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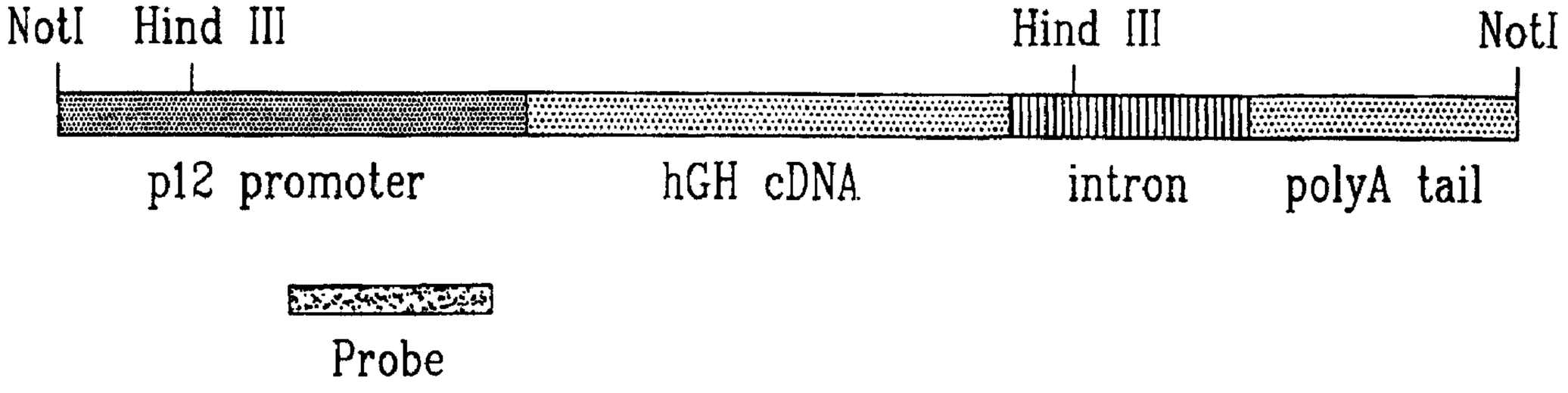

(57) Abrégé/Abstract:
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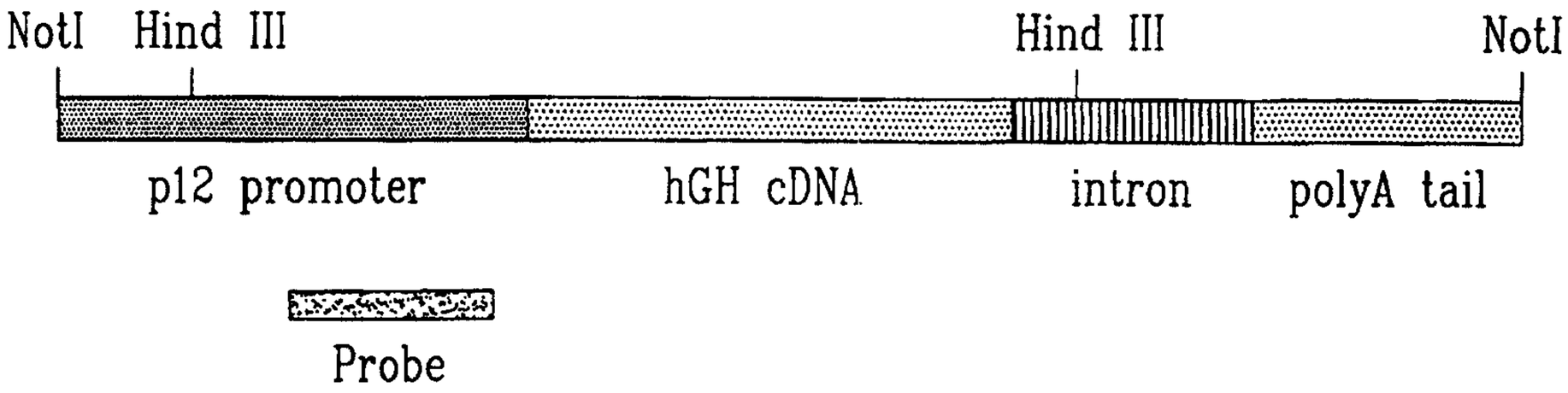


