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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ^{6} **:**
C07K 14/50

A1

(11) International Publication Number: **WO 96/11952**

(43) International Publication Date: 25 April 1996 (25.04.96)

(21) International Application Number: PCT/US95/13099

(22) International Filing Date: 12 October 1995 (12.10.95)

(30) Priority Data:

08/323,339	13 October 1994 (13.10.94)	US
08/487,830	7 June 1995 (07.06.95)	US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/487,830 (CIP)
Filed on 7 June 1995 (07.06.95)

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(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR PURIFYING KERATINOCYTE GROWTH FACTORS

(57) Abstract

The present invention concerns the purification of keratinocyte growth factors.

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METHOD FOR PURIFYING KERATINOCYTE GROWTH FACTORS

Field of the Invention

The present invention relates to the field of protein purification. Specifically, the present invention relates to the field of purifying keratinocyte growth factors.

Background of the Invention

Polypeptide growth factors are important mediators of intercellular communication (Rubin *et al.* (1989), *Proc. Natl. Acad. Sci. USA,* 86:802-806). These molecules are generally released by one cell type and act to influence proliferation of other cell types.

One family of growth factors is the fibroblast growth factors (FGF). There are currently eight known FGF family members which share a relatedness among primary structures: basic fibroblast growth factor, bFGF (Abraham *et al.* (1986), *EMBO J.*, 5:2523-2528); acidic fibroblast growth factor, aFGF (Jaye *et al.* (1986), *Science*, 233:541-545); int-2 gene product, int-2 (Dickson & Peters (1987), *Nature*, 326:833); hst/kFGF (Delli-Bovi *et al.* (1987), *Cell*, 50:729-737, and Yoshida *et al.* (1987), *Proc. Natl. Acad. Sci. USA*, 84:7305-7309); FGF-5 (Zhan *et al.* (1988), *Mol. Cell. Biol.*, 8:3487-3495); FGF-6 (Marics *et al.* (1989), *Oncogene*, 4:335-340); keratinocyte growth factor (Finch *et al.* (1989), *Science*, 24:752-755; Rubin *et al.* (1989), *Proc. Natl. Acad. Sci. USA*, 86:802-806; Ron *et al.* (1993), *The Journal of Biological Chemistry*, 268(4):2984-2988; and Yan *et al.* (1991), *In Vitro Cell. Dev. Biol.*, 27A:437-438); and hisactophilin (Habazzettl *et al.* (1992), *Nature*, 359:855-858).

Among the FGF family of proteins, keratinocyte growth factor (KGF) is a unique effector of non-fibroblast epithelial (particularly keratinocyte) cell proliferation derived from mesenchymal tissues. The term "native KGF" refers to a natural human (hKGF) or recombinant (rKGF) polypeptide (with or without a signal sequence) as depicted by the amino acid sequence presented in SEQ ID NO:2 or an allelic variant thereof. [Unless otherwise indicated, amino acid numbering for molecules described herein shall correspond to that presented for the mature form of the native molecule (i.e., minus the signal sequence), as depicted by amino acids 32 to 194 of SEQ ID NO:2.]

Native KGF may be isolated from natural sources. For example, hKGF can be isolated from medium conditioned by an embryonic lung fibroblast cell line (Rubin *et al.*(1989), *supra*. Three chromatographic steps, namely heparin-Sepharose™ (Pharmacia, Piscataway, NJ) affinity chromatography, HPLC gel filtration, and reverse-phase HPLC, were used to obtain a purified hKGF preparation. Approximately 6 mg of hKGF were recovered from 10 liters of conditioned medium. These chromatographic steps only recovered 0.8% total hKGF based upon a mitogenic activity assay. A further example teaches the use of another chromatographic step using heparin-Sepharose™ affinity and Mono-S™ ion-exchange chromatographys (Pharmacia, Piscataway, NJ) for isolation of rKGF produced in bacteria (Ron *et al.* (1993), *Journal of Biological Chemistry*, 268:2984-2988).

The properties of keratinocyte growth factors suggest a potential for the application thereof as a drug for promoting specific stimulation of epithelial cell growth. It therefore would be desirable to develop a method or methods for obtaining relatively high levels of homogeneous keratinocyte growth factors to provide sufficient quantities of material for comprehensive *in* *vitro* and *in vivo* biological evaluation and for a potential therapeutic application.

It is the object of this invention to provide a novel method for the purification of keratinocyte growth factors.

Summary of the Invention

The present invention is directed to a first method for purifying a keratinocyte growth factor (KGF), the method comprising:

a) obtaining a solution containing KGF;
b) binding KGF from the solution of part (a) to a cation exchange resin;
c) eluting KGF in an eluate solution from the cation exchange resin;
d) passing the eluate solution from part (c) through a molecular weight exclusion matrix; and
e) recovering KGF from the molecular weight exclusion matrix.

The invention is further directed to a second method for purifying a keratinocyte growth factor (KGF), the method comprising:

a) obtaining a solution containing KGF;
b) binding KGF from the solution of part (a) to a cation exchange resin;
c) eluting KGF in an eluate solution from the cation exchange resin;
d) performing hydrophobic interaction chromatography on the eluate solution of part (c); and
e) recovering KGF from the hydrophobic interaction chromatography step of part (d).

Generally, the cation exchange chromatography step of the first or second methods may be conducted with any suitable buffer (e.g., phosphate buffer saline, sodium acetate or tris-HCL) at a pH of preferably between about 6.8-7.5. Suitable columns for use in this step include carboxymethyl cellulose, carboxymethyl agarose and sulfated agarose and cellulose columns (e.g.,columns of S-Sepharose Fast Flow™ resin, Mono-S™ resin and CM-cellulose™ resin, commercially available from Pharmacia, Piscataway, NJ). The flow rate will be variable depending upon the column size.

The gel filtration step of the first method may be conducted in any suitable buffer (e.g., phosphate buffer saline) at a pH of preferably between about 7.0 and 7.5. Suitable columns for use in this step include agarose-based, acrylamide-based, silica-based or polymer-based size-exclusion columns (e.g., columns of Sephadex G-75™ resin and Superdex-75™ resin, commercially available from Pharmacia).

In a particularly preferred embodiment of the second method, free sulfhydryl groups may be oxidized prior to the hydrophobic interaction step, discussed below. Any manner of oxidation may be employed. For example, the protein may be exposed to atmospheric oxygen for a suitable period of time. Alternatively, various oxidation procedures may be employed. One such procedure is particularly suited for keratinocyte growth factors wherein one or more cysteine residues, as compared to the native KGF molecule, are deleted or replaced. In this procedure an oxidizing agent (e.g., cystamine dihydrochloride or another appropriate oxidizing agent, for instance, cystine, oxidized glutathione or divalent copper) may be added to a final concentration, adjusting the pH to preferably between about 7-9.5, with pH 9.0 ± 0.3°C being more preferred when using cystamine dihydrochloride), and holding the temperature at preferably between about 10-30°C, for an appropriate period. The second procedure may be used for oxidizing native KGF and other keratinocyte growth factors with comparable patterns of cysteine residues. In this procedure, oxidation may be accomplished by adding an appropriate amount of an ionic strength modifier (e.g., $(NH_4)_2SO_4)$), adjusting the pH to preferably between about 7.5-9.5, and holding the temperature at preferably between about 23 ± 5°C for an appropriate period.

The hydrophobic interaction step of the second method may be conducted by using any suitable buffer (e.g., sodium phosphate) at a pH of preferably between about 6.0-8.0, more preferably about 7.0, and by eluting with a decreasing linear $(NH_4)_2SO_4$ gradient ranging from 2-0 M. Suitable columns for use in this step include alkyl or phenyl substituted resins (e.g., a column of Butyl-650M Toyopearl™ resin, commercially available from Tosohaas, Inc., Montgomeryville, PA and columns of phenyl Sepharose™ resin and phenyl Superose™ resin, commercially available from Pharmacia).

The process of the present invention may be used to purify KGF. Thus, it should be understood that the terms "keratinocyte growth factor" and "KGF" as employed in this description are intended to include, and to mean interchangeably unless otherwise indicated, native KGF and KGF analog proteins (or "muteins") characterized by a peptide sequence substantially the same as the peptide sequence of native KGF and by retaining some or all of the biological activity of native KGF, particularly non-fibroblast epithelial cell proliferation (e.g., exhibiting at least about 500-fold greater stimulation of BALB/MK keratinocyte cells than that of NIH/3T3 fibroblast cells, and at least about 50-fold greater stimulation of BALB/MK keratinocyte cells than for BS/589 epithelial cells or for CC1208 epithelial cells, as determined by H-thymidine incorporation). By "characterized by a peptide sequence substantially the same as the peptide sequence of native KGF" is meant a peptide sequence which is encoded by a DNA sequence capable of hybridizing to nucleotides 201 to 684 of SEQ ID NO:1, preferably under stringent hybridization conditions.

The determination of a corresponding amino acid position between two amino acid sequences may be determined by aligning the two sequences to maximize matches of residues including shifting the amino and/or carboxyl terminus, introducing gaps as required and/or deleting residues present as inserts in the candidate. Database searches, sequence analysis and manipulations may be performed using one of the well-known and routinely used sequence homology/identity scanning algorithm programs (e.g., Pearson and Lipman (1988), *Proc. Natl. Acad. Sci. U.S.A.*, 85:2444-2448; Altschul *et al.* (1990), *J. Mol. Biol.*, 215:403-410; Lipman and Pearson (1985), *Science*, 222:1435 or Devereux *et al.* (1984), *Nuc. Acids Res.*, 12:387-395).

Stringent conditions, in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents and other parameters typically controlled in hybridization reactions. Exemplary stringent hybridization conditions are hybridization in 4 X SSC at 62-67° C., followed by washing in 0.1 X SSC at 62-67° C. for approximately an hour. Alternatively, exemplary stringent hybridization conditions are hybridization in 45-55% formamide, 4 X SSC at 40-45°C. [*See*, T. Maniatis et. al., *Molecular Cloning* (A Laboratory Manual); Cold Spring Harbor Laboratory (1982), pages 387 to 389].

Thus, the proteins include allelic variations, or deletion(s), substitution(s) or insertion(s) of amino acids, including fragments, chimeric or hybrid molecules of native KGF. One example of KGF includes proteins having residues corresponding to Cys^1 and Cys^{15} of SEQ ID NO:2 replaced or deleted, with the resultant molecule having improved stability as compared with the parent molecule (as taught in commonly owned U.S.S.N. 08/487,825, filed on July 7, 1995). Another example of KGF includes charge-change polypeptides wherein one or more of amino acid residues 41-154 of native KGF (preferably residues Arg^{41}, Gln^{43}, Lys^{55}, Lys^{95}, Lys^{128}, Asn^{137}, Gln^{138}, Lys^{139}, Arg^{144}, Lys^{147}, Gln^{152}, Lys^{153} or Thr^{154}) are deleted or substituted with a neutral residue or negatively charged residue selected to effect a protein with a reduced positive charge (as taught in commonly owned U.S.S.N. 08/323,337, filed on October 13, 1994). A still further example of KGF includes proteins generated by substituting at least one amino acid having a higher loop-forming potential for at least one amino acid within a loop-forming region of Asn^{115}-His^{116}-Tyr^{117}-Asn^{118}-Thr^{119} of native KGF (as taught in commonly owned U.S.S.N. 08/323,473, filed on October 13, 1994). A still yet further example includes proteins having one or more amino acid substitutions, deletions or additions within a region of 123-133 (amino acids 154-164 of SEQ ID NO:2) of native KGF; these proteins may have agonistic or antagonistic activity.

Specifically disclosed proteins include the following KGF molecules (referred to by the residue found at that position in the mature protein (minus signal sequence) set forth in SEQ ID NO:2, followed by that amino acid position in parentheses and then either the substituted residue or "-" to designate a deletion): C(1,15)S, ΔN15-ΔN24, ΔN3/C(15)S, ΔN3/C(15)-, ΔN8/C(15)S, ΔN8/C(15)-, C(1,15)S/R(144)E, C(1,15)S/R(144)Q, ΔN23/R(144)Q, C(1,15,40)S, C(1,15,102)S, C(1,15,102,106)S, ΔN23/N(137)E, ΔN23/K(139)E, ΔN23/K(139)Q, ΔN23/R(144)A, ΔN23/R(144)E, ΔN23/R(144)L,

ΔN23/K(147)E, ΔN23/K(147)Q, ΔN23/K(153)E, ΔN23/K(153)Q, ΔN23/Q(152)E/K(153)E; R(144)Q and H(116)G.

