CGG GAA CAC CGC GCC CTG   979
Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Arg Glu His Arg Ala Leu
                280                 285                 290

CGC ACC TTG GGG CTC ATC ATG GGA ACC TTC ACT CTC TGC TGG TTG CCT  1027
Arg Thr Leu Gly Leu Ile Met Gly Thr Phe Thr Leu Cys Trp Leu Pro
            295                 300                 305

TTC TTT GTG GTC AAC GTG GTG CGC GCC CTC GGG GGC CCC TCT CTG GTG  1075
Phe Phe Val Val Asn Val Val Arg Ala Leu Gly Gly Pro Ser Leu Val
        310                 315                 320

TCC GGC CCC ACT TTC CTC GCC CTT AAC TGG CTG GGC TAT GCC AAC TCT  1123
Ser Gly Pro Thr Phe Leu Ala Leu Asn Trp Leu Gly Tyr Ala Asn Ser
    325                 330                 335

GCC TTC AAC CCG CTC ATC TAC TGC CGC AGC CCG GAC TTT CGG AGC GCC  1171
Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg Ser Ala
340                 345                 350                 355

TTC CGC CGC CTG CTG TGT CGC TGC CGG CCG GAG GAG CAC CTC GCC GCT  1219
Phe Arg Arg Leu Leu Cys Arg Cys Arg Pro Glu Glu His Leu Ala Ala
                360                 365                 370

GCC TCC CCG CCC CGA GCC CCC TCC GGC GCC CCC ACG GCC CTG ACC AGC  1267
Ala Ser Pro Pro Arg Ala Pro Ser Gly Ala Pro Thr Ala Leu Thr Ser
            375                 380                 385

CCC GCT GGC CCC ATG CAG CCC CCA GAG CTC GAC GGG GCT TCC TGC GGA  1315
Pro Ala Gly Pro Met Gln Pro Pro Glu Leu Asp Gly Ala Ser Cys Gly
        390                 395                 400

CTT TCT TAGGCCTTGA AGAAACAACT CCATTGATCC GGAACCTTTG GAAAGCCTCT  1371
Leu Ser
    405
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GGCCGGCCTC GGTTCAGAAT GAGCCCCGTG GAGTTTCCCA GCTGGAAAAC TCTGCCCTCC 1431
CCAGCCTGAC GACTGGGTCC TGGGAGGAGG CGCGGGGGCT GACTGGGGAG GGGAAATCCT 1491
TACCAAGTGG GTTTTCGCTC TCTTTCTGAG AGAAGTTTTC TACACCCCAG CCCTGAACTT 1551
CACCGCTGCC TCAGCAGCTC CCGCGTCTGG TTTCCCATGC CCAGGTGCCC GGGCAGGAGC 1611
TGGGCTGCGT TTAGCCCCGG GACCCGCACC TGTCCCACTC GGGTGCTGTG TGCGCAGGGG 1671
CAAGGCGGGC ACCTTCATTC TGTTCCTTCT GCCGCCCAGA CCCTGAGGAA CCCACCGGGG 1731
TGCTGGAGGC CCAGGCTGAG AAGAGGAAGG TGGGGAAGGT CACGGTTTGG GCTTCTGTCC 1791
CTGGCTTCCT CACTGTAGAC ACACCTACCT CACAGCATTT TCAGGACTTT ACTTTAGCCT 1851
TTGGGGTGGG GGTGGGGGGG CGCTCCTGGT TTCCTGGGAA GGTGAACCAT TAGAATGGGT 1911
CCCTTTTCCT TTTGAAATCA AATTAATAAA TGTTACTGAA TGCAGTTTAA AAAAAAAAAA 1971
AAAAAAAAAA AAAAAAAAAA AAAAAAAAA 2000

(I)

In this sequence, the underlined ATG which occurs at position 107 probably corresponds to the initiation codon for protein synthesis.

There is 85 % homology between the bovine and human nucleotide sequences coding for the β_3-adrenergic receptor, and there is 76 % homology between the bovine and murine nucleotide sequences coding for the β_3-adrenergic receptor.

The said sequence comprises, in particular, the following single restriction sites:

Bpu1102 I, Fok I, EcoR V, Bcg I, Nhe I, BspM I, Afl III, Age I, BstE II, BspH I, Bsg I, Nsp I, Nsp7524 I, NspC I, Sap I, BamH I, BstY I, Asc I, Sty I, Hinc II, Apa I, Bsp120 I, Bbe I, Ehe I, Kas I, Nar I, Ec1136 I, Sac I, Stu I, Fse I, Drd I, Tth111 I, Srf I, Bsu36 I, Sfc I, BstX I, Ase I, Bsm I, Dra I.

The subject of the invention is also the fragments of the said sequence which are useful for expression of the corresponding peptide and/or detection of the bovine gene coding for the bovine β_3-adrenergic receptor.

Among the said fragments, there may be mentioned:

- the 78-base pair fragment which corresponds to nucleotides 218-295 of the sequence of formula I and which codes for the transmembrane region TM1,

- the 72-base pair fragment which corresponds to nucleotides 332-403 of the sequence of formula I and which codes for the transmembrane region TM2,

- the 66-base pair fragment which corresponds to nucleotides 434-499 of the sequence of formula I and which codes for the transmembrane region TM3,

- the 69-base pair fragment which corresponds to nucleotides 572-640 of the sequence of formula I and which codes for the transmembrane region TM4,

- the 72-base pair fragment which corresponds to nucleotides 713-784 of the sequence of formula I and which codes for the transmembrane region TM5,

- the 66-base pair fragment which corresponds to nucleotides 983-1048 of the sequence of formula I and which codes for the transmembrane region TM6,

- the 78-base pair fragment which corresponds to nucleotides 1070-1147 of the sequence of formula I and which codes for the transmembrane region TM7.

The subject of the present invention is also cDNA clones, characterized in that they comprise a sequence fragment coding for the bovine β_3 receptor (Bo-ARβ_3).

According to the invention, the clone designated M13-6.6 comprises 2979 base pairs, includes the sequence of formula I and comprises the following single restriction sites: EcoR V, Bcg I, Nhe I, BstE II, BspH I, Bsg I, Sap I, BamH I, Asc I, Stu I, Fse I, Drd I, Srf I, Sfc I, Ase I, Bsm I, Dra I, Bsp1407 I, Csp6 I, Rsa I, Ssp I, Dra III, Bgl II, Afl II, Spe I, Tfi I, Hpa I, Nde I, EcoN I, BsaB I, Pvu I.

The subject of the present invention is also nucleotide probes, characterized in that they consist of a nucleotide sequence as is defined above, or a

fragment of the latter, labelled using a label such as a radioactive isotope, a suitable enzyme or a fluorochrome.

The said nucleotide probes are characterized in that they hybridize with the nucleotide sequences as are defined above but do not hybridize with the genes coding for the β_1- and β_2-adrenergic receptors, or with the messenger RNA of the said β_1- and β_2-adrenergic receptors.

According to an advantageous embodiment of the said probe, its sequence is homologous with or complementary to that of a segment of at least 10 bp of the sequence I.

For the purpose of the present invention, "homologous sequence" encompasses not only sequences identical to the sequence I, or to a fragment of the latter, but also those which differ therefrom only by the substitution, deletion or addition of a small number of nucleotides, provided that the sequences thus modified have a specificity of hybridization equivalent to that of the sequence (I) or of the unmodified segment in question.

Likewise, "complementary sequence" is understood to mean not only sequences which are strictly complementary to the sequence (I) or to its segments, but also modified sequences, as indicated above, possessing a specificity of hybridization equivalent to that of the said strictly complementary sequences.

The hybridization conditions are defined as follows:

For the shortest probes, that is to say of approximately 10 to approximately 100 nucleotides, suitable hybridization conditions are as follows:
750 mM NaCl, 75 mM Tris-sodium citrate, 50 µg/ml salmon sperm DNA, 50 mM sodium phosphate, 1 mM sodium pyrophosphate, 100 µM ATP, 10 to 25 % formamide, 1 % Ficoll ("PHARMACIA", average molecular weight 400.00), 1 % polyvinylpyrrolidone, 1 % bovine serum albumin, for 14 to 16 h at 42°C.

For the longest probes, that is to say possessing more than approximately 100 nucleotides, suitable hybridization conditions are those stated above for the shortest probes, but in which the medium defined above contains 40 % of formamide instead of 10 to 25 % of formamide.

According to an advantageous arrangement of this embodiment, the said probe can be advantageously defined by any one of the above nucleotide sequences, and in particular by the 2-kbase fragment which corresponds to the whole of the sequence of formula I.

The subject of the present invention is also a peptide and/or a peptide fragment, characterized in that it is encoded by a nucleotide sequence as is defined above, and in that it displays β_3-adrenergic receptor activity.

β_3-Adrenergic receptor activity is that defined in French Patent Application No. 89/00,918, namely that, when the fragment is exposed at the surface of a cell, it is capable of participating in the activation of adenylate cyclase in the presence of one of the following agonists: BRL 28410, BRL 37344, CGP 12177A, (l)-isoproterenol and carazolol; or, it is capable of being recognized by antibodies which do not recognize either the β_1-adrenergic receptor or the β_2-adrenergic receptor; or, it is capable of generating antibodies which do not recognize either the β_1 receptor or the β_2 receptor.

