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(54) **Microbiologically prepared polypeptide comprising the amino acid sequence of human interferon, DNA and plasmids which code for this sequence, microorganisms which contain this genetic information, and processes for their preparation**

(57) Recombinant DNA technology is utilized in the production of interferon and there is provided a microbiologically produced polypeptide, all or part of which comprises the amino acid sequence of human interferon, especially leukocyte (α) interferon; a cDNA which codes for such a polypeptide; a plasmid especially plasmid pBR 322, which comprises the cDNA; and a microorganism, for example, *E. Scherichia coli,* especially *E. coli* K12 or *E. coli* X 1776, which comprises the genetic information required for the production of such a polypeptide. The cDNA is prepared using mRNA obtained from human leukocytes and/or cells of a human lymphoblastoid cell line.

GB 2 071 108 A

SPECIFICATION

Microbiologically prepared polypeptide comprising the amino acid sequence of human interferon, DNA and plasmids which code for this sequence, microorganisms which contain this genetic information, and processes for their preparation

It has been known for more than 20 years that interferon has antiviral activity. Investigations in recent years have shown, *inter alia*, that interferon also inhibits cell division and regulates many reactions of the immunological system. All these results underline the great medical importance of interferon.

The limited amounts of interferon which it has hitherto been possible to use for scientific and therapeutic investigations have been obtained primarily from fibroblast and lymphocyte cultures. However, it was not possible with either process to prepare relatively large amounts of interferon or even sufficient amounts for answering individual questions. This is the reason why the state of knowledge in the field of interferon is progressing very slowly and why very few of the urgently desired investigations in the field of cancer can be carried out. It is therefore imperative to adopt a new approach to the production of interferon.

The present invention relates to an approach utilising recombinant DNA technology.

The present invention accordingly provides a microbiologically prepared polypeptide, all or part of which comprises the amino acid sequence of human interferon, especially human leukocyte interferon (α-interferon).

The invention also provides cDNA which codes for a polypeptide as defined above whenever prepared using mRNA obtained from human leukocytes and/or cells of a human lymphoblastoid cell line, for example, the Namalwa cell line. The invention further provides a plasmid, especially plasmid pBR 322, which comprises cDNA of the invention, and also provides a microorganism, preferably *E.coli*, and especially *E.coli* K12 or *E.coli* X 1776, which comprises a plasmid of the invention. The plasmid may be introduced into the microorganism by transformation.

The invention provides moreover a process for the production of a polypeptide, all or part of which comprises the amino acid sequence of human interferon, especially human leukocyte interferon, which comprises obtaining the genetic information for the biosynthesis of the polypeptide using mRNA from human leukocytes and/or from cells of a human lymphoblastoid cell line, incorporating the resulting gene into microorganisms in a manner which is in itself known, and selecting and culturing, in a manner which is in itself known, those microorganisms which produce the polypeptide. (The term "known" is used herein to denote in actual use in the art or described in the literature of the art.)

The process of the invention generally comprises obtaining from human leukocytes and/or from cells of a human lymphoblastoid cell line mRNA for the polypeptide, producing double stranded cDNA therefrom, incorporating the cDNA into a plasmid, introducing the plasmid into microorganisms, and selecting and culturing those microorganisms that produce the polypeptide.

The invention also provides a process for the preparation of RNA which comprises arranging a caesium chloride-free cell lysate as a layer on an aqueous caesium chloride solution, centrifuging the resulting system, and obtaining the RNA. The lysate is, for example, a lysate of human leukocytes and/or cells of a human lymphoblastoid cell line.

The interferon protein formed in this process possesses the biological and immunological properties which are characteristic of this protein.

The following procedure can be adopted to achieve the aim according to the invention, the means illustrated being merely an example of methods which can be used.

Cells capable of producing human leukocyte interferon are denatured and lysed, generally after induction, for example, with Sendai virus or Newcastle Disease virus and the RNA is obtained as a precipitate after caesium chloride centrifugation.

It has been found advantageous to prepare a cell lysate, to arrange this lysate as a layer on an aqueous caesium chloride solution, and to centrifuge the resulting system. In contrast to methods proposed previously, however, it has now been found that the lysate should be caesium chloride-free to achieve the most complete possible separation of the mRNA from the mixed RNAS in the next reaction step.