As those skilled in the art will also appreciate, a variety of host-vector systems may be utilized to express the KGF protein-coding sequence. These include but are not limited to eucaryotic cell systems such as mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast-containing yeast vectors; or to procaryotic cell systems such as bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA. The expression elements of these vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

Once the protein product of KGF expression has been isolated, purified and assayed for KGF activity (using procedures known to those skilled in the art), it may be formulated in a variety of pharmaceutical compositions. Typically, such compositions include a suitable, usually chemically-defined, carrier or excipient for the therapeutic agent and, depending on the intended form of administration, other ingredients as well. The composition can include aqueous carriers or consist of solid phase formulations in which KGF is incorporated into non-aqueous carriers such as collagens, hyaluronic acid, and various polymers. The composition can be suitably formulated to be administered in a variety of ways, including by injection, orally, topically, intranasally and by pulmonary delivery.

Brief Description of the Drawings

Figure 1 shows the nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of native KGF (the nucleotides encoding the mature form of native KGF is depicted by bases 201 to 684 of SEQ ID NO:1 and the mature form of KGF is depicted by amino acid residues 32 to 194 of SEQ ID NO:2).

Figures 2A, 2B and 2C show the plasmid maps of pCFM1156, pCFM1656 and pCFM3102, respectively.

Figure 3 shows the nucleotide (SEQ ID NO:3) and amino acid (SEQ ID NO:4) sequences of the construct RSH-KGF.

Figure 4 shows the nucleotide (SEQ ID NO:5) and amino acid (SEQ ID NO:6) sequences of the construct contained in plasmid KGF.

Figure 5 shows the chemically synthesized OLIGOs (OLIGO#6 through OLIGO#11; SEQ ID NO:12-17, respectively) used to substitute the DNA sequence between a *KpnI* site and an *EcoRI* site for a *KpnI* site (from amino acid positions 46 to 85 of SEQ ID No:6) in the construct contained plasmid KGF to produce the construct in plasmid KGF(dsd).

Figure 6 shows the chemically synthesized OLIGOs (OLIGO#12 through OLIGO#24; SEQ ID NO:18-30, respectively) used to construct KGF(codon optimized).

Figure 7 shows the nucleotide (SEQ ID NO:31) and amino acid sequences (SEQ ID NO:32) of C(1,15)S, a KGF analog having substitutions of serine for cysteine at amino acid positions 1 and 15 of native KGF.

Figure 8 shows the nucleotide (SEQ ID NO:33) and amino acid sequences (SEQ ID NO:34) of C(1,15)S/R(144)E, a KGF analog having substitutions of serine for cysteine at amino acid positions 1 and 15 and a substitution of glutamic acid for arginine at amino acid position 144 of native KGF.

Figure 9 shows the nucleotide (SEQ ID NO:35) and amino acid (SEQ ID NO:36) sequences of C(1,15)S/R(144)Q, a KGF analog having substitutions of serine for cysteine at amino acid positions 1 and 15 and a substitution of glutamine for arginine at amino acid position 144 of native KGF.

Figure 10 shows the nucleotide (SEQ ID NO:37) and amino acid (SEQ ID NO:38) sequences of ΔN15, a KGF analog having a deletion of the first 15 amino acids of the N-terminus of native KGF.

Figure 11 shows the nucleotide (SEQ ID NO:39) and amino acid (SEQ ID NO:40) sequences of ΔN23, a KGF analog having a deletion of the first 23 amino acids of the N-terminus of native KGF.

Figure 12 shows the nucleotide (SEQ ID NO:41) and amino acid (SEQ ID NO:42) sequences of ΔN23/R(144)Q, a KGF analog having a deletion of the first 23 amino acids of the N-terminus and a substitution of glutamine for arginine at amino acid position 144 of native KGF.

Description of Specific Embodiments

Standard methods for many of the procedures described in the following examples, or suitable alternative procedures, are provided in widely recognized manuals of molecular biology such as, for example, *Molecular Cloning*, Second Edition, Sambrook *et al.*, Cold Spring Harbor Laboratory Press (1987) and *Current Protocols in Molecular Biology*, Ausabel *et al.*, Greene Publishing Associates/Wiley-Interscience, New York (1990).

Example 1: Preparation of DNA Coding for KGF and KGF Analogs

The cloning of the full-length human KGF gene (encoding a polypeptide with the sequence of native KGF) was carried out both by polymerase chain reaction (PCR) of RNA from an animal cell and by PCR of chemically synthesized (*E. coli* optimized codon) oligonucleotides ("OLIGOs"). Both procedures are described below:

PCR amplification using RNA isolated from cells known to produce the polypeptide was performed. Initially, cells from a human fibroblast cell line AG1523A (obtained from Human Genetic Mutant Cell Culture Repository Institute For Medical Research, Camden, New Jersey) were disrupted with guanidium thiocyanate, followed by extraction (according to the method of Chomyzinski *et al.* (1987), *Anal. Biochem.*, 172:156). Using a standard reverse transcriptase protocol for total RNA, the KGF cDNA was generated. PCR (PCR#1) amplification of the KGF gene was carried out using the KGF cDNA as template and primers OLIGO#1 and OLIGO#2 that encode DNA sequences immediately 5' and 3' of the KGF gene [model 9600 Thermocycler (Perkin-Elmer Cetus, Norwalk, CT); 28 cycles; each cycle consisting of one minute at 94°C for denaturation, two minutes at 60°C for annealing, and three minutes at 72°C for elongation]. A small aliquot of the PCR#1 product was then used as template for a second KGF PCR (PCR#2) amplification identical to the cycle conditions described above except for a 50°C annealing temperature. For expression cloning of the KGF gene, nested PCR primers were used to create convenient restriction sites at both ends of the KGF gene. OLIGO#3 and OLIGO#4 were used to modify the KGF DNA product from PCR#2 to include *MluI* and *BamHI* restriction sites at the 5' and 3' ends of the gene, respectively [PCR#3; 30 cycles; each cycle consisting of one minute at 94°C for denaturation, two minutes at 60°C for annealing, and three minutes at 72°C for elongation]. This DNA was subsequently cut with *MluI* and *BamHI*, phenol extracted and ethanol precipitated. It was then resuspended and ligated (using T4 ligase) into a pCFM1156 plasmid (Figure 2A) that contained a "RSH" signal sequence to make construct RSH-KGF (Figure 3). The ligation products were transformed (according to the method of Hanahan (1983), *J. Mol. Biol.*, 166:557) into *E. coli* strain FM5 (ATCC: 53911) and plated onto LB+kanamycin at 28°C. Several transformants were selected and grown in small liquid cultures containing 20 µg/mL kanamycin. The RSH-KGF plasmid was isolated from the cells of each culture and DNA sequenced. Because of an internal *NdeI* site in the KGF gene, it was not possible to directly clone the native gene sequence into the desired expression vector with the bracketed restriction sites of *NdeI* and *BamHI*. This was accomplished as a three-way ligation. Plasmid RSH-KGF was cut with the unique restriction sites of *BsmI* and *SstI*, and a ~3 kbp DNA fragment (containing the 3' end of the KGF gene) was isolated following electrophoresis through a 1% agarose gel. A PCR (PCR#4) was carried out as described for PCR#3 except for the substitution of OLIGO#5 for OLIGO#3. The PCR DNA product was then cut with *NdeI* and *BsmI* and a 311 bp DNA fragment was isolated following electrophoresis through a 4% agarose gel. The third fragment used in the ligation was a 1.8 kbp DNA fragment of pCFM1156 cut with *NdeI* and *SstI* isolated following electrophoresis through a 1% agarose gel. Following ligation (T4 ligase), transformation, kanamycin selection and DNA sequencing as described above, a clone was picked containing the construct in Figure 4, and the plasmid designated KGF. Because of an internal ribosomal binding site that produced truncated products, the KGF DNA sequence between the unique *KpnI* and *EcoRI* sites was replaced with chemically synthesized OLIGOs (OLIGO#6 through OLIGO#11) to minimize the use of the internal start site (Figure 5).

OLIGO#1 (SEQ ID NO:7): 5'-CAATGACCTAGGAGTAACAATCAAC-3'

OLIGO#2 (SEQ ID NO:8): 5'-AAAACAAACATAAATGCACAAGTCCA-3'

OLIGO#3 (SEQ ID NO:9): 5'-ACAACGCGTGCAATGACATGACTCCA-3'

OLIGO#4 (SEQ ID NO:10):

5'-ACAGGATCCTATTAAGTTATTGCCATAGGAA-3'

OLIGO#5 (SEQ ID NO:11):

5'-ACACATATGTGCAATGACATGACTCCA-3'

OLIGO#6 (SEQ ID NO:12):

5'-CTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCC-3'

OLIGO#7 (SEQ ID NO:13):

5'-AAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTT-3'

OLIGO#8 (SEQ ID NO:14):

5'-GCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTG-3'

OLIGO#9 (SEQ ID NO:15):

5'-TCTTGGGTGCCCTTGACTTTGCCGCGTTTGTCGATACGCAGGTAC-3'

OLIGO#10 (SEQ ID NO:16):

5'-ACAGCAACAGTACGGATTTCCATAATATTGTAGTTGTTTTTCATC-3'

OLIGO#11 (SEQ ID NO:17):

5'-AATTCAGATTCAACACCTTTGATTGCAACGATACCA-3'

The OLIGOs were phosphorylated with T4 polynucleotide kinase and then heat denatured. The single-stranded (ss) OLIGOs were then allowed to form a ds DNA fragment by allowing the temperature to slowly decrease to room temperature. T4 ligase was then used to covalently link both the internal OLIGO sticky-ends and the whole ds OLIGO fragment to the KGF plasmid cut with *KpnI* and *EcoRI*. The new plasmid was designated KGF(dsd).

A completely *E. coli* codon-optimized KGF gene was constructed by PCR amplification of chemically synthesized OLIGOs #12 through 24.

OLIGO#12 (SEQ ID NO:18): 5'-AGTTTTGATCTAGAAGGAGG-3'

OLIGO#13 (SEQ ID NO:19): 5'-TCAAAACTGGATCCTATTAA-3'

OLIGO#14 (SEQ ID NO:20):
5'-AGTTTTGATCTAGAAGGAGGAATAACATATGTGCAACGACATGAC-
TCCGGAACAGATGGCTACCAACGTTAACTGCTCCAGCCCGGAACGT-3'

OLIGO#15 (SEQ ID NO:21):
5'-CACACCCGTAGCTACGACTACATGGAAGGTGGTGACATCCGTGTTC-
GTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAA-3'

OLIGO#16 (SEQ ID NO:22):
5'-CGTGGTAAAGTTAAAGGTACCCAGGAAATGAAAAACAACTA-
CAACATCATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAA-3'

OLIGO#17 (SEQ ID NO:23):
5'-GGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAAC-
TGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAA-3'

OLIGO#18 (SEQ ID NO:24):
5'-CTGATCCTGGAAAACCACTACAACACCTACGCATCTGCTAAATGGA-
CCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGT-3'

OLIGO#19 (SEQ ID NO:25):
5'-ATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCT-
CACTTCCTGCCGATGGCAATCACTTAATAGGATCCAGTTTTGA-3'

OLIGO#20 (SEQ ID NO:26):5'-TACGGGTGTGACGTTCCGGG-3'

OLIGO#21 (SEQ ID NO:27):5'-CTTTACCACGTTTGTCGATA-3'

OLIGO#22 (SEQ ID NO:28):5'-ATTCAACACCTTTGATTGCA-3'

OLIGO#23 (SEQ ID NO:29):5'-CCAGGATCAGTTCTTTGAAG-3'

OLIGO#24 (SEQ ID NO:30):5'-GAACCGGGATACCTTTCTGG-3'

OLIGOs #12 through 24 were designed so that the entire DNA sequence encoding native KGF was represented by OLIGOs from either the "Watson" or the "Crick" strand and upon PCR amplification would produce the desired double-stranded DNA sequence (Figure 6) [PCR#5, Model 9600 thermocycler (Perkin-Elmer Cetus); 21 cycles, each cycle consisting of 31 seconds at 94°C for denaturation, 31 seconds at 50°C for annealing, and 31 seconds at 73°C for elongation; following the 21 cycles the PCR was finished with a final elongation step of 7 minutes]. After PCR amplification, the DNA fragment was cut with *XbaI* and *BamHI* and the 521 bp fragment ligated into the expression plasmid pCFM1156 cut with the same enzymes. PCR#5 utilized the outside primers (100 pmoles/100 µl rxn) OLIGO#12 and OLIGO#13 and 1 µl/100 µl rxn of a KGF template derived by ligation (by T4 ligase) of OLIGOs #14 through #19 (OLIGOs#15 through OLIGOs#18 were phosphorylated with T4 polynucleotide kinase) using OLIGOs#20 through OLIGOs#24 as band-aid oligos (Jayaraman *et al.* (1992), *Biotechniques*, 12:392) for the ligation. The final construct was designated KGF(codon optimized).

