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(71) Applicant (*for all designated States except US*): **AVESTHA GENGRAINE TECHNOLOGIES PVT LTD.** [IN/IN]; "Discoverer", 9th Floor, Unit 3, International Tech Park, Whitefield Road, Bangalore 560066, Karnataka (IN).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): **MORAWALA, Patell, Villoo** [IN/IN]; "Discoverer" 9th Floor, Unit 3, International Tech Park, Whitefield Road, Bangalore 560066, Karnataka (IN).

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**(54) Title:** A METHOD FOR THE PRODUCTION OF A MONOCLONAL ANTIBODY TO CD20 FOR THE TREATMENT OF B-CELL LYMPHOMA

**(57) Abstract:** The present invention relates to the recombinant method used for the production of soluble form of an antibody that binds to CD20 for treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma (NHL). The treatment will comprise the use of immunologically active anti-CD20 antibodies; or radiolabeled anti-CD20 antibodies and or cooperative strategies where both labeled and non-labeled antibodies will be used for treatment of NHL. The procedure describes the de novo synthesis of the nucleic acid sequence encoding anti-CD20, transformation of the constructed nucleic acid sequences into competent bacteria and the sub-cloning of the same into mammalian expression vectors for expression of the desired protein. DNA constructs comprising the control elements associated with the gene of interest has been disclosed. The nucleic acid sequence of interest has been codon optimized to permit expression in the suitable mammalian host cells.

WO 2006/126069 A2

# A method for the production of a monoclonal antibody to CD20 for the treatment of B-cell lymphoma.

## FIELD OF THE INVENTION:

The present invention relates to the recombinant method used for the production of soluble form of an antibody that binds to CD20 for treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma (NHL). The treatment will comprise the use of immunologically active anti-CD20 antibodies; or radiolabeled anti-CD20 antibodies and or cooperative strategies where both labeled and non-labeled antibodies will be used for treatment of NHL. The procedure describes the de novo synthesis of the nucleic acid sequence encoding anti-CD20, transformation of the constructed nucleic acid sequences into competent bacteria and the sub-cloning of the same into mammalian expression vectors for expression of the desired protein. DNA constructs comprising the control elements associated with the gene of interest has been disclosed. The nucleic acid sequence of interest has been codon optimized to permit expression in the suitable mammalian host cells.

## BACKGROUND OF THE INVENTION:

Antibodies have been considered as a powerful tool to recognize and target almost any molecule with a high degree of specificity and affinity. Monoclonal antibodies (mAbs) have been used as *in vitro* diagnostics hence enabling worldwide standardisation of reagents for RIA, ELISA immunocytopathology and flow cytometry. Monoclonal antibodies have also been extensively used for *in vivo* localisation of tumour antigens and in the immunotherapy of cancer.

This problem has been addressed by the development of antibodies of two basic types. The first type, referred to as chimeric antibodies, wherein the murine constant domains only are replaced by equivalent domains of human origin (Morrison et al, P.N.A.S., 1984, 81, 6851-6855; Boulianne et al, Nature, 1985, 314, 268-270; and Neuberger et al, Nature, 1985, 314, 268-270). The second type is where the murine constant domains and the

murine framework regions are all replaced by equivalent domains and regions of human origin. This second type of antibody is referred to as a humanized or CDR-grafted antibody (Jones et al, Nature, 1986, 321, 522-525; and Riechmann et al, Nature, 1988, 332, 323-327). For example, for therapeutic purposes, human IgG1 and rat IgG2b are currently favored isotypes. Further, of the human IgG isotypes,IgG1 and IgG3 appear to be most effective for complement and cell mediated lysis, and therefore for killing tumour cells. A human antibody would of course avoid the need for "humanization", however cell lines, which secrete human antibodies, are very unstable and have generally proven unsuitable for commercial scale production.

To generate sufficient quantities of antibody for full clinical use it is desirable to employ an efficient recombinant expression system. Since myeloma cells represent a natural host specialized for antibody production and secretion, cell lines derived from these have been used for the expression of recombinant antibodies. Often, complex vector design, based around immunoglobulin gene regulatory elements, is required, and final expression levels have been reported which are highly variable (Winter et al, Nature, 1988, 332, 323-327; Weidle et al, Gene, 1987, 60, 205-216; Nakatani et al, Bio/Technology, 1989, 7, 805-810; and Gillies et al, Bio/Technology, 1989, 7, 799-804). An alternative mammalian expression system is that offered by the use of Chinese hamster ovary (CHO) cells. The use of these cells has enabled the production of large quantities of several therapeutic proteins for research and clinical use (Kaufman et al, Mol.Cell.Biol, 1985, 5, 1750-1759; and Zettlmeissl et al, Bio/Technology, 1987, 5, 720-725). There are, however, very few instances of the use of these cells for the expression of antibodies and the levels of expression of murine antibodies reported to date are low of the order of 0.01-0.1. mu.g/ml (Weidle et al, Gene, 1987, 51, 21-29; and Feys et al, Int.J.Cancer, 1988, 2, 26-27).