According to an advantageous embodiment of the said peptide, it comprises 405 amino acids and possesses the amino acid sequence (SEQ ID No. 2) of the following formula II:

```
Met Ala Pro Trp Pro Pro Gly Asn Ser Ser Leu Thr Pro Trp Pro Asp
 1               5                  10                  15

Ile Pro Thr Leu Ala Pro Asn Thr Ala Asn Ala Ser Gly Leu Pro Gly
            20                  25                  30

Val Pro Trp Ala Val Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Val
        35              → TM1   40                  45

Leu Ala Thr Val Gly Gly Asn Leu Leu Val Ile Val Ala Ile Ala Arg
    50                  55                  60          TM1 ←

Thr Pro Arg Leu Gln Thr Met Thr Asn Val Phe Val Thr Ser Leu Ala
65                  70                  75  → TM2           80

Thr Ala Asp Leu Val Val Gly Leu Leu Val Val Pro Pro Gly Ala Thr
                85                  90                  95

Leu Ala Leu Thr Gly His Trp Pro Leu Gly Val Thr Gly Cys Glu Leu
      TM2 ← 100                 105                 110
                                                → TM3
Trp Thr Ser Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu
        115                 120                 125

Cys Ala Leu Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg
    130 TM3←            135                 140

Tyr Gly Ala Leu Val Thr Lys Arg Arg Ala Leu Ala Ala Val Val Leu
145                 150                 155 → TM4           160

Val Trp Val Val Ser Ala Ala Val Ser Phe Ala Pro Ile Met Ser Lys
                165                 170                 175
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Trp Trp Arg Ile Gly Ala Asp Ala Glu Ala Gln Arg Cys His Ser Asn
TM4 ← 180 185 190

Pro Arg Cys Cys Thr Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser
195 200 →TM5 205

Ser Ser Val Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr
210 215 220

Ala Arg Val Phe Val Val Ala Thr Arg Gln Leu Arg Leu Leu Arg Arg
225 TM5← 230 235 240

Glu Leu Gly Arg Phe Pro Pro Glu Glu Ser Pro Pro Ala Pro Ser Arg
245 250 255

Ser Gly Ser Pro Gly Leu Ala Gly Pro Cys Ala Ser Pro Ala Gly Val
260 265 270

Pro Ser Tyr Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Arg Glu His
275 280 285

Arg Ala Leu Arg Thr Leu Gly Leu Ile Met Gly Thr Phe Thr Leu Cys
290 →TM6 295 300

Trp Leu Pro Phe Phe Val Val Asn Val Val Arg Ala Leu Gly Gly Pro
305 310 TM6← 315 320

Ser Leu Val Ser Gly Pro Thr Phe Leu Ala Leu Asn Trp Leu Gly Tyr
→TM7 325 330 335

Ala Asn Ser Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe
340 345 TM7← 350

Arg Ser Ala Phe Arg Arg Leu Leu Cys Arg Cys Arg Pro Glu Glu His
355 360 365

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Leu Ala Ala Ala Ser Pro Pro Arg Ala Pro Ser Gly Ala Pro Thr Ala
    370                 375                 380

Leu Thr Ser Pro Ala Gly Pro Met Gln Pro Pro Glu Leu Asp Gly Ala
385                 390                 395                 400

Ser Cys Gly Leu Ser
                405
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(II)

This peptide is designated hereinafter bovine β_3-adrenergic receptor (Bo-ARβ_3).

The invention also comprises the peptides which are variants of those defined above, which contain certain mutations, without the peptides losing the β_3-adrenergic receptor properties.

Among these variants, there may be mentioned those which are recognized by antibodies that recognize the transmembrane regions, as well as those which are recognized by antibodies that recognize the regions other than the transmembrane regions.

The subject of the present invention is also fragments or combinations of fragments of Bo-ARβ_3, according to the invention, and in particular:

- a fragment of 26 amino acids corresponding to the segment 38-63 of the formula II and constituting the transmembrane region TM1,

- a fragment of 24 amino acids corresponding to the segment 76-99 of the formula II and constituting the transmembrane region TM2,

- a fragment of 22 amino acids corresponding to the segment 110-131 of the formula II and constituting the transmembrane region TM3,

- a fragment of 23 amino acids corresponding to the segment 156-178 of the formula II and constituting the transmembrane region TM4,

- a fragment of 24 amino acids corresponding to the segment 203-226 of the formula II and constituting

the transmembrane region TM5,

- a fragment of 22 amino acids corresponding to the segment 293-314 of the formula II and constituting the transmembrane region TM6,

- a fragment of 26 amino acids corresponding to the segment 322-347 of the formula II and constituting the transmembrane region TM7.

The said fragments may advantageously be obtained by synthesis, in particular by the Merrifield method.

The subject of the present invention is also a recombinant cloning and/or expression vector, characterized in that it comprises a nucleotide sequence according to the invention.

For the purpose of the present invention, recombinant vector is understood to mean either a plasmid, a cosmid or a phage.

According to an advantageous embodiment of the said vector, it consists of a suitable recombinant vector comprising, in particular, an origin of replication in a suitable host microorganism, in particular a bacterium or a eukaryotic cell, at least one gene whose expression permits selection either of the bacteria or of the eukaryotic cells which have received the said vector, and a suitable regulatory sequence, in particular a promoter permitting expression of the genes in the said bacteria or eukaryotic cells, into which vector is inserted a nucleotide sequence or a sequence fragment as are defined above, which vector is a vector for the expression of a peptide, of a peptide fragment or of a combination of peptide fragments having bovine $ß_3$-adrenergic receptor activity.

According to an advantageous arrangement of this embodiment, the said vector consists of an expression vector pRc/CMV into which is inserted, in the multisite linker, at least the fragment coding for the bovine $ß_3$-adrenergic receptor; such a plasmid has been designated pRc/CMV-Boß3-ADR, and has been

deposited with the Collection Nationale de Cultures de Microorganismes [National Collection of Microorganism Cultures] (CNCM) held by the PASTEUR INSTITUTE, dated 15th April 1993, under No. I-1297.

The subject of the present invention is also a suitable host cell obtained by genetic transformation, characterized in that it is transformed with an expression vector according to the invention.

Such a cell is capable of expressing a peptide of bovine origin having β_3-adrenergic receptor acitivity.

According to an advantageous embodiment, the host cell consists, in particular, of cells of the CHO (Chinese Hamster Ovary) line.

Another of the microorganisms used can consist of a bacterium, in particular *Escherichia coli*.

It was not obvious that cattle have β_3-adrenergic receptors whose activation unexpectedly enables the amount and quality of the fats to be regulated, thereby enabling the quality of bovine meat to be improved.

Advantageously, the bovine β_3-adrenergic receptors according to the invention constitute a tool for the selection of ligands participating in the activation of these receptors, and make it possible to identify and select ß-adrenergic ligands which are specific for the β_3-adrenergic receptors, and especially ligands having more affinity and which are more selective for the bovine β_3-adrenergic receptor than for the human β_3-adrenergic receptor.

According to the invention, the method for the selection and identification of substances capable of behaving as a specific ligand with respect to a peptide (bovine β_3-adrenergic receptor) according to the invention comprises:

- bringing the said substance into contact with a host cell previously transformed with an expression vector as defined above, which host cell expresses the said bovine peptide (bovine β_3-adrenergic receptor), if

necessary after suitable physical or chemical induction, and which contacting is carried out under conditions permitting the formation of a bond between at least one of the specific sites and the said substance if circumstances are appropriate, and

- detecting the possible formation of a complex of the ligand-peptide type.

Such a process makes it possible to select either ligands specific for the β_3-adrenergic receptor, or ligands specific for the bovine β_3-adrenergic receptor exclusively.

Besides the foregoing arrangements, the invention also comprises other arrangements which will become apparent from the description which follows, reference being made to the attached drawings wherein:

- Figure 1a shows the restriction map of the 2,000 bp coding fragment which corresponds to the formula I;

- Figure 1b shows the restriction map of the clone 6.6 which contains the bovine β_3-adrenergic gene and comprises 3 kb in all;

- Figure 2 is a comparison of the human, murine (mouse and rat) and bovine β_3 receptors;

- Figure 3 shows a comparison of the coding sequences for Hu-ARβ_3 and bovine ARβ_3;

- Figure 4 shows the expression vector pRc/CMV-Boß3-ADR;

- Figure 5 shows the expression vector pRc/CMV including a multisite linker comprising the following single restriction sites; Hind III, BstX I, Not I, Xba I and Apa I.

It should, however, be clearly understood that these examples are given only by way of illustration of the subject of the invention, and in no way constitute a limitation thereof.

Example 1: Isolation and identification of the bovine β_3-adrenergic gene

- Preparation of RNA:

The bovine β_3-adrenergic gene was isolated from

a cDNA library of calf brown adipose tissue, constructed in bacteriaphage λgt11.

To this end, the total RNA is extracted from calf brown adipose tissue by the guanidinium thiocyanate method, and the poly(A)$^+$ messenger RNA is then purified using oligo(dT) columns (Pharmacia Ref.: 27-9258-01).

The total RNA and messenger RNA were analysed by Northern blotting to verify the presence and size of the messengers of the desired gene. After electrophoresis, the RNA was transferred onto a positively charged nylon membrane (Amersham Hybond®-N+ ref. RPN 203B). This membrane is then hybridized with a radiolabelled probe (radiolabelling: see screening of recombinant phages, below), consisting of a 2900-base pair DNA fragment containing the whole of the murine β_3-adrenergic gene previously isolated in the laboratory (NAHMIAS et al., 1991, EMBO J., 10, 3721-3727; International Application WO 92/12,246). After hybridization with the radiolabelled probe, the filters are washed and exposed for several days to autoradiography film (KODAK X-OMAT AR); an approximately 2.0-kilobase fragment is observed, both in the total RNA fraction and in the purified messenger RNA fraction. This confirms that the gene corresponding to the β_3-adrenergic receptor is expressed in calf brown adipose tissue, and that the cDNA library can be constructed from this purified poly(A)$^+$ messenger RNA.

To verify that the RNA source is indeed brown adipose tissue, the Northern blot obtained above was hybridized with a radiolabelled probe corresponding to the gene for human uncoupling protein (hUCP). This protein is only present in this type of adipose tissue, and may be regarded as a kind of "marker" for brown adipose tissue. With this probe, a strongly positive signal is detected.

- Synthesis of cDNA:

The corresponding cDNA is then synthesized, taking as template the purified poly(A)$^+$ messenger RNA

and as primer for the synthesis of the first strand an oligo(dT)$_{15}$ primer originating from the "RiboClone cDNA synthesis system" kit (Promega ref. C 2100). The synthesis of the first cDNA strand takes place in the presence of AMV reverse transcriptase, and the synthesis of the second strand is carried out using two enzymes acting simultaneously (*E. coli* polymerase I and *E. coli* RNase H). The double-stranded cDNA is then treated with T4 DNA polymerase so as to obtain blunt ends. The Promega C 2100 kit is used for all of these reactions.

Next, adaptors containing EcoR I sites are added so as to be able to insert the cDNA obtained into bacteriaphage λgt11 under the following conditions, described in the EcoR I Adaptor Ligation System I kit (Promega ref. C 1900):

the cDNA is centrifuged through a Sephacryl® S-400 matrix (kit) to remove small molecules; the adaptors are then added to the cDNA by ligation in the presence of T4 DNA ligase, the mixture is left overnight and a second centrifugation is performed through a Sephacryl® S-400 column so as to remove unbound adaptors.

Before inserting the cDNA thus treated into the λgt11 vector, the adaptors are phosphorylated in the presence of T4 polynucleotide kinase.

Insertion of the cDNA into bacteriaphage λgt11

The bacteriaphage λgt11 used as vector originates from the "Protoclone Lambda gt11 System" kit Promega ref. T 301/0-2). The DNA of the phage is digested with EcoR I and dephosphorylated. Dephosphorylation prevents the vector from closing up again.

Several ligations are performed with variable amounts of the cDNA obtained, with 0.5 µg of vector DNA, under the following conditions, for each ligation: for 3 hours at room temperature in the presence of T4 DNA ligase (Promega kit C1900).

An *in vitro* encapsidation is then performed

using the "Packagene" extracts present in Promega kit T301/0-2.

After incubation at 22°C for 2 hours, the reconstituted phage particles are used to infect bacteria, in particular the strain Y1090(r-) (Genotype: Δ(lacU169), proA+, Δ(lon), araD139, strA, supF, (trpC22::Tn10), (pMC9), hsd(r-, m+)), under the following conditions: the encapsidated phages are very greatly diluted (1/1,000 or 1/10,000); each dilution of phages is incubated with Y1090(r-) cells at 37°C for 30 minutes, and these infected bacteria are then plated out on a nutrient medium (LB agar) contained in Petri dishes. The dishes are incubated overnight at 37°C, and the next day lytic plaques are observed; each plaque corresponds to a recombinant phage. By counting the number of lytic plaques and multiplying by the given dilution factor, the titer of the cDNA library is determined, and is approximately 4 million recombinant phages. The background of the vector alone without insert is 3.5 %, which is entirely acceptable.

- <u>Screening of recombinant phages</u>

On the basis of the results obtained, approximately 200,000 phages were plated out on Petri dishes (LB agar medium) so as to be able to screen them with a radiolabelled probe under the following conditions:

- bacterial strain used: LE 392 (Genotype: F-, hsdR 574 (r-, m+), supE44, supF58, lacY1 or Δ(lacIZY)6, galk2, galT22, metB1, trpR55, λ-);

- probe: 2900-base pair DNA fragment (murine β_3-adrenergic gene), as specified above for Northern blotting, radiolabelled by random priming (Boehringer kit ref. 1004 760), incorporating 50 μCi of [α-^{32}P]dATP and 50 μCi of [α-^{32}P]dCTP (Amersham references PB 10204 and PB 10205).

After transfer of the DNA from the lytic plaques onto Hybond®-N+ membranes (Amersham ref. RPN 132B), the latter are hybridized with the radiolabelled probe, then washed and exposed overnight to

autoradiography film.

17 hybridization signals were observed, 11 of which subsequently proved to be false positives. The 6 remaining clones (1, 3, 5, 6, 8 and 9) were purified by four successive isolations, followed by a hybridization with the murine β_3-adrenergic probe described above.