All of the KGF analogs described herein are composed in part from DNA sequences found in KGF(dsd) or KGF(codon optimized), or a combination of the two. The sequences are further modified by the insertion into convenient restrictions sites of DNA sequences that encode the particular KGF analog amino acids made utilizing one or more of the above-described techniques for DNA fragment synthesis. Any of the analogs can be generated in their entirety by either of the above described techniques. However, as a part of the general OLIGO design optimized *E. coli* codons were used where appropriate, although the presence of *E. coli* optimized codons in part or *in toto* of any of the genes where examined did not significantly increase the yield of protein that could be obtained from cultured bacterial cells. Figures 7 to 12 set forth by convenient example particular KGF analog nucleotide and amino acid sequence constructions: C(1,15)S (Figure 7); C(1,15)S/R(144)E (Figure 8); C(1,15)S/R(144)Q (Figure 9); ΔN15 (Figure 10); ΔN23 (Figure 11) and ΔN23/R(144)Q (Figure 12). All the KGF analog constructions described herein were DNA sequence confirmed.

Example 2: Purification from *E. coli*

Three different expression plasmids were utilized in the cloning of the KGF analog genes. They were pCFM1156 (ATCC 69702), pCFM1656 (ATCC 69576), and pCFM3102 (Figures 2A, 2B and 2C, respectively). The plasmid p3102 can be derived from the plasmid pCFM1656 by making a series of site directed base changes with PCR overlapping oligo mutagenesis. Starting with the *BglII* site (pCFM1656 plasmid bp #180) immediately 5' to the plasmid replication promoter, P_{copB}, and proceeding toward the plasmid replication genes, the base pair changes are as follows:

pCFM1656 bp #	bp in pCFM1656	bp changed to in pCFM3102
# 204	T/A	C/G
# 428	A/T	G/C
# 509	G/C	A/T
# 617	- -	insert two G/C bp
# 677	G/C	T/A
# 978	T/A	C/G
# 992	G/C	A/T
# 1002	A/T	C/G
# 1005	C/G	T/A
# 1026	A/T	T/A
# 1045	C/G	T/A
# 1176	G/C	T/A
# 1464	G/C	T/A
# 2026	G/C	bp deletion
# 2186	C/G	T/A
# 2479	A/T	T/A
# 2498-2501	AGTG TCAC	GTCA CAGT
# 2641-2647	TCCGAGC AGGCTCG	bp deletion
# 3441	G/C	A/T
# 3452	G/C	A/T
# 3649	A/T	T/A
# 4556	--	insert bps (SEQ ID NO:43) 5'-GAGCTCACTAGTGTCGACCTGCAG-3' (SEQ ID NO:44) 5'-CTCGAGTGATCACAGCTGGACGTC-3'

As seen above, pCFM1156, pCFM1656 and pCFM3102 are very similar to each other and contain many of the same restriction sites. The plasmids were chosen by convenience, and the vector DNA components can be easily exchanged for purposes of new constructs. The host used for all cloning was *E. coli* strain FM5 (ATCC: 53911) and the transformations were carried out (according to the method of Hanahan (1983), *supra*) or by electroelution with a Gene Pulser™ transfection apparatus (BioRad Laboraties, Inc., Hercules, CA), according to the manufacturer's protocol.

Initially, a small, freshly-cultured inoculum of the desired recombinant *E. coli* clone harboring the desired construct on one of the three pCFM vectors was started by transferring 0.1 mL of a frozen glycerol stock of the appropriate strain into a 2 L flask containing 500 mL of Luria broth. The culture was shaken at 30°C for 16 hours. Thereafter the culture was transferred to a 15 L fermentor containing 8 L of sterile batch medium (Tsai, *et al.* (1987), *J. Industrial Microbiol.*, 2:181-187).

Feed batch fermentation starts with the feeding of Feed # 1 medium (Tsai, *et al.* (1987.), *supra*). When the OD600 reached 35, expression of the desired KGF analog was induced by rapidly raising the culture temperature to 37°C to allow the amplification of plasmid. After two hours at 37°C, the culture temperature was quickly raised to 42°C to denature the CI repressor and the addition of Feed 1 was discontinued in favor of Feed 2, the addition rate of which was initiated at 300 mL/hr. Feed 2 comprised 175 g/L trypticase-peptone, 87.5 g/L yeast extract, and 260 g/L glucose. After one hour at 42°C, the culture temperature was decreased to 36°C, where this temperature was then maintained for another 6 hours.

The fermentation was then halted and the cells were harvested by centrifugation into plastic bags placed within 1 L centrifuge bottles. The cells were pelleted by centrifugation at 400 rpm for 60 minutes, after which the supernatants were removed and the cell paste frozen at -90°C.

Following expression of the various KGF analogs in *E. coli*, native KGF, C(1,15)S, C(1,15)S/R(144)E, C(1,15)S/R(144)Q, ΔN15, ΔN23, and ΔN23/R(144)Q protein were purified using the following procedure. Cell paste from a high cell density fermentation was suspended at 4°C in 0.2 M NaCl, 20 mM $NaPO_4$, pH 7.5 as a 10-20% solution (weight per volume) using a suitable high shear mixer. The suspended cells were then lysed by passing the solution through a homogenizer (APV Gaulin, Inc., Everett, MA) three times. The outflowing homogenate was cooled to 4-8°C by using a suitable heat exchanger. Debris was then removed by centrifuging the lysate in a J-6B™ centrifuge (Beckman Instruments, Inc., Brea, CA) equipped with a JS 4.2 rotor at 4,200 rpm for 30-60 min. at 4°C. Supernatants were then carefully decanted and loaded onto a previously prepared 450 mL (5 cm x 23 cm) column of S-Sepharose Fast Flow™ resin (Pharmacia) column equilibrated with 0.2 M NaCl, 20 mM $NaPO_4$, pH 7.5 at 4°C. Next, the column was washed with five column volumes (2250 mL) of 0.4 M NaCl, 20 mM $NaPO_4$, pH 7.5 at 4°C. The desired protein was eluted by washing the column with 5 L of 0.5 M NaCl, 20 mM $NaPO_4$, pH 7.5. Again, 50 mL fractions were collected and the A_{280} of the effluent was continuously monitored. Fractions identified by A_{280} as containing eluted material were then analyzed by SDS-PAGE through 14% gels to confirm the presence of the desired polypeptide.

Those fractions containing proteins of interest were then pooled, followed by the addition of an equal volume of distilled water. The diluted sample was then loaded onto a previously prepared 450 mL (5 cm x 23 cm) column of S-Sepharose Fast Flow equilibrated with 0.4 M NaCl, 20 mM $NaPO_4$, pH 6.8 at 4°C. The column was washed with 2250 mL of 0.4 M NaCl, 20 mM $NaPO_4$, pH 6.8 and the protein eluted using a 20 column volume linear gradient ranging from 0.4 M NaCl, 20 mM $NaPO_4$, pH 6.8 to 0.6 M NaCl, 20 mM $NaPO_4$, pH 6.8. Again, 50 mL fractions were collected under constant A_{280} monitoring of the effluent. Those fractions containing the protein (determined by 14% SDS-PAGE) were then pooled, followed by concentration through a YM-10 membrane (10,000 molecular weight cutoff) in a 350cc stirring cell (Amicon, Inc. Mayberry, MA) to a volume of 30-40 mL.

The concentrate was then loaded onto a previously generated 1,300 mL (4.4 cm x 85 cm) column of Superdex-75™ resin (Pharmacia) equilibrated in column buffer comprising 1X PBS (Dulbecco's Phosphate Buffered Saline, "D-PBS," calcium and magnesium-free) or 0.15 M NaCl, 20 mM $NaPO_4$, pH 7.0. After allowing the sample to run into the column, the protein was eluted from the gel filtration matrix using column buffer. Thereafter, 10 mL fractions were recovered and those containing the analog (determined by 14% SDS-PAGE) were pooled. Typically, the protein concentration was about 5-10 mg/mL in the resultant pool. All of the above procedures were performed at 4-8°C, unless otherwise specified.

An alternative purification procedure was used to purify native KGF, C(1,15)S and ΔN23. The procedure involves the following steps and, unless otherwise specified, all procedures, solutions and materials were conducted at 23 ± 5°C.

Upon completion of the production phase of a bacterial fermentation, the cell culture was cooled to 4-8°C and the cells were harvested by centrifugation or a similar process. On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, was suspended in a mild buffer solution, 20 mM $NaPO_4$, 0.2 M NaCl, pH 7.5, weighing about five times that of the cell paste to be suspended. The cells were dispersed to a homogeneous solution using a high shear mixer. The temperature of the cell paste dispersion was maintained at 4-8°C during homogenization.

The cells were then lysed by pressure, for example by passing the cell paste dispersion twice through an appropriately sized cell homogenizer. The homogenate was kept chilled at 5 ± 3°C. To clarify the cell lysate, a previously prepared depth filter housing (Cuno, Inc., Meriden, CT) equipped with a filter having an appropriate amount of filter surface area, equilibrated with a suitable volume of 0.2 M NaCl, 20 mM $NaPO_4$, pH 7.5 was employed. The equilibration and clarification were performed at 5 ± 3°C. Prior to clarification, an appropriate amount of a suitable filter aid was used to pre-coat the filter and be thoroughly mixed with the cell lysate, after which the lysate was clarified by passing the solution through the filter apparatus. The filter was washed with 0.2 M NaCl, 20 mM $NaPO_4$, pH 7.5. The filtrate and any subsequent wash were collected in a chilled container of suitable capacity, all the while being maintained at less than 10°C.

Following clarification the lysate was then passed through a previously prepared column of SP-Sepharose Fast Flow containing at least 1 mL of resin per 2 g of cell paste. The column of SP-Sepharose Fast Flow was equilibrated with cold (5 ± 3°C), 0.2 M NaCl, 20 mM $NaPO_4$, pH 7.5. The temperature of the column was maintained at less than 10°C. The clarified lysate (5 ± 3°C) was then loaded onto the ion exchange column, with the absorbance at 280 nm (A_{280}) of eluate being continuously monitored. After sample loading, the column was washed with cold 0.2 M NaCl, 20 mM $NaPO_4$, pH 7.5, followed by washing with 0.3 M NaCl, 20 mM $NaPO_4$, pH 7.5 at 23 ± 5°C.

To elute the desired protein, a linear gradient ranging from 0.2-1 M NaCl, 20 mM $NaPO_4$, pH 7.5 was used. Bulk product was collected in several fractions on the basis of the A_{280} of the eluate. Following elution, these fractions were pooled and the volume noted.

To oxidize free sulfhydryl groups, an oxidation step was performed. For proteins with altered cysteine patterns, as compared to native KGF, an oxidizing agent (e.g., cystamine dihydrochloride or another appropriate oxidizing agent, for instance, cystine, oxidized glutathione or divalent copper) was added to a final concentration of 1-20 mM and the pH was adjusted to 7-9.5, with a pH of 9.0 ± 0.3 when cystamine dihydrochloride was used. The oxidation was conducted at 10 - 30°C for an appropriate period. For the native KGF protein, oxidation was accomplished by adding an appropriate amount of $(NH_4)_2SO_4$ such as 1-2 M $(NH_4)_2SO_4$, adjusting the pH to 7.5-9.5, and holding the temperature at 23 ± 5°C for an appropriate period.

After oxidation, the pH of the solution was adjusted to between 6.5 and 9.5. If necessary, solid $(NH_4)_2SO_4$ was added to the solution to a final concentration of 2 M. To remove particulates, the solution was passed through appropriate clarification filters.

The filtered, oxidized product was then subjected to hydrophobic interaction chromatography (HIC). The HIC matrice was Butyl-650M Toyopearl™ resin (Tosohaas, Inc., Montgomeryville, PA). The protein-containing solution was loaded onto the column, which had been previously equilibrated with 2 M $(NH_4)_2SO_4$, 0.15 M NaCl, 20 mM $NaPO_4$, pH 7.0. After sample loading, the column was washed with 2 M $(NH_4)_2SO_4$, 0.15 M NaCl, 20 mM $NaPO_4$, pH 7.0. The desired protein was then eluted using a decreasing linear $(NH_4)_2SO_4$ gradient ranging from 2-0 M developed in 0.15 M NaCl, 20 mM NaPO4, pH 7.0. When the desired protein began to elute, as indicated by an increase in the A_{280} of the eluate, fractions were collected. Aliquots of each fraction were then analyzed by SDS-PAGE. Those fractions containing the desired protein were then pooled, thoroughly mixed, and the volume of the pool determined, as was the concentration of the protein therein.

The pooled HIC protein-containing eluate was then concentrated and the elution buffer exchanged. Typically, proteins were concentrated to 5.0-10.0 mg/mL. Ultrafiltration was conducted using an ultrafiltration system equipped with a Pellicon™ cassette system (Millipore, Inc., Bedford, MA) with an appropriately sized cut-off membrane

After concentration, the sample was diafiltered against an appropriate buffer. The retentate from the concentration step was diafiltered against 0.15 M NaCl, 20 mM $NaPO_4$, pH 7.0 until the conductivity of the retentate was within 5% of the conductivity of the 0.15 M NaCl, 20 mM $NaPO_4$, pH 7.0 solution.