The anti-CD20 antibody binds specifically to the antigen CD20 (human B lymphocyte restricted differentiation antigen, Bp35), a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD located on pre B and mature B lymphocytes. The antigen is also expressed on > 90% of B cell non Hodgkin's lymphomas (NHL), but is not found on hematopoietic stem cells, pro B cells, normal plasma cells or other normal tissues. CD20 regulates an early step(s) in the activation process for cell cycle initiation

and differentiation, and possibly functions as a calcium ion channel. CD20 is not shed from the cell surface and does not internalize upon antibody binding. Free CD20 antigen is not found in the circulation. The anti-CD20 antibody works by recruiting the body's natural defenses to attack and kill the B cell to which it binds via the CD20 antigen. The host cell killing takes place by two mechanisms: 1) the complement-dependent cytotoxicity (CDC) pathway and 2) the antibody dependent cell mediated cytotoxicity (ADCC) pathway.

The anti-CD20 antibody is a genetically engineered chimeric murine/human monoclonal antibody. The antibody is an $IgG_1$ kappa immunoglobulin containing murine light- and heavy-chain variable region sequences and human constant region sequences. The anti-CD20 antibody is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids (based on cDNA analysis) and has an approximate molecular weight of 145 kD. The anti-CD20 antibody has a binding affinity for the CD20 antigen of approximately 8.0 nM. The chimeric anti-CD20 antibody is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium containing the antibiotic gentamicin. The anti-CD20 antibody is purified by affinity and ion exchange chromatography.

The present invention relates to the construction, cloning, expression, purification and production of antibodies that can bind to CD20. The antibody will be a targeted therapy indicated for the treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma (NHL).

**SUMMARY OF THE INVENTION:**

The present invention is directed to the transformation of nucleic acid sequence encoding the polypeptide anti-CD20.

According to an aspect of the invention there is provided the nucleic acid sequences encoding the heavy and the light chains of the anti-CD 20 molecule. According to an

further aspect of the invention there is provided the corresponding amino-acid sequence encoded by the nucleic acid sequences.

A particular aspect of the invention relates to the de novo synthesis of the variable regions of the heavy and the light chain of the anti-CD 20 molecule. Further disclosed is the construction of the vector constructs with the nucleic acid sequence of interest, transformation of the vector constructs into competent bacteria and subcloning of the anti-CD 20 chains into mammalian expression vectors.

## DETAILED DESCRIPTION OF THE INVENTION:

The anti-CD20 antibody is a mouse-human chimeric antibody. It is comprised of two chains – 1) the light chain which is made up of the variable domain derived form the light chain of the mouse monoclonal antibody 2B8 and the human kappa constant domain and 2) the heavy chain which is made of the variable domain of the heavy chain of the mouse monoclonal antibody 2B8 and the human IgG1 constant domain. The antibody sequence is delineated in the patent WO 94/11026.

A *de novo* approach has been followed in terms of synthesis of the coding regions of cDNA-construct because the hybridoma 2B8 that secretes the anti-CD20 mouse monoclonal antibody is not available in the public domain. De novo gene synthesis would also enable codon optimization with respect to the particular mammalian cell line to be used for protein expression.

The nucleotide sequence encoding the light chain of the anti-CD20 antibody has been represented in SEQ ID 1.

The Codon-optimized version of the nucleotide sequence encoding the light chain of the anti-CD20 antibody has been represented in SEQ ID 2.

The codons in the coding DNA sequence of the light chain of the anti-CD20 antibody that have been altered as part of the codon-optimization process to ensure optimal recombinant protein expression in mammalian cell lines such as CHO K1 and HEK 293. The Nucleotide sequence encoding the heavy chain of the anti-CD20 antibody has been represented in SEQ ID 3.

The Codon-optimized version of the nucleotide sequence encoding the heavy chain of the anti-CD20 antibody has been depicted in SEQ ID 4.

The codons in the coding DNA sequence of the heavy chain of the anti-CD20 antibody that have been altered as part of the codon-optimization process to ensure optimal recombinant protein expression in mammalian cell lines such as CHO K1 and HEK 293.

**Choice of the Expression Vector:**

The design of the mammalian expression vector for the expression of the anti-CD20 antibody can be based on one of the commercially available vectors (eg: *pcDNA* or *pIRES* from Invitrogen or BD Biosciences respectively), modified to include the following features:

(a) A multiple cloning site for insertion of cDNA encoding both the light and the heavy antibody chains of the anti-CD20 antibody in separate expression cassettes in the same vector.