(57) Abstract

The present invention relates to a method for the production and secretion into animal's semen of an exogenous recombinant protein comprising the steps of: a) producing a transgenic animal characterized by an expression system comprising a promoter specific for the genital tract or accessory glands operatively linked to an exogenous DNA sequence coding for the recombinant protein through a DNA sequence coding for a signal peptide effective in secreting and maturing the recombinant protein in genital tract tissue; b) collecting semen produced by said transgenic animal; and c) isolating the exogenous recombinant protein from the semen.

TRANSGENIC EXPRESSION IN GENITAL TRACT AND SEXUAL ACCESSORY GLANDS

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to the production of recombinant proteins in animal's semen. Particularly, this invention relates to an expression system which comprises at least a semen-specific protein promoter operatively linked to a DNA sequence coding for a signal peptide and a desired recombinant protein product. When such a system is transgenically incorporated into an animal, the recombinant protein is expressed in the semen of the animal. This invention also relates to the transgenic animal that produces the desired recombinant product in its semen. Recombinant products produced by the expression systems and transgenically altered animals of this invention can be produced at significantly less cost than by conventional recombinant protein production techniques. There is also a potential to alter specific characteristics related to sperm viability and potential storage systems.

(b) Description of Prior Art

Recombinant DNA technology has enabled the cloning and expression of genes encoding medically and agriculturally important proteins and glycoproteins. Such products include, "for example, insulin, growth hormone, growth hormone releasing factor, somatostatin, tissue plasminogen activator, tumor necrosis factor, lipocortin, coagulation factors VIII and IX, erythropoietin, the interferons, colony stimulating factor, the interleukins and urokinase, antibodies.

Many of these important proteins, however, are large (molecular weights in excess of 30 Kd), secreted, require sulfhydryl bonds to maintain proper folding, are glycosylated and are sensitive to proteases. As a

result, the recombinant production of such products in prokaryotic cells has proven to be less than satisfactory because the desired recombinant proteins are incorrectly processed, lack proper glycosylation or are improperly formed. Accordingly, resort has been had to the production of those recombinant proteins in cultured eukaryotic cells. This technique has proven to be both expensive and often unreliable due the variability of cell culture methods. For example, average yields are 10 mg of recombinant protein per liter of culture media, with the resulting cost typically for exceeding one thousand dollars per gram of recombinant protein. Accordingly, resort has been had to the production of those recombinant proteins in cultured eukaryotic cells. It is believed that the use of the genital tract as a tissue for expression overcomes, either wholly or to a satisfactory degree, this potential source of difficulty. Several examples using mammary glands of transgenic mammals as bioreactors have demonstrated their potential to produce recombinant protein products.

Harvesting from body fluids as opposed to solid tissue is desirable, because such routes, are by and large renewable, and most proteins of biomedical importance are themselves secreted into body fluids. Secretion into the bloodstream is a possible route, either from liver or B lymphocytes, but the coagulating properties of blood and the presence of biologically active peptides and antigenic molecules may prove a hindrance to subsequent downstream processing.

It would be highly desirable to be provided with a means to produce recombinant proteins in large quantities.

SUMMARY OF THE INVENTION

The above difficulties may be overcome in accordance with the present invention, as it is the case, for example, for the production of recombinant proteinin milk, by the use of the genital tract as a tissue of expression. Semen is readily collected, available in large 'quantities in several animal species and well characterized biochemically. Further, several proteins are present at high concentrations in this body fluid.