- Analysis of positive clones:

To identify the clone(s) containing the entire bovine β_3-adrenergic gene, that is to say the cDNA corresponding to the coding region for the whole protein, 2 methods were used: amplification by PCR and cleavage with a restriction endonuclease, with the object of finding among the positive clones the one which contains the largest insert.

1) Amplification by PCR was carried out in lysate of phages (encapsidated phage particles) using the following two primers:

1218: 24-mer λgt11 primer (sense strand) of formula: 5′ d(GGTGGCGACGACTCCTGGAGCCCG)3′, and

1222: λgt11 primer (antisense strand), also 24-mer, of formula: 5′ d(TTGACACCAGACCAACTGGTAATG)3′ (New England Biolabs).

In view of the fact that these primers hybridize on both sides of the insertion site of the cDNA into the phage, it was possible in this way to find out the size of the fragments inserted into the different positive clones.

2) The DNA of the 6 phages of interest was prepared and cut with the restriction enzyme EcoR I so as to verify the size of the inserts; hybridization with the murine β_3-adrenergic probe enabled the clone containing the largest positive insert to be detected.

The outcome of these two approaches was that clone No. 6 was chosen for a more exhaustive analysis in view of the fact that it contains the largest insert of desired cDNA (3 kilobases).

After cleavage of the phage λ with the restriction enzyme EcoR I, the fragment containing cDNA was inserted into bacteriophage M13tg131 so as to be

able to sequence the gene.

EXAMPLE 2: Sequencing of the bovine β_3-adrenergic gene

The approximately 3-kb DNA fragment bounded by the EcoR I enzyme sites was sequenced.

This DNA fragment was purified from the DNA of clone 6 and subcloned into the EcoR I site of the vector M13tg131. The M13 clones which had integrated the DNA fragment in the 2 opposing orientations (6.3 and 6.6) were identified and sequenced.

To perform the sequencing reactions, the USB Sequenase Version 2.0 kit (United States Biochemical ref. 70770) was used.

The sequence was produced using specific primers, which hybridize with the sense strand (clone M13-6.6) or with the antisense strand (clone M13-6.3) according to the method of Sanger (MANIATIS et al., *Molecular Cloning*, 2nd edition, pages 13.3-13.10).

The results obtained from the sequence of the 3-kilobase EcoR I fragment show the nucleotide sequence of bovine ARβ_3 (1215 bp) and non-coding regions (106 bp at the 5′ end and 638 bp at the 3′ end) (formula I). The restriction sites contained in the 2,000-bp fragment are positioned on Figure 1a (bov 6.6 short (pA)).

Figure 1b shows the single restriction sites contained in the 3-kb fragment which was sequenced.

Comparison of the coding regions of the human and bovine β_3 genes (Figure 3) shows a strong homology (85 % in respect of the nucleotide sequences between bovine and human AR; comparison of the coding regions of the bovine and murine β_3 genes also shows strong homology (76 % in respect of the nucleotide sequences between bovine AR and murine AR).

The bovine β_3 gene codes for the peptide of 405 amino acids which displays a very large homology with the human β_3 peptide or the murine β_3 peptide (Figure 2), as indicated above.

EXAMPLE 3: Construction of a vector for the

expression of bovine ARβ_3

The restriction map of the 2-kb fragment which was sequenced (Figure 1a) shows the presence of a site of cleavage by the enzyme Srf I at position 1598, that is to say 270 nucleotides upstream of the coding region of the bovine β_3 gene. DNA of the clone M13-6.6 was digested with the enzymes EcoR I and Srf I to liberate the 1598-base pair fragment containing the coding region of the bovine β_3 gene and a portion of the untranslated 3' region. This DNA fragment was purified and then inserted into the expression vector pRc/CMV at the Hind III and Xba I cleavage sites (Figure 5).

Since the ends generated by the enzymes Hind III and Xba I on the one hand, and EcoR I and Srf I on the other hand, are not compatible, care was taken to treat the EcoR I and Srf I ends of the insert on the one hand with the Klenow fragment of polymerase I, and the Hind III and Xba I ends of the vector on the other hand with the Klenow fragment of polymerase I, so as to obtain blunt ends (MANIATIS et al., *Molecular Cloning*, 2nd edition, pages 5.40-5.42).

The recombinant plasmid pRc/CMV-Boß3-ADR shown in Figure 4 was thereby obtained.

EXAMPLE 4: Pharmacological properties of the expression product of the bovine β_3 gene

a) Transfection of CHO-K1 cells

To characterize better the bovine β_3-adrenergic receptor, the bovine β_3 gene is expressed at the surface of eukaryotic cells, which possess all the elements needed for transduction of the signal.

The recombinant plasmid pRc/CMV of Example 3 was transfected into CHO-K1 cells by a lypofectin transfection method; the transfected cells are selected with G418 (neomycin derivative).

More specifically, the said transfection method is carried out as follows:

CHO-K1 cells (ATCC CCL 61) are cultured to confluence in a culture medium containing; 45 % DMEM medium, 45 % F12-Ham medium, 10 % heat-inactivated

foetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin.

1 µg of DNA of bovine β_3 plasmid pRc/CMV is mixed with 5 µl of lipofectin (Gibco) and 1,000 µl of the abovementioned culture medium without serum.

This mixture is added to cells in culture, which are left to incubate at 37°C for 5 hours.

The medium is replaced with a culture medium, mentioned above, containing serum, and the cells are incubated again at 37°C for 48 hours. The cells are then distributed in 96 wells according to variable dilutions and incubated in the presence of geneticin (G418, Gibco) at a concentration of 400 µg/ml in complete medium for approximately 10 days, the medium being changed every other day.

The different colonies obtained are then subcloned, first in 48 wells and then in 6 wells, before screening.

Stable colonies are screened for their capacity to bind specifically to [^{125}I]cyanopindolol and also for their capacity to stimulate adenylate cyclase in the presence of isoproterenol, in accordance with protocol described in TATE et al., Eur. J. Biochem., 191, 196, 357-361.

Transfected cells stably expressing ARβ_3 bind [^{125}I]cyanopindolol with an affinity equivalent to that of the corresponding ARβ_3 in man or in mouse and also obtained by cloning.

A set of subclones were selected from the stable clones; one of them, designated 62-26, was used for the pharmacological evaluation of bovine ARβ_3.

b) Pharmacological characteristics of the bovine ARβ_3 receptor

Pharmacological characterization of the bovine ARβ_3 receptor was carried out using the stable clones 62-26. The pharmacological properties of 10 β_3-adrenergic ligands were determined by studies of stimulation of adenylate cyclase and of binding of [^{125}I]cyanopindolol.

Adenylate cyclase activation experiments were carried out according to the protocol detailed in BLIN et al., Mol. Pharmacol., 1991, 44, 1094-1104.

Briefly, preconfluent cells in six-well plates (0.6×10^6 cells/well) are placed in contact or otherwise with increasing doses of ligands for 30 minutes at 37°C. Reaction is stopped by washing in PBS at 4°C and adding 500 µl of 1N NaOH. After centrifugation and neutralization with 1N acetic acid, the cell lysates are recovered and the total amount of cAMP accumulated is determined using a commercial assay kit.