(b) The design of the expression vector can also accommodate an independent (bi-cistronic) IRES-mediated co-expression of the green fluorescent protein which would allow rapid screening of highly expressing transfectants using fluorescence assisted cell sorting.

(c) Cloning of the chimeric light and heavy antibody chains in two separate mammalian expression systems having different selectable markers.

**Sub-cloning of the RTX antibody chains in mammalian expression vectors**

The *de novo* synthesized anti-CD20 antibody-heavy chain (RTX-HC) and anti-CD20 antibody-light chain (RTX-LC) were obtained as cDNA fragments cloned into the pBSKII vector and construct referred as pBSKII-RTX-LC and pBSKII-RTX-HC respectively. The DNA was transformed into DH10b *E. coli* cells and plated onto LB agar plates containing ampicillin. A colony from the plate was inoculated in liquid medium and a DNA mini prep was carried out. The sequence of the two antibody chains was confirmed by sequencing.

The full-length anti-CD20 antibody light and heavy chains were cloned sub-cloned into mammalian expression vectors pCAIN and pCAID respectively. The pBSKII/ RTX-LC clone and the pCAIN vector were digested with BglII and EcoRI. The resultant construct referred to as pCAIN/RTX-LC. The insert (700 bp) from the former and the vector backbone from the latter were gel purified and ligated (Fig. 5). The ligation mix was transformed into heat shock competent DH10b cells and plated onto LB agar plates containing ampicillin as the selection antibiotic. A few transformants were picked and a DNA mini prep was carried out. The clones were checked by restriction enzyme digestion (Fig. 6).

The pBSKII/ RTX-HC clone and the pCAID vector were digested with BamHI and EcoRI and resultant construct referred as pCAID/RTX-HC. The insert (1429 bp) from the former and the vector backbone from the latter were gel purified and ligated (Fig. 7). The ligation mix was transformed into heat shock competent DH10b cells and plated onto LB agar plates containing ampicillin as the selection antibiotic. A few transformants were picked and a DNA mini prep was carried out. The clones were checked by restriction enzyme digestion (Fig. 8).

**DNA sequencing and analysis:**

The final clones of RTX-HC and RTX-LC cloned into pCAID and pCAIN mammalian expression vectors respectively were sequenced and their sequence accuracy confirmed.

**Maintenance and propagation of the genes encoding the anti-CD20 antibody antibody chains:**

The cDNA construct encoding the chimeric light and heavy chain of the anti-CD20 antibody will be maintained and propagated in a standard bacterial cell line such as Top 10 (Invitrogen).

**Transient / stable recombinant protein expression in CHO-K1:**

(a) Transient / stable expression of the construct will be done using the Chinese hamster ovary cells (CHO) a mammalian cell line that is FDA approved for industrial applications. Transient expression is useful to check the expression of a construct and to rapidly obtain small quantities of a recombinant protein.
(b) Subsequently, CHO cells that display a stable and high expression of the desired monoclonal antibody will be developed using standard procedures.

**Purification of the anti-CD20 antibody:**

The mature chimeric antibody the anti-CD20 antibody is comprised of two antibody heavy chains (451 x 2 amino acids) and two antibody light chains (213 x 2 amino acids) and has an approximate molecular weight of 145 kDa. Both the chains have an N-terminal 20 amino acid leader sequence is cleaved off prior to the secretion of the hormone.

Subsequent to the establishment of reproducible bioactivity in accordance with the recommended functional / binding assays mentioned above, efforts will be made to optimize the purification procedures. The purification strategies will aim at process economics, speed to market, scalability, reproducibility, and maximum purity of the

product with functional stability and structural integrity as the major objectives. To this effect, a combinatorial approach with both filtration (normal and tangential flow filtration) and chromatography would be explored. The process qualification requirements and acceptance criteria studies will be conducted on 3 batches.

Accordingly, the current invention envisages the following steps in the purification process:

a. Initial clarification using COHC / A1HC / 0.45 μ depth filters
b. Concentration using Pellicon XL Biomax 50 kDa cut-off filter based on tangential flow filtration
c. Chromo step – I: Affinity chromatography using Prosep VA Ultra for serum based (2 % fetal calf serum [FCS]) / and Prosep VA for serum free culture supernatants.
d. Chromo step – II: Strong cation exchanger such as SP Sepharose
e. Chromo step – III: Flow through based strong anion exchanger such as Cellufine Q (a cellulose based medium) for the removal of host cell proteins and nucleic acids.
f. Virus removal using size exclusion filtration and leached protein A using Cellufine sulfate
g. Sterile filltration
h. Endotoxin removal using either Remtox / Cellufine ET chromatography
h. Formulation