The present invention is a new method to solve such problems by providing an efficient means of producing large quantities of recombinant protein products in the semen of transgenically altered animals.

According to one embodiment of the present invention, a DNA sequence coding for a desired protein is operatively linked in an expression system to a genital tract-specific protein promoter, or any promoter sequence specifically activated in male genital tissue, through a DNA sequence coding for a signal peptide that permits secretion and maturation of the desired protein in the genital tract tissue. More preferably, the expression system also includes a 3' untranslated region downstream of the DNA sequence coding for the desired recombinant protein. This untranslated region may stabilize the rDNA transcript of the expression system. Optionally, the expression system also includes a 5' untranslated region upstream of the DNA sequence coding for the signal peptide.

The expression system is transgenetically introduced into a host genome. As a result, one or more copies of the construct or system become incorporated into the genome of the transgenic animal. The presence of the expression system will permit the male species to produce and to secrete the recombinant protein product, into or along with its semen. Such method permits the

low cost, high level production of the desired proteins.

The expression "operatively linked" as used herein is intended to mean the linking of a genital tract-specific promoter or a promoter specifically activated in genital tract tissue to a DNA sequence coding for a desired protein so as to permit and control expression of that DNA sequence and production of that protein.

The expression "recombinant protein" as used herein is intended to mean a protein or peptide coded for by a DNA sequence which is not endogenous to the native genome of the animal in whose semen it is produced in accordance with this invention or a protein or peptide coded for by a DNA sequence which is endogenous to the native genome of the animal in whose semen it is produced does not lead to the production of that protein or peptide in its semen at the same level that the transgenic animal of this invention produces that protein in its semen.

The expression "genital tract" as used herein is intended to mean the reproductive anatomical male system whole or in part involving the prostate gland, the seminal vesicle, epididymis, seminiferous tubules, ampule, vas deferens, and the bulbourethral gland.

In accordance with the present invention there is provided a method for the production and secretion into animal's semen of an exogenous recombinant protein comprising the steps of:

a) producing a transgenic animal characterized by an expression system comprising a promoter specific for the genital tract or accessory glands operatively linked to an exogenous DNA sequence coding for the recombinant protein through a DNA sequence coding for a signal peptide effective

in secreting and maturing the recombinant protein in genital tract tissue;

b) collecting semen produced by the transgenic animal; and

c) isolating the exogenous recombinant protein from the semen.

More specifically, the invention concerns a method for the production and secretion into a mouse's semen of an exogenous recombinant protein comprising the steps of:

a) producing a transgenic mouse whose genome comprises an expression system comprising in operable association a p12 promoter, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the mouse's semen to a detectable level;

b) collecting semen produced by said transgenic mouse; and

c) isolating the exogenous recombinant protein from the semen.

The invention is also directed to a method for the production and secretion into a pig's semen of an exogenous recombinant protein comprising the steps of:

a) producing a transgenic pig whose genome comprises an expression system comprising in operable association a p12 promoter, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the pig's semen to a detectable level;

b) collecting semen produced by said transgenic pig; and

c) isolating the exogenous recombinant protein from the semen.

The expression system used in accordance with the present invention may also include a 3' untranslated region downstream of the DNA sequence coding for the recombinant protein or a 5' untranslated region between the promoter and the DNA sequences coding for the signal peptide.

In accordance with another embodiment of the present invention, the promoter may be selected from the group consisting of p12, p25, kallikreins, PSA, SBP-C and secretory protein IV promoters.

In accordance with another embodiment of the present invention, the recombinant protein may be selected from the group consisting of mono- and bi-specific antibodies, immunoglobulins, cytokines, coagulation factors, tissue plasminogen activator, GM-CSF, erythropoietin, thrombopoietin, alpha-1 anti-trypsin, animal growth hormones, cell surface proteins, insulin, interferons, lipases, antiviral protein, antibacterial protein, bacteriocins, peptide hormones, lipocortins and epidermal growth factor.