Study of the competitive binding of ligands was carried out on intact cells according to the protocol detailed in BLIN et al., Mol. Pharmacol., 1991, 44, 1094-1104. Briefly, 10^5 cells are incubated with 0.5 nM [^{125}I]cyanopindolol in the presence or absence of increasing concentrations of competitors for 30 minutes at 37°C. Reaction is stopped by dilution with ice-cold PBS, the cells are filtered off and the radioactivity is measured in a gamma counter. The results were analysed using Graph-Pad® software.

Among the ligands tested, four are described as β_1-, β_2- and β_3-AR-receptor agonists: (-)-isoproterenol, (-)-epinephrine, (-)-norepinephrine, BRL 37344; three are described as specific for the ARβ_3 receptor (β_1-, β_2-AR antagonists): CGP12177A, ICI201651, bucindolol. Bupranolol was also tested since it is described as an antagonist of the three subtypes of receptor (BLIN et al., Br. J. Pharmacol., 1994, in press). Lastly, (-)-propranolol, described as a partial agonist of the human ARβ_3 receptor and antagonist of the mouse ARβ_3 receptor (NAHMIAS et al., EMBO J., 1991, 10, 3721-3727), was tested.

The values of the adenylate cyclase activation constants (K_{act}), of the inhibition constants (K_i) and of the intrinsic activity (IA) corresponding to the ratio of the effect of the ligand at 10^{-4} M to the effect of isoproterenol at 10^{-4} M, which are obtained

for the different ligands, are presented in the table below. The four ligands which are agonists of the three subtypes of receptors ($ß_1$-, $ß_2$-, $ß_3$-AR) have K_{act} and K_i values close to those obtained for the human AR$ß_3$ receptor (BLIN et al., Br. J. Pharmacol., 1994, in press), mouse AR$ß_3$ receptor (NAHMIAS et al., EMBO J., 1991, 10, 3721-3727) and rat AR$ß_3$ receptor (GRANNEMAN et al., J. Pharmacol. Exp. Therap., 1991, 40, 895-899; MUZZIN et al., J. Biol. Chem., 1991, 266, 24053-24058).

The specific ligands for the AR$ß_3$ receptor all have a smaller K_{act} value for the bovine AR$ß_3$ receptor compared to the human and mouse AR$ß_3$ receptors, and hence improved efficacy in stimulating adenylate cyclase. (-)-Propranolol is a partial agonist at bovine AR$ß_3$, as for the human AR$ß_3$ receptor. In contrast, bupranolol, which is described as a potent antagonist for human and murine AR$ß_3$ receptors, is a partial agonist at the bovine AR$ß_3$ receptor.

LIGANDS	Mouse RAß3-CHO			Human RAß3-CHO			BovineRAß3-CHO		
	Binding Ki (nM)	Accumul. Kact (nM)	cAMP IA	Binding Ki (nM)	accumul. Kact (nM)	cAMP IA	Binding Ki(nM)	accumul. Kact (nM)	cAMP IA
agonists ß1/ß2/ß3									
(-) isoproterenol	-	99±44	1.4±0.1	620	4	0.9	84±81	14±2	0.9±0.1
(-) epinephrine	4,600±1,850	23±0.3	0.91±0.03	20,650±2.810	49±5	1.00±0.04	11,105±7.345	50.7±3.7	0.8±0.3
(-) norepinephrine	1,840±600	13±4	1.06±0.06	475±75	6.32±0.7	1.00	423±255	54±4.3	1.00±0.5
BRL 37344	290±136	0.4±0.1	1.07±0.08	287±92	15±3	1.11±0.12	2.13±1.4	0.3±0.07	0.84±0.1
ß1/ß2 antagonists/ ß3 agonists									
CGP 12177A	152±19	41±9	0.75±0.08	88±22	139±44	0.68±0.02	218±161	1.41±0.5	0.93±0.20
ICI 201651	239±104	15±1	1.02±0.02	85±12	20±9	1.14±0.14	27.7±24	1.1±0.9	0.85±0.1
Bucindolol	21±5	40±14	1.11±0.06	23±10	7.0±1.2	1.01±0.10	73±42	12.8±5.0	0.99±0.10
partial agonist/ antagonist									
(-) propranolol	150±22	antagonist 406±98	-	145±8	1,490±550	0.51±0.12	589±74	661±78	0.71±0.08
antagonists ß1/ß2/ß3									
(-) bupranolol	42±19	antagonist 12±1	-	50±14	antagonist	-	85±40	507±75	0.34±0.01

As emerges from the foregoing, the invention is in no way limited to those of its embodiments and modes of implementation and application which have just been described more explicitly; it encompasses, on the contrary, all variants which may occur to the specialist in the field, without departure from the scope or range of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT:

(A) NAME: VETIGEN

(B) STREET: 66 rue de Javel

(C) CITY: PARIS

(E) COUNTRY: FRANCE

(F) POSTAL CODE: 75015

(A) NAME: VIRBAC

(B) STREET: lere Avenue - 2065 M - L.I.D.

(C) CITY: CARROS

(E) COUNTRY: FRANCE

(F) POSTAL CODE: 06516

(ii) TITLE OF INVENTION: NUCLEOTIDE SEQUENCES CODING FOR THE BOVINE BETA$_3$-ADRENERGIC RECEPTOR AND THEIR APPLICATIONS.

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: Patent Release #1.0, Version #1.25 (EPO)

(vi) DATA OF THE PREVIOUS APPLICATION

(A) NUMBER OF THE FILING: FR 93 04670

(B) DATE OF FILING: 21 APR 1993

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2000 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(A) NAME/KEY: CDS
(B) LOCATION: 107..1324
(D) OTHER INFORMATION:/function = Bovine beta-3 receptor"/products = "Adrenergic, Beta Receptor"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

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                                                    Met Ala Pro
                                                      1

TGG CCT CCT GGG AAC AGC TCT CTG ACC CCG TGG CCA GAT ATC CCC ACC      163
Trp Pro Pro Gly Asn Ser Ser Leu Thr Pro Trp Pro Asp Ile Pro Thr
      5                  10                  15

CTG GCA CCC AAT ACT GCC AAC GCG AGT GGG CTG CCA GGG GTG CCC TGG      211
Leu Ala Pro Asn Thr Ala Asn Ala Ser Gly Leu Pro Gly Val Pro Trp
 20                  25                  30                  35

GCG GTG GCG CTG GCG GGG GCG CTG TTG GCG CTA GCG GTG CTG GCC ACC      259
Ala Val Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Val Leu Ala Thr
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                    40                  45                  50

GTG GGA GGC AAC CTG CTG GTA ATC GTG GCC ATC GCC CGG ACG CCG AGA      307
Val Gly Gly Asn Leu Leu Val Ile Val Ala Ile Ala Arg Thr Pro Arg
                55                  60                  65

CTC CAG ACC ATG ACC AAC GTG TTC GTG ACT TCG CTG GCC ACA GCC GAC      355
Leu Gln Thr Met Thr Asn Val Phe Val Thr Ser Leu Ala Thr Ala Asp
            70                  75                  80

CTG GTG GTG GGG CTC CTG GTC GTG CCC CCG GGG GCC ACG TTG GCG CTG      403
Leu Val Val Gly Leu Leu Val Val Pro Pro Gly Ala Thr Leu Ala Leu
        85                  90                  95

ACC GGC CAC TGG CCC CTG GGC GTC ACC GGT TGC GAG CTG TGG ACC TCA      451
Thr Gly His Trp Pro Leu Gly Val Thr Gly Cys Glu Leu Trp Thr Ser
100                 105                 110                 115

GTG GAC GTG CTG TGT GTG ACC GCC AGC ATC GAA ACC CTG TGC GCC CTG      499
Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Ala Leu
                120                 125                 130

GCG GTG GAC CGC TAC CTG GCC GTG ACC AAC CCG CTG CGC TAC GGC GCG      547
Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg Tyr Gly Ala
            135                 140                 145

CTG GTC ACC AAA CGC CGC GCC CTA GCA GCC GTG GTC CTG GTG TGG GTG      595
Leu Val Thr Lys Arg Arg Ala Leu Ala Ala Val Val Leu Val Trp Val
        150                 155                 160

GTG TCC GCC GCG GTG TCG TTT GCG CCC ATC ATG AGC AAA TGG TGG CGC      643
Val Ser Ala Ala Val Ser Phe Ala Pro Ile Met Ser Lys Trp Trp Arg
    165                 170                 175

ATC GGG GCC GAT GCC GAG GCG CAG CGT TGC CAC TCC AAC CCG CGC TGC      691
Ile Gly Ala Asp Ala Glu Ala Gln Arg Cys His Ser Asn Pro Arg Cys
180                 185                 190                 195

TGC ACC TTC GCC TCC AAC ATG CCC TAC GCG CTG CTC TCC TCC TCG GTC      739
Cys Thr Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser Ser Ser Val
                200                 205                 210

TCG TTC TAT CTT CCG CTC CTG GTG ATG CTC TTC GTC TAC GCA CGA GTT      787
Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr Ala Arg Val
            215                 220                 225
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TTC GTG GTG GCC ACG CGC CAG CTG CGC TTG CTG CGC CGG GAG CTG GGT        835