After further precipitation, the mRNA is separated from the resulting mixed RNAS using affinity chromatography, especially an oligo-dT-cellulose column. The mRNA obtained is completed to give a RNA-DNA double strand with the aid of the enzyme reverse transcriptase. After digestion of the RNA strand, the DNA strand (complementary DNA = cDNA) is completed to give a DNA-DNA double strand (dsDNA) with again using reverse transcriptase, or using the enzyme polymerase I.

This double-stranded DNA must now be incorporated into a plasmid. For this purpose, it is necessary to lengthen the 3′ end of the DNA, for example with dCMP residues. A plasmid, for example pBR 322 or pBR 325 is cut with a restriction endonuclease, for example, PstI, and lengthened with, for example, dGMP residues. When the DNA lengthened in this way is brought together with the correspondingly lengthened plasmid, base pairing takes place between the DNA and the plasmid. This circularized molecule is then introduced by transformation into microorganisms in particular bacteria, preferably *E. coli*, for example, *E. coli* K 12 or *E. coli* X 1776; the bonds which are not yet closed are covalently linked by means of the enzymes of the microorganism. The transformed microorganisms are plated on agar and selected on the basis of antibiotic resistances which are determined by the chosen plasmid and the restriction nuclease used.

Those clones which express interferon-containing proteins are selected with the aid of immunological and biological tests. These clones are bred and the microorganism mass is centrifuged off, extracted

and tested for interferon content. The solution obtained on digestion of the interferon-producing clones contains interferon protein.

The following Examples illustrate the invention.

1. PROCESS FOR THE ISOLATION OF RNA

a) *Preparation of interferon-producing lymphoblastoid cells*

Cells of the lymphoblastoid cell line Namalva are reproduced, in a manner which is in itself known, until a cell concentration of 1 - 6 x 10^6 cells/ml is reached. For the interferon induction, the cells were taken up in fresh medium and infected at a concentration of 1 - 10 x 10^6 cells/ml with Newcastle Disease Virus or Sendai virus (multiplicity of the infection: <1.0). After incubation for 4 - 24 hours at 37°C, the cells are centrifuged off.

b) *Preparation of interferon-producing leukocytes*

To prepare interferon-producing leukocytes, leukocyte suspensions are obtained from the blood of healthy donors. For this purpose, the white cell layer at the boundary between the erythrocytes and the serum of centrifuged blood samples was suspended in suitable buffer solutions and separated from the erythrocytes and thrombocytes by fractional sedimentation, for example settling at 1 G or low-speed centrifugation. The leukocytes were then suspended in a conventional nutrient medium and induced as described above.

c) *Preparation of RNA*

The cells obtained are taken up in a "denaturing medium" (4 M in respect of guanidinium thiocyanate, 1 M in respect of mercaptoethanol and 0.15 M in respect of sodium acetate - pH 5.5) and homogenized for 1 minute at 0°C using an Ultra-Turrax. The homogenized mixture is centrifuged at 20,000 rpm for 15 minutes at 4°C.

The supernatant is then arranged in a layer on 5 ml of a solution ("cushion") which is 5.7 M in respect of CsCl, 10 mM in respect of tris-hydroxyaminomethane (tris) and 10 mM in respect of ethylenediaminetetraacetic acid (EDTA), and which has a pH of 7.6, and is centrifuged in a Beckman-SW 27 Rotor for 36 hours at 20°C and 22,000 rpm.

It was found that, in contrast to data in the literature, the addition of CsCl to the lysate must be prevented in order to achieve the most complete separation possible of the mRNA in the following reaction step. After centrifugation has ended, the small Polyallomer tubes are frozen in liquid nitrogen and the bottom of the small tube, where the RNA precipitate is located, is cut off. The precipitate is taken up in a solution which is 10 mM in respect of tris and 10 mM in respect of EDTA and contains 1% (w/v) of "Sarcosyl" (Na salt of N-lauryl-sarcosine), and which has a pH of 7.6, and the suspension is centrifuged at 20,000 rpm (40,000G) for 20 minutes. The resulting precipitate is again taken up in the same buffer, and the mixture is warmed for 5 minutes at 65°C and centrifuged again. The combined supernatants are made 0.3 M in respect of sodium acetate, 2.5 times the volume of ethanol is added and the mixture is kept at −20°C.