In addition, to remove precipitates and bacterial endotoxin that might be present, the concentrated diafiltered protein-containing sample was passed through a 0.1 µm Posidyne™ filter (Pall, Inc., Cortland, NY). After determining the protein concentration of the solution and on the basis of the desired concentration of the final bulk product, the solution was diluted with 0.15 M NaCl, 20 mM sodium phosphate, pH 7.0, to the desired final concentration. A final aseptic filtration through a 0.22 µm filter, was then performed as the final bulk product was transferred to a pyrogen-free container for storage (at about 5°C) for further formulation.

Example 3: Purification from Mammalian Cell Culture

This example describes the expression, isolation, and characterization of two biologically active recombinant KGF (rKGF) forms produced in a mammalian expression system.

The human KGF gene was isolated by PCR amplification of cDNA made from normal dermal human fibroblast cells (Clonetec, Inc., Palo Alto, CA). Following the making of cDNA by reverse transcriptase, PCR was used to amplify the KGF gene. OLIGO#25 and OLIGO#26 were used to amplify the gene out of the cDNA and OLIGO#27 and OLIGO#28 were used to place *Hind*III and *Bgl*II restriction sites at the fragment ends by a second PCR amplification, as set forth in Figure 1.

OLIGO#25 (SEQ ID NO:45): 5'-CAATCTACAATTCACAGA-3'
OLIGO#26 (SEQ ID NO:46): 5'-TTAAGTTATTGCCATAGG-3'
OLIGO#27 (SEQ ID NO:47): 5'-AACAAAGCTTCTACAATTCACAGATAGGA-3'
OLIGO#28 (SEQ ID NO:48): 5'-AACAAGATCTTAAGTTATTGCCATAGG-3'

Following cloning and DNA sequence confirmation, the KGF gene DNA was then used. Amplification was effected using two primers:

OLIGO#29 (SEQ. ID. NO:49):
5'-CGGTCTAGACCACCATGCACAAATGGATACTGACATGG-3'
OLIGO#30 (SEQ. ID. NO:50):
5'-GCCGTCGACCTATTAAGTTATTGCCATAGGAAG-3'

The sense primer, OLIGO#29, included an *Xba*I site and a consensus Kozak translation sequence (5'-CCACC-3') upstream of the start codon, ATG. The antisense primer, OLIGO#30, included a *Sal*I cloning site and an additional stop codon. After 18 cycles of PCR amplification (30 sec. denaturation at 94°C, 40 sec. annealing at 55°C, and 40 sec. elongation at 72°C), the product was digested with *Xba*I and *Sal*I and ligated with a similarly digested DNA of pDSRα2 (according to the methods of Bourdrel *et al.* (1993), *Protein Exp. & Purif.*, 4:130-140 and Lu *et al.* (1992), *Arch. Biochem. Biophys.*, 298:150-158). This resulted in plasmid KGF/pDSRα2 which placed the human KGF gene between the SV40 early promoter and the α-FSH polyadenylation sequences. Two clones were picked and DNA sequence analysis confirmed construction of the desired vector.

Two micrograms of KGF/pDSRα2 DNA were then linearized with *PvuI*. Chinese hamster ovary (CHO) cells, seeded the day before at 0.8 x 10^6 cells/60 mm culture dish, were then transfected with the treated DNA using a standard calcium phosphate precipitation method (Bourdrel *et al.*, *supra*). Two weeks later, individual colonies were picked and transferred into 24-well plates. The conditioned media was considered serum-free when the cells reached confluency and aliquots thereof were analyzed by Western blotting using a polyclonal rabbit antiserum reactive against *E. coli*-expressed human KGF.

Westerns were performed by running samples through 12.5% (w/v) SDS polyacrylamide gels, followed by electroblotting for 1 hr. at 400 mA onto nitrocellulose membranes using a semidry transfer apparatus (Hoefer Scientific Instruments, San Francisco, CA). 20 mM Tris, 150 mM glycine, 20% methanol served as the transfer buffer. The nitrocellulose sheets were blocked by incubation with 10% normal goat serum in PBS. Rabbit anti-serum raised against *E. coli*-derived KGF was used as primary antibody. For use, it was diluted 1/10,000 in 1% normal goat serum in PBS and incubated with the blocked nitrocellulose sheets for 12 hr. at room temperature, after which excess antibody was removed by three 30 min. washes in PBS. The nitrocellulose membranes were then incubated in 100 mL of 1% normal goat serum in PBS containing Vectastain™ biotinylated goat anti-rabbit IgG (secondary antibody, Vector Labs, Burlingame, CA), for 30 minutes at room temperature. After three 10 minute washes in PBS, a 30 minute room temperature incubation was performed in a 100 mL solution of 1% normal goat serum containing streptavidin and biotinylated peroxidase, prepared according to manufacturer's directions (Vector Labs). Following three washes in PBS, KGF cross-reactive material was visualized by incubation in a mixture of 60 µL of 30% (w/v) H_2O_2 in 100 mL of PBS and 50 mg of 4-chloronapthol in 20 mL of methanol. The reaction was stopped by rinsing in water after 10 minutes.

Analysis of the blots revealed that the KGF-specific antibody associated with three distinct protein bands, two being closely related with molecular weights of about 25-29 kDa and one with an estimated molecular weight of about 17 kDa, as compared to the expected molecular weight of approximately 18.8 of the 163 amino acid mature protein. Additionally, several high expressing clones secreting more than 2.0 mg of rKGF per liter, as judged by Western analysis, were selected and expanded into roller bottles (according to the method of Lu *et al.*, *supra*) to generate large volumes of serum-free conditioned medium for purification of KGF by cationic exchange chromatography and gel filtration, as set forth below.

KGF from 3 L of serum-free conditioned medium was purified applying the medium directly to a cation exchange column (5 x 24 cm) packed with 450 mL of sulfoethyl column of SP-Sepharose Fast Flow(Pharmacia) pre-equilibrated with 20 mM sodium phosphate, pH 7.5. After washing with five column volumes of 20 mM sodium phosphate, 0.2 M NaCl, pH 7.5, rKGF was eluted using a 20 column volume linear gradient of 0.2 to 1.0 M NaCl in 20 mM sodium phosphate, pH 7.5. 50 mL fractions were collected with continuous A_{280} monitoring. KGF protein was detected by analyzing aliquots of each fraction by SDS-PAGE. SDS-PAGE was performed on an electrophoresis system (Novex, San Diego, CA) using precast 14% Tris-glycine precast gels (according to the method of Laemmli (1970), *Nature*, 227:680-685). Samples were mixed with non-reducing SDS sample buffer without heating before loading. The proteins were detected by either Coomassie blue or silver staining. Two late-eluting peaks were seen to contain protein bands corresponding to the 25-29 kDa and 17 kDa bands detected by Western blot. The fractions containing each of these peaks were separately concentrated to a volume of less than 1.0 mL and subjected to gel filtration.

The gel filtrations employed columns of Superdex-75™ resin (HR 10/30, Pharmacia) pre-equilibrated with PBS, pH 7.2, and calibrated with the following known molecular weight standards (BioRad, San Francisco, CA): thyroglobulin (670 kDa), gamma globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and vitamin B-12 (1.4 kDa). These purification steps resulted in an approximate 2000-fold purification of rKGF, specifically including a 17 kDa and a 30 kDa material, as estimated by silver staining.

In the instance of the higher molecular weight material, rKGF eluted as a major symmetrical peak, which was called KGF-a. Upon SDS-PAGE analysis of a lesser amount of this material, 3 µg/lane versus 6 µg/lane, two bands with a 1-2 kDa molecular weight difference were resolved. In the instance of the lower molecular weight material, termed KGF-b, gel filtration resulted in a protein preparation having the expected mobility. For both KGF-a and KGF-b, the overall yield after purification was approximately 30-40%.

Amino acid sequences from KGF-a and KGF-b were also analyzed. These analyses were performed on an automatic sequencer (Model 477A or 470A, Applied Biosystems, Inc., Foster City, CA) equipped with a Model 120A on-line PTH-amino acid analyzer and a Model 900A data collection system (according to the method of Lu *et al.* (1991), *J. Biol. Chem.*, 266:8102-8107). Edman sequence analysis of KGF-a revealed a major N-terminal sequence of X_1-N-D-M-T-P-E-Q-M-A-T-N-V-X_2-X_3-S- (SEQ ID

NO:51). A minor sequence starting from the third N-terminal amino acid, aspartic acid, was also present in 1.6% of the total sequenceable protein. X_1, X_2, and X_3 were the unassigned due to the absence of phenylthiohydantoinyl (PTH) amino acid signals during sequence analysis.

Interestingly, N-terminal sequence analysis of KGF-b revealed an N-terminal amino acid sequence of S-Y-D-Y-M-E-G-G-D-I-R-V- (SEQ ID NO:52), indicating that it is an N-terminally truncated form of KGF that has been proteolytically cleaved at the Arg^{23}-Ser^{24} peptide bond.

To further characterize purified KGF-a and KGF-b, the protein was subjected to glycosidases (neuraminidase, O-glycanase, and/or N-glycanase), using known techniques (Sasaki *et al.* (1987), *J. Biol. Chem.*, 262:12059-12076; Takeuchi *et al.* (1988), *J. Biol. Chem.*, 263:3657-3663; Zsebo *et al.* (1990), *Cell*, 63:195-201). These data indicate that KGF-a contains N- and O-linked carbohydrates, although the lower molecular weight form of KGF-a probably contains only N-linked sugar. Glycosidase treatment did not cause molecular weight reduction for KGF-b, indicating that the molecule is unglycosylated.

Example 4: Biological Activity

Each KGF analog was diluted and assayed for biological activity by measuring the [^{3}H]-thymidine uptake of Balb/MK cells (according to the method of Rubin *et al.* (1989), *supra*). The samples were first diluted in a bioassay medium consisting of 50% customer-made Eagle's MEM, 50% customer-made F12, 5 µg/mL transferrin, 5 ng/mL sodium selenite, 0.0005% HSA and 0.005% Tween 20. KGF samples were then added into Falcon primeria 96-well plates seeded with Balb/MK cells. Incorporation of [^{3}H]-Thymidine during DNA synthesis was measured and converted to input native KGF concentration by comparison to a native KGF standard curve. Each of the tested analogs exhibited mitogenic activity.

Interaction with the KGF receptor was examined using isolated KGF receptor membrane preparations prepared from Balb/MK mouse epidermal keratinocytes (by the procedure described by Massague (19932), *J. Biol. Chem.*, 258:13614-13620). Specifically, various forms of KGF were diluted with 50 mM Tris-HCl, pH 7.5, containing 0.2% bovine serum albumin so as to range in concentration from 0.8 ng to 100 ng per 50 µL. They were individually incubated with the membrane preparation (75 ng/mL) and ^{125}I-labeled *E. coli*-derived KGF (1.5 ng). Receptor binding and competition experiments were performed at 4°C for 16 hr., after which time samples were taken, centrifuged, and washed twice with the above diluent buffer to remove unbound and non-specifically bound, labeled KGF. Samples were then counted for the remaining radioactivity. Competition curves for receptor binding between KGF samples and labeled KGF were constructed by plotting percent uncompetition versus concentrations of each KGF sample. Radioreceptor assay uncompetition experiments indicated that *E. coli*-derived KGF, KGF-a, and KGF-b have similar receptor binding activity.

While the present invention has been described above both generally and in terms of preferred embodiments, it is understood that other variations and modifications will occur to those skilled in the art in light of the description above.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Amgen Inc.