Phe Val Val Ala Thr Arg Gln Leu Arg Leu Leu Arg Arg Glu Leu Gly
        230                 235                 240

CGC TTC CCG CCA GAG GAG TCT CCG CCG GCT CCT TCT CGC TCC GGA TCC        883
Arg Phe Pro Pro Glu Glu Ser Pro Pro Ala Pro Ser Arg Ser Gly Ser
    245                 250                 255

CCT GGC CTG GCG GGG CCG TGC GCC TCG CCC GCG GGG GTG CCC TCC TAC        931
Pro Gly Leu Ala Gly Pro Cys Ala Ser Pro Ala Gly Val Pro Ser Tyr
260                 265                 270                 275

GGC CGG CGG CCG GCG CGC CTT CTG CCT CTG CGG GAA CAC CGC GCC CTG        979
Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Arg Glu His Arg Ala Leu
                280                 285                 290

CGC ACC TTG GGG CTC ATC ATG GGA ACC TTC ACT CTC TGC TGG TTG CCT       1027
Arg Thr Leu Gly Leu Ile Met Gly Thr Phe Thr Leu Cys Trp Leu Pro
            295                 300                 305

TTC TTT GTG GTC AAC GTG GTG CGC GCC CTC GGG GGC CCC TCT CTG GTG       1075
Phe Phe Val Val Asn Val Val Arg Ala Leu Gly Gly Pro Ser Leu Val
        310                 315                 320

TCC GGC CCC ACT TTC CTC GCC CTT AAC TGG CTG GGC TAT GCC AAC TCT       1123
Ser Gly Pro Thr Phe Leu Ala Leu Asn Trp Leu Gly Tyr Ala Asn Ser
    325                 330                 335

GCC TTC AAC CCG CTC ATC TAC TGC CGC AGC CCG GAC TTT CGG AGC GCC       1171
Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg Ser Ala
340                 345                 350                 355

TTC CGC CGC CTG CTG TGT CGC TGC CGG CCG GAG GAG CAC CTC GCC GCT       1219
Phe Arg Arg Leu Leu Cys Arg Cys Arg Pro Glu Glu His Leu Ala Ala
                360                 365                 370

GCC TCC CCG CCC CGA GCC CCC TCC GGC GCC CCC ACG GCC CTG ACC AGC       1267
Ala Ser Pro Pro Arg Ala Pro Ser Gly Ala Pro Thr Ala Leu Thr Ser
            375                 380                 385

CCC GCT GGC CCC ATG CAG CCC CCA GAG CTC GAC GGG GCT TCC TGC GGA       1315
Pro Ala Gly Pro Met Gln Pro Pro Glu Leu Asp Gly Ala Ser Cys Gly
        390                 395                 400

CTT TCT TAGGCCTTGA AGAAACAACT CCATTGATCC GGAACCTTTG GAAAGCCTCT        1371
Leu Ser
    405
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GGCCGGCCTC GGTTCAGAAT GAGCCCCGTG GAGTTTCCCA GCTGGAAAAC TCTGCCCTCC 1431

CCAGCCTGAC GACTGGGTCC TGGGAGGAGG CGCGGGGGCT GACTGGGGAG GGGAAATCCT 1491

TACCAAGTGG GTTTTCGCTC TCTTTCTGAG AGAAGTTTTC TACACCCCAG CCCTGAACTT 1551

CACCGCTGCC TCAGCAGCTC CCGCGTCTGG TTTCCCATGC CCAGGTGCCC GGGCAGGAGC 1611

TGGGCTGCGT TTAGCCCCGG GACCCGCACC TGTCCCACTC GGGTGCTGTG TGCGCAGGGG 1671

CAAGGCGGGC ACCTTCATTC TGTTCCTTCT GCCGCCCAGA CCCTGAGGAA CCCACCGGGG 1731

TGCTGGAGGC CCAGGCTGAG AAGAGGAAGG TGGGGAAGGT CACGGTTTGG GCTTCTGTCC 1791

CTGGCTTCCT CACTGTAGAC ACACCTACCT CACAGCATTT TCAGGACTTT ACTTTAGCCT 1851

TTGGGGTGGG GGTGGGGGGG CGCTCCTGGT TTCCTGGGAA GGTGAACCAT TAGAATGGGT 1911

CCCTTTTCCT TTTGAAATCA AATTAATAAA TGTTACTGAA TGCAGTTTAA AAAAAAAAAA 1971

AAAAAAAAAA AAAAAAAAAA AAAAAAAAA 2000

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 405 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

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Ile Pro Thr Leu Ala Pro Asn Thr Ala Asn Ala Ser Gly Leu Pro Gly
20 25 30

Val Pro Trp Ala Val Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Val
35 40 45

Leu Ala Thr Val Gly Gly Asn Leu Leu Val Ile Val Ala Ile Ala Arg
50 55 60

Thr Pro Arg Leu Gln Thr Met Thr Asn Val Phe Val Thr Ser Leu Ala
65 70 75 80

Thr Ala Asp Leu Val Val Gly Leu Leu Val Val Pro Pro Gly Ala Thr
85 90 95

Leu Ala Leu Thr Gly His Trp Pro Leu Gly Val Thr Gly Cys Glu Leu
100 105 110

Trp Thr Ser Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu
115 120 125

Cys Ala Leu Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg
130 135 140

Tyr Gly Ala Leu Val Thr Lys Arg Arg Ala Leu Ala Ala Val Val Leu
145 150 155 160

Val Trp Val Val Ser Ala Ala Val Ser Phe Ala Pro Ile Met Ser Lys
165 170 175

Trp Trp Arg Ile Gly Ala Asp Ala Glu Ala Gln Arg Cys His Ser Asn
180 185 190

Pro Arg Cys Cys Thr Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser
195 200 205

Ser Ser Val Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr
210 215 220

Ala Arg Val Phe Val Val Ala Thr Arg Gln Leu Arg Leu Leu Arg Arg
225 230 235 240

Glu Leu Gly Arg Phe Pro Pro Glu Glu Ser Pro Pro Ala Pro Ser Arg
245 250 255

Ser Gly Ser Pro Gly Leu Ala Gly Pro Cys Ala Ser Pro Ala Gly Val
260 265 270

Pro Ser Tyr Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Arg Glu His
275 280 285

Arg Ala Leu Arg Thr Leu Gly Leu Ile Met Gly Thr Phe Thr Leu Cys
290 295 300

Trp Leu Pro Phe Phe Val Val Asn Val Val Arg Ala Leu Gly Gly Pro
305 310 315 320

Ser Leu Val Ser Gly Pro Thr Phe Leu Ala Leu Asn Trp Leu Gly Tyr
325 330 335

Ala Asn Ser Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe
340 345 350

Arg Ser Ala Phe Arg Arg Leu Leu Cys Arg Cys Arg Pro Glu Glu His
355 360 365

Leu Ala Ala Ala Ser Pro Pro Arg Ala Pro Ser Gly Ala Pro Thr Ala
370 375 380

Leu Thr Ser Pro Ala Gly Pro Met Gln Pro Pro Glu Leu Asp Gly Ala
385 390 395 400

Ser Cys Gly Leu Ser
405

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 12, line 12-13 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

Collection Nationale de Cultures de Microorganismes

Address of depositary institution (including postal code and country) [4]

28 rue du Docteur Roux, 75724 PARIS CEDEX 15

Date of deposit [5]	Accession Number [6]
15 April 1993	I-1297

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

As regards the designations in which an application is made for a European patent, a sample of the microorganism deposited shall, until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, be made available only by the issue of a sample to an expert nominated by the requester (Rule 28 (4) of the EPC).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

Australia
Canada
USA
Europe
Japan
New Zealand

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was

(Authorized Officer)

AUSTRALIAN PATENT OFFICE

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS :

1. An isolated and purified nucleotide sequence, which comprises the cDNA coding for the bovine β_3-adrenergic receptor, comprising the nucleotide sequence SEQ ID No. 1.

2. Sequence according to Claim 1, characterized in comprising the following single restriction sites:

Bpu1102 I, Fok I, EcoR V, Bcg I, Nhe I, BspM I, Afl III, Age I, BstE II, BspH I, Bsg I, Nsp I, Nsp7524 I, NspC I, Sap I, BamH I, BstY I, Asc I, Sty I, Hinc II, Apa I, Bsp120 I, Bbe I, Ehe I, Kas I, Nar I, Ec1136 I, Sac I, Stu I, Fse I, Drd I, Tth111 I, Srf I, Bsu36 I, Sfc I, BstX I, Ase I, Bsm I, Dra I.

3. Fragment of the sequence according to Claim 1 or claim 2, consisting of a 72-base pair segment which corresponds to nucleotides 332-403 of sequence ID No. 1, which fragment codes for the transmembrane region TM2.

4. Fragment of the sequence according to any one of Claim 1 or Claim 2, consisting of a 69-base pair segment which corresponds to nucleotides 572-640 of sequence ID No. 1, which fragment codes for the transmembrane region TM4.

5. Fragment of the sequence according to any one of Claim 1 or Claim 2, consisting of a 66-base pair segment which corresponds to nucleotides 983-1048 of sequence ID No. 1, which fragment codes for the transmembrane region TM6.

6. Fragment of the sequence according to any one of Claim 1 or Claim 2, consisting of a 78-base pair segment which corresponds to nucleotides 1070-1147 of sequence ID No. 1, which fragment codes for the transmembrane region TM7.

7. An isolated and purified cDNA clone, comprising a sequence coding for the bovine β_3-adrenergic receptor (BO-ARβ_3) according to any one of Claims 1 to 6.