2. PREPARATION OF mRNA

The RNA is separated from the ethanolic solution by centrifugation (20,000 rpm, (40,000G), 30 minutes, −5°C) and dissolved in a solution which is 0.5 M in respect of NaCl and 10 mM in respect of tris and which has a pH of 7.5. The resulting solution is charged onto an oligo-dT-cellulose column (manufacturer: Collaborative Research, type 3), which has been equilibrated with the same buffer, and is eluted with 1 mM tris solution of pH 7.6 or with distilled water. The elution of the RNA containing poly-A (mRNA) can be followed by measuring the extinction at 260 nm. The mRNA thus obtained is treated with sodium acetate until a final concentration of 0.3 M is reached, 2.5 times the volume of ethanol is added and the solution is kept at −20°C.

3. PREPARATION OF THE cDNA

a) *Preparation of single-stranded cDNA*

The conversion of the mRNA into the corresponding DNA (cDNA) is carried out with the aid of the enzyme reverse transcriptase. The incubation batch is: 50 mM in respect of tris, pH 8.3, 10 mM in respect of $MgCl_2$, 30 mM in respect of 2-mercaptoethanol and 0.5 mM in respect of all 4 commonly occurring deoxyribonucleoside triphosphates (corresponding triphosphates of adenine, guanine, cytosine and thymidine), and contains 100 µg/ml of oligo-dT_{12-18} (manufacturer: Boehringer Mannheim), about 100 µg/ml of polyadenylated RNA and 800 units/ml of reverse transcriptase (manufacturer: Life Science Inc., St. Petersburg, USA). To follow the reaction, a deoxyribonucleoside triphosphate labeled by ^{32}P in the α-position (specific activity: 50 Ci/mmole) can be added to the batch. The mixture is incubated for 60 minutes at 42°C and the reaction is then stopped by adding 20 mM EDTA solution. The resulting solution is extracted with an equal volume of phenol saturated with water, the phenol is extracted by shaking with ether and the ether residues are evaporated off with nitrogen. Non-incorporated deoxyribonucleoside triphosphates are separated off by column chromatography on Sephadex G50 in a solution which is 10 mM in respect of tris, pH 9.0, 100 mM in respect of NaCl and 1mM in respect of EDTA. The eluted cDNA is precipitated by adding 0.3 M sodium acetate solution and 2.5 times the volume of ethanol.

After centrifugation, the precipitate is taken up in 0.1 M NaOH solution and incubated for 20 minutes at 70°C, or is incubated in 0.3 M NaOH solution overnight at room temperature. The mixture is brought to pH 7.6 with 1 M HCl and 1 M tris solution.

b) *Formation of double-stranded cDNA (dsDNA)*

For the synthesis of the second DNA strand, again the enzyme reverse transcriptase can be used, or the enzyme polymerase I can be used.

Formation of the dsDNA with reverse transcriptase

The incubation batch (pH 8.3) is 50 mM in respect of tris, 10 mM in respect of $MgCl_2$, 30 mM in respect of 2-mercaptoethanol and 0.5 mM in respect of the 4 abovementioned deoxyribonucleoside triphos-

phates, and contains 50 μg/ml of cDNA and 800 units/ml of reverse transcriptase. The reaction is carried out for 120 minutes at 42°C and is stopped by adding EDTA until a final concentration of 20 mM is reached. The phenol extraction and gel permeation chromatography on Sephadex G50 are then carried out as described above.

Formation of the dsDNA with polymerase I

The incubation batch (pH 6.9) is 200 mM in respect of Hepes (N-2-hydroxyethylpiperazine-N -2-ethanesulphonic acid) 0.5 mM in respect of the 4 abovementioned deoxyribonucleoside triphosphates, 10 mM in respect of $MgCl_2$, 30 mM in respect of 2-mercaptoethanol and 70 mM in respect of KCl. The reaction can be followed by adding deoxyribonucleotides, labeled by ^{32}P (in the α-positions), to the incubation batch. The reaction is started with 800 units/ml of polymerase I and carried out for 2 hours at 15°C. The reaction is stopped by adding a solution containing 0.1% (w/v) of sodium dodecyl-sulfate and 100 μg/ml of RNA. The resulting solution is extracted as described. The solution obtained after the extraction is charged onto a Sephadex G50 column and the chromatography is carried out with a solution which is 10 mM in respect of tris, pH 9.0, 100 mM in respect of NaCl and 1 mM in respect of EDTA. The eluted DNA can be determined by the radioactivity and is precipitated by adding 0.3 M sodium acetate solution and 2.5 times the volume of ethanol. The ethanol precipitate is taken up in a solution (pH 4.5) which is 300 mM in respect of NaCl, 30 mM in respect of sodium acetate and 3mM in respect of $ZnCl_2$, and 1,500 units/ml of S1 nuclease (Boehringer Mannheim) are added. The reaction is stopped after one hour at 37°C by adding EDTA until a final concentration of 20 mM is reached. Extraction with phenol and precipitation with ethanol are then carried out as described above.

