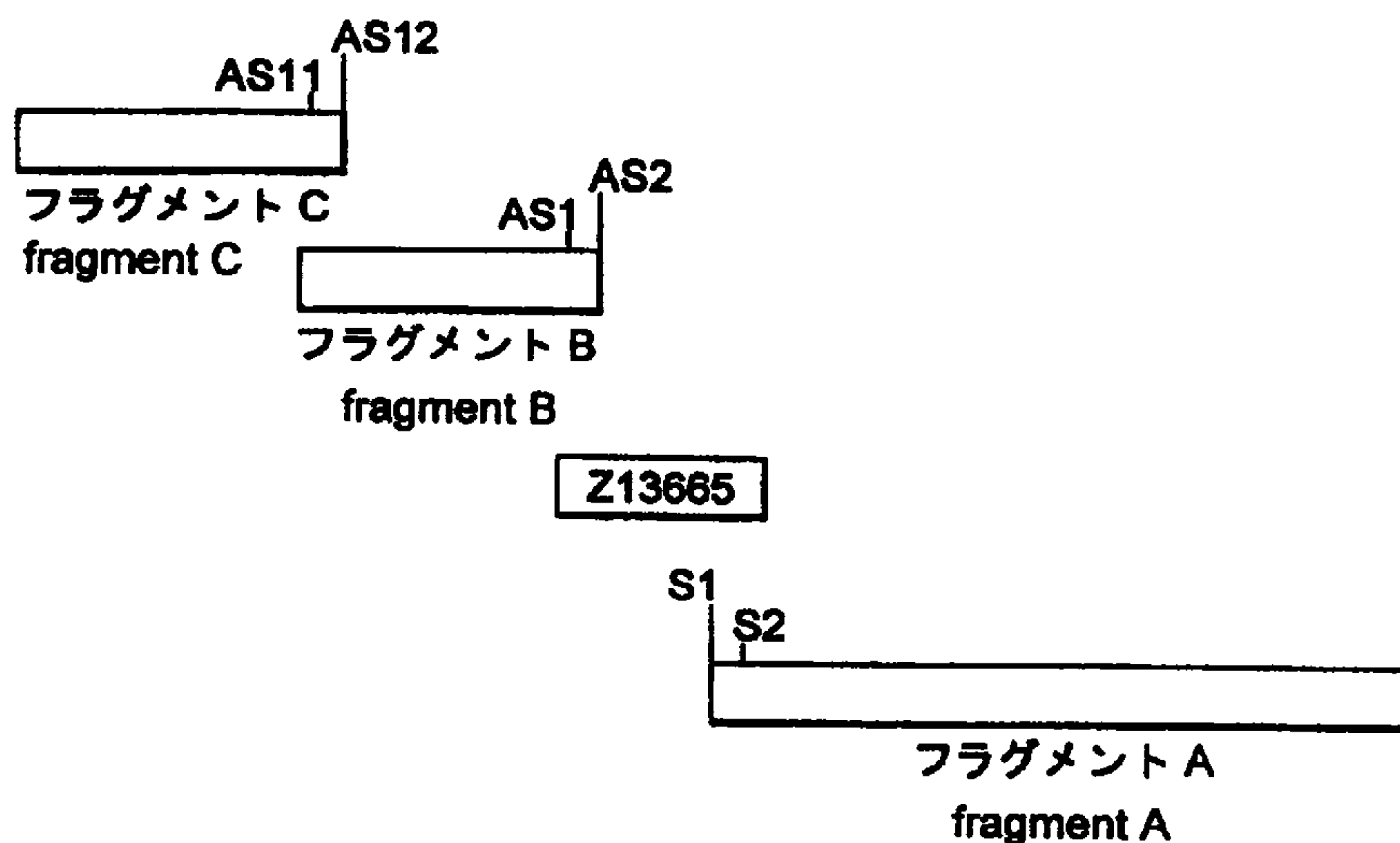




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(51) Int.Cl.⁶ C12N 15/12, C07K 14/46, C07K 16/18, C12P 21/08, C12P 21/02
(30) 1997/09/24 (9/258689) JP
(54) **PROTEINE DE VERRUES, POLYNUCLEOTIDE CODANT
CETTE PROTEINE, POLYNUCLEOTIDE ANTISENS DE
CELUI-CI ET ANTICORPS RECONNAISSANT LA PROTEINE**
(54) **WARTS PROTEIN, POLYNUCLEOTIDE ENCODING THE
SAME, ANTISENSE POLYNUCLEOTIDE THEREOF, AND
ANTIBODY RECOGNIZING THE PROTEIN**



(57) Cette invention concerne une protéine homologue qui est codée par un gène homologue à un gène de verrues de type d, et qui est isolée à partir d'un vertébré puis séquencée. Cette invention concerne également un polynucléotide codant cette protéine homologue, lequel contient un ARN, etc., qui comprend la séquence de base correspondant au gène homologue, ainsi que le polynucléotide antisens de celui-ci. Cette invention concerne enfin un anticorps capable de reconnaître la protéine homologue susmentionnée.

(57) A homologous protein encoded by a gene homologous to a d-warts gene and isolated from a vertebrate followed by sequencing; a polynucleotide encoding the homologous protein which contains an RNA, etc. comprising the base sequence corresponding to the homologous gene and the antisense polynucleotide thereof; and an antibody recognizing the above homologous protein.



ABSTRACT

This invention realizes the isolation of a homologous gene of d-warts gene from a verberate, the determination of a base sequence thereof, and provides a homologous protein encoded by the gene. The invention also provides a polynucleotide that encodes the homologous protein, and that includes such as RNA comprising a base sequence which corresponds to the homologous gene, as well as provides an antisense polynucleotides thereof. The invention further provides an antibody that recognize the homologous protein.

DESCRIPTION

WARTS PROTEIN, POLYNUCLEOTIDE ENCODING THE PROTEIN,
ANTISENSE POLYNUCLEOTIDE THEREOF AND ANTIBODY RECOGNIZING
THE PROTEIN

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Technical Field

This invention relates to a homologous protein of
Drosophila warts protein in vertebrate, particularly in
mammal as well as to a polynucleotide encoding the protein.
The invention also relates to an antisense polynucleotide
of the polynuceotide and an antibody recognizing the
homologous protein.

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Background Art

The nervous system of human beings is the most highly
differentiated one among those of vertebrates. However,
the analysis of the human nervous system has lagged behind
due to its complicated mechanisms. In particular, the
progress of analysis of tumors developing in the cranial
nerve system is slower than its counterpart analysis of other
tumors. Not only malignant tumors but also benign tumors
developing in the cranial nerve system may jeopardize the
patient's life, depending on the locations at which the
tumors develop. Therefore, improved understanding of the
developmental mechanisms of cranial nerve tumors is
desired.

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On the other hand, many mutants have been obtained in *Drosophila* during the analysis of neural development, and genetic analysis thereof has been progressing. *Drosophila* warts protein (which may be referred to as "d-warts" hereafter) encoded by *Drosophila* warts gene (which may be referred to as "d-warts gene" hereafter) has homology to DM protein kinase (DM-PK), a serine/threonine kinase; and its involvement in the signal transduction system has been suggested. The deletion of d-warts gene is known to cause the abnormal proliferation of epithelial cells and the hyperplasia of imaginal discs.

Disclosure of the Invention

It can not immediately be predicted for sure that homologous genes of the *Drosophila* gene also exist in vertebrates, particularly mammals; if homologous genes of d-warts gene do exist in vertebrates, particularly mammals, the genes can possibly be utilized to elucidate the developmental mechanisms of tumors that develop in the cranial nerve system of the vertebrates, particularly the mammals.

An object of this invention is to isolate a homologous gene of the d-warts gene from a vertebrate, to determine the base sequence of the gene, and to provide a homologous protein encoded by said gene.

Another object of the invention is to provide a

polynucleotide containing RNA or the like comprising a base sequence that corresponds to the homologous gene, which encodes the homologous protein, as well as to provide an antisense polynucleotide thereof.

5 A still further object of the invention is to provides an antibody recognizing the homologous protein.

 Specifically, the invention provides h-warts, which is a human homologous protein of d-warts, and a polynucleotide encoding the protein. Here,
10 representatives of the "polynucleotide" are DNA and RNA.

 The present inventors have searched EST database on the basis of the base sequence of d-warts gene to prepare primers, performed PCR by using the primers and a human fetal brain cDNA library as a template, isolated a h-warts gene
15 which is a human homologous gene of the d-wart gene, and determined a base sequence thereof. Further, the inventors have determined the amino acid sequence of h-warts which is a human homologous protein of d-warts.

 This invention also provides a protein (h-warts
20 variant) comprising an amino acid sequence wherein one or more amino acids of the base sequence of h-warts have been substituted or deleted, or one or more amino acids have been added to the amino acid sequence of h-warts. In these cases, homology between the variant and h-warts is preferred to
25 be 75% or greater. It is also preferred that there be no variation in the conserved portion between d-warts and

h-warts. Specifically, there should preferably be no variation in any of the following portions of amino acid sequence of h-warts (as set forth in SEQ ID NO: 1 in the Sequence Listing): from the 354th Tyr to the 366th His; from
5 the 407th Gln to the 427th Lys; from the 464th Arg to the 482nd Asn; from the 488th Leu to the 509th Asp; from the 522nd Glu to the 531st Glu; from the 538th Ser to the 577th His; from the 626th Leu to the 642nd Leu; from the 662nd Glu to the 672nd Ala; and from the 752nd His to the 764th
10 Pro.

The invention also discloses an antisense polynucleotide comprising a base sequence complementary to the base sequence of polynucleotide encoding h-warts or that encoding a h-warts variant. Here, representatives of the
15 "antisense polynucleotide" include antisense DNAs and antisense RNAs.

The invention also discloses a polynucleotide that is the whole polynucleotide encoding h-warts or a variant thereof, or a portion thereof comprising 12 or more bases.

20 This polynucleotide can be used to prepare either a partial protein of h-warts or that of the h-warts variant if it is a part of the coding region. It can also be used as a probe.

The invention further discloses an antisense
25 polynucleotide that is the whole antisense polynucleotide of h-warts or of a h-warts variant, or a portion thereof

comprising 12 or more bases.

This antisense polynucleotide can be used to inhibit the biosynthesis of either h-warts or a variant thereof. It can also be used as a probe.

5 The invention still further discloses an polynucleotide or an antisense polynucleotide that has been obtained by chemically modifying each of the polynucleotide and the antisese polynucleotide.

10 Further, the invention discloses a method whereby a portion of the polynucleotide encoding h-warts or a variant thereof or a portion of the antisese polynucleotide of h-warts or a variant thereof (including single-stranded DNA, RNA or their chemically modified forms) is used as the probe to obtain cDNA from a cDNA library of an animal other than
15 humans wherein said cDNA hybridizes to said probe and is a homologous gene of h-warts gene (also a homologous gene of d-warts gene) in an animal other than humans.

20 Further, the invention discloses cDNA obtained by the aforementioned method for acquiring the homologous gene of h-warts gene. In this specification, this homologous gene of h-warts gene is referred to as "warts gene."

25 Furthermore, the invention discloses a homologous protein of h-warts encoded by the homologous gene of h-warts gene, which is also a homologous protein of d-warts. In this specification, this homologous protein of h-warts is referred to as "warts." The "warts" can be produced by

a transformant into which the gene has been introduced.

Furthermore, the invention discloses an antibody that recognizes any of h-warts, the h-warts variant and warts.

5 **Brief Description of the Drawings**

Fig. 1 shows a comparison between the amino acid sequences of h-warts and d-warts.

10 Fig. 2 shows the positional relationship of EST, the respective primers and cDNA fragments obtained in a process to acquire h-wart gene.

Fig. 3 is a photograph of electrophoresis showing the results of northern blotting hybridization where cDNA of h-warts is hybridized to mRNAs of various human tissues.

15 Fig. 4 is a photograph of electrophoresis showing the results of northern blotting hybridization where cDNA of h-warts is hybridized to mRNAs of various human tissues.

Fig. 5 is a photograph of electrophoresis showing the results of northern blotting hybridization where cDNA of h-warts is hybridized to mRNAs of various human cell lines.