(ii) TITLE OF INVENTION: Method for Purifying Keratinocyte Growth Factors

(iii) NUMBER OF SEQUENCES: 52

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
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(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: U.S.A.
(F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/487,830
(B) FILING DATE:
(C) CLASSIFICATION: not yet known

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 862 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CAATCTACAA TTCACAGATA GGAAGAGGTC AATGACCTAG GAGTAACAAT CAACTCAAGA      60

TTCATTTTCA TTATGTTATT CATGAACACC CGGAGCACTA CACTATAATG CACAAATGGA     120

TACTGACATG GATCCTGCCA ACTTTGCTCT ACAGATCATG CTTTCACATT ATCTGTCTAG     180

TGGGTACTAT ATCTTTAGCT TGCAATGACA TGACTCCAGA GCAAATGGCT ACAAATGTGA     240

ACTGTTCCAG CCCTGAGCGA CACACAAGAA GTTATGATTA CATGGAAGGA GGGGATATAA     300
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GAGTGAGAAG ACTCTTCTGT CGAACACAGT GGTACCTGAG GATCGATAAA AGAGGCAAAG    360

TAAAAGGGAC CCAAGAGATG AAGAATAATT ACAATATCAT GGAAATCAGG ACAGTGGCAG    420

TTGGAATTGT GGCAATCAAA GGGGTGGAAA GTGAATTCTA TCTTGCAATG AACAAGGAAG    480

GAAAACTCTA TGCAAAGAAA GAATGCAATG AAGATTGTAA CTTCAAAGAA CTAATTCTGG    540

AAAACCATTA CAACACATAT GCATCAGCTA AATGGACACA CAACGGAGGG GAAATGTTTG    600

TTGCCTTAAA TCAAAAGGGG ATTCCTGTAA GAGGAAAAAA AACGAAGAAA GAACAAAAAA    660

CAGCCCACTT TCTTCCTATG GCAATAACTT AATTGCATAT GGTATATAAA GAACCCAGTT    720

CCAGCAGGGA GATTTCTTTA AGTGGACTGT TTTCTTTCTT CTCAAAATTT TCTTTCCTTT    780

TATTTTTTAG TAATCAAGAA AGGCTGGAAA AACTACTGAA AAACTGATCA AGCTGGACTT    840

GTGCATTTAT GTTTGTTTTA AG                                             862
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met His Lys Trp Ile Leu Thr Trp Ile Leu Pro Thr Leu Leu Tyr Arg
1               5                   10                  15

Ser Cys Phe His Ile Ile Cys Leu Val Gly Thr Ile Ser Leu Ala Cys
            20                  25                  30

Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Cys Ser Ser
        35                  40                  45

Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile
    50                  55                  60

Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg Ile Asp
65                  70                  75                  80

Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn Tyr Asn
                85                  90                  95

Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile Lys Gly
            100                 105                 110

Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys Leu Tyr
        115                 120                 125
```

Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu Ile Leu
130 135 140

Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His Asn Gly
145 150 155 160

Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val Arg Gly
165 170 175

Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro Met Ala
180 185 190

Ile Thr

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 595 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATCGATTTGA TTCTAGAAGG AGGAATAACA TATGAAAAAG CGCGCACGTG CTATCGCCAT 60

TGCTGTGGCT CTGGCAGGTT TCGCAACTAG TGCACACGCG TGCAATGACA TGACTCCAGA 120

GCAAATGGCT ACAAATGTGA ACTGTTCCAG CCCTGAGCGA CACACAAGAA GTTATGATTA 180

CATGGAAGGA GGGGATATAA GAGTGAGAAG ACTCTTCTGT CGAACACAGT GGTACCTGAG 240

GATCGATAAA AGAGGCAAAG TAAAAGGGAC CCAAGAGATG AAGAATAATT ACAATATCAT 300

GGAAATCAGG ACAGTGGCAG TTGGAATTGT GGCAATCAAA GGGGTGGAAA GTGAATTCTA 360

TCTTGCAATG AACAAGGAAG GAAAACTCTA TGCAAAGAAA GAATGCAATG AAGATTGTAA 420

CTTCAAAGAA CTAATTCTGG AAAACCATTA CAACACATAT GCATCAGCTA AATGGACACA 480

CAACGGAGGG GAAATGTTTG TTGCCTTAAA TCAAAAGGGG ATTCCTGTAA GAGGAAAAAA 540

AACGAAGAAA GAACAAAAAA CAGCCCACTT TCTTCCTATG GCAATAACTT AATAG 595

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 186 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Lys Lys Arg Ala Arg Ala Ile Ala Ile Ala Val Ala Leu Ala Gly
1               5                   10                  15

Phe Ala Thr Ser Ala His Ala Cys Asn Asp Met Thr Pro Glu Gln Met
            20                  25                  30

Ala Thr Asn Val Asn Cys Ser Ser Pro Glu Arg His Thr Arg Ser Tyr
        35                  40                  45

Asp Tyr Met Glu Gly Gly Asp Ile Arg Val Arg Arg Leu Phe Cys Arg
    50                  55                  60

Thr Gln Trp Tyr Leu Arg Ile Asp Lys Arg Gly Lys Val Lys Gly Thr
65                  70                  75                  80

Gln Glu Met Lys Asn Asn Tyr Asn Ile Met Glu Ile Arg Thr Val Ala
                85                  90                  95

Val Gly Ile Val Ala Ile Lys Gly Val Glu Ser Glu Phe Tyr Leu Ala
            100                 105                 110

Met Asn Lys Glu Gly Lys Leu Tyr Ala Lys Lys Glu Cys Asn Glu Asp
        115                 120                 125

Cys Asn Phe Lys Glu Leu Ile Leu Glu Asn His Tyr Asn Thr Tyr Ala
    130                 135                 140

Ser Ala Lys Trp Thr His Asn Gly Gly Glu Met Phe Val Ala Leu Asn
145                 150                 155                 160

Gln Lys Gly Ile Pro Val Arg Gly Lys Lys Thr Lys Lys Glu Gln Lys
                165                 170                 175

Thr Ala His Phe Leu Pro Met Ala Ile Thr
            180                 185
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 499 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: unknown
    (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TATGTGCAAT GACATGACTC CAGAGCAAAT GGCTACAAAT GTGAACTGTT CCAGCCCTGA     60

GCGACACACA AGAAGTTATG ATTACATGGA AGGAGGGGAT ATAAGAGTGA GAAGACTCTT    120

CTGTCGAACA CAGTGGTACC TGAGGATCGA TAAAAGAGGC AAAGTAAAAG GGACCCAAGA    180
```

```
GATGAAGAAT AATTACAATA TCATGGAAAT CAGGACAGTG GCAGTTGGAA TTGTGGCAAT      240

CAAAGGGGTG GAAAGTGAAT TCTATCTTGC AATGAACAAG GAAGGAAAAC TCTATGCAAA      300

GAAAGAATGC AATGAAGATT GTAACTTCAA AGAACTAATT CTGGAAAACC ATTACAACAC      360

ATATGCATCA GCTAAATGGA CACACAACGG AGGGGAAATG TTTGTTGCCT TAAATCAAAA      420

GGGGATTCCT GTAAGAGGAA AAAAAACGAA GAAAGAACAA AAAACAGCCC ACTTTCTTCC      480

TATGGCAATA ACTTAATAG                                                   499
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Cys Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Cys
1               5                   10                  15

Ser Ser Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly
            20                  25                  30

Asp Ile Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg
        35                  40                  45

Ile Asp Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn
    50                  55                  60

Tyr Asn Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile
65                  70                  75                  80

Lys Gly Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys
                85                  90                  95

Leu Tyr Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu
            100                 105                 110

Ile Leu Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His
        115                 120                 125

Asn Gly Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val
    130                 135                 140

Arg Gly Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro
145                 150                 155                 160

Met Ala Ile Thr
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAATGACCTA GGAGTAACAA TCAAC 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AAAACAAACA TAAATGCACA AGTCCA 26

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACAACGCGTG CAATGACATG ACTCCA 26

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACAGGATCCT ATTAAGTTAT TGCCATAGGA A 31

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACACATATGT GCAATGACAT GACTCCA 27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGCGTATCG ACAAACGCGG CAAAGTCAAG GGCACCC 37

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGAGATGAA AAACAACTAC AATATTATGG AAATCCGTAC TGTT 44

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTGTTGGTA TCGTTGCAAT CAAAGGTGTT GAATCTG 37

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCTTGGGTGC CCTTGACTTT GCCGCGTTTG TCGATACGCA GGTAC 45

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ACAGCAACAG TACGGATTTC CATAATATTG TAGTTGTTTT TCATC 45

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
AATTCAGATT CAACACCTTT GATTGCAACG ATACCA                                36
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 20 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: unknown
    (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
AGTTTTGATC TAGAAGGAGG                                                  20
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 20 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: unknown
    (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
TCAAAACTGG ATCCTATTAA                                                  20
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 91 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: unknown
    (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
AGTTTTGATC TAGAAGGAGG AATAACATAT GTGCAACGAC ATGACTCCGG AACAGATGGC      60

TACCAACGTT AACTGCTCCA GCCCGGAACG T                                     91
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CACACCCGTA GCTACGACTA CATGGAAGGT GGTGACATCC GTGTTCGTCG TCTGTTCTGC 60

CGTACCCAGT GGTACCTGCG TATCGACAAA 90

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGTGGTAAAG TTAAAGGTAC CCAGGAAATG AAAAACAACT ACAACATCAT GGAAATCCGT 60

ACTGTTGCTG TTGGTATCGT TGCAATCAAA 90

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGTGTTGAAT CTGAATTCTA CCTGGCAATG AACAAAGAAG GTAAACTGTA CGCAAAAAAA 60

GAATGCAACG AAGACTGCAA CTTCAAAGAA 90

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTGATCCTGG AAAACCACTA CAACACCTAC GCATCTGCTA AATGGACCCA CAACGGTGGT 60

GAAATGTTCG TTGCTCTGAA CCAGAAAGGT 90

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATCCCGGTTC GTGGTAAAAA AACCAAAAAA GAACAGAAAA CCGCTCACTT CCTGCCGATG 60

GCAATCACTT AATAGGATCC AGTTTTGA 88

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TACGGGTGTG ACGTTCCGGG 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTTACCACG TTTGTCGATA 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATTCAACACC TTTGATTGCA 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCAGGATCAG TTCTTTGAAG 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAACCGGGAT ACCTTTCTGG 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
ATGTCTAATG ATATGACTCC GGAACAGATG GCTACCAACG TTAACTCCTC CTCCCCGGAA        60

CGTCACACGC GTTCCTACGA CTACATGGAA GGTGGTGACA TCCGCGTACG TCGTCTGTTC       120

TGCCGTACCC AGTGGTACCT GCGTATCGAC AAACGCGGCA AAGTCAAGGG CACCCAAGAG       180

ATGAAAAACA ACTACAATAT TATGGAAATC CGTACTGTTG CTGTTGGTAT CGTTGCAATC       240

AAAGGTGTTG AATCTGAATT CTACCTGGCA ATGAACAAAG AAGGTAAACT GTACGCAAAA       300

AAAGAATGCA ACGAAGACTG CAACTTCAAA GAACTGATCC TGGAAAACCA CTACAACACC       360

TACGCATCTG CTAAATGGAC CCACAACGGT GGTGAAATGT TCGTTGCTCT GAACCAGAAA       420

GGTATCCCGG TTCGTGGTAA AAAAACCAAA AAAGAACAGA AAACCGCTCA CTTCCTGCCG       480

ATGGCAATCA CTTAA                                                        495
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Ser Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Ser
1               5                   10                  15

Ser Ser Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly
            20                  25                  30

Asp Ile Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg
        35                  40                  45

Ile Asp Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn
    50                  55                  60
```

```
Tyr Asn Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile
65                  70                  75                  80

Lys Gly Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys
                85                  90                  95

Leu Tyr Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu
            100                 105                 110

Ile Leu Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His
        115                 120                 125

Asn Gly Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val
    130                 135                 140

Arg Gly Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro
145                 150                 155                 160

Met Ala Ile Thr
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
ATGTGCAATG ATATGACTCC TGAACAAATG GCTACCAATG TCAACTGTTC CTCTCCGGAG    60

CGCCACACCC GGAGTTACGA TTACATGGAA GGTGGGGATA TTCGCGTACG TCGTCTGTTC   120

TGCCGTACCC AGTGGTACCT GCGTATCGAC AAACGCGGCA AAGTCAAGGG CACCCAAGAG   180

ATGAAAAACA ACTACAATAT TATGGAAATC CGTACTGTTG CTGTTGGTAT CGTTGCAATC   240

AAAGGTGTTG AATCTGAATT CTATCTTGCA ATGAACAAGG AAGGAAAACT CTATGCAAAG   300

AAAGAATGCA ATGAAGATTG TAACTTCAAA GAACTAATTC TGGAAAACCA TTACAACACA   360

TATGCATCTG CTAAATGGAC CCACAACGGT GGTGAAATGT TCGTTGCTCT GAACCAGAAA   420

GGTATCCCTG TTCAAGGTAA GAAAACCAAG AAAGAACAGA AACCGCTCA CTTCCTGCCG   480

ATGGCAATCA CTTAA                                                    495
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Met Cys Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Cys
1               5                   10                  15

Ser Ser Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly
            20                  25                  30

Asp Ile Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg
        35                  40                  45

Ile Asp Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn
    50                  55                  60

Tyr Asn Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile
65                  70                  75                  80

Lys Gly Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys
                85                  90                  95

Leu Tyr Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu
            100                 105                 110

Ile Leu Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His
        115                 120                 125

Asn Gly Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val
    130                 135                 140

Gln Gly Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro
145                 150                 155                 160

Met Ala Ile Thr
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATGTCTAATG ATATGACTCC GGAACAGATG GCTACCAACG TTAACTCCTC CTCCCCGGAA 60

CGTCACACGC GTTCCTACGA CTACATGGAA GGTGGTGACA TCCGCGTACG TCGTCTGTTC 120

TGCCGTACCC AGTGGTACCT GCGTATCGAC AAACGCGGCA AAGTCAAGGG CACCCAAGAG 180

ATGAAAAACA ACTACAATAT TATGGAAATC CGTACTGTTG CTGTTGGTAT CGTTGCAATC 240

AAAGGTGTTG AATCTGAATT CTATCTTGCA ATGAACAAGG AAGGAAAACT CTATGCAAAG 300

AAAGAATGCA ATGAAGATTG TAACTTCAAA GAACTAATTC TGGAAAACCA TTACAACACA 360

TATGCATCTG CTAAATGGAC CCACAACGGT GGTGAAATGT TCGTTGCTCT GAACCAGAAA 420

GGTATCCCTG TTCAAGGTAA GAAAACCAAG AAAGAACAGA AAACCGCTCA CTTCCTGCCG 480

ATGGCAATCA CTTAA 495

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Ser Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Ser
1 5 10 15

Ser Ser Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly
20 25 30

Asp Ile Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg
35 40 45

Ile Asp Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn
50 55 60

Tyr Asn Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile
65 70 75 80

Lys Gly Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys
85 90 95

Leu Tyr Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu
100 105 110

Ile Leu Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His
115 120 125