8. A clone according to Claim 7, comprising 2979

base pairs, including sequence ID No. 1 according to Claim 2 and comprising the following single restriction sites:

EcoR V, Bcg I,

Nhe I, BstE II, BspH I, Bsg I, Sap I, BamH I, Asc I, Stu I, Fse I, Drd I, Srf I, Sfc I, Ase I, Bsm I, Dra I, Bsp1407 I, Csp6 I, Rsa I, Ssp I, Dra III, Bgl II, Afl II, Spe I, Tfi I, Hpa I, Nde I, EcoN I, BsaB I, Pvu I.

9. A nucleotide probe, which hybridizes with a nucleotide sequence according to any one of Claims 1 to 6 or with the complementary sequence under conditions of hybridization such that it does not hybridize with the genes or messenger RNA coding for the β_1- or β_2-adrenergic receptors, wherein said probe is selected from the group of nucleotide probes consisting of:

the nucleic acid sequence which corresponds to nucleotides 332-403 of the sequence ID No. 1,

the nucleic acid sequence which corresponds to nucleotides 572-640 of the sequence ID No. 1,

the nucleic acid sequence which corresponds to nucleotides 983-1048 of the sequence ID No. 1, and

the nucleic acid sequence which corresponds to nucleotides1070-1147 of the sequence ID No. 1.

10. An isolated and purified protein which is encoded by a nucleotide sequence according to Claim 1, wherein said protein displays β3-adrenergic receptor activity and comprises the sequence of 405 amino acids of SEQ ID No. 2.

11. A peptide comprising a fragment of 24 amino acids corresponding to the segment 76-99 of sequence ID No. 2 and constituting the transmembrane region TM2.

12. A peptide comprising a fragment of 23 amino acids corresponding to the segment 156-178 of sequence Id No. 2 and constituting the transmembrane region TM4.

13. A peptide comprising a fragment of 22 amino acids corresponding to the segment 293-314 of sequence Id No. 2

and constituting the transmembrane region TM6.

14. A peptide comprising a fragment of 26 amino acids corresponding to the segment 322-347 of sequence ID No. 2 and constituting the transmembrane region TM7.

15. A recombinant plasmid comprising a nucleotide sequence according to any one of Claims 1 to 6.

16. A recombinant plasmid according to Claim 15, comprising an origin of replication in a host cell at least one gene whose expression permits selection of said host cell which has received the said plasmid and a regulatory sequence, including a promoter permitting expression of a protein having bovine β_3-adrenergic receptor activity in said host cell.

17. A recombinant plasmid according to Claim 16, wherein said plasmid is pRc/CMV into which is inserted, in the poly-linker, at least a sequence coding for the bovine β_3-adrenergic receptor, and has been deposited with the collection National de Cultures de Microorganismes [National Collection of Microorganism Cultures] (CNCM) held by the PASTEUR INSTITUTE, dated 15 April 1993, under No. I-1297.

18. A host cell transformed by an recombinant plasmid according to any one of claims 15 to 17, comprising regulatory elements allowing expression of the nucleotide sequence coding for the protein having bovine β_3-adrenergic receptor activity.

19. A host cell according to Claim 18, wherein said host cell is a CHO cell.

20. A host cell according to Claim 18, wherein said host cell is *Escherichia coli*.

21. A method for detecting the binding of an agonist or antagonist to a protein according to Claim 10, comprising the steps of :

- placing the said agonist or antagonist in contact with a host cell previously transformed by a vector as defined in any one of Claims 15 to 17;

- detecting the formation of a complex of formed between said agonist or antagonist and said protein.

Dated this 8th day of January 1998

VETIGEN AND VIRBAC

By their Patent Attorneys

GRIFFITH HACK

Fellows Institute of Patent

Attorneys of Australia

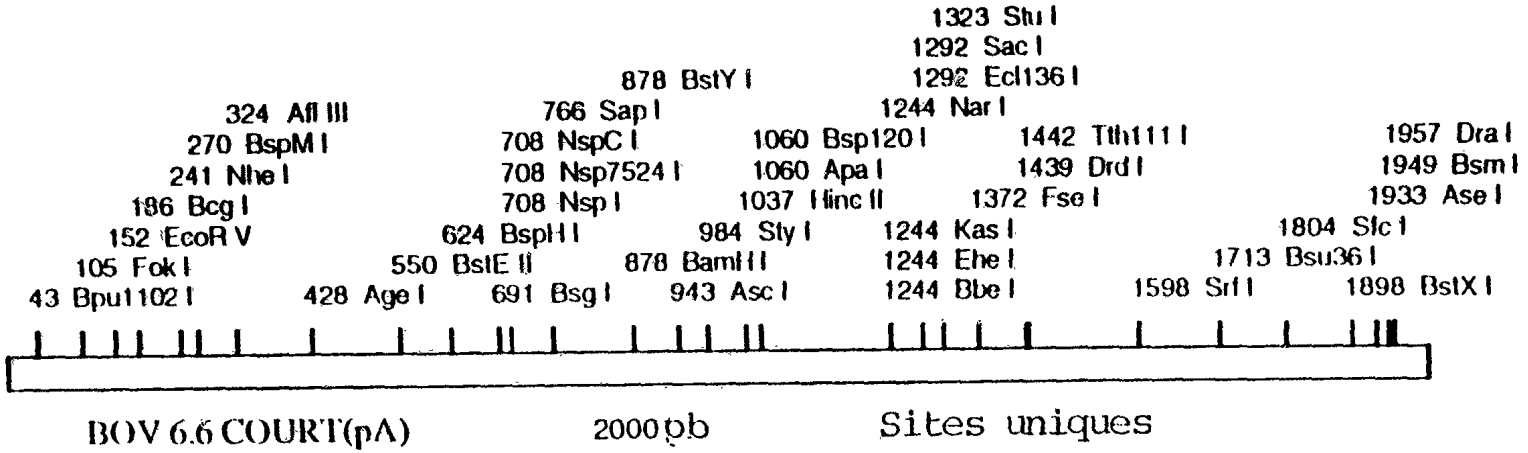

1323 Stu I
1292 Sac I
878 BstY I
1292 Ecl136 I
324 Afl III
766 Sap I
1244 Nar I
270 BspM I
708 NspC I
1060 Bsp120 I
1442 Tth111 I
1957 Dra I
241 Nhe I
708 Nsp7524 I
1060 Apa I
1439 Drd I
1949 Bsm I
186 Bcg I
708 Nsp I
1037 Hinc II
1372 Fse I
1933 Ase I
152 EcoR V
624 BspH I
984 Sty I
1244 Kas I
1804 Sfc I
105 Fok I
550 BstE II
878 BamH I
1244 Ehe I
1713 Bsu36 I
43 Bpu1102 I
428 Age I
691 Bsg I
943 Asc I
1244 Bbe I
1598 Srf I
1898 BstX I
BOV 6.6 COURT(pA)
2000 pb
Sites uniques

FIGURE 1a

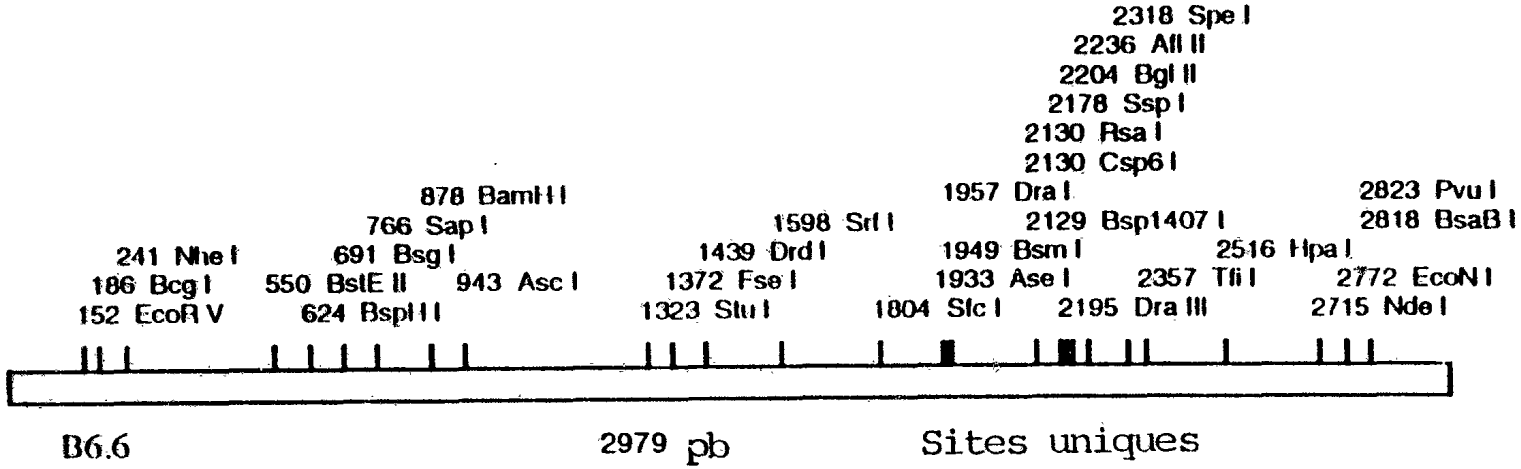

2318 Spe I
2236 Afl II
2204 Bgl II
2178 Ssp I
2130 Rsa I
2130 Csp6 I
878 BamH I
766 Sap I
241 Nhe I
691 Bsg I
186 Bcg I
550 BstE II
943 Asc I
152 EcoR V
624 BspH I
1598 Srf I
1439 Drd I
1372 Fse I
1323 Stu I
1957 Dra I
2129 Bsp1407 I
1949 Bsm I
1933 Ase I
1804 Sfc I
2195 Dra III
2823 Pvu I
2818 BsaB I
2516 Hpa I
2357 Tfi I
2772 EcoN I
2715 Nde I
B6.6
2979 pb
Sites uniques

FIGURE 1b

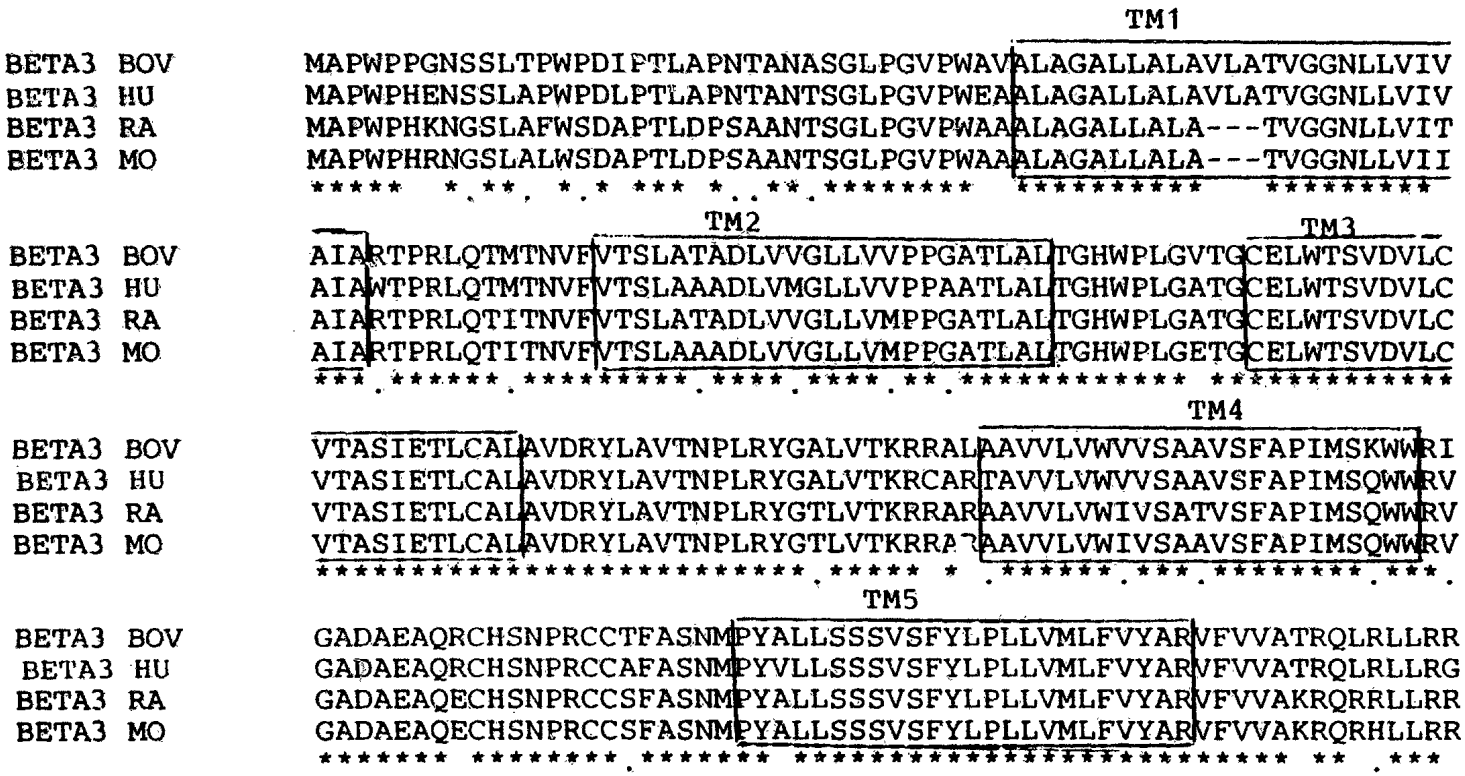

TM1
BETA3 BOV MAPWPPGNSSLTPWPDIPTLAPNTANASGLPGVPWAVALAGALLALAVLATVGGNLLVIV
BETA3 HU MAPWPHENSSLAPWPDLPTLAPNTANTSGLPGVPWEAALAGALLALAVLATVGGNLLVIV
BETA3 RA MAPWPHKNGSLAFWSDAPTLDPSAANTSGLPGVPWAAALAGALLALA---TVGGNLLVIT
BETA3 MO MAPWPHRNGSLALWSDAPTLDPSAANTSGLPGVPWAAALAGALLALA---TVGGNLLVII
TM2
TM3
BETA3 BOV AIARTPRLQTMTNVFVTSLATADLVVGLLVVPPGATLALTGHWPLGVTGCELWTSVDVLC
BETA3 HU AIAWTPRLQTMTNVFVTSLAAADLVMGLLVVPPAATLALTGHWPLGATGCELWTSVDVLC
BETA3 RA AIARTPRLQTITNVFVTSLATADLVVGLLVMPPGATLALTGHWPLGATGCELWTSVDVLC
BETA3 MO AIARTPRLQTITNVFVTSLAAADLVVGLLVMPPGATLALTGHWPLGETGCELWTSVDVLC
TM4
BETA3 BOV VTASIETLCALAVDRYLAVTNPLRYGALVTKRRALAAVVLVWVVSAAVSFAPIMSKWWRI
BETA3 HU VTASIETLCALAVDRYLAVTNPLRYGALVTKRCARTAVVLVWVVSAAVSFAPIMSQWWRV
BETA3 RA VTASIETLCALAVDRYLAVTNPLRYGTLVTKRRARAAVVLVWIVSATVSFAPIMSQWWRV
BETA3 MO VTASIETLCALAVDRYLAVTNPLRYGTLVTKRRARAAVVLVWIVSAAVSFAPIMSQWWRV
TM5
BETA3 BOV GADAEAQRCHSNPRCCTFASNMPYALLSSSVSFYLPLLVMLFVYARVFVVATRQLRLLRR
BETA3 HU GADAEAQRCHSNPRCCAFASNMPYVLLSSSVSFYLPLLVMLFVYARVFVVATRQLRLLRG
BETA3 RA GADAEAQECHSNPRCCSFASNMPYALLSSSVSFYLPLLVMLFVYARVFVVAKRQRRLLRR
BETA3 MO GADAEAQECHSNPRCCSFASNMPYALLSSSVSFYLPLLVMLFVYARVFVVAKRQRHLLRR

FIGURE 2.1

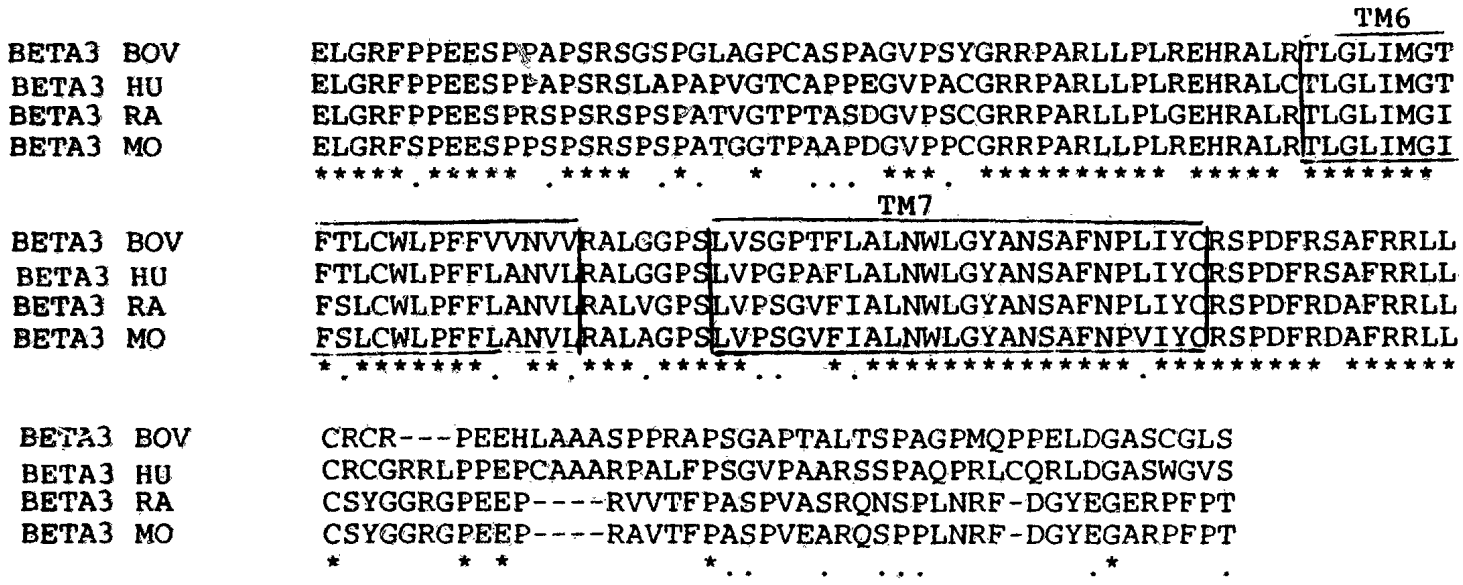

TM6
BETA3 BOV ELGRFPPEESPPAPSRSGSPGLAGPCASPAGVPSYGRRPARLLPLREHRALRTLGLIMGT
BETA3 HU ELGRFPPEESPPAPSRSLAPAPVGTCAPPEGVPACGRRPARLLPLREHRALCTLGLIMGT
BETA3 RA ELGRFPPEESPRSPSRSPSPATVGTPTASDGVPSCGRRPARLLPLGEHRALRTLGLIMGI
BETA3 MO ELGRFSPEESPPSPSRSPSPATGGTPAAPDGVPPCGRRPARLLPLREHRALRTLGLIMGI
TM7
BETA3 BOV FTLCWLPFFVVNVVRALGGPSLVSGPTFLALNWLGYANSAFNPLIYCRSPDFRSAFRRLL
BETA3 HU FTLCWLPFFLANVLRALGGPSLVPGPAFLALNWLGYANSAFNPLIYCRSPDFRSAFRRLL
BETA3 RA FSLCWLPFFLANVLRALVGPSLVPSGVFIALNWLGYANSAFNPLIYCRSPDFRDAFRRLL
BETA3 MO FSLCWLPFFLANVLRALAGPSLVPSGVFIALNWLGYANSAFNPVIYCRSPDFRDAFRRLL
BETA3 BOV CRCR---PEEHLAAASPPRAPSGAPTALTSPAGPMQPPELDGASCGLS
BETA3 HU CRCGRRLPPEPCAAARPALFPSGVPAARSSPAQPRLCQRLDGASWGVS
BETA3 RA CSYGGRGPEEP----RVVTFPASPVASRQNSPLNRF-DGYEGERPFPT
BETA3 MO CSYGGRGPEEP----RAVTFPASPVEARQSPPLNRF-DGYEGARPFPT

FIGURE 2.2

ADN Huβ3 épissé (en haut); ADN β3 bov : cadre de lecture ouvert (en bas)

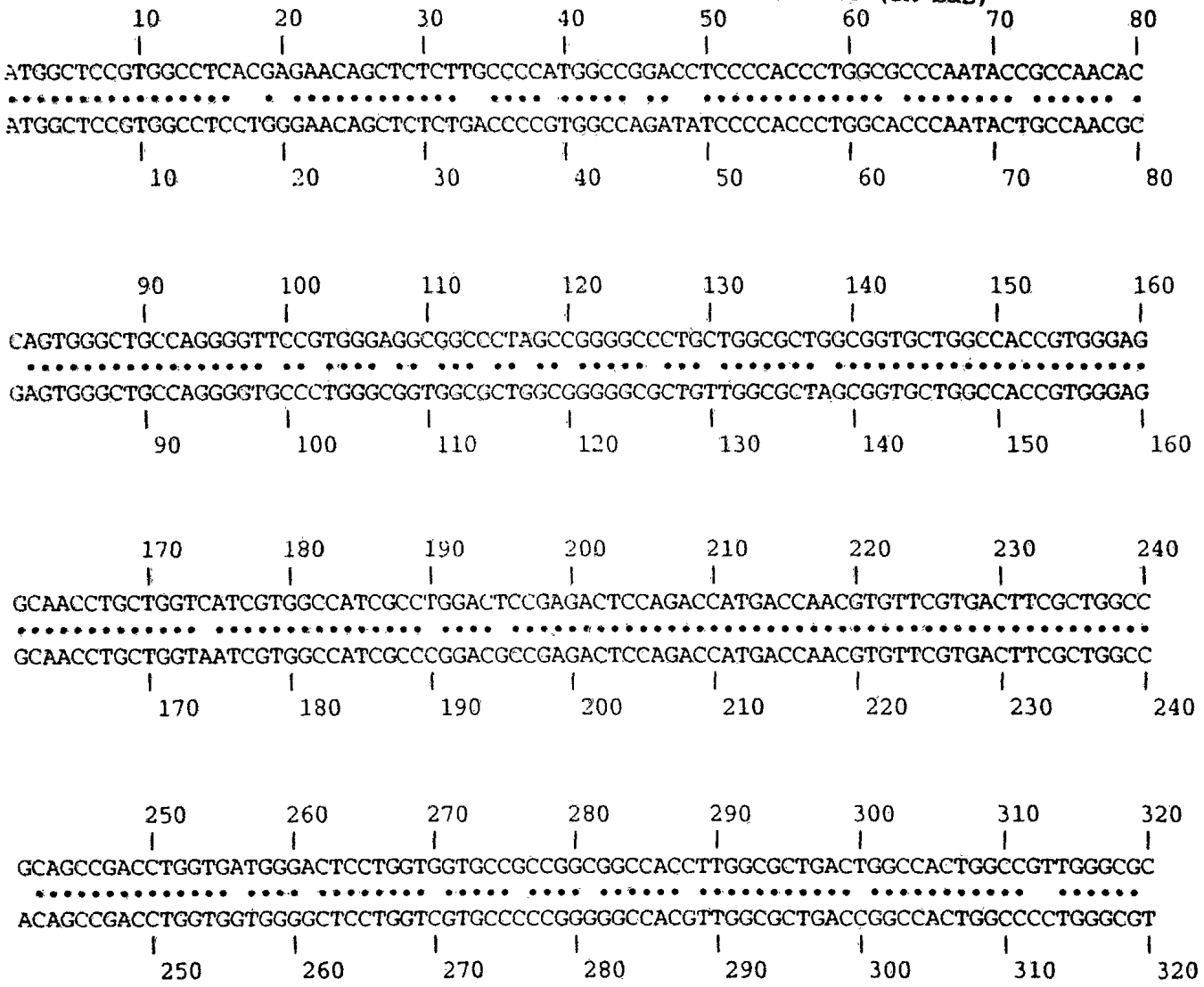
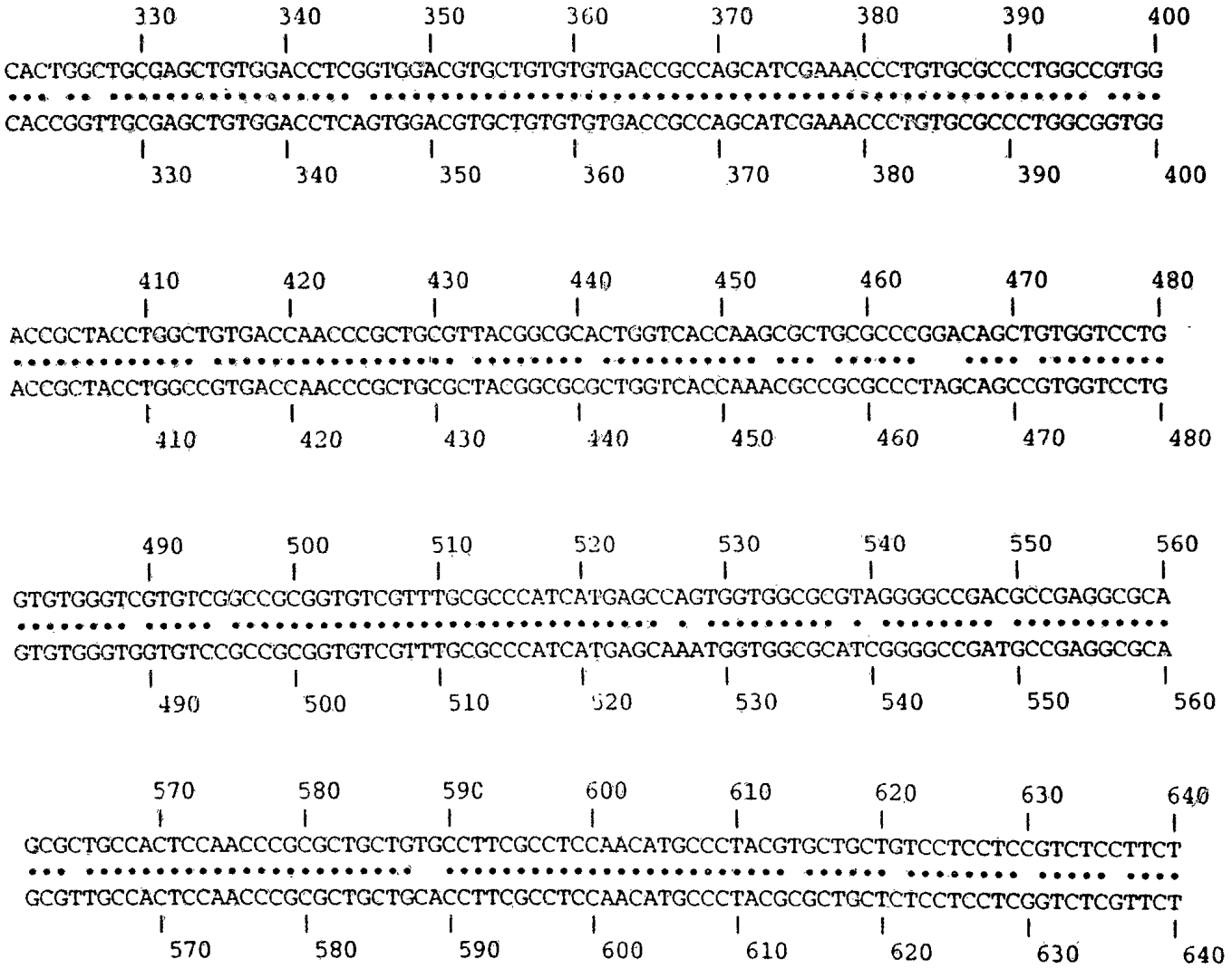


FIGURE 3.1


330 340 350 360 370 380 390 400
CACTGGCTGCGAGCTGTGGACCTCGGTGGACGTGCTGTGTGTGACCGCCAGCATCGAAACCCTGTGCGCCCTGGCCGTGG
CACCGGTTGCGAGCTGTGGACCTCAGTGGACGTGCTGTGTGTGACCGCCAGCATCGAAACCCTGTGCGCCCTGGCGGTGG
330 340 350 360 370 380 390 400
410 420 430 440 450 460 470 480
ACCGCTACCTGGCTGTGACCAACCCGCTGCGTTACGGCGCACTGGTCACCAAGCGCTGCGCCCGGACAGCTGTGGTCCTG
ACCGCTACCTGGCCGTGACCAACCCGCTGCGCTACGGCGCGCTGGTCACCAAACGCCGCGCCCTAGCAGCCGTGGTCCTG
410 420 430 440 450 460 470 480
490 500 510 520 530 540 550 560
GTGTGGGTCGTGTCGGCCGCGGTGTCGTTTGCGCCCATCATGAGCCAGTGGTGGCGCGTAGGGGCCGACGCCGAGGCGCA
GTGTGGGTGGTGTCCGCCGCGGTGTCGTTTGCGCCCATCATGAGCAAATGGTGGCGCATCGGGGCCGATGCCGAGGCGCA