**Establishment of the identity of the target protein using biochemical, immunological and physico-chemical methods:**

The percent recovery of the total protein at each stage will be quantitated using bicinchoninic acid procedure (BCA) / Bradford dye binding method. The target protein concentration at each stage of purification will be probed using highly specific and reliable enzyme based immunoassays such as direct or indirect sandwich ELISA

Qualitative and target specific western analysis will be followed at each stage. Reversed phase chromatography, isoelectric focusing and two-dimensional gel electrophoresis will be employed to evaluate the purified product. Secondary structural analysis would be examined using far UV circular dichroism. Molecular mass and oligomeric status will be investigated using size exclusion and MALDI-TOF. The investigations will also focus on the stability of the protein in relation to pH and temperature. As NESP is a hyperglycosylated protein, glycosylation patterns of the purified protein would be documented using gas chromatography (GC) analysis.

7. Assays for in vitro and in vivo activity of the anti-CD20 antibody:

Bioassays for detecting *in vitro* CD20 binding of the anti-CD20 antibody and the effector function of the antibody will be done using:

a) Human C1q binding and CD-20 positive SB cells in a flow cytometry assay using fluoroscin labelled C1q
b) Complement dependent cell lysis of CD20 positive SB cells
c) Antibody dependent cellular cytotoxicity effector assay using CD20 positive cells and CD20 negative cells

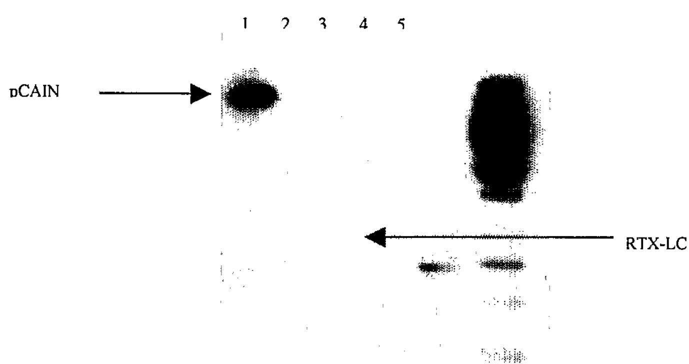
Pre-clinical *in vivo* bioactivity of the anti-CD20 antibody will be tested on non-human primates (cynomolgus monkeys) for:

a) efficacy of B cell depletion from peripheral blood lymph nodes and bone marrow.
b) evaluation of any toxicity associated with the chimeric antibody

We Claim:

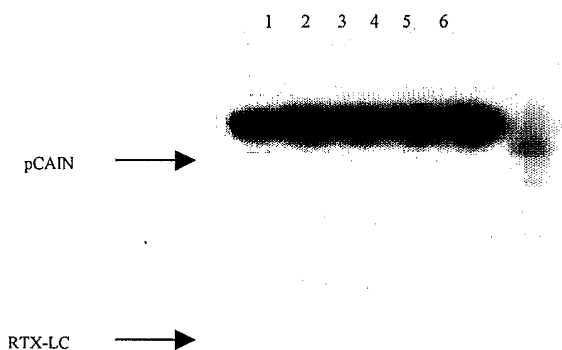
1. The process of preparing in vivo biologically active anti- CD 20 monoclonal antibody comprising the steps:
   - A step of *De novo* synthesis of the light and the heavy chains of the anti-CD20 monoclonal antibody
   - A step of construction of the full-length kappa light chain of the anti-CD 20 antibody
   - A step of construction of the full length IgG1 heavy chain of the anti-CD 20 antibody
   - A step of construction of vectors comprising the nucleic acid sequences encoding the light and the heavy polypeptide chains of the anti-CD 20 molecule.
   - A step of subcloning of the anti-CD 20 antibody chains in mammalian expression vectors for production of the biologically active antibody molecule.
2. A method according to claim 1, wherein the nucleotide sequence encoding the light chain of the anti-CD20 antibody has been represented in SEQ ID 1.
3. A method according to claim 1, wherein the nucleotide sequence encoding the heavy chain of the anti-CD 20 antibody has been represented in SEQ ID 2.
4. A method according to claim 1, wherein the vector comprising the nucleic acid fragment encoding the heavy chain of the anti-CD20 is subjected to site-directed mutagenesis.
5. A method according to claim 1, wherein the full-length anti-CD20 heavy and light chain have been subcloned into mammalian vectors
6. A method of preparation of an in vivo biologically active anti-CD20 monoclonal antibody comprising steps of transforming a host cell with a vector construct of FIG No. and isolating said product from said host cell or the medium of its growth.
7. A pharmaceutical composition comprising a therapeutically effective amount of anti-CD20 antibody and a pharmaceutically acceptable diluent, adjuvant or carrier, wherein said antibody is purified from mammalian cells grown in culture

FIG 1.