```
Asn Gly Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val
    130                 135                 140

Gln Gly Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro
145                 150                 155                 160

Met Ala Ile Thr
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 450 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
ATGTCTTCTC CTGAACGTCA TACGCGTTCC TACGACTACA TGGAAGGTGG TGACATCCGC      60

GTACGTCGTC TGTTCTGCCG TACCCAGTGG TACCTGCGTA TCGACAAACG CGGCAAAGTC     120

AAGGGCACCC AAGAGATGAA AAACAACTAC AATATTATGG AAATCCGTAC TGTTGCTGTT     180

GGTATCGTTG CAATCAAAGG TGTTGAATCT GAATTCTACC TGGCAATGAA CAAAGAAGGT     240

AAACTGTACG CAAAAAAAGA ATGCAACGAA GACTGCAACT TCAAAGAACT GATCCTGGAA     300

AACCACTACA ACACCTACGC ATCTGCTAAA TGGACCCACA ACGGTGGTGA AATGTTCGTT     360

GCTCTGAACC AGAAAGGTAT CCCGGTTCGT GGTAAAAAAA CCAAAAAAGA ACAGAAAACC     420

GCTCACTTCC TGCCGATGGC AATCACTTAA                                      450
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 149 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
Met Ser Ser Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly
1               5                   10                  15

Gly Asp Ile Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu
            20                  25                  30
```

```
Arg Ile Asp Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn
        35                  40                  45

Asn Tyr Asn Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala
    50                  55                  60

Ile Lys Gly Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly
65                  70                  75                  80

Lys Leu Tyr Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu
                85                  90                  95

Leu Ile Leu Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr
            100                 105                 110

His Asn Gly Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro
        115                 120                 125

Val Arg Gly Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu
    130                 135                 140

Pro Met Ala Ile Thr
145
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 426 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
ATGTCCTACG ACTACATGGA AGGTGGTGAC ATCCGCGTAC GTCGTCTGTT CTGCCGTACC     60

CAGTGGTACC TGCGTATCGA CAAACGCGGC AAAGTCAAGG GCACCCAAGA GATGAAAAAC    120

AACTACAATA TTATGGAAAT CCGTACTGTT GCTGTTGGTA TCGTTGCAAT CAAAGGTGTT    180

GAATCTGAAT TCTACCTGGC AATGAACAAA GAAGGTAAAC TGTACGCAAA AAAAGAATGC    240

AACGAAGACT GCAACTTCAA AGAACTGATC CTGGAAAACC ACTACAACAC CTACGCATCT    300

GCTAAATGGA CCCACAACGG TGGTGAAATG TTCGTTGCTC TGAACCAGAA AGGTATCCCG    360

GTTCGTGGTA AAAAAACCAA AAAAGAACAG AAAACCGCTC ACTTCCTGCC GATGGCAATC    420

ACTTAA                                                               426
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
Met Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile Arg Val Arg Arg Leu
1               5                   10                  15

Phe Cys Arg Thr Gln Trp Tyr Leu Arg Ile Asp Lys Arg Gly Lys Val
            20                  25                  30

Lys Gly Thr Gln Glu Met Lys Asn Asn Tyr Asn Ile Met Glu Ile Arg
        35                  40                  45

Thr Val Ala Val Gly Ile Val Ala Ile Lys Gly Val Glu Ser Glu Phe
    50                  55                  60

Tyr Leu Ala Met Asn Lys Glu Gly Lys Leu Tyr Ala Lys Lys Glu Cys
65                  70                  75                  80

Asn Glu Asp Cys Asn Phe Lys Glu Leu Ile Leu Glu Asn His Tyr Asn
                85                  90                  95

Thr Tyr Ala Ser Ala Lys Trp Thr His Asn Gly Gly Glu Met Phe Val
            100                 105                 110

Ala Leu Asn Gln Lys Gly Ile Pro Val Arg Gly Lys Lys Thr Lys Lys
        115                 120                 125

Glu Gln Lys Thr Ala His Phe Leu Pro Met Ala Ile Thr
    130                 135                 140
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 426 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
ATGTCCTACG ACTACATGGA AGGTGGTGAC ATCCGCGTAC GTCGTCTGTT CTGCCGTACC    60

CAGTGGTACC TGCGTATCGA CAAACGCGGC AAAGTCAAGG GCACCCAAGA GATGAAAAAC   120

AACTACAATA TTATGGAAAT CCGTACTGTT GCTGTTGGTA TCGTTGCAAT CAAAGGTGTT   180

GAATCTGAAT TCTATCTTGC AATGAACAAG GAAGGAAAAC TCTATGCAAA GAAAGAATGC   240

AATGAAGATT GTAACTTCAA AGAACTAATT CTGGAAAACC ATTACAACAC ATATGCATCT   300

GCTAAATGGA CCCACAACGG TGGTGAAATG TTCGTTGCTC TGAACCAGAA AGGTATCCCT   360

GTTCAAGGTA AGAAAACCAA GAAAGAACAG AAAACCGCTC ACTTCCTGCC GATGGCAATC   420

ACTTAA                                                              426
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Met Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile Arg Val Arg Arg Leu
1               5                   10                  15

Phe Cys Arg Thr Gln Trp Tyr Leu Arg Ile Asp Lys Arg Gly Lys Val
            20                  25                  30

Lys Gly Thr Gln Glu Met Lys Asn Asn Tyr Asn Ile Met Glu Ile Arg
        35                  40                  45

Thr Val Ala Val Gly Ile Val Ala Ile Lys Gly Val Glu Ser Glu Phe
    50                  55                  60

Tyr Leu Ala Met Asn Lys Glu Gly Lys Leu Tyr Ala Lys Lys Glu Cys
65                  70                  75                  80

Asn Glu Asp Cys Asn Phe Lys Glu Leu Ile Leu Glu Asn His Tyr Asn
                85                  90                  95

Thr Tyr Ala Ser Ala Lys Trp Thr His Asn Gly Gly Glu Met Phe Val
            100                 105                 110

Ala Leu Asn Gln Lys Gly Ile Pro Val Gln Gly Lys Lys Thr Lys Lys
        115                 120                 125

Glu Gln Lys Thr Ala His Phe Leu Pro Met Ala Ile Thr
    130                 135                 140
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GAGCTCACTA GTGTCGACCT GCAG 24

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: complement (1..24)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTGCAGGTCG ACACTAGTGA GCTC 24

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAATCTACAA TTCACAGA 18

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TTAAGTTATT GCCATAGG 18

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

AACAAAGCTT CTACAATTCA CAGATAGGA 29

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

AACAAGATCT TAAGTTATTG CCATAGG 27

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CGGTCTAGAC CACCATGCAC AAATGGATAC TGACATGG 38

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
GCCGTCGACC TATTAAGTTA TTGCCATAGG AAG                                33
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```
Xaa Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Xaa Xaa Ser
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```
Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile Arg Val
1               5                   10
```

WHAT IS CLAIMED IS:

1. A method for purifying a keratinocyte growth factor (KGF), the method comprising:
   a) obtaining a solution comprising KGF;
   b) binding KGF from the solution of part (a) to a cation exchange resin;
   c) eluting KGF in an eluate solution from the cation exchange resin;
   d) passing the eluate solution from part (c) through an appropriate molecular weight exclusion matrix; and
   e) recovering KGF from the molecular weight exclusion matrix.

2. The method according to Claim 1 wherein the KGF is produced in procaryotic cells.

3. The method according to Claim 1 wherein the KGF is produced in *E. coli*.

4. The method according to Claim 1 wherein the KGF is produced in mammalian cells.

5. The method according to Claim 4 wherein the KGF is produced in Chinese hamster ovary cells.

6. A method for purifying a keratinocyte growth factor (KGF), the method comprising:
   a) obtaining a solution comprising KGF;
   b) binding KGF from the solution of part (a) to a cation exchange resin;
   c) eluting KGF in an eluate solution from the cation exchange resin;

d) performing hydrophobic interaction chromatography on the eluate solution of part (c); and

e) recovering KGF from the hydrophobic interaction chromatography step of part (d).

7. A method according to Claim 6 further comprising oxidation of free sulfhydryl groups in KGF.

8. The method according to Claim 6 wherein the KGF is produced in procaryotic cells.

9. The method according to Claim 7 wherein the KGF is produced in *E. coli*.

10. The method according to Claim 6 wherein the KGF is produced in mammalian cells.

11. The method according to Claim 10 wherein the KGF is produced in Chinese hamster ovary cells.

Figure 1

human KGF (+ signal sequence)

```
  |---OLIGO#25-----|          |-------OLIGO#1---------|
5'CAATCTACAATTCACAGA 3'      5'CAATGACCTAGGAGTAACAATCAAC 3'
5'CAATCTACAATTCACAGATAGGAAGAGGTCAATGACCTAGGAGTAACAATCAACTCAAGA-
  ---------+---------+---------+---------+---------+---------+ 60

 -TTCATTTTCATTATGTTATTCATGAACACCCGGAGCACTACACTATAATGCACAAATGGA-
  ---------+---------+---------+---------+---------+---------+ 120
                                                  M  H  K  W  I

 -TACTGACATGGATCCTGCCAACTTTGCTCTACAGATCATGCTTTCACATTATCTGTCTAG-
  ---------+---------+---------+---------+---------+---------+ 180
     L  T  W  I  L  P  T  L  L  Y  R  S  C  F  H  I  I  C  L  V

 -TGGGTACTATATCTTTAGCTTGCAATGACATGACTCCAGAGCAAATGGCTACAAATGTGA-
  ---------+---------+---------+---------+---------+---------+ 240
     G  T  I  S  L  A  C  N  D  M  T  P  E  Q  M  A  T  N  V  N

 -ACTGTTCCAGCCCTGAGCGACACACAAGAAGTTATGATTACATGGAAGGAGGGGATATAA-
  ---------+---------+---------+---------+---------+---------+ 300
     C  S  S  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R

 -GAGTGAGAAGACTCTTCTGTCGAACACAGTGGTACCTGAGGATCGATAAAAGAGGCAAAG-
  ---------+---------+---------+---------+---------+---------+ 360
     V  R  R  L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V

 -TAAAAGGGACCCAAGAGATGAAGAATAATTACAATATCATGGAAATCAGGACAGTGGCAG-
  ---------+---------+---------+---------+---------+---------+ 420
     K  G  T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V

 -TTGGAATTGTGGCAATCAAAGGGGTGGAAAGTGAATTCTATCTTGCAATGAACAAGGAAG-
  ---------+---------+---------+---------+---------+---------+ 480
     G  I  V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G

 -GAAAACTCTATGCAAAGAAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGG-
  ---------+---------+---------+---------+---------+---------+ 540
     K  L  Y  A  K  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E

 -AAAACCATTACAACACATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTTTG-
  ---------+---------+---------+---------+---------+---------+ 600
     N  H  Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V

 -TTGCCTTAAATCAAAAGGGGATTCCTGTAAGAGGAAAAAAACGAAGAAAGAACAAAAAA-
  ---------+---------+---------+---------+---------+---------+ 660
     A  L  N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T