20

Best Mode for Carrying Out the Invention

As a probe that is used to obtain a warts gene in a certain kind of vertebrate from a cDNA library of the animal, it is possible to use DNA containing a highly homologous portion between d-warts and h-warts, wherein said portion encodes the amino acid sequence of a portion well conserved

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across the species.

Referring to Fig. 1, the well conserved portions in amino acid sequence between d-warts and h-warts include those of the amino acid sequence of h-warts (amino acid sequence set forth in SEQ ID NO: 1 in the Sequence Listing) enumerated in the following: from the 354th Tyr to the 366th His; from the 407th Gln to the 427th Lys; from the 464th Arg to the 482nd Asn; from the 488th Leu to the 509th Asp; from the 522nd Glu to the 531st Glu; from the 538th Ser to the 577th His; from the 626th Leu to the 642nd Leu; from the 662nd Glu to the 672nd Ala; and from the 752nd His to the 764th Pro. In Fig. 1, a numeral at the right end of a line indicates the position of a right end amino acid at the line.

DNAs encoding these portions are portions of the base sequence of h-warts gene (base sequence set forth in SEQ ID NO: 2 in the Sequence Listing) enumerated in the following: from the 1069th T to the 1107th T; from the 1228th C to the 1290th G; from the 1389th C to the 1455th T; from the 1471th C to the 1536th T; from the 1573rd G to the 1602nd A; from the 1621st A to the 1740th C; from the 1885th C to the 1935th G; from the 1993rd G to the 2025th A; and from the 2263rd C to the 2301st T.

By using DNA comprising the base sequence that contain any of the aforementioned portions as a probe, a warts gene in a certain kind of vertebrate can thus be obtained from

a cDNA library of the animal. Particularly, warts gene in a certain kind of mammal can preferably be obtained a cDNA library of the mammal. RNA comprising a base sequence that corresponds to the aforementioned DNA can also be used as the probe.

A method for acquiring warts gene from a cDNA library of a certain kind of vertebrate will be illustrated below.

1. Construction of cDNA library

A cDNA library of a target vertebrate is constructed from mRNA of the animal. Alternatively, a commercially available cDNA library may be used. Plaques on the order of 10^6 are prepared on a NZY agar medium and this library is fixed on a nitrocellulose membrane following the method disclosed in Molecular Cloning 2nd Edition (Cold Spring Harbor Laboratory Press, 1989 p.9.38-9.41). Subsequently, the membrane is washed with 2xSSC at 60 °C.

2. Acquisition of warts gene

As the probe there may be used cDNA comprising a base sequence ranging from the 1621st A to the 1740th C of the base sequence set forth in SEQ ID NO: 2 in the Sequence Listing, and a full-length warts gene may be obtained from the cDNA library according to the following protocol:

(1) Label DNA to be the probe; for example, Takara MEGA LABEL Kit (registered trademark, Takara Co. Ltd.) may be usable.

(2) Prepare then a DNA reaction solution having the

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composition described below, incubate the solution at 37 °C for 30 min, and thereafter subject the solution to heat treatment at 70 °C for 10 min to deactivate enzyme.

5	DNA probe (2 pmol/ μ l)	2 μ l
	10-fold concentrated phosphorylation buffer	2 μ l
	[γ - ³² P] ATP (370 MBq/ml) (Amersham Pharmacia Biotech Inc.)	5 μ l
	T4 polynucleotide kinase	1 μ l
10	total	10 μ l

(3) Pack SephadexG-25 (registered trademark, Amersham Pharmacia Biotech Inc.) equilibrated with T50E (50mM Tris-Cl pH 8.0, 1mM EDTA) into a 1.5ml Poly-Prep column (Bio-Rad Inc.) to make a bed volume of 1 ml, and charge the column with the DNA reaction solution heat-treated in
15
aforementioned step (2);

(4) Let each 200 μ l of T50E to flow through the column 4 times thereafter and obtain a labeled DNA probe from the
20
fraction eluted by the second 200 μ l.

(5) Hybridize 150 μ l of the above-obtained labeled DNA probe fraction to a cDNA probe of the plaques fixed on the nitrocellulose membrane under the following conditions.

25 A. Prehybridization

6 $\times$ SSC

5 × Denhardt's

0.05% sodium pyrophosphate

100 μ g/ml denatured herring sperm DNA

0.5% SDS

5 total liquid quantity: 50ml

reaction temperature: 37 °C

reaction time: 1 h

B. Hybridization

6 × SSC

10 1 × Denhardt's

0.05% sodium pyrophosphate

100 μ g/ml denatured herring sperm DNA

1×10⁶cpm/ml cDNA probe

total liquid quantity: 50ml

15 reaction temperature: 42 °C

reaction time: 18 h

(6) After hybridization, wash the nitrocellulose
membrane once under conditions C and once under conditions
20 D as described below.

C. 6 × SSC, 0.1% SDS 500 ml

temperature: 42 °C

time: 20 min

25 D. 3 × SSC, 0.1% SDS 500ml

temperature: 42 °C

time: 20 min

(7) Expose the washed nitrocellulose membrane to a Kodak AR5 film at -80°C overnight, and take an autoradiograph.

5 (8) Determine the location of positive plaques from the autoradiograph and recover the corresponding plaques on agar into a SM solution;

(9) Use the recovered plaques to form plaques again on a NZY agar medium by conventional means and fix them onto
10 a nitrocellulose membrane.

(10) Repeat steps (5) through (9) for 3 times to make positive plaques be single, recover this plaque, suspend it into $100\mu\text{l}$ of the SM solution, and stabilize the phage. It is thus possible to isolate the warts gene from the plaque.

15 3. Large-scale preparation of warts gene

(1) Mix $50\mu\text{l}$ of the phage of the plaque suspended in the SM solution with $20\mu\text{l}$ of *E. coli* Y1090r, and maintain the mixture at 37°C for 15 min.

(2) Transfer then the mixed solution to 10 ml of NZY
20 medium containing $100\mu\text{g/ml}$ ampicillin, and culture it at 37°C for 6 h.

(3) Carry out centrifugation at 8000rpm for 5 min, then collect the supernatant;

(4) Dissolve the supernatant by adding 1 ml of 5M NaCl
25 and 1.1 g of polyethylene glycol 6000 thereto.

(5) Hold this solution on ice for 1 h, and then subject

it to centrifugation at 10000 rpm at 4 °C for 20 min.

(6) Collect a precipitate and suspend it in 700 μ l of SM solution;

5 (7) Add 500 μ l of chloroform and stir the suspension to dissolve the residual *E. coli*;

(8) Carry out centrifugation at 5000 rpm for 10 min and recover the aqueous layer.

10 (9) Add each 1 μ l of 1 mg/ml RNaseA and 5mg/ml DNaseI (both available from Sigma Inc.) to the recovered aqueous layer, hold the solution at 37 °C for 1 h, and then add 600 μ l of 20% polyethylene glycol 6000 (0.8 M NaCl), and hold the solution on ice for 30 min.

(10) Carry out centrifugation at 15000rpm at 4 °C for 20 min, and collect a precipitate.

15 (11) Add 500 μ l of SM solution, 50 μ l of 5M NaCl and 50 μ l of 0.5 M EDTA to the collected precipitate, further add 400 μ l of phenol, and stir the mixture to dissolve the phage and cause cDNA to liberate.

20 (12) Subject the solution to centrifugation at 15000 rpm, at room temperature for 5 min, recover the aqueous layer, and then add 1 ml of ethanol to the aqueous layer, carry out centrifugation at 15000 rpm for 20 min and discard the liquid layer.

25 (13) Wash the precipitate with 1 ml of 70% ethanol 1ml, and then dissolve the precipitate in 100 μ l of TE solution (Tris-Cl pH 8.0, 10mM, 1mM EDTA) to obtain a DNA solution.

4. Determination of the base sequence of warts gene

The full base sequence of warts gene may be determined by the dye terminator method, when an autosequencer can be used.

5 In the case where it is found as the result of sequencing that DNA containing a whole coding region (may be referred to as "full-length DNA") could not be obtained, a combination of DNAs can be chosen so as to cover the coding region. The DNAs may be cut at suitable restriction enzyme sites which are contained in the overlapping portions, and ligated to form the full-length DNA. Alternatively, primers may be designed such that they cover the whole coding region, and the primers may be used to perform PCR with a cDNA library from the target vertebrate, preferably a mammal, as the template, whereby the full-length DNA may also be obtained.

5. Determination of amino acid sequence

The amino acid sequence of warts may be determined from the base sequence determined in aforementioned step 4. For example, this can be done by the use of a commercially available program (e.g., GENETYX®-CD program ver.34 (Software Development Inc.)).

6. Preparation of transformant

As has been described above, warts gene may be obtained. This warts gene can be inserted into a vector, which can be then introduced into a host to prepare a transformant. The transformant may be cultured in a suitable medium to

prepare a protein.

Natural mutation or artificial mutation (for example, method as described in Molecular Cloning 2nd Edition (Cold Spring Harbor Laboratory Press, 1989) pp. 15. 1-15. 113) can alter a polynucleotide without changing the principal functions of a polypeptide encoded by the polynucleotide. For h warts of this invention, it is also possible to use this method to prepare a protein, namely a h-warts variant comprising an amino acid sequence obtained by making substitution, deletion or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO:1 in the Sequence Listing.

When the technique of site-directed mutagenesis is employed to prepare a variant, a single operation normally allows several amino acids to be substituted, deleted or added. If this operation is repeated plural times, more amino acids can be substituted, deleted or added to prepare a desired variant. A h-warts variant of this invention preferably has homology of 75% or greater to the amino acid of h-warts, more preferably 90% or greater, and most preferably 95% or greater.

The amino acid sequence of h-warts variant of this invention may be determined on the basis of the base sequence of gene encoding the variant. For example, this can be done by the use of a commercially available program (e.g., GENETYX GENETYX®-CD program ver.34 (Software Development

Inc.).

Because of degeneracy in genetic codes, it is possible to substitute at least a part of bases in the base sequence of a polynucleotide with other kinds of bases without
5 altering the amino acid sequence of the polypeptide produced by the polynucleotide. Therefore, the polynucleotides of this invention that encode warts include all those containing any patterns of degeneracy.

DNA comprising the base sequence set forth in SEQ ID
10 NO: 2 in the Sequence Listing is a naturally existing h-warts gene.

This invention includes antisense polynucleotides having base sequences of antisense strands of polynucleotides encoding the foregoing h-warts, and
15 derivatives thereof. The antisense polynucleotide can be hybridized to the polynucleotide encoding h-warts, and it possibly inhibits the biosynthesis of a polypeptide encoded by the polynucleotide when the hybridized polynucleotide is a polynucleotide of the encoding region.

20 The antisense polynucleotide for inhibiting the biosynthesis of a polypeptide preferably comprises 12 or more bases. On the other hand, if the length is too long, it is unsuitable for incorporating the full-length antisense polynucleotide into a cell. The antisense
25 polynucleotide used for inhibiting the biosynthesis of warts by being incorporated into a cell comprises 12 to 30,

preferably 15 to 25, more preferably 18 to 22 bases.

The antisense polynucleotides can also be used as probes.

5 The antisense polynucleotides or portions thereof according to this invention embrace all of those in which a plurality of nucleotides comprising bases, phosphoric acids, and sugars are bonded, including those not present in nature. Typical examples of antisense polynucleotides include antisense DNAs and antisense RNAs.

10 Various kinds of antisense polynucleotide derivatives having high binding strength with target DNAs and mRNAs, tissue selectivity, cell permeability, nuclease resistance and intracellular stability can be obtained by applying known antisense technology to the antisense polynucleotides
15 of this invention.

In the viewpoint of ease of hybridization, it is generally considered advantageous that an antisense polynucleotide or a derivative thereof is designed to have a base sequence complementary to the base sequence of a
20 region which forms a stem loop. The antisense polynucleotide and a derivative thereof according to this invention can form a stem loop if necessary.