c) *Preparation of cDNA in a "one-pot process"*

As an alternative to the reactions described, the dsDNA can also be obtained from the mRNA in a "one-pot process". In this process, the reaction of the reverse transcriptase is carried out as described, but 140 mM KCl solution is also added to the incubation batch. After the reaction has ended, the sample is heated at 100°C for 4 minutes and the precipitate formed is centrifuged off. 0.4 M Hepes buffer (pH 6.9) is added to the supernatant in an equal volume, the 4 abovementioned deoxyribonucleotides being present in the buffer at a concentration of 0.5 mM. The reaction is carried out at 15°C, as described, by adding 800 units/ml of polymerase I and is stopped by adding sodium dodecylsulfate and RNA. The procedure under 3.b), "Formation of the dsDNA with polymerase I", is then followed.

4. LENGTHENING OF THE 3' ENDS OF cDNA WITH dCTP (DEOXYCYTOSINE TRIPHOSPHATE)

For incorporation into the plasmid, it is necessary to lengthen the 3' end of the DNA with one of the 4 abovementioned deoxynucleotides. Furthermore, the 3' ends of the cut plasmid are lengthened with the complementary deoxynucleotide. On bringing the DNA and the plasmid together, base pairing then takes place between the DNA and the plasmid. This re-circularized molecule can be used for the transformation of bacteria; the bonds which are not yet closed are covalently linked by means of an enzyme system in the bacterium.

For example, the 3' ends of the cDNA can be lengthened with dCMP residues (deoxycytosine monophosphate residues) and the plasmid can be lengthened with dGMP residues (deoxyguanidine monophosphate residues). On using these deoxynucleotides in the manner described, and on opening the plasmid by means of the restriction enzyme Pst I, a Pst I cleavage site is re-formed at every insertion site, after incorporation of the alien DNA, so that, after reproduction of the plasmid, the inserted DNA can easily be cut out again for further examination, using the restriction enzyme Pst I.

The precipitate from the alcohol precipitation, after degradation with S1 nuclease, is dissolved in an aqueous solution (pH 6.7) which is 30 mM in respect of tris, 1 mM in respect of $CoCl_2$, 140 mM in respect of potassium cacodylate (cacodylic acid is dimethyl arsenous acid) and 150 μM in respect of dCTP, contains 100 μg per ml of autoclaved gelatin hydrolysate and is 0.1 M in respect of dithioerythritol. ^{32}P-labeled dCTP should be used to follow the reaction. The reaction is started by adding terminal deoxynucleotidyl transferase and carried out for 10 minutes at 37°C. After the reaction time has elapsed, the sample is placed in ice and the number of incorporated dCMP residues is determined by radioactivity measurement in the precipitate which can be formed with trichloroacetic acid. In the reaction, about 10% of the nucleotides originally present should have been added; if this is not the case, the reaction can be continued by adding further enzyme. If too large a number of dCMP residues have been added, the resulting chain can be shortened with the aid of the enzyme S1 nuclease.

5. PROCESS FOR THE INTEGRATION OF DNA INTO A PLASMID

A suitable circular plasmid, for example pBR 322, is cut with a restriction endonuclease which only recognizes one sequence on the plasmid. It is advantageous if this cleavage site lies behind a strong or inducible promoter and/or is located such that an antibiotic resistance is influenced.

The plasmid converted to the linear form in this way is then lengthened at the 3' ends by one of the four deoxynucleotides.