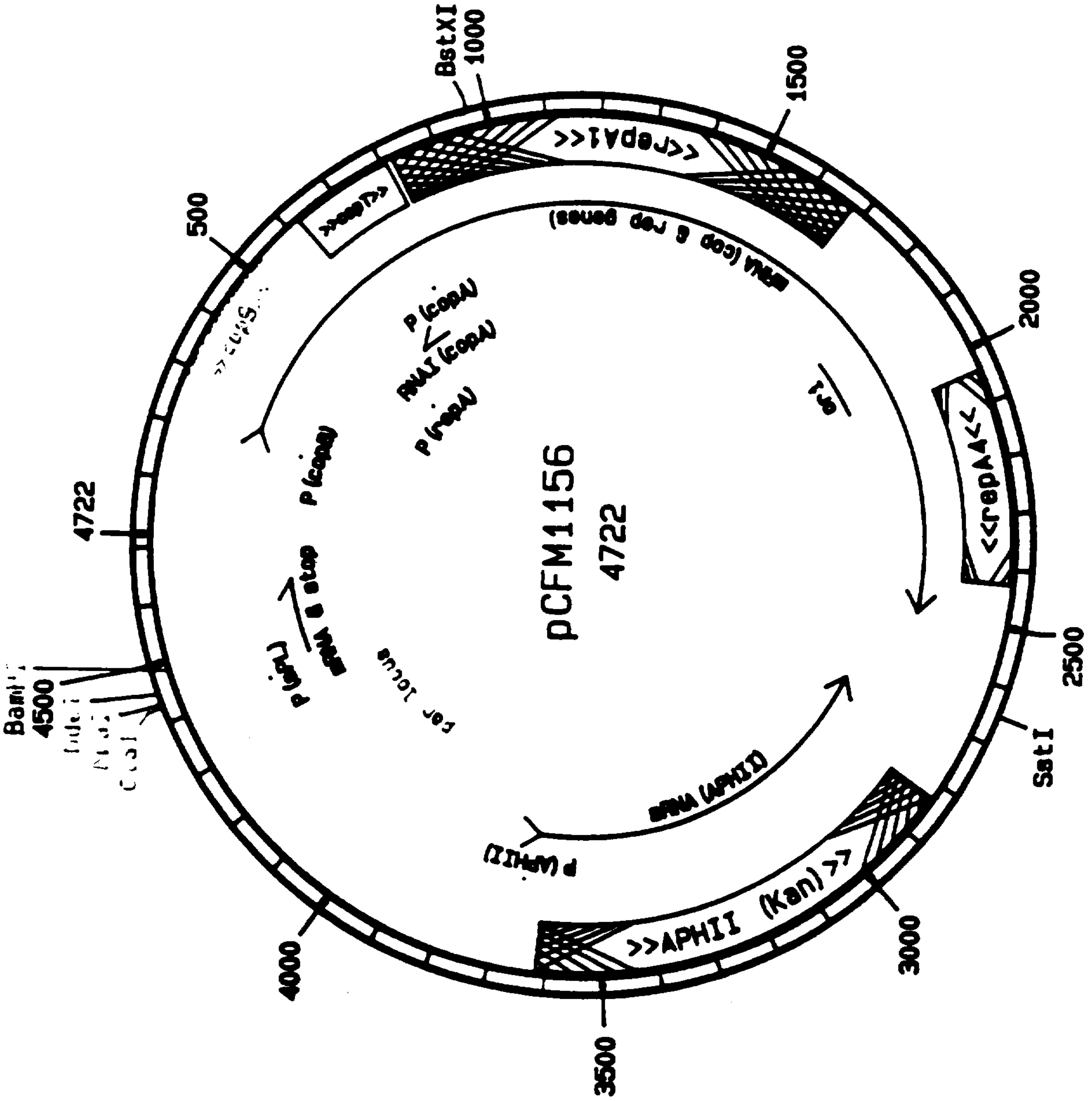
 -CAGCCCACTTTCTTCCTATGGCAATAACTTAATTGCATATGGTATATAAAGAACCCAGTT
  ---------+---------+---------+---------+---------+---------+ 720
     A  H  F  L  P  M  A  I  T  *
              3'GGATACCGTTATTGAATT 5'
                |----OLIGO#26----|
```

2/15
(continued)

```
-CCAGCAGGGAGATTTCTTTAAGTGGACTGTTTTCTTTCTTCTCAAAATTTTCTTTCCTTT
 ---------+---------+---------+---------+---------+---------+ 780

-TATTTTTTAGTAATCAAGAAAGGCTGGAAAAACTACTGAAAAACTGATCAAGCTGGACTT
 ---------+---------+---------+---------+---------+---------+ 840
                                                     3'ACCTGAA-
                                                        |------

-GTGCATTTATGTTTGTTTTAAG 3'
 ---------+---------+-- 862
-CACGTAAATACAAACAAAA 5'
 ---OLIGO#2--------|
```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1000
1500
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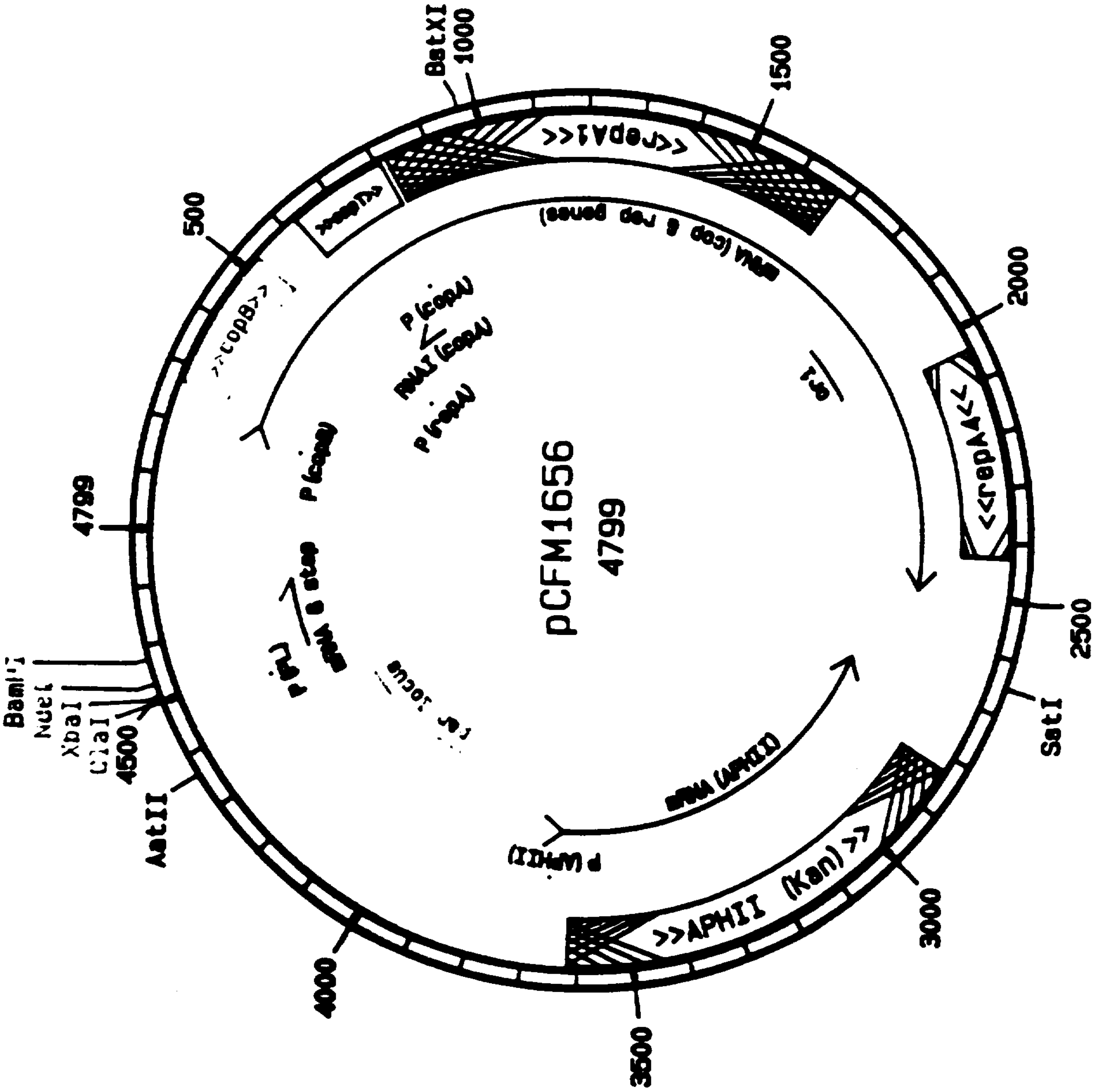
P (copA)
RNAI (copA)
P (repA)
P (copB)
2000
<<repA4<<
2500
SstI
3000
>>APHII (Kan)>>
mRNA (APHII)
P (APHII)
3500
4000
P (par)
mRNA & stop
cer locus
4722
500
BamHI
4500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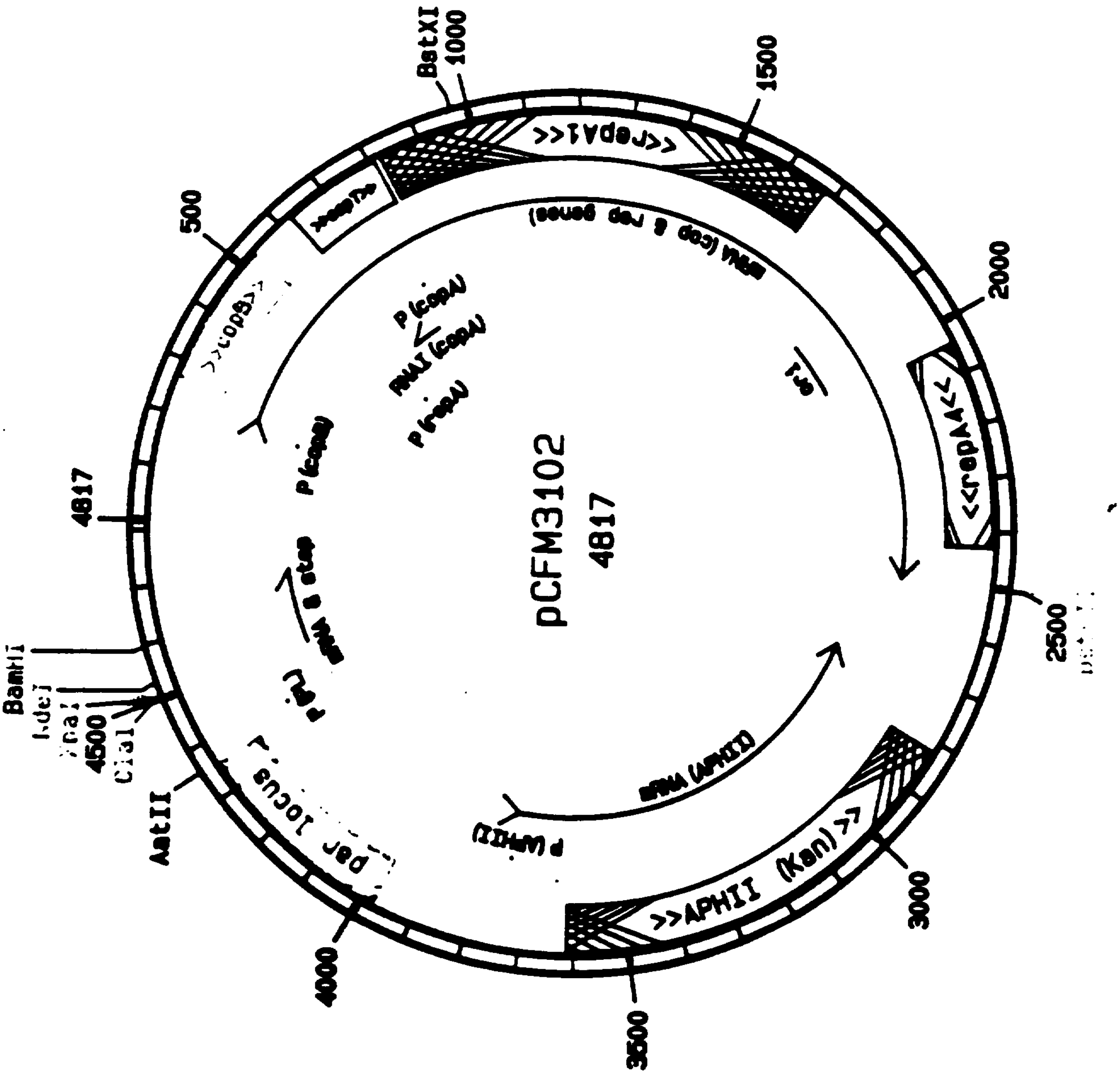
P (copA)
RNAI (copA)
P (repA)
P (copB)
ori
mRNA (APHII)
P (APHII)
par locus
P (PL)
mRNA & stop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BstXI
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1500
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2500
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4000
4500
4817
500
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<<repA4<<
>>APHII (Kan)>>
par locus
mRNA (APHII)
P (APHII)
mRNA (cop & rep genes)
P (copA)
RNAI (copA)
P (repA)
P (copB)
mRNA B stop
P(pL)
T1
BamHI
NdeI
XbaI
ClaI
AatII

figure 3

RSH-KGF

```
plasmid DNA         ClaI         XbaI             NdeI
sequence         5'-ATCGATTTGATTCTAGAAGGAGGAATAACATATGAAAAAG-
                                                     M  K  K

                 RSH signal sequence                          MluI
-CGCGCACGTGCTATCGCCATTGCTGTGGCTCTGGCAGGTTTCGCAACTAGTGCACA-3'
 R  A  R  A  I  A  I  A  V  A  L  A  G  F  A  T  S  A  H  A  -


 MluI
5'CGCGTGCAATGACATGACTCCAGAGCAAATGGCTACAAATGTGAACTGTTCCAGCCCTGA-
  ---------+---------+---------+---------+---------+---------+  60
  -  C  N  D  M  T  P  E  Q  M  A  T  N  V  N  C  S  S  P  E