It is also expected that the antisense polynucleotides having sequences complementary to sequences existing in the
25 vicinity of the translation start codon, or at a ribosomal binding site, the capping site or a splicing site are

generally provided with a great inhibitory effect on expression. Thus, a great expression inhibitory effect is expected in the antisense polynucleotides or derivatives thereof according to this invention which contain sequences
5 complementary to sequences existing in the vicinity of the translation start codon, or at a ribosomal binding site, the capping site, or a splicing site of a gene encoding warts or those of mRNA.

At present, it is generally known that preferable
10 derivatives are those which have enhanced performance in at least one of nuclease resistance, tissue selectivity, cell permeability, and binding strength and that especially preferable derivatives are polynucleotide derivatives which have phosphorothioate bonds as their skeleton
15 structures. The polynucleotides or derivatives thereof according to this invention also embrace derivatives having these functions and structures.

If the antisense polynucleotides of the this invention are of natural type, they can be prepared by synthesis using
20 a chemical synthesizer or by the PCR method that employs the gene encoding h-warts as a template. Also, among the derivatives, there are ones that can be synthesized using a chemical synthesizer, such as the methylphosphonate type and the phosphorothioate type. In these cases, the
25 synthetic operation may be conducted according to a manual attached to the chemical synthesizer and the synthesized

products as obtained may be purified by HPLC method using reverse phase chromatography or the like to produce the desired antisense polynucleotides or derivatives thereof.

5 The polynucleotide encoding h-warts of this invention, an antisense polynucleotide thereof, or a polynucleotide that is a part of any of the foregoing (polynucleotide having a base sequence comprising 12 or more consecutive bases) can be used as a probe for screening cDNA libraries for warts genes. Here, those with GC contents of from 30 to 70% are
10 preferably usable. Further, the polynucleotides having base sequences comprising 15 or more consecutive bases are particularly preferable. The polynucleotides that are used as probes may be derivatives thereof. The sequences comprising a greater number of bases than those described
15 above are recognized as specific sequences. With respect to cDNA libraries that are used for screening with the aid of said probes, those prepared from mRNAs can preferably be used. A group of cDNAs selected by random sampling from these cDNA libraries may be employed as samples for screening.
20 Commercially available cDNAs are also usable.

The polynucleotide encoding h-warts of this invention, an antisense polynucleotide thereof, or a polynucleotide that is a part of any of the foregoing (polynucleotide having a base sequence comprising 12 or more consecutive bases)
25 can be used as a probe. The tissues with the expression of mRNAs derived from h-warts gene can be discovered by means

of northern blotting hybridization of mRNA derived from each tissue by using the polynucleotide as the probe.

When DNA or RNA is chemically synthesized, it is well known to subject the DNA or RNA to chemical modification, such as methylation or biotinylation of the side chain, or substitution of oxygen of the phosphate group by sulfur. For example, when DNA set forth in SEQ ID NO: 2 in the Sequence Listing is to be chemically synthesized, it is possible to synthesize DNA different from the DNA set forth in the Sequence Listing itself by carrying out the aforementioned chemical modification.

In addition, cDNAs can be labeled with radioisotopes even if they are derived from cDNA libraries.

Accordingly, the DNAs and RNAs of this invention encompass within their scope the chemically modified DNAs and RNAs. The chemically modified DNAs and RNAs are both able to perform a function of coding proteins as well as a function as probes. The chemically modified antisense polynucleotides are able to perform a function of inhibiting the biosynthesis of proteins as well as a function as probes.

According to the method known in the art, it is possible to obtain a transformant by introducing the plasmid into a suitable host such as *E. coli*. Warts can be produced by culturing the resulting transformant, amplifying the gene, and expressing protein. Likewise, a warts variant can be produced by culturing a transformant into which DNA encoding

the warts variant has been introduced.

The culture that contains warts or a variant thereof which the transformant has been allowed to produce is recovered, and through manipulations such as concentration, solubilization, dialysis, and various chromatographic techniques, as occasion calls, warts or a variant thereof according to this invention can be purified.

A variety of references are available for the cultivation of a transformant, and the expression of warts or a variant thereof on the basis of the base sequences described in the this invention may be carried out by a known method. In this case, any of bacteria such as *E. coli*, yeast, and animal cells is usable as a host. Particularly, animal cells are preferable. For the introduction of genes into cells, the liposome method, the electroporation method, and the like may be used.

For purification of warts or a variant thereof from the resulting cultured product, there are methods, such as immunoprecipitation, salting out, ultrafiltration, isoelectric precipitation, gel filtration, electrophoresis, ion-exchange chromatography, a variety of affinity chromatography such as hydrophobic chromatography and antibody chromatography, chromatofocusing, adsorption chromatography, and reverse phase chromatography. Purification may be carried out by selecting any of the aforementioned methods as appropriate.

Also, in the production stage, warts or a variant thereof to be produced may be prepared by a transformant as a fusion peptide with other polypeptides. In this case, the purification step requires a manipulation in which warts
5 or a variant thereof is cut out by treatment with a chemical substance such as bromocyanogen or with an enzyme such as protease.

An antibody that recognizes warts (warts antibody) or an antibody that recognizes a warts variant (warts variant
10 antibody) can be obtained, respectively, by immunizing an animal other than humans and other than that from which warts is derived, with warts, a variant thereof, or a polypeptide comprising an amino acid sequence specific to warts or a variant thereof.

15 The western blotting method, ELISA method, immunostaining method (for example, measurement by means of FACS) or the like can confirm that the warts antibody or the warts variant antibody each recognizes warts or the warts variant.

20 Also, the method using a portion of protein, which may be bound to another carrier protein (such as bovine serum albumin) as an immunogen is often employed. The portion of protein may be synthesized by using, for example, a peptide synthesizer. It is preferred that this portion of protein
25 comprise 8 or more amino acid residues.

It is well known that for the substance with apparent

antigenicity, if a polyclonal antibody can be obtained by immunization, a monoclonal antibody can be produced by a hybridoma using the lymphocyte of an immunized animal. Therefore, the antibodies of this invention also embrace
5 monoclonal antibodies within their scope.

In the this invention, the antibody also embraces active fragments. The active fragment is a fragment of the antibody having antigen-antibody reactivity, such as $F(ab')_2$, Fab' , Fab and Fv .

10 For example, $F(ab')_2$ is obtained if an antibody of this invention is digested with pepsin; and Fab is obtained if digested with papain. Fab' is obtained if $F(ab')_2$ is reduced by an reagent such as 2-mercaptoethanol and alkylated by monoiodoacetic acid. Fv is a univalent
15 antibody active fragment wherein a heavy chain variable region and a light chain valiable region are bonded to each other via a linker.

Chimera antibodies may be obtained by maintaining the above-stated active fragments while substituting
20 fragments of other animals for the other parts.

The antibodies of this invention are usable for the elucidation of functions of warts expression or the like, as well as for the elucidation of generation mechanisms of malignant tumors.

25 Detection of warts includes the method using an antibody or the method employing enzyme reaction.

Specific examples of the method using an antibody include (1) a method using a labeled warts antibody to detect warts; and (2) a method using a warts antibody and a labeled secondary antibody thereof to detect warts. The
5 markers used here include, for example, radioisotopes (RI), enzymes, avidin or biotin, or fluorescent substances (FITC, Rhodamine, etc.).

Examples of the method employing enzyme reaction include the ELISA method, immunoagglutination, western
10 blotting method, identification of immunoreactive molecules using flow cytometry, or the methods similar thereto.

EXAMPLES

This invention will be hereinbelow explained in detail
15 by way of the following examples; however, the invention is not to be limited to these examples.

(Example 1) Isolation of h-warts gene and determination of its base sequence

1. Searching EST Database

20 When EST database (database wherein sequences of mRNA fragments are registered,
<http://www.ncbi.nlm.nih.gov/dbEST>) on Internet was searched for the sequence of any homologue of the d-warts gene from the sequences (EST) registered in the database,
25 a sequence registered as Z13665 was hit.

2. Synthesis of primers

In order to acquire a DNA fragment comprising a base sequence which is a part of the base sequence of the Z13665 from human cDNA library, the following primers were synthesized.

5 Warts S1 primer 5'-TATTCTCCTCAAGCATTTAAATTC-3' (base
sequence set forth in SEQ. ID. NO: 6 in the Sequence Listing)
and warts S2 primer 5'-TTTATGGAGCAACATGTAGAAAAT -3' (base
sequence set forth in SEQ. ID. NO: 7 in the Sequence Listing)
were designed and synthesized with a DNA synthesizer (ABI
10 Model 392) as a 5' primer and a 3' primer, respectively.
Each of the synthesized primers was dissolved in distilled
water to give a concentration of 20 pmol/ μ l. These
solutions were used as PCR primers to perform PCR operations
as described below.

15 3. PCR (1)

PCR operation using a human fetal brain cDNA library,
Matathon-Ready cDNA library, (Clontech Inc.) was performed
under the following conditions.

20 PCR only towards the 3' end was performed by employing
only warts S1 primer as the primer. The PCR operation
included the steps of charging a solution having the
composition below into a test tube, overlaying mineral oil
(15 μ l) on the solution, and allowing it to stand at 94 °C
for 5 min.

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	cdNA library ($1\mu\text{g}/\mu\text{l}$)	2 μl
	dNTP mixed solution (respective NTPs were dissolved in distilled water at concentrations of 20 pmol/ μl)	
		1 μl
5	warts S1 Primer (20 pmol/ μl)	1 μl
	10-fold concentrated PCR buffer (400 mM Tris-HCl (pH 8.3), 1M KCl, 100 mM MgCl ₂ , 100 mM DTT)	
		1.5 μl
	distilled water	9 μl
10	Taq polymerase (5 units/ μl)	0.5 μl
	total	15 μl

Subsequently, the following cycle was repeated 50 times for reaction: at 60 °C for 30 sec, followed by at 72 °C for 1.5 min, and followed by at 94 °C for 30 sec.

Then, reaction was allowed to proceed at 55 °C for 2 min, finally at 72 °C for 10 min to carry out the extension reaction of fragment, thus completing the PCR operation.

Next, by employing warts S2 primer and AP2 primer of the cdNA, the PCR product obtained above was used as a template to perform a second PCR. The second PCR operation included the steps of charging a solution having the composition below into a test tube, overlaying mineral oil (20 μl) on the solution, and allowing it to stand at 94 °C for 5 min.

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	Above-obained PCR product ($1\mu\text{g}/1\mu\text{l}$)	5 μl
	dNTP mixed solution	2 μl
	warts S2 Primer (20 pmol/ μl)	1 μl
	AP2 Primer (20 pmol/ μl)	1 μl
5	10-fold concentrated PCR buffer (400 mM Tris-HCl (pH 8.3), 1 M KCl, 100 mM MgCl_2 , 100 mM DTT))	2 μl
	distilled water	8.5 μl
	Taq polymerase (5 units/ μl)	0.5 μl
	total	20 μl

10

Subsequently, the following cycle was repeated 40 times for reaction: at 60 °C for 30 sec, followed by at 72 °C for 1 min, and followed by at 94 °C for 30 sec.

Then, reaction was allowed to proceed at 55 °C for 2 min and finally at 72 °C for 10 min to carry out the extension reaction of fragment, thus completing the PCR operation.

4. Determination of DNA base sequence (1)

The DNA fragment (may be referred to as fragment A hereafter) obtained from the above-described 3'- extension reaction was subjected to mini gel electrophoresis (0.75% agarose gel), and the band of fragment A was cut out from the gel. Fragment A was recovered by means of Gene Clean (available from BIO 101 Inc.), and the band was ascertained on the mini gel electrophoresis.

Fragment A (1 μl) was taken up and diluted in 99 μl of TE. Absorbance (A_{260}) of the diluted solution was

measured at 260 nm and the DNA concentration was computed (A260, 1.0=50 μ g/ml). Fragment A was further diluted with TE so as to give a DNA concentration of 1 μ g/ μ l.

For this diluted solution, DNA sequencing was conducted by the dye-terminator method using warts S2 primer and AP2 primer with the aid of an auto-sequencer (ABI Model 373A), and the base sequence of fragment A was determined. This base sequence is set forth in SEQ ID NO: 3 in the Sequence Listing.