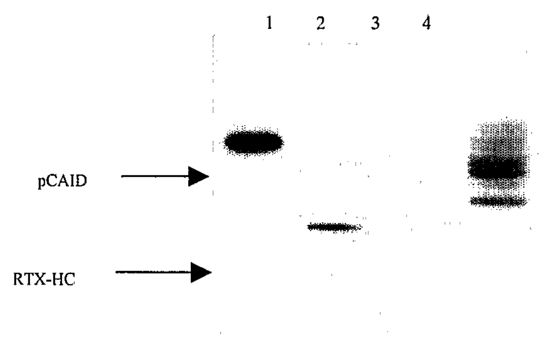

Restriction digested and gel purified RTX-LC and pCAIN for ligation reaction.
Lane 1 – pCAIN digested with BglII and EcoRI
Lane 2 – RTX-LC digested with BglII and EcoRI
Lane 5 –1 kb DNA ladder

FIG 2




Restriction digestion of the pCAIN/ RTX-LC clones with BglII and EcoRI for identification of the transformants containing the insert. For all the clones tested a fall-out fragment corresponding to the size of the ALZ-LC antibody fragment was observed (~700 bp).
Lane1-5 – pCAIN/ RTX-LC clones 1, 2, 3, 4, 5 digested with BglII and EcoRI
Lane 6- 1 kb DNA ladder
Lane 7 – uncut pCAIN/ ALZ-LC clone 1

FIG 3




Restriction digested and gel purified RTX-HC and pCAID for ligation reaction.
Lane 1 – pCAID digested with BamHI and EcoRI
Lane 3 – RTX-HC digested with BamHI and EcoRI
Lane 4 – 1 kb DNA ladder

FIG 4.

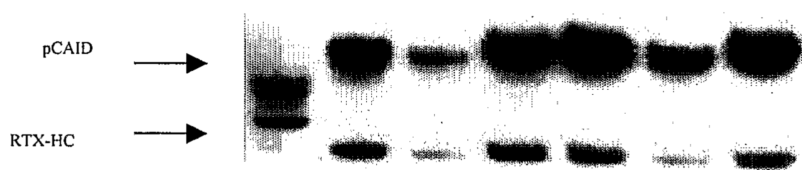



**Fig. 8: Restriction digestion of the pCAID/ RTX-HC clones with BamHI and EcoRI for identification of the transformants containing the insert. For all the clones tested a fall-out fragment corresponding to the size of the RTX-HC antibody fragment was observed (~1429 bp).**

Lanes 2- 8 - pCAID/ RTX-HC clones 1, 2, 3, 4, 5, and 6 digested with BamHI and EcoRI