-GCGACACACAAGAAGTTATGATTACATGGAAGGAGGGGATATAAGAGTGAGAAGACTCTT-
  ---------+---------+---------+---------+---------+---------+ 120
  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

                 KpnI       ClaI
-CTGTCGAACACAGTGGTACCTGAGGATCGATAAAAGAGGCAAAGTAAAAGGGACCCAAGA-
  ---------+---------+---------+---------+---------+---------+ 180
  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

-GATGAAGAATAATTACAATATCATGGAAATCAGGACAGTGGCAGTTGGAATTGTGGCAAT-
  ---------+---------+---------+---------+---------+---------+ 240
  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

                  EcoRI
-CAAAGGGGTGGAAAGTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAA-
  ---------+---------+---------+---------+---------+---------+ 300
  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

             BsmI
-GAAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACAC-
  ---------+---------+---------+---------+---------+---------+ 360
  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

 NdeI
-ATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTTTGTTGCCTTAAATCAAAA-
  ---------+---------+---------+---------+---------+---------+ 420
  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

-GGGGATTCCTGTAAGAGGAAAAAAAACGAAGAAAGAACAAAAAACAGCCCACTTTCTTCC-
  ---------+---------+---------+---------+---------+---------+ 480
  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

                 BamHI
-TATGGCAATAACTTAATAG 3'                  -plasmid DNA
  ---------+---------+--- 503            -sequence
  M  A  I  T  *  *
```

Figure 4

KGF

```
 NdeI
5'TATGTGCAATGACATGACTCCAGAGCAAATGGCTACAAATGTGAACTGTTCCAGCCCTGA-
  ---------+---------+---------+---------+---------+---------+ 60
   M  C  N  D  M  T  P  E  Q  M  A  T  N  V  N  C  S  S  P  E

 -GCGACACACAAGAAGTTATGATTACATGGAAGGAGGGGATATAAGAGTGAGAAGACTCTT-
  ---------+---------+---------+---------+---------+---------+ 120
   R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

                    KpnI       ClaI
 -CTGTCGAACACAGTGGTACCTGAGGATCGATAAAAGAGGCAAAGTAAAAGGGACCCAAGA-
  ---------+---------+---------+---------+---------+---------+ 180
   C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

 -GATGAAGAATAATTACAATATCATGGAAATCAGGACAGTGGCAGTTGGAATTGTGGCAAT-
  ---------+---------+---------+---------+---------+---------+ 240
   M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

                     EcoRI
 -CAAAGGGGTGGAAAGTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAA-
  ---------+---------+---------+---------+---------+---------+ 300
   K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

       BsmI
 -GAAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACAC-
  ---------+---------+---------+---------+---------+---------+ 360
   K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

  NdeI
 -ATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTTTGTTGCCTTAAATCAAAA-
  ---------+---------+---------+---------+---------+---------+ 420
   Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

 -GGGGATTCCTGTAAGAGGAAAAAAAACGAAGAAAGAACAAAAAACAGCCCACTTTCTTCC-
  ---------+---------+---------+---------+---------+---------+ 480
   G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

                      BamHI
 -TATGGCAATAACTTAATAG 3'
  ---------+---------+---   503
   M  A  I  T  *
```

Figure 5

substitution of KpnI to EcoRI sequence to make KGF(dsd)

```
 KpnI
      |-----------OLIGO#6-----------------||------OLIGO#7---------
5'    CTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAACAACTACAAT-
3'CATGGACGCATAGCTGTTTGCGCCGTTTCAGTTCCCGTGGGTTCTCTACTTTTTGTTGATGTTA-
  |---------------OLIGO#9---------------------||--OLIGO#10--------
   -  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  N  Y  N  -

                                                              EcoRI
   ---------------------||---------OLIGO#8-------------------|
5'-ATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTG     3'
3'-TAATACCTTTAGGCATGACAACGACAACCATAGCAACGTTAGTTTCCACAACTTAGACTTAA 5'
   -------------------------||----OLIGO#11----------------------|
   I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  E  S  E  -
```

Figure 6

KGF (codon optimized)

```
           XbaI
  |-----OLIGO#12-----|
5'AGTTTTGATCTAGAAGGAGG 3'
  |--------------------------OLIGO#14-------------------------
5'AGTTTTGATCTAGAAGGAGGAATAACATATGTGCAACGACATGACTCCGGAACAGATGGCT-

  -----------------------------||--------------OLIGO#15--------
 -ACCAACGTTAACTGCTCCAGCCCGGAACGTCACACCCGTAGCTACGACTACATGGAAGGTG-
                  3' GGGCCTTGCAGTGTGGGCAT 5'
                     |-----OLIGO#20-----|

  ---------------------OLIGO#15----------------------------||-
 -GTGACATCCGTGTTCGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACG-
                                              3' ATAGCTGTTTGC-
                                                 |-OLIGO#21--

  ---------------------OLIGO#16---------------------------------
 -TGGTAAAGTTAAAGGTACCCAGGAAATGAAAAACAACTACAACATCATGGAAATCCGTACT-
 -ACCATTTC 5'
  -------|

  -------------------------||-------------OLIGO#17------------
 -GTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACA-
               3' ACGTTAGTTTCCACAACTTA 5'
                  |-----OLIGO#22-----|

  -----------------OLIGO#17------------------------------||----
 -AAGAAGGTAAACTGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGAT-
                                           3'GAAGTTTCTTGACTA-
                                             |---OLIGO#23---

  ---------------------OLIGO#18---------------------------------
 -CCTGGAAAACCACTACAACACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATG-
 -GGACC 5'
  ----|

  ----------------------||------------------OLIGO#19---------
 -TTCGTTGCTCTGAACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGA-
            3' GGTCTTTCCATAGGGCCAAG 5'
               |-----OLIGO#24-----|

  -------OLIGO#19-----------------------------------|
 -AAACCGCTCACTTCCTGCCGATGGCAATCACTTAATAGGATCCAGTTTTGA 3'
                            3' AATTATCCTAGGTCAAAACT 5'
                               |----OLIGO#13------|
                                     BamHI
```

Figure 7

KGF C(1,15)S

```
5'ATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGTTAACTCCTCCTCCCCGGAA-
  ---------+---------+---------+---------+---------+---------+ 60
  M  S  N  D  M  T  P  E  Q  M  A  T  N  V  N  S  S  S  P  E

 -CGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTC-
  ---------+---------+---------+---------+---------+---------+ 120
  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

 -TGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAG-
  ---------+---------+---------+---------+---------+---------+ 180
  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

 -ATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATC-
  ---------+---------+---------+---------+---------+---------+ 240
  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

 -AAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAA-
  ---------+---------+---------+---------+---------+---------+ 300
  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

 -AAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACC-
  ---------+---------+---------+---------+---------+---------+ 360
  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

 -TACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAA-
  ---------+---------+---------+---------+---------+---------+ 420
  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

 -GGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCG-
  ---------+---------+---------+---------+---------+---------+ 480
  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

 -ATGGCAATCACTTAA 3'
  ---------+----- 495
  M  A  I  T  *
```

Figure 8

KGF R(144)Q

```
5'ATGTGCAATGATATGACTCCTGAACAAATGGCTACCAATGTCAACTGTTCCTCTCCGGAG-
  ---------+---------+---------+---------+---------+---------+ 60
  M  C  N  D  M  T  P  E  Q  M  A  T  N  V  N  C  S  S  P  E

 -CGCCACACCCGGAGTTACGATTACATGGAAGGTGGGGATATTCGCGTACGTCGTCTGTTC-
  ---------+---------+---------+---------+---------+---------+ 120
  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

 -TGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAG-
  ---------+---------+---------+---------+---------+---------+ 180
  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

 -ATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATC-
  ---------+---------+---------+---------+---------+---------+ 240
  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

 -AAAGGTGTTGAATCTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAAG-
  ---------+---------+---------+---------+---------+---------+ 300
  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

 -AAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACACA-
  ---------+---------+---------+---------+---------+---------+ 360
  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

 -TATGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAA-
  ---------+---------+---------+---------+---------+---------+ 420
  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

 -GGTATCCCTGTTCAAGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCG-
  ---------+---------+---------+---------+---------+---------+ 480
  G  I  P  V  Q  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

 -ATGGCAATCACTTAA 3'
  ---------+----- 495
  M  A  I  T  *
```

Figure 9

KGF C(1,15)S/R(144)Q

```
5'ATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGTTAACTCCTCCTCCCCGGAA-
  ---------+---------+---------+---------+---------+---------+ 60
  M  S  N  D  M  T  P  E  Q  M  A  T  N  V  N  S  S  S  P  E

 -CGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTC-
  ---------+---------+---------+---------+---------+---------+ 120
  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

 -TGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAG-
  ---------+---------+---------+---------+---------+---------+ 180
  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

 -ATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATC-
  ---------+---------+---------+---------+---------+---------+ 240
  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

 -AAAGGTGTTGAATCTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAAG-
  ---------+---------+---------+---------+---------+---------+ 300
  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

 -AAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACACA-
  ---------+---------+---------+---------+---------+---------+ 360
  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

 -TATGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAA-
  ---------+---------+---------+---------+---------+---------+ 420
  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

 -GGTATCCCTGTTCAAGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCG-
  ---------+---------+---------+---------+---------+---------+ 480
  G  I  P  V  Q  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

 -ATGGCAATCACTTAA 3'
  ---------+----- 495
 M  A  I  T  *
```

Figure 10

KGF ΔN15

```
5'ATGTCTTCTCCTGAACGTCATACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGC-
  ---------+---------+---------+---------+---------+---------+ 60
  M  S  S  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R

 -GTACGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTC-
  ---------+---------+---------+---------+---------+---------+ 120
  V  R  R  L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V

 -AAGGGCACCCAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTT-
  ---------+---------+---------+---------+---------+---------+ 180
  K  G  T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V

 -GGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGT-
  ---------+---------+---------+---------+---------+---------+ 240
  G  I  V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G

 -AAACTGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAA-
  ---------+---------+---------+---------+---------+---------+ 300
  K  L  Y  A  K  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E

 -AACCACTACAACACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTT-
  ---------+---------+---------+---------+---------+---------+ 360
  N  H  Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V

 -GCTCTGAACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACC-
  ---------+---------+---------+---------+---------+---------+ 420
  A  L  N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T

 -GCTCACTTCCTGCCGATGGCAATCACTTAA 3'
  ---------+---------+---------+ 450
  A  H  F  L  P  M  A  I  T  *
```

Figure 11

KGF ΔN23

```
5'ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
  ---------+---------+---------+---------+---------+---------+ 60
   M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T

 -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
  ---------+---------+---------+---------+---------+---------+ 120
   Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N

 -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
  ---------+---------+---------+---------+---------+---------+ 180
   N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V

 -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
  ---------+---------+---------+---------+---------+---------+ 240
   E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C

 -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
  ---------+---------+---------+---------+---------+---------+ 300
   N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S

 -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCG -
  ---------+---------+---------+---------+---------+---------+ 360
   A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P

 -GTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
  ---------+---------+---------+---------+---------+---------+ 420
   V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I

 -ACTTAA 3'
  ------   426
   T  *
```

Figure 12

KGF ΔN23/R(144)Q

```
5'ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
  ---------+---------+---------+---------+---------+---------+ 60
  M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T

 -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
  ---------+---------+---------+---------+---------+---------+ 120
  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N

 -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
  ---------+---------+---------+---------+---------+---------+ 180
  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V

 -GAATCTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAAGAAAGAATGC-
  ---------+---------+---------+---------+---------+---------+ 240
  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C-

 -AATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACACATATGCATCT-
  ---------+---------+---------+---------+---------+---------+ 300
  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S

 -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
  ---------+---------+---------+---------+---------+---------+ 360
  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P

 -GTTCAAGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
  ---------+---------+---------+---------+---------+---------+ 420
  V  Q  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I

 -ACTTAA 3'
  ------ 426
  T  *
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/13099

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/50

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 6 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	FEBS LETTERS, vol. 328, no. 1,2, - August 1993 AMSTERDAM NL, pages 17-20, M.SUZUKI E A 'Spleen-derived growth factor, SDGF-3, is identified as keratinocyte growth factor (KGF)' see page 18 ---	1-5
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 4, 5 February 1993 MD US, pages 2984-2988, D.RON ET AL 'Expression of biologically active recombinant KGF' cited in the application see the whole document --- -/--	1-11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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1

Date of the actual completion of the international search 28 February 1996	Date of mailing of the international search report 15.03.96
Name and mailing address of the ISA 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	Authorized officer Groenendijk, M

INTERNATIONAL SEARCH REPORT

Intern ' nal Application No
PC1/US 95/13099

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Categ y *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,90 08771 (RUBIN JEFFREY S ;FINCH PAUL W (US); AARONSON STUART A (US)) 9 August 1990 cited in the application The whole document;see especially p.13, lines 2-7, p.40 to 43; page 66, lines 15-25;Tab. I-1 -----	1-11

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr al Application No
PCT/US 95/13099

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