5. PCR(2)

In a manner similar to the aforementioned PCR, warts AS1 primer 5'-TGAAGTTGTAATCTGTTTCTTTTC-3' (base sequence set forth in SEQ. ID. NO: 8 in the Sequence Listing), which would serve as the 5' primer in the 5'-direction of Z13665, and warts AS2 primer 5'-CTTGTTTTTCCTAACAGTAATAGG -3' (base sequence set forth in SEQ. ID. NO: 9 in the Sequence Listing) as the 3' primer were designed and synthesized with a DNA synthesizer (ABI Model 392). Each of the synthesized primers was dissolved in distilled water to give a concentration of 20 pmol/ μ l. These solutions were used as PCR primers to perform a PCR operation as described below.

PCR operation using a human fetal brain cDNA library, Matathon-Ready cDNA library (Clontech Inc.) was performed under the following conditions.

PCR only towards the 5' end was performed by employing only warts AS2 primer as the primer. The PCR operation

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included the steps of charging a solution having the composition below into a test tube, overlaying mineral oil (15 μ l) on the solution, and allowing it to stand at 94 °C for 5 min.

5

cDNA library (1 μ g/ μ l)	2 μ l
dNTP mixed solution (respective NTPs were dissolved in distilled water at concentrations of 20 pmol/ μ l)	1 μ l
10 warts AS2 Primer (20 pmol/ μ l)	1 μ l
10-fold concentrated PCR buffer (400 mM Tris-HCl (pH 8.3), 1M KCl, 100 mM MgCl ₂ , 100 mM DTT)	1.5 μ l
distilled water	9 μ l
15 Taq polymerase (5 units/ μ l)	0.5 μ l
total	15 μ l

20

Subsequently, the following cycle was repeated 50 times for reaction: at 60 °C for 30 sec, followed by at 72 °C for 1.5 min, and followed by at 94 °C for 30 sec.

Then, reaction was allowed to proceed at 55 °C for 2 min, finally at 72 °C for 10 min to carry out the extension reaction of fragment, thus completing the PCR operation.

25

Next, by employing warts AS1 primer and AP2 primer of the cDNA, the PCR product obtained above was used as a template to perform a second PCR operation. The second PCR

operation included the steps of charging a solution having the composition below into a test tube, overlaying mineral oil (20 μ l) on the solution, and allowing it to stand at 94 °C for 5 min.

5

	Above-obtained PCR product (1 μ g/1 μ l)	5 μ l
	dNTP mixed solution	2 μ l
	warts AS1 Primer (20 pmol/ μ l)	1 μ l
	AP2 Primer (20 pmol/ μ l)	1 μ l
10	10-fold concentrated PCR buffer (400 mM Tris-HCl (pH 8.3), 1 M KCl, 100 mM MgCl ₂ , 100 mM DTT))	
		2 μ l
	distilled water	8.5 μ l
	Taq polymerase (5 units/ μ l)	0.5 μ l
15	total	20 μ l

Subsequently, the following cycle was repeated 40 times for reaction: at 60 °C for 30 sec, followed by at 72 °C for 1 min, and followed by at 94 °C for 30 sec.

20

Then, reaction was allowed to proceed at 55 °C for 2 min and finally at 72°C for 10 min to carry out the extension reaction of fragment, thus completing the PCR operation.

6. Determination of base Sequence (2)

25

The DNA fragment (may be referred to as fragment B hereafter) obtained from the above-described 5'- extension reaction was subjected to mini gel electrophoresis (0.75%

agarose gel), and the band of fragment B was cut out from the gel. Fragment B was recovered by means of Gene Clean (available from BIO 101 Inc.), and the band was ascertained on the mini gel electrophoresis.

5 Fragment B (1 μ l) was taken up and diluted in 99 μ l of TE. Absorbance (A260) of the diluted solution was measured at 260 nm and the DNA concentration was computed (A260, 1.0=50 μ g/ml). Fragment B was further diluted with TE so as to give a DNA concentration of 1 μ g/ μ l.

10 For this diluted solution, DNA sequencing was conducted by the dye-terminator method using warts AS1 primer and AP2 primer with the aid of an auto-sequencer (ABI Model 373A), and the base sequence of fragment B was determined. This base sequence is set forth in SEQ ID NO:
15 4 in the Sequence Listing.

7. PCR (3)

 In a manner similar to the aforementioned PCR, warts AS11 primer 5'-CTGGAGCAGAAGATGACTGAGGCC-3' (base sequence set forth in SEQ. ID. NO: 10 in the Sequence Listing), which
20 would serve as the 5' primer for the 5' end of fragment B, and warts AS12 primer 5'-CATGCCCACTGCTCGGGGATGACT-3' (base sequence set forth in SEQ. ID. NO: 11 in the Sequence Listing) as the 3' primer were designed and synthesized with a DNA synthesizer (ABI Model 392). Each of the synthesized
25 primers was dissolved in distilled water to give a concentration of 20 pmol/ μ l. These solutions were used as

PCR operation using a human fetal brain cDNA library, Matathon-Ready cDNA library (Clontech Inc.) was performed under the following conditions.

cDNA library (1 μ g/ μ l)	2 μ l
dNTP mixed solution (respective NTPs were dissolved in distilled water at concentrations of 20 pmol/ μ l)	1 μ l
warts AS12 Primer (20 pmol/ μ l)	1 μ l
10-fold concentrated PCR buffer (400 mM Tris-HCl (pH 8.3), 1M KCl, 100 mM MgCl ₂ , 100 mM DTT)	1.5 μ l
distilled water	9 μ l
Taq polymerase (5 units/ μ l)	0.5 μ l
total	15 μ l

Subsequently, the following cycle was repeated 50 times for reaction: at 60 °C for 30 sec, followed by at 72 °C for 1.5 min, and followed by at 94 °C for 30 sec.

Then, reaction was allowed to proceed at 55 °C for 2 min, finally at 72 °C for 10 min to carry out the extension reaction of fragment, thus completing the PCR operation.

Next, by employing warts AS11 primer and AP2 primer of the cDNA, the PCR product obtained above was used as a template to perform a second PCR operation. The second PCR operation included the steps of charging a solution having the composition below into a test tube, overlaying mineral oil (20 μ l) on the solution, and allowing it to stand at 94 °C for 5 min.

	Above-obtained PCR product (1 μ g/1 μ l)	5 μ l
	dNTP mixed solution	2 μ l
	warts AS11 Primer(20 pmol/ μ l)	1 μ l
15	AP2 Primer (20 pmol/ μ l)	1 μ l
	10-fold concentrated PCR buffer (400 mM Tris-HCl (pH 8.3), 1 M KCl, 100 mM MgCl ₂ , 100 mM DTT))	2 μ l
	distilled water	8.5 μ l
20	Taq polymerase (5 units/ μ l)	0.5 μ l
	total	20 μ l

Subsequently, the following cycle was repeated 40 times for reaction: at 60 °C for 30 sec, followed by at 72 °C for 1 min, and followed by at 94 °C for 30 sec.

Then, reaction was allowed to proceed at 55 °C for 2

min and finally at 72°C for 10 min to carry out the extension reaction of fragment, thus completing the PCR operation.

8. Determination of base Sequence (3)

The DNA fragment (may be referred to as fragment C hereafter) obtained from the above-described 5'-extension reaction was subjected to mini gel electrophoresis (0.75% agarose gel), and the band of fragment C was cut out from the gel. Fragment B was recovered by means of Gene Clean (BIO 101 Inc.), and the band was ascertained on the mini gel electrophoresis.

Fragment C (1 μ l) was taken up and diluted in 99 μ l of TE. Absorbance (A₂₆₀) of the diluted solution was measured at 260 nm and the DNA concentration was computed (A₂₆₀, 1.0=50 μ g/ml). Fragment C was further diluted with TE so as to give a DNA concentration of 1 μ g/ μ l.

For this diluted solution, DNA sequencing was conducted by the dye-terminator method using warts AS11 primer and AP2 primer with the aid of an auto-sequencer (ABI Model 373A), and the base sequence of fragment C was determined. This base sequence is set forth in SEQ ID NO: 5 in the Sequence Listing.

9. Acquisition of h-warts gene

S17 sense primer, 5'-TCTGGAAACATGGAATACGTAATC-3' (base sequence set forth in SEQ ID NO: 12 in the Sequence Listing) corresponding to a part of fragment C, and AS22 antisense primer, 5'-TATGGGTAGCCATTGTCATCAAAA-3' (base

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sequence set forth in SEQ ID NO: 13 in the Sequence Listing) corresponding to a part of fragment A were synthesized with a DNA synthesizer (ABI Model 392) as a 5'-primer and a 3'-primer, respectively.

5 PCR reaction was performed by using these primers and using a human fetal brain cDNA library (Clontech Inc.) as the template. A solution having the composition below was charged into a test tube; mineral oil (20 μ l) was overlaid on the solution; and it is allowed to stand at 94 ° C for
10 5 min.

human fetal brain cDNA library (1 μ g/ μ l)	5 μ l
dNTP mixed solution (respective NTPs were dissolved in distilled water at concentrations of 20 pmol/ μ l)	
	1 μ l
S22 sense primer (20 pmol/ μ l)	1 μ l
AS22 antisense primer (20 pmol/ μ l)	1 μ l
10-fold concentrated PCR buffer (400 mM Tris-HCl (pH 8.3), 1M KCl, 100 mM MgCl ₂ , 100 mM DTT)	
	2 μ l
Taq polymerase (5 units/ μ l)	0.5 μ l
distilled water	9.5 μ l
total	20 μ l

25 Subsequently, the following cycle was repeated 40 times for reaction: at 60 °C for 30 sec, followed by at 72 °C

for 2 min, and followed by at 94 °C for 30 sec. Then, reaction was allowed to proceed at 55 °C for 2 min, finally at 72 °C for 10 min, thus completing the PCR operation.

5 After the reaction, the PCR product was subjected to mini gel electrophoresis (0.75% agarose gel). As a result, a band of about 2440 kbp was observed.

Thus, the h-warts gene was isolated.

The base sequence of h-warts gene is set forth in SEQ ID NO: 2 in the Sequence Listing.

10 The positional relationship among the full-length h-warts gene, fragment A, fragment B, fragment C, and Z13665 is shown in Fig. 2.

10. Determination of amino acid sequence

15 The amino acid sequence of h-warts was determined based on the above-described base sequence. This amino acid sequence is set forth in SEQ ID NO: 1 in the Sequence Listing. Homology between the h-warts protein and the d-warts protein is about 70%, and the proteins are well conserved between these species. A comparison of both proteins is shown in
20 Fig. 1. In Fig. 1, "h-wts" denotes h-warts and "d-wts" denotes d-warts. DM-PK domain of h-warts corresponds to a portion ranging from the 405th Leu to the 768th Trp of the amino acid set forth in SEQ ID NO: 1 in the Sequence Listing. Homology between h-warts and DM-PK is about 50%.

25 The primary structure of h-warts suggests the involvement of h-warts in signal transmission and

differentiation and proliferation, particularly in malignant tumors.

11. Preparation of transformant

A full-length h-warts gene comprising the above-mentioned base sequence was inserted into a pBj-Myc vector and a transformant was prepared by introducing the vector to *E. coli* (DH5 α). This transformant was designated "myc-warts" and deposited on September 24, 1997 in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry locating at 1-1-3 Higashi, Tsukuba, Ibaragi, Japan 305-8566 (Accession No. FERM P-16441), and it was transferred on August 24, 1998 to International Deposition (Accession No. FERM BP-6470) under Budapest Treaty.

(Example 2) Expression of h-warts in a variety of human tissues

The fragment A that had been labeled through manipulations as described in (1) to (4) below was hybridized under high stringency conditions to each membrane of Human Tissue Northern Blot I, II and Human Cell Line Multiple Tissue Northern Blot (Clontech Inc.) in which 2 μ g of poly(A)+RNA (mRNA) from a variety of human tissues or from a variety of human cells was blotted therein according to the description in Molecular Cloning: A Laboratory Manual 2nd Edition, p 7.39-7.52. Analysis was conducted by

northern blotting hybridization.

(1) Fragment A of the h-warts gene was labeled with a Takara MEGA Labeling Kit (registered trademark, Takara Co. Ltd.).