This is carried out, for example, in the following manner:

30 μg of plasmid are incubated with 50 units of Pst I restriction endonuclease in the presence of a solution which is 50 mM in respect of NaCl, 6 mM in respect of tris, 6 mM in respect of $MgCl_2$ and 6 mM in respect of 2-mercaptoethanol and contains 0.1 mg/ml of autoclaved gelatin hydrolysate, for 40 minutes at 37°C and at a pH value of 7.5. Extraction is then carried out with phenol and ether as described and the cut plasmid is precipitated with ethanol in the presence of 0.3 M sodium acetate solution. The lengthening of the 3' ends of the plasmid with dGTP

(deoxyguanidine triphosphate) is carried out analogously to the lengthening of the cDNA with dCTP, described above.

The lengthened plasmid-DNA is then mixed with the lengthened ds cDNA in a solution (pH 8.0) which is 0.1 M in respect of NaCl, 10 mM in respect of tris and 1 mM in respect of EDTA, and the mixture is heated at 56°C for 2 minutes, incubated at 42°C for 2 hours and then cooled slowly to room temperature. The hybrid DNA obtained in this way is then used for the transformation of E. coli.

6. CLONING OF THE HYBRID PLASMID IN E. COLI

The bacteria, for example E. coli X 1776, are left to grow, at 37°C in 50 ml of a customary nutrient medium, until an optical density of $A_{600} = 0.5 - 0.6$ is reached, and they are sedimented, washed once with 10 mM tris solution of pH 7.5, then resuspended in 40 ml of a solution which is 75 mM in respect of $CaCl_2$, 5 mM in respect of $MgCl_2$ and 5 mM in respect of tris, and which has a pH of 8.0, and incubated for 20 minutes in ice. The cells are then sedimented and resuspended in 2 ml of a solution which is 75 mM in respect of $CaCl_2$, 5 mM in respect of $MgCl_2$ and 5 mM in respect of tris and which has a pH of 8.0.

0.2 ml of this bacterial suspension is then mixed with 0.1 ml of hybrid DNA solution and the mixture is incubated on ice for 45 minutes. It is then warmed at 42°C for 90 seconds and subsequently mixed with 0.2 ml of nutrient medium.

50 µl-75 µl of this suspension are then plated on agar containing tetracyclin and ampicillin and selection is made on the basis of antibiotic resistance.

7. ISOLATION OF STRAINS WHICH PRODUCE INTERFERON-CONTAINING PROTEINS

a) *Immunological detection*

With the aid of a test system developed by Broome and Gilbert (S. Broome and E. Gilbert, PNAS, *75*, 2,746, 1978), the clones are examined for the expression of interferon-containing proteins. Clones which express interferon-containing proteins can be detected, as a result of the binding of a radioactive antibody to these proteins, with subsequent autoradiography, by the fact that the X-ray film is darkened at this site.

For this purpose, up to 50 clones per nitrocellulose filter are left to grow for 2 days at 37°C. The filters are then laid on an agar block which contains 1 mg/ml of lysozyme; a PVC film coated with interferon antibodies is applied to the bacterial colony and incubation is then carried out for 2 - 3 hours at 4°C. The PVC film is then incubated for 15 hours at 4°C in a solution of ^{132} iodine-labeled interferon antibodies. The specific activity of the solution about 1×10^6 cpm/ml. After washing and drying, the films are subjected to autoradiography.

b) *Biological detection*

The clones are bred overnight in 50 ml of a conventional nutrient medium at 37°C. The bacteria are then sedimented, washed twice with a cold solution which is 10 mM in respect of tris, pH 8.0, and 30 mM in respect of NaCl, and resuspended in a solution which 30 mM in respect of tris, pH 8.0, and 1 mM in respect of EDTA and contains 20% of sucrose. The suspension is then shaken at room temperature for 10 minutes, the bacteria are sedimented and resuspended in ice-cold H_2O and the suspension is incubated in an ice bath for 10 minutes. The bacteria are then sedimented and the supernatant is tested in a virus inhibition test for the content of interferon-containing protein, in a manner which is in itself known.

8. PREPARATION OF INTERFERON PROTEIN

The clones which show interferon activity in the various tests are bred in the conventional nutrient media and the bacteria are centrifuged off and extracted with aqueous buffers. The extraction solution contains the interferon protein.

WHAT WE CLAIM IS:-

1. A microbiologically prepared polypeptide, all or part of which comprises the amino acid sequence of human interferon.

2. A microbiologically prepared polypeptide as claimed in claim 1, all or part of which comprises the amino acid sequence of human leukocyte interferon.