490 500 510 520 530 540 550 560
570 580 590 600 610 620 630 640
GCGCTGCCACTCCAACCCGCGCTGCTGTGCCTTCGCCTCCAACATGCCCTACGTGCTGCTGTCCTCCTCCGTCTCCTTCT
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570 580 590 600 610 620 630 640

FIGURE 3.2

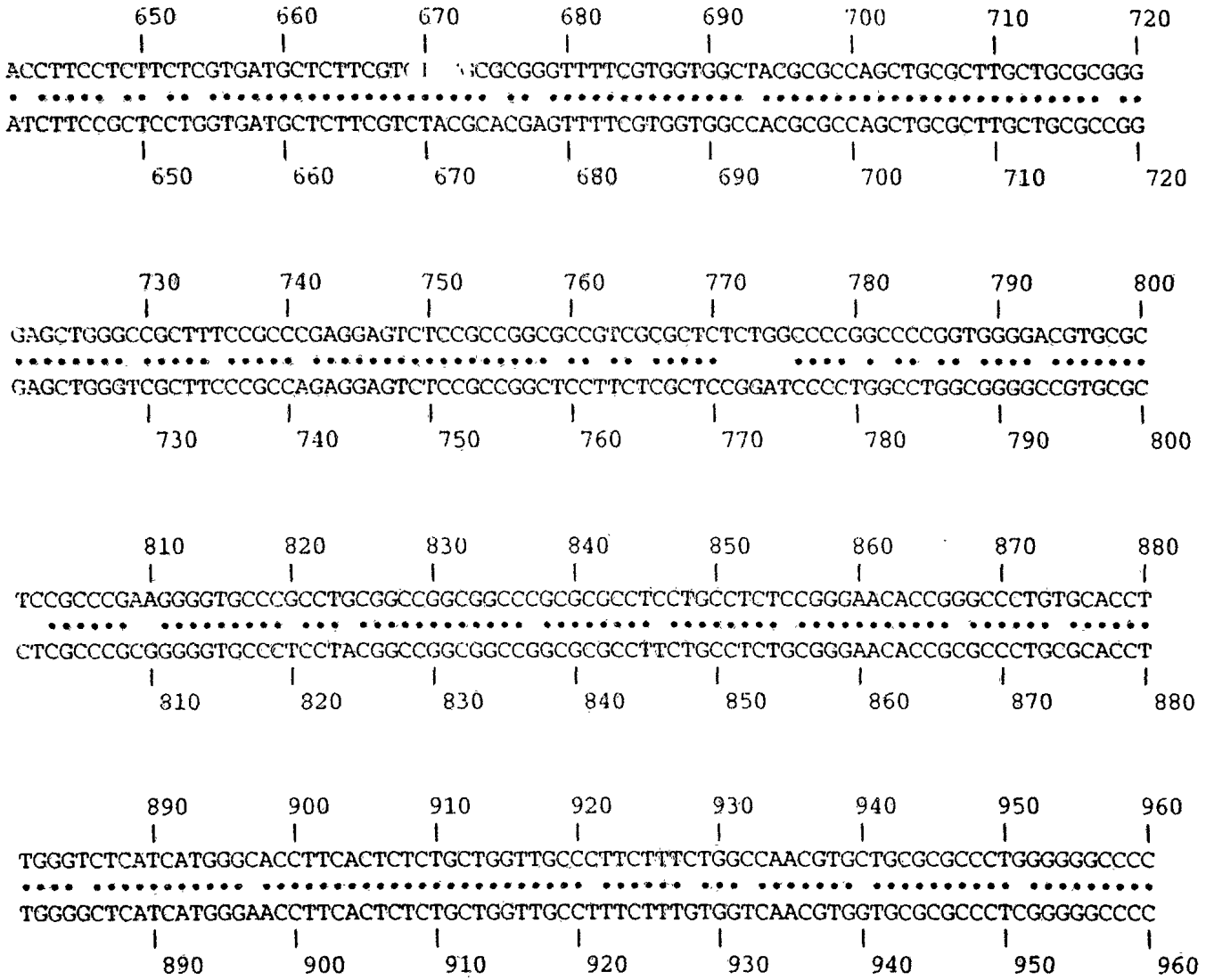
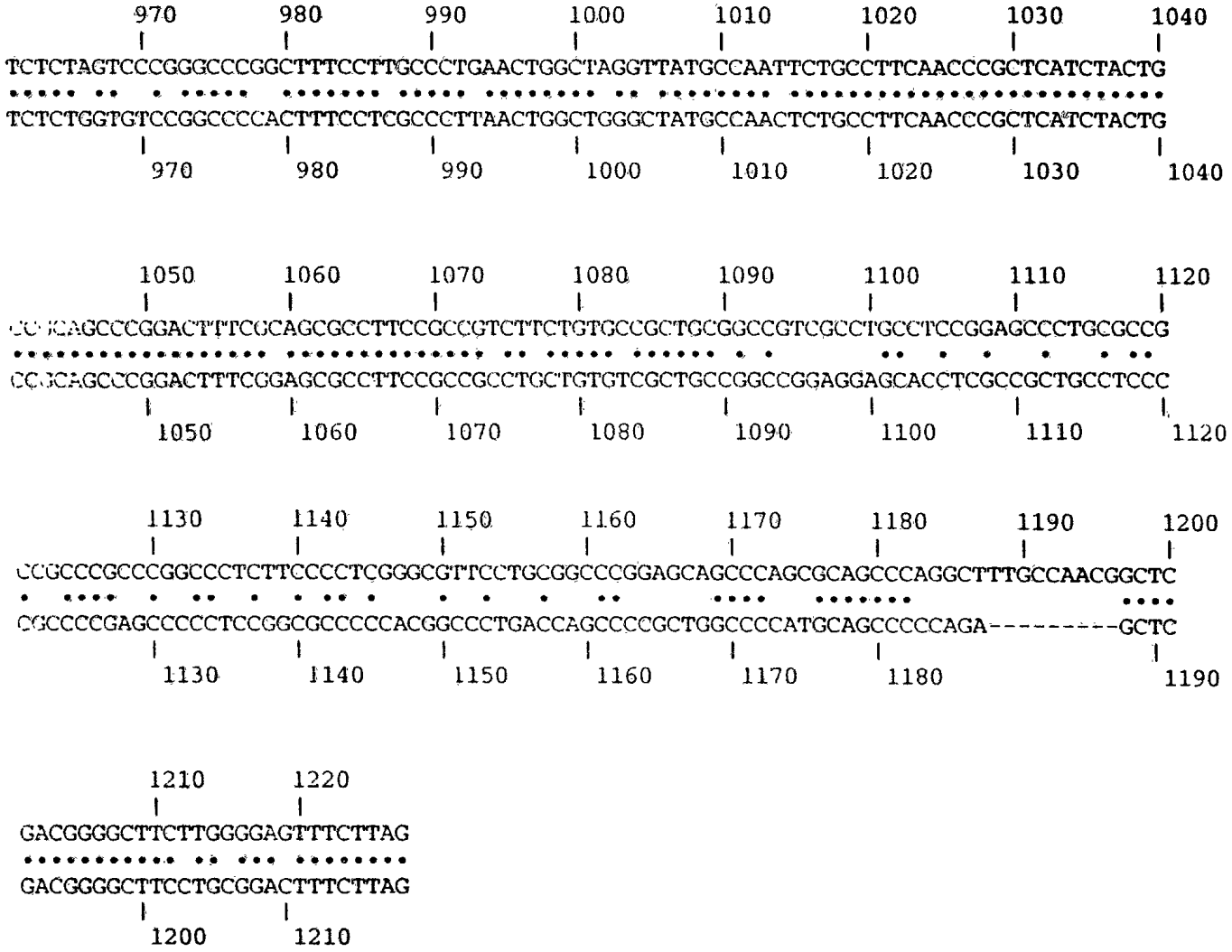

650 660 670 680 690 700 710 720
ACCTTCCTCTTCTCGTGATGCTCTTCGTC GCGCGGGTTTTCGTGGTGGCTACGCGCCAGCTGCGCTTGCTGCGCGGG
ATCTTCCGCTCCTGGTGATGCTCTTCGTCTACGCACGAGTTTTCGTGGTGGCCACGCGCCAGCTGCGCTTGCTGCGCCGG
650 660 670 680 690 700 710 720
730 740 750 760 770 780 790 800
GAGCTGGGCCGCTTTCCGCCCGAGGAGTCTCCGCCGGCGCCGTCGCGCTCTCTGGCCCCGGCCCCGGTGGGGACGTGCGC
GAGCTGGGTCGCTTCCCGCCAGAGGAGTCTCCGCCGGCTCCTTCTCGCTCCGGATCCCCTGGCCTGGCGGGGCCGTGCGC
730 740 750 760 770 780 790 800
810 820 830 840 850 860 870 880
TCCGCCCGAAGGGGTGCCCGCCTGCGGCCGGCGGCCCGCGCGCCTCCTGCCTCTCCGGGAACACCGGGCCCTGTGCACCT
CTCGCCCGCGGGGGTGCCCTCCTACGGCCGGCGGCCGGCGCGCCTTCTGCCTCTGCGGGAACACCGCGCCCTGCGCACCT
810 820 830 840 850 860 870 880
890 900 910 920 930 940 950 960
TGGGTCTCATCATGGGCACCTTCACTCTCTGCTGGTTGCCCTTCTTTCTGGCCAACGTGCTGCGCGCCCTGGGGGGCCCC
TGGGGCTCATCATGGGAACCTTCACTCTCTGCTGGTTGCCTTTCTTTGTGGTCAACGTGGTGCGCGCCCTCGGGGGCCCC
890 900 910 920 930 940 950 960

FIGURE 3.3


970 980 990 1000 1010 1020 1030 1040
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TCTCTGGTGTCCGGCCCCACTTTCCTCGCCCTTAACTGGCTGGGCTATGCCAACTCTGCCTTCAACCCGCTCATCTACTG
970 980 990 1000 1010 1020 1030 1040
1050 1060 1070 1080 1090 1100 1110 1120
CCGCAGCCCGGACTTTCGCAGCGCCTTCCGCCGTCTTCTGTGCCGCTGCGGCCGTCGCCTGCCTCCGGAGCCCTGCGCCG
CCGCAGCCCGGACTTTCGGAGCGCCTTCCGCCGCCTGCTGTGTCGCTGCCGGCCGGAGGAGCACCTCGCCGCTGCCTCCC
1050 1060 1070 1080 1090 1100 1110 1120
1130 1140 1150 1160 1170 1180 1190 1200
CCGCCCGCCCGGCCCTCTTCCCCTCGGGCGTTCCTGCGGCCCGGAGCAGCCCAGCGCAGCCCAGGCTTTGCCAACGGCTC
CGCCCCGAGCCCCCTCCGGCGCCCCCACGGCCCTGACCAGCCCCGCTGGCCCCATGCAGCCCCCAGA---------GCTC
1130 1140 1150 1160 1170 1180 1190
1210 1220
GACGGGGCTTCTTGGGGAGTTTCTTAG
GACGGGGCTTCCTGCGGACTTTCTTAG
1200 1210

FIGURE 3.4

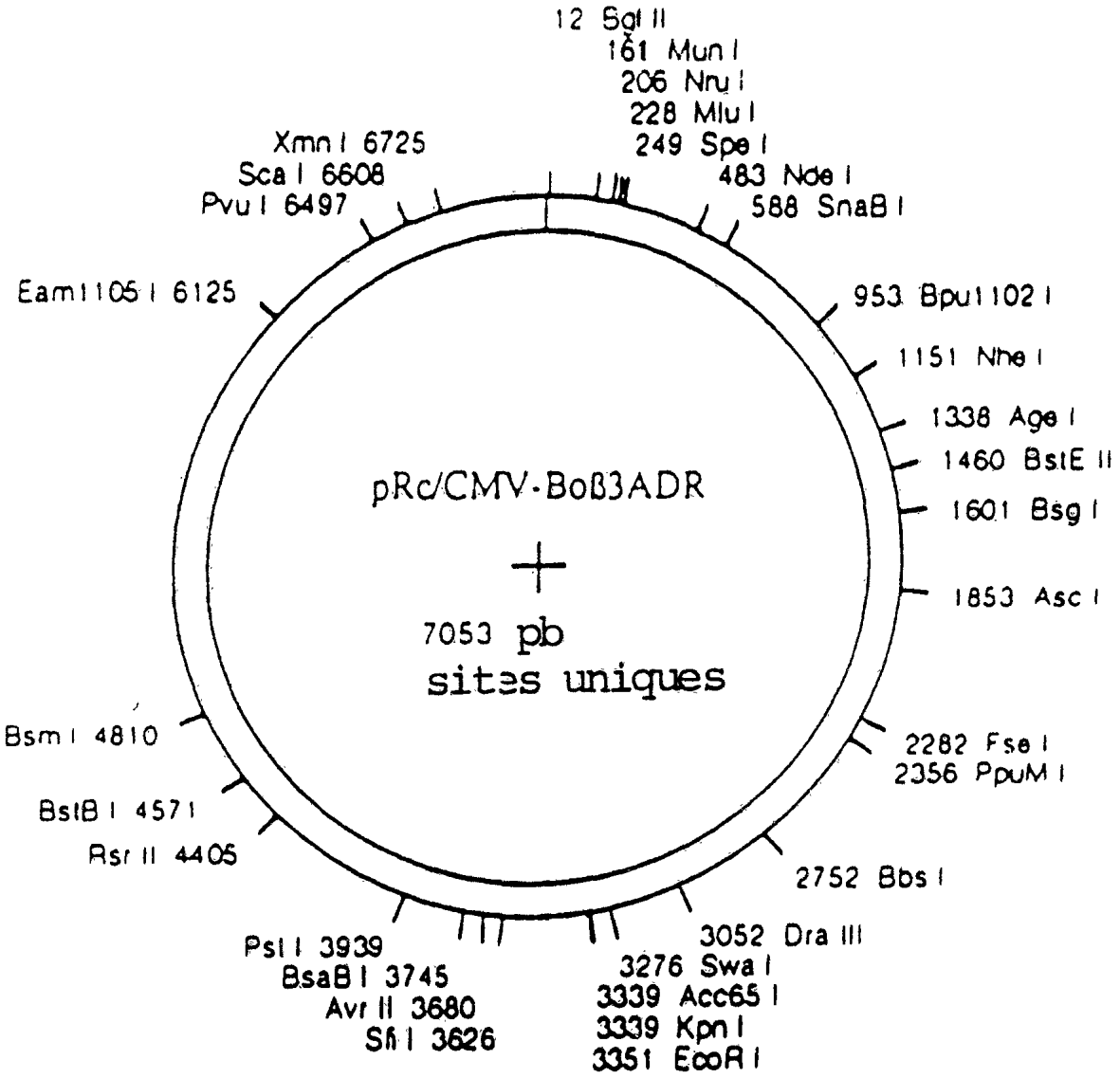
12 Sgf II
161 Mun I
206 Nru I
228 Mlu I
249 Spe I
483 Nde I
588 SnaB I
953 Bpu1102 I
1151 Nhe I
1338 Age I
1460 BstE II
1601 Bsg I
1853 Asc I
2282 Fse I
2356 PpuM I
2752 Bbs I
3052 Dra III
3276 Swa I
3339 Acc65 I
3339 Kpn I
3351 EcoR I
Psi I 3939
BsaB I 3745
Avr II 3680
Sfi I 3626
BstB I 4571
Rsr II 4405
Bsm I 4810
Eam1105 I 6125
Pvu I 6497
Sca I 6608
Xmn I 6725
pRc/CMV-Boß3ADR
7053 pb
sites uniques

FIGURE 4

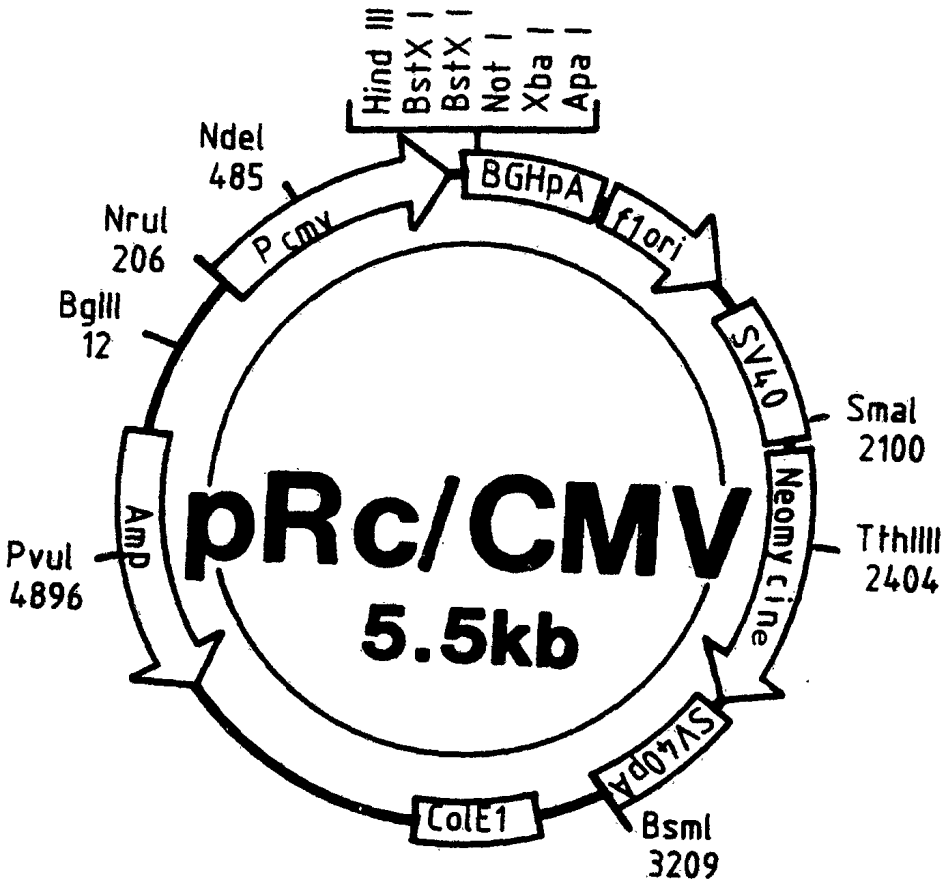
Hind III
BstX I
BstX I
Not I
Xba I
Apa I
BGHpA
f1 ori
SV40
Neomycine
SV40PA
ColE1
AmP
P cmv
Ndel
485
Nrul
206
BglII
12
Pvul
4896
SmaI
2100
TthIIII
2404
BsmI
3209
pRc/CMV
5.5kb

FIGURE 5

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/FR 94/00447

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K15/00 C12N15/12 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 12246 (CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE) 23 July 1992 cited in the application see the whole document ---	1-30
X	FR,A,2 642 075 (INSTITUT PASTEUR) 27 July 1990 see the whole document & WO,A,90 08775 cited in the application ---	1-30
X	EMBL DATA LIBRARY Acc.Nr.: X67214, 18 janviers 1993, B.Stoffel et al., "Bovine beta3-adrenergic receptor, partial genomic sequence". --- -/--	4,5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 1994	18 -08- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FR 94/00447

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 351 921 (MERCK & CO. INC) 24 January 1990 see the whole document	1-30

-.-

-.-.-.-.-.-

INTERNATIONAL SEARCH REPORT

...rmation on patent family members

Interna... l Application No
PCT/FR 94/00447

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9212246	23-07-92	FR-A-	2671559	17-07-92
		EP-A-	0567577	03-11-93
		JP-T-	6504915	09-06-94
FR-A-2642075	27-07-90	DE-D-	69005620	10-02-94
		DE-T-	69005620	21-07-94
		EP-A-	0455682	13-11-91
		WO-A-	9008775	09-08-90
		JP-T-	4504354	06-08-92
		US-A-	5288607	22-02-94
WO-A-9008775	09-08-90	FR-A-	2642075	27-07-90
		DE-D-	69005620	10-02-94
		DE-T-	69005620	21-07-94
		EP-A-	0455682	13-11-91
		JP-T-	4504354	06-08-92
		US-A-	5288607	22-02-94
EP-A-0351921	24-01-90	JP-A-	2084121	26-03-90

RAPPORT DE RECHERCHE INTERNATIONALE

Dema ternationale No
PCT/FR 94/00447

A. CLASSEMENT DE L'OBJET DE LA DEMANDE
CIB 5 C07K15/00 C12N15/12 C12N5/10

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)
CIB 5 C07K

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si cela est réalisable, termes de recherche utilisés)

C. DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie *	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
X	WO,A,92 12246 (CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE) 23 Juillet 1992 cité dans la demande voir le document en entier ---	1-30
X	FR,A,2 642 075 (INSTITUT PASTEUR) 27 Juillet 1990 voir le document en entier & WO,A,90 08775 cité dans la demande ---	1-30
X	EMBL DATA LIBRARY Acc.Nr.: X67214, 18 janviers 1993, B.Stoffel et al., "Bovine beta3-adrenergic receptor, partial genomic sequence". --- -/--	4,5

[X] Voir la suite du cadre C pour la fin de la liste des documents

[X] Les documents de familles de brevets sont indiqués en annexe

* Catégories spéciales de documents cités:

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

"E" document antérieur, mais publié à la date de dépôt international ou après cette date

"L" document pouvant jeter un doute sur une revendication de priorité ou cité pour déterminer la date de publication d'une autre citation ou pour une raison spéciale (telle qu'indiquée)

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"P" document publié avant la date de dépôt international, mais postérieurement à la date de priorité revendiquée

"T" document ultérieur publié après la date de dépôt international ou la date de priorité et n'appartenenant pas à l'état de la technique pertinent, mais cité pour comprendre le principe ou la théorie constituant la base de l'invention

"X" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive par rapport au document considéré isolément

"Y" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme impliquant une activité inventive lorsque le document est associé à un ou plusieurs autres documents de même nature, cette combinaison étant évidente pour une personne du métier

"&" document qui fait partie de la même famille de brevets

Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
26 Juillet 1994	18 -08- 1994

Nom et adresse postale de l'administration chargée de la recherche internationale	Fonctionnaire autorisé
Office Européen des Brevets, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	De Kok, A

Formulaire PCT/ISA/210 (deuxième feuille) (juillet 1992)

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale No
PCT/FR 94/00447

C.(suite) DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie *	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
A	EP,A,0 351 921 (MERCK & CO. INC.) 24 Janvier 1990 voir le document en entier -----	1-30

RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs aux membres de familles de brevets

Demande internationale No
PCT/FR 94/00447

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)		Date de publication
WO-A-9212246	23-07-92	FR-A-	2671559	17-07-92
		EP-A-	0567577	03-11-93
		JP-T-	6504915	09-06-94
FR-A-2642075	27-07-90	DE-D-	69005620	10-02-94
		DE-T-	69005620	21-07-94
		EP-A-	0455682	13-11-91
		WO-A-	9008775	09-08-90
		JP-T-	4504354	06-08-92
		US-A-	5288607	22-02-94
WO-A-9008775	09-08-90	FR-A-	2642075	27-07-90
		DE-D-	69005620	10-02-94
		DE-T-	69005620	21-07-94
		EP-A-	0455682	13-11-91
		JP-T-	4504354	06-08-92
		US-A-	5288607	22-02-94
EP-A-0351921	24-01-90	JP-A-	2084121	26-03-90