5 (2) Then, a DNA reaction solution having the following composition was prepared, and after this solution was incubated at 37 °C for 30 min, it was subjected to heat treatment at 70 °C for 10 min to deactivate enzyme.

DNA probe (2pmol/ μ l)	2 μ l
10-fold concentrated phosphorylation buffer	2 μ l
[γ - ³² P]ATP(370 MBq/ml) (Amersham Pharmacia Biotech Inc.)	5 μ l
T4 polynucleotide kinase	1 μ l
total	10 μ l

15 (3) SephadexG-25 (registered trademark, Amersham Pharmacia Biotech Inc.) equilibrated by T50E (50mM Tris-Cl pH 8.0, 1mM EDTA) was packed into 1.5ml Poly-Prep column (Bio-Rad Inc.) to obtain a bed volume of 1 ml, and the DNA reaction solution that had been subjected to heat treatment
20 in the aforementioned step (2) was charged on the column.

(4) Thereafter, each 200 μ l of T50E was poured through the column 4 times to obtain a labeled DNA probe from the fraction eluted by the second 200 μ l.

25 Photographs showing the results with respect to a variety of tissues are shown in Figs. 3 and 4. The expression of mRNA of h-warts (a band of 9.5 kb) was found in all the

tissues other than the lung and kidney from these photographs.

Fig. 5 is a photograph showing the results with respect to a variety of cells. From this photograph, the expression was recognized in all the cells other than A549 cell derived from the lung cancer.

(Example 3) Production of recombinant h-warts protein

The full-length h-warts gene was inserted into a pBJ-Myc vector, and a transformant was prepared by introducing the vector to a COS cell (ATCC CRL-1651).

This transformant (1×10^6) was added into a cell lysing solution (100 μ l) comprising 50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 50 mM iodoacetamide, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1% NaN_3 , 10 μ g/ml soybean trypsin inhibitor, 1 μ g/ml aprotinin, 1 mM PMSF (phenyl methyl sulfonyl fluoride) and 1 μ g/ml leupeptin, and was dissolved by stirring thoroughly. After allowing to stand on ice for 60 min, the solution was centrifuged at 15,000 rpm for 10 min and the supernatant was recovered.

To the recovered supernatant was added 50 ml of CNBr-activated Sepharose beads (Amersham Pharmacia Biotech Inc.) to which 1 mg of IgG from a non-immunized rabbit (antibody concentration of 1 mg/1 ml resin) had been bound. After overnight reaction at 4 °C, it was centrifuged to remove nonspecific binding proteins bound to the beads and the supernatant was recovered.

This supernatant (10 μ l) was mixed with an equal quantity of SDS-PAGE sample buffer and 1 μ l of 2-mercaptoethanol (2ME), and heat treatment was carried out at 95 °C for 5 min. Then, the mixture was electrophoresed on a 5-20% gradient gel.

After electrophoresis, the gel was stained with coomassie brilliant blue. As a result, a band at the position of about 88kD was observed and production of the recombinant h-warts was ascertained.

(Example 4) Chromosomal mapping

The chromosomal mapping of the full-length h-warts gene was determined by PCR using radiation hybrid cells. Primer wts S6, 5'-ACACAGGCTCAGAGATTAAAA-3' (base sequence set forth in SEQ ID NO: 14 in the Sequence Listing) and primer wts AS7, 5'-CCTCGAATTCCAGGCCCTTTTACAA-3' (base sequence set forth in SEQ ID NO: 15 in the Sequence Listing) were designed out of the warts sequence. Then, PCR was performed against 93 radiation hybrid clones of human whole genome (Research Genetics Inc.). As a result, the section numbers of panels to which the aforementioned primers (wts S6 and wts AS7) had been hybridized were reported via e-mail to the Whitehead Institute/MIT Center for Genome Research (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>.) and the information on the chromosomal positions of the panels and base sequences of the clone was acquired. When EST database was searched for the base sequences, it

turned out that plural base sequences registered with EST overlapped a part of the base sequence of the h-warts gene. Consequently, it was determined that h-warts was located between D6S311 and WI5215 both of which had been registered with EST database, i.e., on Chromosome 6 long arm, 6q25.

INDUSTRIAL APPLICABILITY

This invention has, for the first time, revealed that a homologous protein of *Drosophila* warts exists in vertebrate. It is anticipated that warts of the invention is involved in signal transmission, as well as in the differentiation and abnormal proliferation of epithelial cells. Accordingly, warts of the invention, a gene therefor, and an antibody recognizing warts are effective for analysing the developmental mechanisms of tumors. The h-warts gene of the invention has higher homology to warts genes of other vertebrates from cDNA libraries thereof, particularly those of mammals as compared to d-warts. Thus, polynucleotides encoding h-warts, antisense polynucleotides thereof, or portions thereof are suited as probes with which warts genes of the other vertebrates, particularly those of mammals may be obtained.

SEQUENCE LISTING

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<120> A warts protein, a polynucleotide coding the protein, an antisense polynucleotide to the polynucleotide and an antibody recognizing the protein

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<150> JP 9-258689

<151> 1997-09-24

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35 40 45

Pro Ile Ile Met Gln Ser Ser Ser Lys Phe Asn Phe Pro Ser Gly Arg

50 55 60

Pro Gly Met Gln Asn Gly Thr Gly Gln Thr Asp Phe Met Ile His Gln

65 70 75 80

Asn Val Val Pro Ala Gly Thr Val Asn Arg Gln Pro Pro Pro Pro Tyr

85 90 95

Pro Leu Thr Ala Ala Asn Gly Gln Ser Pro Ser Ala Leu Gln Thr Gly

100 105 110

Gly Ser Ala Ala Pro Ser Ser Tyr Thr Asn Gly Ser Ile Pro Gln Cys

115 120 125

Met Met Val Pro Asn Arg Asn Ser His Asn Met Glu Leu Tyr Asn Ile

130 135 140

Ser Val Pro Gly Leu Gln Thr Asn Trp Arg Gln Ser Ser Ser Ala Pro

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290	295	300
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485

490

495

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500

505

510

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515

520

525

Ile Ala Glu Leu Thr Cys Ala Val Glu Ser Val His Lys Met Gly Phe

530

535

540

Ile His Arg Asp Ile Lys Pro Asp Asn Ile Leu Ile Asp Arg Asp Gly

545

550

555

560

His Ile Lys Leu Thr Asp Phe Gly Leu Cys Thr Gly Phe Arg Trp Thr

565

570

575

His Asp Ser Lys Tyr Tyr Gln Ser Gly Asp His Pro Arg Gln Asp Ser

580

585

590

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595

600

605

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Gln Thr Pro Leu Glu Thr Gln Met Lys Val Ile Asn Trp Gln Thr Ser			
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15

20

25

30

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35

40

45

aga caa cca atc atc atg cag agt tct agc aaa ttt aac ttt cca tca 195

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50

55

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65

70

75

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His Gln Asn Val Val Pro Ala Gly Thr Val Asn Arg Gln Pro Pro Pro

80

85

90

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95

100

105

110

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Thr Gly Gly Ser Ala Ala Pro Ser Ser Tyr Thr Asn Gly Ser Ile Pro

115

120

125

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gct cca gcc cag tca tcc ccg agc agt ggg cat gaa atc cct aca tgg 531			
Ala Pro Ala Gln Ser Ser Pro Ser Ser Gly His Glu Ile Pro Thr Trp			
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Thr Ala Ile Thr Pro Ala Pro Ile Gln Gln Pro Val Lys Ser Met Arg			
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Val Leu Lys Pro Glu Leu Gln Thr Ala Leu Ala Pro Thr His Pro Ser			
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Trp Ile Pro Gln Pro Ile Gln Thr Val Gln Pro Ser Pro Phe Pro Glu

240

245

250

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255

260

265

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275

280

285

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290

295

300

gat cag cca agc ttg ccc aag gaa gat gag agt gaa aag agt tat gaa 963

Asp Gln Pro Ser Leu Pro Lys Glu Asp Glu Ser Glu Lys Ser Tyr Glu

305

310

315

aat gtt gat agt ggg gat aaa gaa aag aaa cag att aca act tca cct 1011

Asn Val Asp Ser Gly Asp Lys Glu Lys Lys Gln Ile Thr Thr Ser Pro

320

325

330

att act gtt agg aaa aac aag aaa gat gaa gag cga agg gaa tct cgt 1059

Ile Thr Val Arg Lys Asn Lys Lys Asp Glu Glu Arg Arg Glu Ser Arg

335	340	345	350	
att caa agt tat tct cct caa gca ttt aaa ttc ttt atg gag caa cat				1107
Ile Gln Ser Tyr Ser Pro Gln Ala Phe Lys Phe Phe Met Glu Gln His				
	355	360	365	
gta gaa aat gta ctc aaa tct cat cag cag cgt cta cat cgt aaa aaa				1155
Val Glu Asn Val Leu Lys Ser His Gln Gln Arg Leu His Arg Lys Lys				
	370	375	380	
caa tta gag aat gaa atg atg cgg gtt gga tta tct caa gat gcc cag				1203
Gln Leu Glu Asn Glu Met Met Arg Val Gly Leu Ser Gln Asp Ala Gln				
	385	390	395	
gat caa atg aga aag atg ctt tgc caa aaa gaa tct aat tac atc cgt				1251
Asp Gln Met Arg Lys Met Leu Cys Gln Lys Glu Ser Asn Tyr Ile Arg				
	400	405	410	
ctt aaa agg gct aaa atg gac aag tct atg ttt gtg aag ata aag aca				1299
Leu Lys Arg Ala Lys Met Asp Lys Ser Met Phe Val Lys Ile Lys Thr				
415	420	425	430	
cta gga ata gga gca ttt ggt gaa gtc tgt cta gca aga aaa gta gat				1347
Leu Gly Ile Gly Ala Phe Gly Glu Val Cys Leu Ala Arg Lys Val Asp				
	435	440	445	

act aag gct ttg tat gca aca aaa act ctt cga aag aaa gat gtt ctt 1395

Thr Lys Ala Leu Tyr Ala Thr Lys Thr Leu Arg Lys Lys Asp Val Leu

450

455

460

ctt cga aat caa gtc gct cat gtt aag gct gag aga gat atc ctg gct 1443

Leu Arg Asn Gln Val Ala His Val Lys Ala Glu Arg Asp Ile Leu Ala

465

470

475

gaa gct gac aat gaa tgg gta gtt cgt cta tat tat tca ttc caa gat 1491

Glu Ala Asp Asn Glu Trp Val Val Arg Leu Tyr Tyr Ser Phe Gln Asp

480

485

490

aag gac aat tta tac ttt gta atg gac tac att cct ggg ggt gat atg 1539

Lys Asp Asn Leu Tyr Phe Val Met Asp Tyr Ile Pro Gly Gly Asp Met

495

500

505

510

atg agc cta tta att aga atg ggc atc ttt cca gaa agt ctg gca cga 1587

Met Ser Leu Leu Ile Arg Met Gly Ile Phe Pro Glu Ser Leu Ala Arg

515

520

525

ttc tac ata gca gaa ctt acc tgt gca gtt gaa agt gtt cat aaa atg 1635

Phe Tyr Ile Ala Glu Leu Thr Cys Ala Val Glu Ser Val His Lys Met

530

535

540

ggt ttt att cat aga gat att aaa cct gat aat att ttg att gat cgt 1683

Gly Phe Ile His Arg Asp Ile Lys Pro Asp Asn Ile Leu Ile Asp Arg

545	550	555	
gat ggt cat att aaa ttg act gac ttt ggc ctc tgc act ggc ttc aga			1731
Asp Gly His Ile Lys Leu Thr Asp Phe Gly Leu Cys Thr Gly Phe Arg			
560	565	570	
tgg aca cac gat tct aag tac tat cag agt ggt gac cat cca cgg caa			1779
Trp Thr His Asp Ser Lys Tyr Tyr Gln Ser Gly Asp His Pro Arg Gln			
575	580	585	590
gat agc atg gat ttc agt aat gaa tgg ggg gat ccc tca agc tgt cga			1827
Asp Ser Met Asp Phe Ser Asn Glu Trp Gly Asp Pro Ser Ser Cys Arg			
	595	600	605
tgt gga gac aga ctg aag cca tta gag cgg aga gct gca cgc cag cac			1875
Cys Gly Asp Arg Leu Lys Pro Leu Glu Arg Arg Ala Ala Arg Gln His			
610	615	620	
cag cga tgt cta gca cat tct ttg gtt ggg act ccc aat tat att gca			1923
Gln Arg Cys Leu Ala His Ser Leu Val Gly Thr Pro Asn Tyr Ile Ala			
625	630	635	
cct gaa gtg ttg cta cga aca gga tac aca cag ttg tgt gat tgg tgg			1971
Pro Glu Val Leu Leu Arg Thr Gly Tyr Thr Gln Leu Cys Asp Trp Trp			
640	645	650	