Lane 8 – 1 kb DNA ladder

# FIG. 5

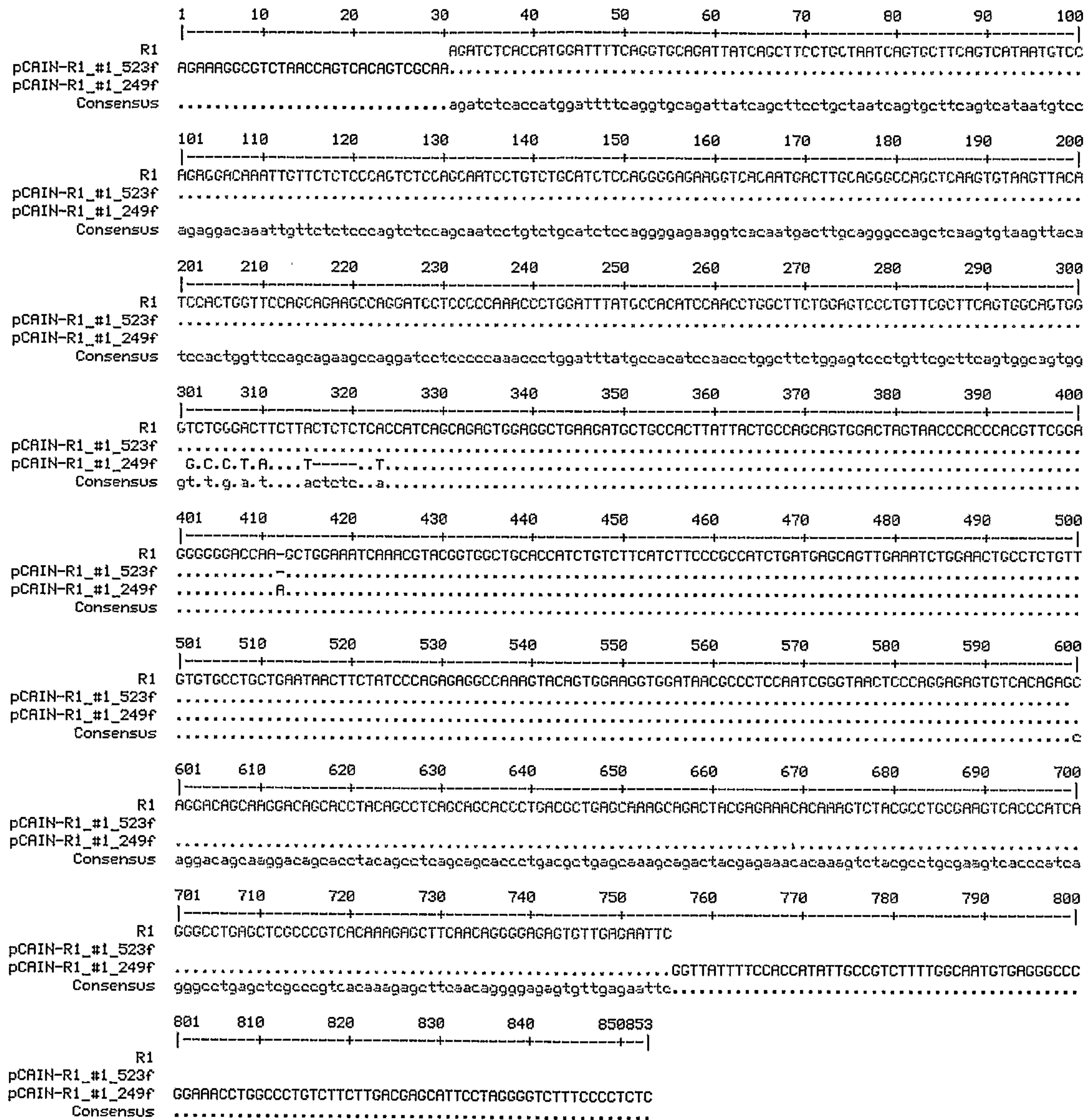


FIG. 6

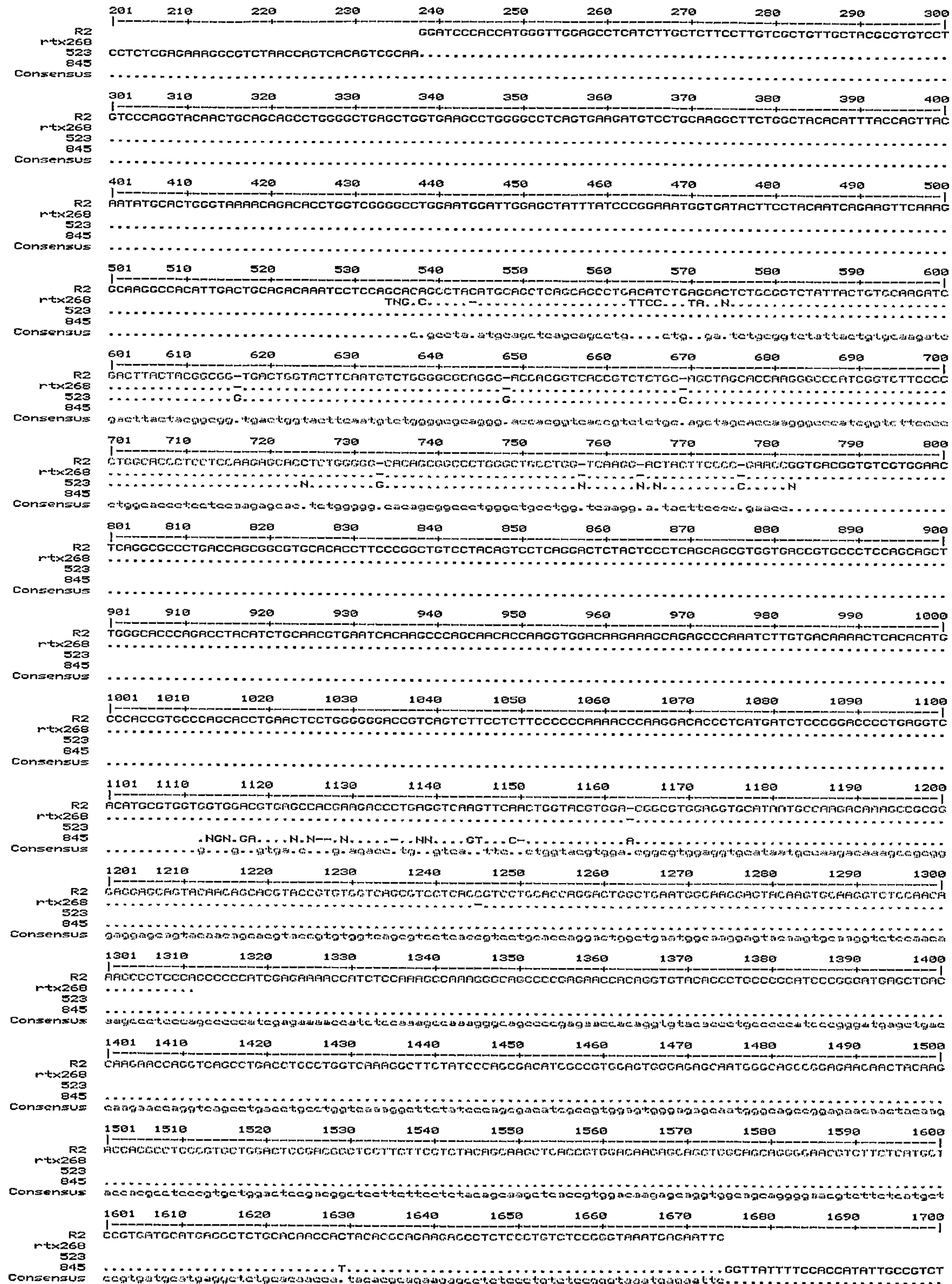