3. A cDNA code for the amino amid sequence as claimed in claim 1 or claim 2, whenever prepared using mRNA obtained from human leukocytes and/or cells from a human lymphoblastoid cell line.

4. A plasmid which comprises cDNA as claimed in claim 3.

5. Plasmid pBR 322 which comprises cDNA as claimed in claim 3.

6. A microorganism which comprises the genetic information required for the biosynthesis of a polypeptide as claimed in claim 1 or claim 2.

7. A microorganism as claimed in claim 6, being *E. coli*.

8. A microorganism as claimed in claim 7, wherein the *E. coli* is *E. coli* K12 or *E. coli* X 1776.

9. A microorganism as claimed in any one of claims 6 to 8, wherein the genetic information comprises cDNA as claimed in claim 3.

10. A microorganism as claimed in any one of claims 6 to 8, wherein the genetic information comprises a plasmid as claimed in claim 4 or claim 5.

11. A process for the preparation of a microorganism as claimed in claim 10, which comprises introducing into the microorganism by transformation a plasmid as claimed in claim 4 or claim 5.

12. A process for the preparation of a polypeptide as claimed in claim 1 or claim 2, which comprises obtaining the genetic information for the biosynthesis of the polypeptide using leukocyte mRNA and/or mRNA obtained from cells of a human lymphoblastoid cell line, incorporating the resulting gene into microorganisms in a manner which is in itself known, and selecting and culturing, in a manner which is in itself known, those microorganisms which produce this polypeptide.

13. A process for the preparation of a polypeptide as claimed in claim 1 or claim 2, which comprises obtaining from human leukocytes and/or cells of a human lymphoblastoid cell line mRNA for the polypeptide, producing double stranded cDNA therefrom, incorporating the cDNA into a plasmid, introducing the plasmid into microorganisms, and

selecting and culturing those microorganisms that produce the polypeptide.

14. A process as claimed in claim 13, wherein a human lymphoblastoid cell line is the Namalwa cell line.

15. A process as claimed in claim 13 or claim 14, wherein the leukocytes and/or lymphoblastoid cells are induced using a virus.

16. A process as claimed in claim 15, wherein the virus is the Sendai virus or the Newcastle disease virus.

17. A process as claimed in any one of claims 13 to 16, wherein the plasmid is plasmid pBR 322.

18. A process as claimed in any one of claims 12 to 17, wherein the microorganism is *E. coli*.

19. A process as claimed in claim 18, wherein the microorganism is *E. coli* K12 or *E. coli* X 1776.

20. A process as claimed in any one of claims 13 to 19, wherein the plasmid is introduced by transformation.

21. A process as claimed in any one of claims 13 to 20, wherein the RNA is isolated from the leukocytes and/or cells of a human lymphoblastoid cell line by arranging a caesium chloride-free lysate of the leukocytes and/or cells of a human lymphoblastoid cell line on an aqueous solution of caesium chloride, centrifuging the resulting system, and obtaining the RNA.

22. A process as claimed in claim 21, wherein the mRNA is isolated by affinity chromatography.

23. A process as claimed in claim 22, wherein the chromatography is carried out on an oligo-dT-cellulose column.

24. A process as claimed in claim 12 or claim 13, carried out substantially as described in the Example herein.

25. A polypeptide as claimed in claim 1 or claim 2, whenever produced by a process as claimed in any one of claims 12 to 24.

26. A process for the preparation of RNA which comprises arranging a caesium chloride-free lysate as a layer on an aqueous caesium chloride solution, centrifuging the resulting system, and obtaining the RNA.

27. A process as claimed in claim 26, wherein the lysate is a lysate of human leukocytes and/or cells of a human lymphoblastoid cell line.

28. A process for the preparation of cDNA as claimed in claim 3, which comprises obtaining RNA from human leukocytes and/or from cells of a human lymphoblastoid cell line, isolating the mRNA therefrom, and using the mRNA to prepare cDNA.

29. A process as claimed in claim 28, wherein the RNA is obtained by a process as claimed in claim 26 or claim 27.

30. A process for the preparation of a plasmid as claimed in claim 4 or claim 5, which comprises introducing cDNA as claimed in claim 3 into a plasmid.

31. A process as claimed in claim 30, wherein the plasmid is plasmid pBR 322 and restriction endonuclease Pst I is used.

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