agt gtt ggt gtt att ctt ttt gaa atg ttg gtg gga caa cct cct ttc 2019

Ser Val Gly Val Ile Leu Phe Glu Met Leu Val Gly Gln Pro Pro Phe

655 660 665 670

ttg gca caa aca cca tta gaa aca caa atg aag gtt atc aac tgg caa 2067

Leu Ala Gln Thr Pro Leu Glu Thr Gln Met Lys Val Ile Asn Trp Gln

675 680 685

aca tct ctt cac att cca cca caa gct aaa ctc agt cct gaa gct tct 2115

Thr Ser Leu His Ile Pro Pro Gln Ala Lys Leu Ser Pro Glu Ala Ser

690 695 700

gat ctt att att aaa ctt tgc cga gga ccc gaa gat cgc tta ggc aag 2163

Asp Leu Ile Ile Lys Leu Cys Arg Gly Pro Glu Asp Arg Leu Gly Lys

705 710 715

aat ggt gct gat gaa ata aaa gct cat cca ttt ttt aaa aca att gat 2211

Asn Gly Ala Asp Glu Ile Lys Ala His Pro Phe Phe Lys Thr Ile Asp

720 725 730

ttc tcc agt gac ctg aga cag cag tct gct tca tac att cct aaa atc 2259

Phe Ser Ser Asp Leu Arg Gln Gln Ser Ala Ser Tyr Ile Pro Lys Ile

735 740 745 750

aca cac cca aca gat aca tca aat ttt gat cct gtt gat cct gat aaa 2307

Thr His Pro Thr Asp Thr Ser Asn Phe Asp Pro Val Asp Pro Asp Lys

755

760

765

tta tgg agt gat gat aac gag gaa gaa aat gta aat gac act ctc aat 2355

Leu Trp Ser Asp Asp Asn Glu Glu Glu Asn Val Asn Asp Thr Leu Asn

770

775

780

ggg atg gta taa aaatggaaag catcctgaac atgcattcta tgaatttacc 2407

Gly Met Val

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1

5

10

15

gta ctc aaa tct cat cag cag cgt cta cat cgt aaa aaa caa tta gag 96

Val Leu Lys Ser His Gln Gln Arg Leu His Arg Lys Lys Gln Leu Glu

20

25

30

aat gaa atg atg cgg gtt gga tta tct caa gat gcc cag gat caa atg 144

Asn Glu Met Met Arg Val Gly Leu Ser Gln Asp Ala Gln Asp Gln Met

35

40

45

aga aag atg ctt tgc caa aaa gaa tct aat tac atc cgt ctt aaa agg 192

Arg Lys Met Leu Cys Gln Lys Glu Ser Asn Tyr Ile Arg Leu Lys Arg

50

55

60

gct aaa atg gac aag tct atg ttt gtg aag ata aag aca cta gga ata 240

Ala Lys Met Asp Lys Ser Met Phe Val Lys Ile Lys Thr Leu Gly Ile

65

70

75

80

gga gca ttt ggt gaa gtc tgt cta gca aga aaa gta gat act aag gct 288

Gly Ala Phe Gly Glu Val Cys Leu Ala Arg Lys Val Asp Thr Lys Ala

85

90

95

ttg tat gca aca aaa act ctt cga aag aaa gat gtt ctt ctt cga aat 336

Leu Tyr Ala Thr Lys Thr Leu Arg Lys Lys Asp Val Leu Leu Arg Asn

100

105

110

caa gtc gct cat gtt aag gct gag aga gat atc ctg gct gaa gct gac 384

Gln Val Ala His Val Lys Ala Glu Arg Asp Ile Leu Ala Glu Ala Asp

115

120

125

aat gaa tgg gta gtt cgt cta tat tat tca ttc caa gat aag gac aat 432

Asn Glu Trp Val Val Arg Leu Tyr Tyr Ser Phe Gln Asp Lys Asp Asn

130

135

140

tta tac ttt gta atg gac tac att cct ggg ggt gat atg atg agc cta 480

Leu Tyr Phe Val Met Asp Tyr Ile Pro Gly Gly Asp Met Met Ser Leu

145

150

155

160

tta att aga atg ggc atc ttt cca gaa agt ctg gca cga ttc tac ata 528

Leu Ile Arg Met Gly Ile Phe Pro Glu Ser Leu Ala Arg Phe Tyr Ile

165

170

175

gca gaa ctt acc tgt gca gtt gaa agt gtt cat aaa atg ggt ttt att 576

Ala Glu Leu Thr Cys Ala Val Glu Ser Val His Lys Met Gly Phe Ile

180

185

190

cat aga gat att aaa cct gat aat att ttg att gat cgt gat ggt cat 624

His Arg Asp Ile Lys Pro Asp Asn Ile Leu Ile Asp Arg Asp Gly His

195

200

205

att aaa ttg act gac ttt ggc ctc tgc act ggc ttc aga tgg aca cac 672

Ile Lys Leu Thr Asp Phe Gly Leu Cys Thr Gly Phe Arg Trp Thr His

210

215

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gat tct aag tac tat cag agt ggt gac cat cca cgg caa gat agc atg 720

Asp Ser Lys Tyr Tyr Gln Ser Gly Asp His Pro Arg Gln Asp Ser Met
 225 230 235 240

gat ttc agt aat gaa tgg ggg gat ccc tca agc tgt cga tgt gga gac 768
 Asp Phe Ser Asn Glu Trp Gly Asp Pro Ser Ser Cys Arg Cys Gly Asp
 245 250 255

aga ctg aag cca tta gag cgg aga gct gca cgc cag cac cag cga tgt 816
 Arg Leu Lys Pro Leu Glu Arg Arg Ala Ala Arg Gln His Gln Arg Cys
 260 265 270

cta gca cat tct ttg gtt ggg act ccc aat tat att gca cct gaa gtg 864
 Leu Ala His Ser Leu Val Gly Thr Pro Asn Tyr Ile Ala Pro Glu Val
 275 280 285

ttg cta cga aca gga tac aca cag ttg tgt gat tgg tgg agt gtt ggt 912
 Leu Leu Arg Thr Gly Tyr Thr Gln Leu Cys Asp Trp Trp Ser Val Gly
 290 295 300

gtt att ctt ttt gaa atg ttg gtg gga caa cct cct ttc ttg gca caa 960
 Val Ile Leu Phe Glu Met Leu Val Gly Gln Pro Pro Phe Leu Ala Gln
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 Thr Pro Leu Glu Thr Gln Met Lys Val Ile Asn Trp Gln Thr Ser Leu
 325 330 335

cac att cca cca caa gct aaa ctc agt cct gaa gct tct gat ctt att 1056

His Ile Pro Pro Gln Ala Lys Leu Ser Pro Glu Ala Ser Asp Leu Ile

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345

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att aaa ctt tgc cga gga ccc gaa gat cgc tta ggc aag aat ggt gct 1104

Ile Lys Leu Cys Arg Gly Pro Glu Asp Arg Leu Gly Lys Asn Gly Ala

355

360

365

gat gaa ata aaa gct cat cca ttt ttt aaa aca att gat ttc tcc agt 1152

Asp Glu Ile Lys Ala His Pro Phe Phe Lys Thr Ile Asp Phe Ser Ser

370

375

380

gac ctg aga cag cag tct gct tca tac att cct aaa atc aca cac cca 1200

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385

390

395

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aca gat aca tca aat ttt gat cct gtt gat cct gat aaa tta tgg agt 1248

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405

410

415

gat gat aac gag gaa gaa aat gta aat gac act ctc aat ggg atg gta 1296

Asp Asp Asn Glu Glu Glu Asn Val Asn Asp Thr Leu Asn Gly Met Val

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5

10

15

tcc ccg agc agt ggg cat gaa atc cct aca tgg caa cct aac ata cca 96

Ser Pro Ser Ser Gly His Glu Ile Pro Thr Trp Gln Pro Asn Ile Pro

20

25

30

gtg agg tca aat tct ttt aat aac cca tta gga aat aga gca agt cac 144

Val Arg Ser Asn Ser Phe Asn Asn Pro Leu Gly Asn Arg Ala Ser His

35

40

45

tct gct aat tct cag cct tct gct aca aca gtc act gca att aca cca 192

Ser Ala Asn Ser Gln Pro Ser Ala Thr Thr Val Thr Ala Ile Thr Pro

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gct cct att caa cag cct gtg aaa agt atg cgt gta tta aaa cca gag 240			
Ala Pro Ile Gln Gln Pro Val Lys Ser Met Arg Val Leu Lys Pro Glu			
65	70	75	80
cta cag act gct tta gca cct aca cac cct tct tgg ata cca cag cca 288			
Leu Gln Thr Ala Leu Ala Pro Thr His Pro Ser Trp Ile Pro Gln Pro			
	85	90	95
att caa act gtt caa ccc agt cct ttt cct gag gga acc gct tca aat 336			
Ile Gln Thr Val Gln Pro Ser Pro Phe Pro Glu Gly Thr Ala Ser Asn			
	100	105	110
gtg act gtg atg cca cct gtt gct gaa gct cca aac tat caa gga cca 384			
Val Thr Val Met Pro Pro Val Ala Glu Ala Pro Asn Tyr Gln Gly Pro			
	115	120	125
cca cca ccc tac cca aaa cat ctg ctg cac caa aac cca tct gtt cct 432			
Pro Pro Pro Tyr Pro Lys His Leu Leu His Gln Asn Pro Ser Val Pro			
	130	135	140
cca tac gag tca atc agt aag cct agc aaa gag gat cag cca agc ttg 480			
Pro Tyr Glu Ser Ile Ser Lys Pro Ser Lys Glu Asp Gln Pro Ser Leu			
145	150	155	160

ccc aag gaa gat gag agt gaa aag agt tat gaa aat gtt gat agt ggg 528

Pro Lys Glu Asp Glu Ser Glu Lys Ser Tyr Glu Asn Val Asp Ser Gly

165

170

175

gat aaa gaa aag aaa cag att aca act tca cct att act gtt agg aaa 576

Asp Lys Glu Lys Lys Gln Ile Thr Thr Ser Pro Ile Thr Val Arg Lys

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185

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gctcacccta gcaacggaca gagagtgaac cccccaccac cactcaagt aaggagtgtt 180

actcctccac cacctccaag aggccagact cccctccaa gaggtacaac tccacctccc 240

ccttcatggg aaccaaactc tcaaacaag cgctattctg gaaac atg gaa tac gta 297

Met Glu Tyr Val

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atc tcc cga atc tct cct gtc cca cct ggg gca tgg caa gag ggc tat 345