FIG 7

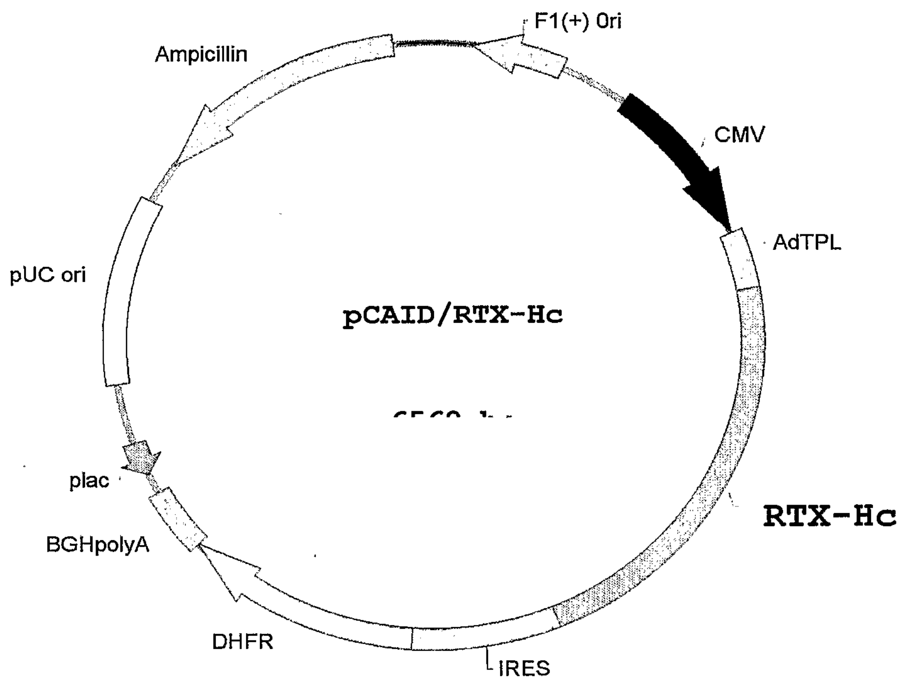
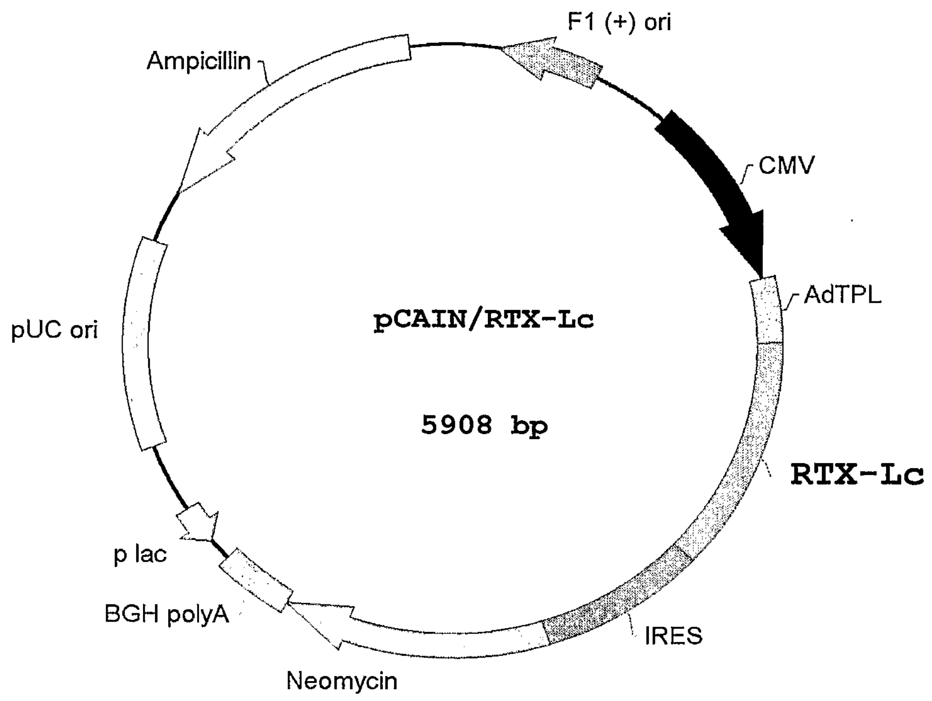