Ile Ser Arg Ile Ser Pro Val Pro Pro Gly Ala Trp Gln Glu Gly Tyr

5 10 15 20

cct cca cca ctt ttc aac act tcc ccc atg aat cct cct aat caa gga 393

Pro Pro Pro Leu Phe Asn Thr Ser Pro Met Asn Pro Pro Asn Gln Gly

25 30 35

cag aga ggc att agt tct gtt cct gtt ggc aga caa cca atc atc atg 441

Gln Arg Gly Ile Ser Ser Val Pro Val Gly Arg Gln Pro Ile Ile Met

40 45 50

cag agt tct agc aaa ttt aac ttt cca tca ggg aga cct gga atg cag 489

Gln Ser Ser Ser Lys Phe Asn Phe Pro Ser Gly Arg Pro Gly Met Gln

55 60 65

aat ggt act gga caa act gat ttc atg ata cac caa aat gtt gtc cct 537

Asn Gly Thr Gly Gln Thr Asp Phe Met Ile His Gln Asn Val Val Pro

70 75 80

gct ggc act gtg aat cgg cag cca cca cct cca tat cct ctg aca gca 585

Ala Gly Thr Val Asn Arg Gln Pro Pro Pro Pro Tyr Pro Leu Thr Ala

85

90

95

100

gct aat gga caa agc cct tct gct tta caa aca ggg gga tct gct gct 633

Ala Asn Gly Gln Ser Pro Ser Ala Leu Gln Thr Gly Gly Ser Ala Ala

105

110

115

cct tcg tca tat aca aat gga agt att cct cag tgt atg atg gtg cca 681

Pro Ser Ser Tyr Thr Asn Gly Ser Ile Pro Gln Cys Met Met Val Pro

120

125

130

aac aga aat agt cat aac atg gaa cta tat aac att agt gta cct gga 729

Asn Arg Asn Ser His Asn Met Glu Leu Tyr Asn Ile Ser Val Pro Gly

135

140

145

ctg caa aca aat tgg cgt cag tca tct tct gct cca gcc cag tca tcc 777

Leu Gln Thr Asn Trp Arg Gln Ser Ser Ser Ala Pro Ala Gln Ser Ser

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155

160

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Pro Ser Ser Gly His Glu

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170

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24

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24

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<400> 11

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24

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24

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24

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21

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cctcgaattc caggcccttt taaa

25

CLAIMS

1. A protein comprising an amino acid sequence set forth in SEQ ID NO: 1 in the Sequence Listing.

5 2. A protein comprising an amino acid sequence obtained by deleting or substituting one or more amino acids of the amino acid sequence set forth in SEQ ID NO:1 in the Sequence Listing, or adding different amino acid(s) to the amino acid sequence set forth in SEQ ID NO: 1 in the Sequence Listing.

10 3. A polynucleotide encoding the protein according to claim 1 or 2.

4. An antisense polynucleotide comprising a base sequence of an antisense strand of the polynucleotide according to claim 3 or a derivative thereof.

15 5. A polynucleotide or an antisense polynucleotide comprising 12 or more consecutive bases which is a part of the polynucleotide according to claim 3 or which is a part of the antisense polynucleotide according to claim 4.

20 6. The polynucleotide or the antisense polynucleotide according to any of claims 3 - 5, wherein said polynucleotide or said antisense polynucleotide is chemically modified.

25 7. A method for acquiring a cDNA homologous to a DNA comprising a base sequence set forth in SEQ ID NO: 2 in the Sequence Listing, said method using the polynucleotide or the antisense polynucleotide according to claim 5 or 6 as a probe to acquire the cDNA hybridizing to said probe from a cDNA library of a vertebrate.

8. A cDNA acquired by the method according to claim 7, said cDNA being homologous to the DNA comprising a base sequence set forth in SEQ ID NO: 2 in the Sequence Listing.

5 9. A protein homologous to the protein according to claim 1 and comprising an amino acid sequence encoded by the cDNA according to claim 8.

10. An antibody recognizing the protein according to any of claims 1, 2 and 9.

SEI 98-32

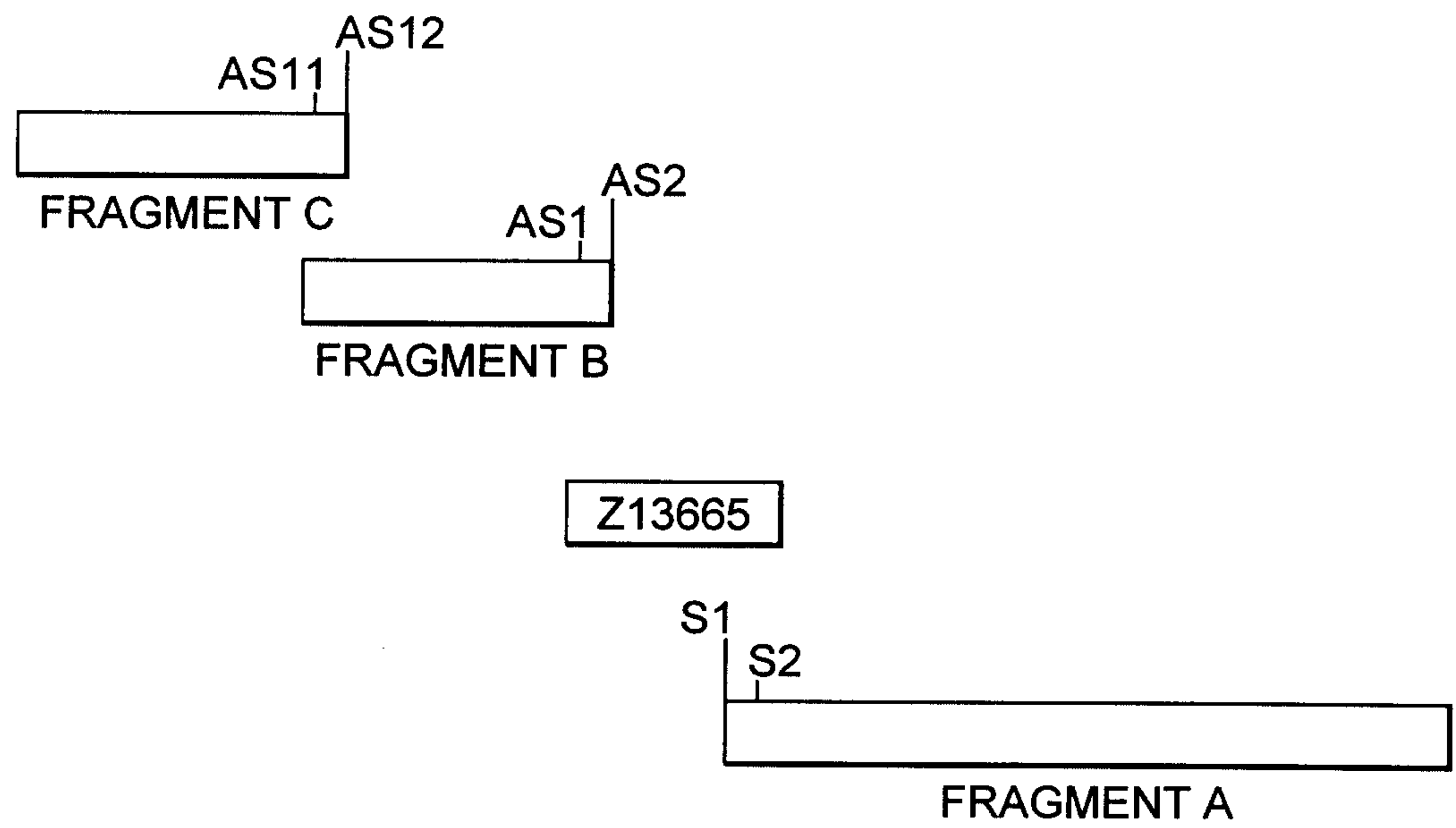
Fig. 1

1 h-wt	-----	-----	-----	-----	MEYVISR-----ISP	VPPGANEQGYPPPL	NTSP-----ANPPNQ--	35
2 d-wt	MHPAGEKRGRENK	YTAEALESIKQDLTR	FEVQNNRNNQNYTP	LRYTATNGRNDALTP	DYTHAKQPMPPPSA	SPAPDVVIPPPIAIV	90	
1 h-wt	GQGISSVPV-----	-----GRQPIIX-	-----	-----	-----	-----QSSSKFNFPSCR	65	
2 d-wt	GQPGAGSISVSGV	GVVGVANGRVPQMT	ALMPNKLIRKPSIER	DTASSHYLRCSALD	SGAGSSRSDSPSHM	THQPSR-----TVGNP	177	
1 h-wt	GMQNGTGQTDFAHQ	NVPA-----GTV	NRQPPPPYPLT-----	-----AANGQEPSA	---LQTGGSAAPESSY	TNG-SIPQFMVPNR	134	
2 d-wt	CGXGGSFSPSPGSE	VAPPAPPRNPTASS	AATPPPPVPTSQAY	VKRRSPALNRRPPAI	APPTQRCNS--PVIT	QNGLNPOOQLTQQL	265	
1 h-wt	NSIDMELYNISVPL	QTNMPQ-----S	SSAPAQSSPSSGHEI	PTWQ-PNIPVRSNSF	NNPLGNRASHSANSQ	PSATTVTAITPAPI-	214	
2 d-wt	KSLN--LYPCGGGA	VVEPPPPYLIQGCAG	GAAAPPPPPSYTASH	QSRQSPQSQQSDYR	KSPSSGIYSATSAGS	PEPITVS-LPPAPLA	352	
1 h-wt	-----QQP-	-----VKSMRVLXPEL	QTAALAPTHPSWIP--	-QPIQTVQSP-FPE	GTASNVTVPVPAEA	PNYQPPPPYPKILL	284	
2 d-wt	KPQPRVYQARSQPI	IMQSVKSTQVQKPL	QTAVAPQSPSSASAS	NSPVHVLAAAPPBPQ	KSAVVVQQQQAALAA	AKQQ-----QHQQH	435	
1 h-wt	HQN-----PSVPPYES	I-SKPSKEDQPLPK	EDESE-----	-----	-----	-----	314	
2 d-wt	QOSKPATPTTPPLVG	LNSKPNCLEPPSYAX	SMQAKAATVQQQQQ	QQQQQQVQQQVQQQ	QQQQQQQLQALRVLQ	AAQRRERDQRRERD	525	
1 h-wt	-----	-----	-----	-----	-----K	BYENVDSGDKEKQI	330	
2 d-wt	QQKLANGNPGROMLP	PPPYQSNMNNNSEIK	PPSCNNNNIQISNSN	LATTPPIPPAKYNNN	SSNTGANSSGGNGS	TGTTASSSTSCCKIK	615	
1 h-wt	TTSPITVRN---KX	DEERRESRIQSYSPQ	AFKFFMEQIVENVLK	SHOQLRUKKQLENE	MNRVGLSQDAQDOMR	KMLCKESNYIRLKR	417	
2 d-wt	HASPIPERKXISKEK	EEERKEFRIQSYSPQ	AFKFFMEQIHENVIK	SYRQRTYRKQLEKE	MIKVGLPDQTIEMR	KMLNCKESNYIRLKR	705	
1 h-wt	AKMDKSMFVKIKTLG	IGAFGEVCLARKVDT	KA-LYATKTLRKQDV	LLRNQVAHVKAERDI	LAZADNEHVRLYYG	FQDKDNLVFMVYIP	506	
2 d-wt	AKMDKSMFVKIKPIG	VGAFGEVTLVSKIDT	SNHLYAMKTLRKADV	LKRNQVAHVKAERDI	LAZADNVMVVKLYG	FQDKDNLVFMVYIP	795	
1 h-wt	GGDMMSLLIRMGIPP	ESLARFYIAELTCAV	ESVHKMGFIHRDIKP	DNILIDRDGHIKLTD	FGLCTGFRWTHDSKC	YYQSGDHPRQDSMDF	596	
2 d-wt	GGDLMSLLIKLGIPE	EELARFYIAEVTCAV	D6VHKMGFIHRDIKP	DNILIDRDGHIKLTD	FGLCTGFRWTHNSKY	YQENGHRSRQDSME-	884	
1 h-wt	SNEMGDPSSCRGDR	LXSLERRAARQHQR	LAHSLVGTTPNYIAPE	VLLRAGYTQLCDHWS	VGVIILFEMLVGQPPF	LAQTPLETQMKVINW	686	
2 d-wt	--PHEYS--ENGPK	PTVLERRRRRDHQRV	LAHSLVGTTPNYIAPE	VLSRGYTQLCDYNS	VGVIILFEMLVGQPPF	LANSPLETQCKVINW	970	
1 h-wt	QTSLHIPPQAKLSPE	ASDLIIXLCRGPEDR	LGKNGADEIKAPFF	KTIDPSSDLRQQSAS	YIPKITHPTDTSNFD	PVDPDKLHSDNEEE	776	
2 d-wt	EKTLHIPPQAKLSRE	ATDLIRLCLASADKR	LGKS-VDEVKSHDFP	XGIDFA-DKRXQKAP	YIPEIKHPTDTSNFD	PVDPEKLSRSDSTMS	1058	
1 h-wt	NVND-----TLNGM	V-----	-----	-----	-----	-----	786	
2 d-wt	SGDDVDQNDRTFHGT	FEFTFRFFDDKQPP	DMTDDQAPVTV	-----	-----	-----	1099	

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SEI 98-32

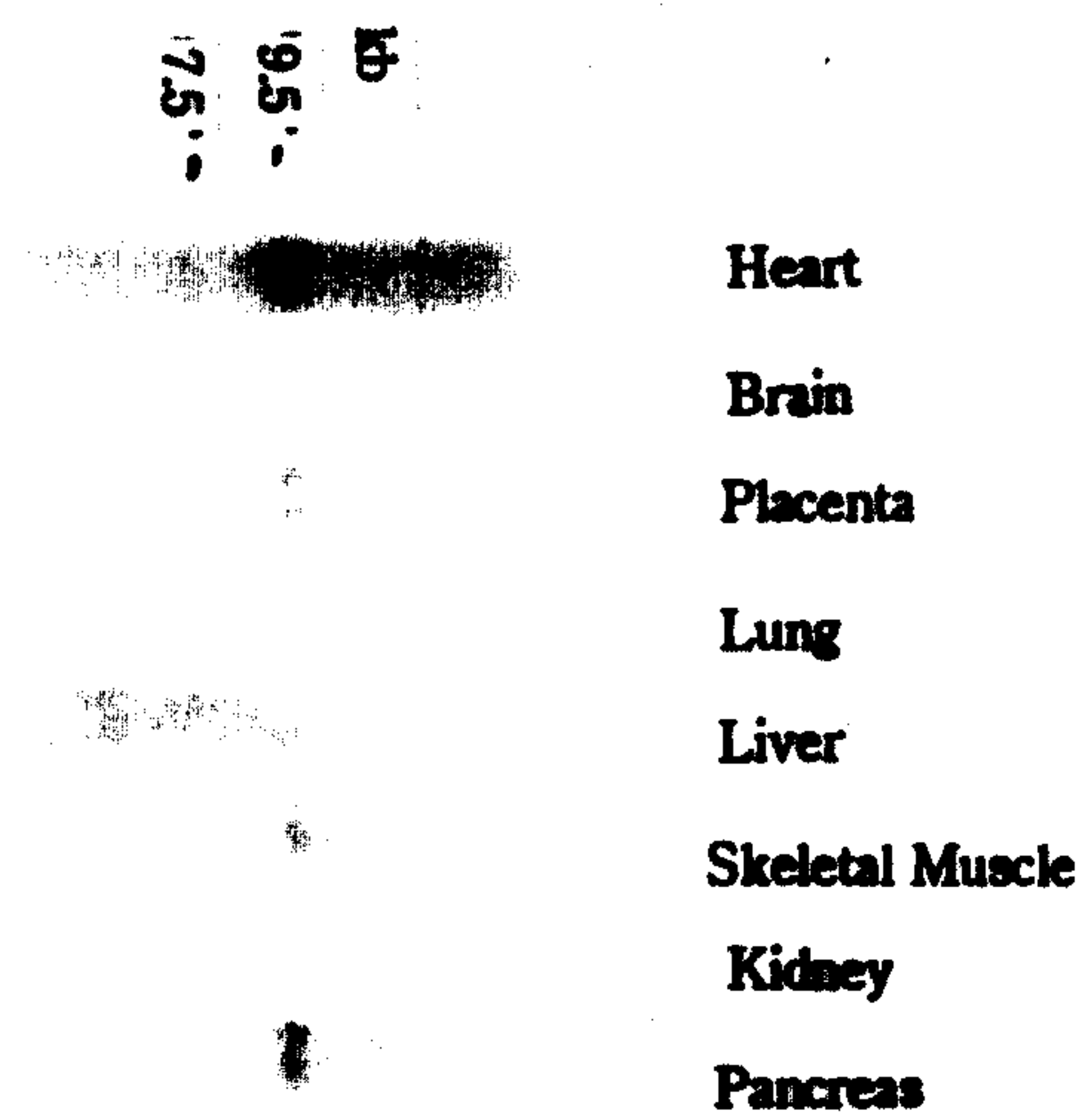
Fig.2



Marks & Clerk

SEI 98-32

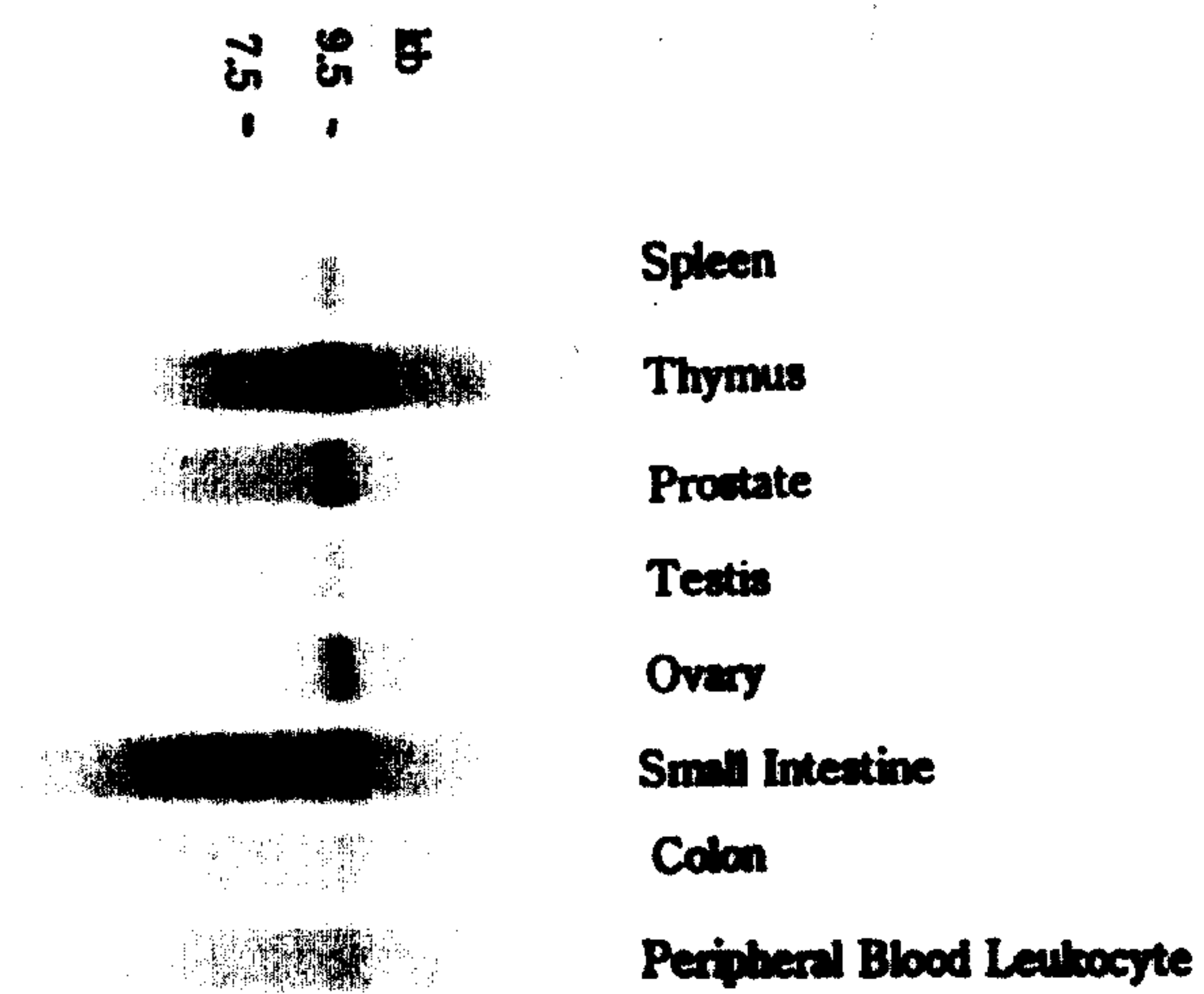
Fig.3



Marks + Clerk

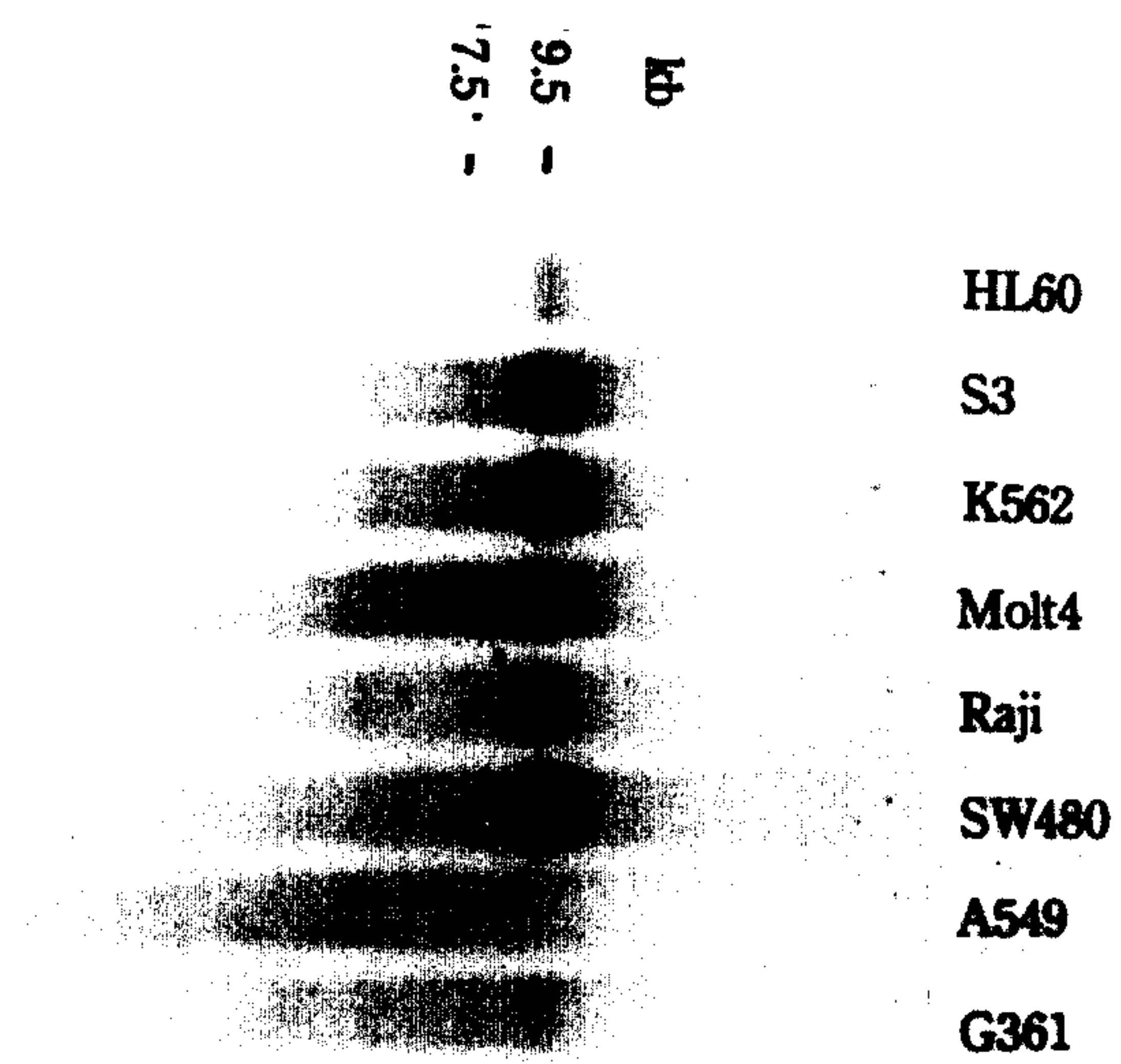
SEI 98-32

Fig.4



SEI 98-32

Fig.5



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