FIG 8



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Val Leu Ser Gln Val Gln Leu Gln Gln Leu Gly Ala Glu Leu Val Lys
            20                  25                  30

cct ggc gcc tcc gtg aag atg tcc tgc aaa gca tcc ggc tac acc ttc      144
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

acc agt tac aac atg cat tgg gtg aag caa act ccg ggc aga ggc ctg      192
Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu
    50                  55                  60

gaa tgg ata ggg gcg atc tac ccc ggg aac ggt gac acg agt tac aac      240
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn
65                  70                  75                  80

cag aag ttc aag ggc aag gcg acc ctc acc gcc gac aag tcg tcc tcg      288
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
                85                  90                  95

acc gct tac atg cag ctg tcc tcc ctg acg tcg gag gac tcg gcc gtg      336
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
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                                                        ritu
                 100                         105                         110

tac tac tgc gcc cgc tcg acc tac tac ggc ggc gac tgg tac ttc aac          384
Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn
        115                     120                     125

gtg tgg ggg gcc ggc acc acc gtg acc gtg agt gcc gcc tcc acc aag          432
Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys
    130                     135                     140

ggg ccg tcc gtg ttc cca ctc gca cca tca tcc aag tcc acc agt ggg          480
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145                     150                     155                     160

ggc act gcc gca tta ggg tgc ctg gtc aag gat tac ttt ccc gaa cct          528
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
                165                     170                     175

gtg acc gtc tct tgg aat tcc ggg gcc ctc acg tcc ggg gtc cat acc          576
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
            180                     185                     190

ttc cca gct gtg ttg cag tcg tcg ggg ctg tac tcc ctg tcc tcg gtt          624
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
        195                     200                     205

gtt acc gtc ccc tcg tca agt ctg ggc acc cag acc tac atc tgc aac          672
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
    210                     215                     220

gtc aat cac aag ccc tcg aac acc aag gtc gac aag aag gcc gaa ccc          720
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro
225                     230                     235                     240

aag tcg tgc gac aag acg cac aca tgt cct ccc tgc ccc gct ccc gaa          768
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
                245                     250                     255

ctg ctg ggc gga cct agt gtg ttc ctg ttc cct cca aag ccc aag gac          816
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
            260                     265                     270

acc ctc atg atc tca cgc acc cca gag gtg acc tgc gtc gtc gtg gat          864
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
        275                     280                     285

gtg tcg cac gag gat ccc gag gtt aag ttc aac tgg tac gtg gac ggc          912
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    290                     295                     300

gtg gag gtt cac aac gct aaa acc aag cca cgg gag gag cag tat aac          960
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305                     310                     315                     320

tcc acg tac cgc gtg gtg tca gtg ctc aca gtg ctt cac caa gac tgg         1008
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
                325                     330                     335

ctc aac ggg aag gag tac aag tgc aag gtg tcc aac aag gcc ctt ccc         1056
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
            340                     345                     350

gcc ccc atc gag aag act atc tcc aag gct aag gga cag ccg cga gag         1104
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                                        ritu
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        355                 360                 365

cct cag gtg tac acc ctg cct ccc tcg cgc gac gag tta aca aag aac      1152
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
    370                 375                 380

cag gta tcg ctc act tgc ctg gtc aaa ggt ttc tac cct tcc gac atc      1200
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385                 390                 395                 400

gcc gtc gag tgg gag agt aac ggg cag ccc gag aac aac tat aag act      1248
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
                405                 410                 415

aca ccc ccc gtg cta gat tcc gat ggg tcc ttc ttc ctg tac tcg aag      1296
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
            420                 425                 430

ctc aca gtg gat aag agt cgc tgg cag cag ggg aac gtc ttc agt tgc      1344
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
        435                 440                 445

tcc gtg atg cac gag gcc ctg cac aac cac tac acg cag aag tcc ctg      1392
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
    450                 455                 460

agc ctg tcg ccc ggc aag tga                                          1413
Ser Leu Ser Pro Gly Lys
465                 470
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