In accordance with another embodiment of the present invention, there is provided a method to increase sperm viability and semen storage which comprises the steps of:

a) producing a transgenic animal characterized by an expression system comprising a promoter specific for the genital tract or accessory glands operatively linked to an exogenous DNA sequence coding for a recombinant protein capable of improving sperm viability and semen storage through a DNA sequence coding for a signal peptide effective in secreting and maturing the recombinant protein in genital tract tissue; and

b) collecting semen produced by the transgenic animal;

whereby the semen has an improved storage capability and containing sperms of increased viability.

More specifically, the invention concerns a a method to increase sperm viability and semen storage which comprises the steps of:

a) producing a transgenic mouse whose genome comprises an expression system comprising in operable association a promoter specific for

the genital tract or accessory glands, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the mouse's semen to a detectable level; and

b) collecting semen produced by said transgenic mouse;

whereby said semen has an improved storage capability and containing sperms of increased viability, and

wherein said protein heterologous to the promoter is selected from the group consisting of catalase, superoxide dismutase, calcitonin, antibiotics, and epididymal fertility proteins.

Also, the invention is directed to a method to increase sperm viability and semen storage which comprises the steps of:

a) producing a transgenic pig whose genome comprises an expression system comprising in operable association a promoter specific for the genital tract or accessory glands, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the pig's semen to a detectable level; and

b) collecting semen produced by said transgenic pig;

whereby said semen has an improved storage capability and containing sperms of increased viability,

and wherein said protein heterologous to the promoter is selected from the group consisting of catalase, superoxide dismutase, calcitonin, antibiotics, and epididymal fertility proteins.

In accordance with another embodiment of the present invention, the recombinant protein may be selected from the group consisting of catalase, superoxide dismutase, calcitonin, antibiotics such as gentamycin, and epididymal fertility proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a chimeric construct containing the mouse pl2 gene promoter linked to the human growth hormone coding sequence in accordance with one embodiment of the present invention;

Fig. 2 illustrates a Southern blot analysis of the integration of the transgene into the genomic DNA of the transgenic mice; and

Fig. 3 illustrates the recombinant hGH assay with samples from pl2-hGH transgenic mice and wt controls.

DETAILED DESCRIPTION OF THE INVENTION

The present invention solves such problems by providing new efficient means of producing large quantities of recombinant protein products in the semen of transgenically altered animal. This invention relates to processes, DNA sequences, compositions of matter and transgenic animals for the production of recombinant proteins. More specifically, this invention relates to the transgenic incorporation of one or more copies of a construct comprising a genital tract-specific protein

promoter or any promoter sequence specifically activated in genital tract tissue, operatively linked to a DNA sequence coding for a desired recombinant protein through a DNA sequence coding for a signal peptide that permits the secretion and maturation of the desired recombinant protein in the genital tract tissue. The construct is transgenically incorporated into animal embryos or stem cells or adult cells used for cloning and the recombinant protein product is subsequently expressed and secreted into or along with the semen of the transgenic animal.

Any animal may be usefully employed in this invention. Preferably, animal that produce large volumes of semen and have frequent ejaculating periods are preferred. Preferred animal are mammals, such as pigs. Of course, each of these animals may not be as effective as the others with respect to any given expression sequence of this invention. For example, a particular genital tract-specific promoter or signal sequence may be more effective in one animal than in others. However, one of skill in the art may easily make such choices by following the teachings of this invention.

Among the genital tract-specific protein promoters useful in the various embodiments of this invention are the p12, p25, kallikreins, PSA, PSSP-C and secretory protein IV promoters. The genital tract specific protein promoter or the promoters that are specifically activated in genital tract tissue may be derived from either cDNA or genomic sequences. Preferably, they are genomic in origin.

Among the signal peptides that are useful in accordance with this invention are genital tract-specific signal peptides or other signal peptides useful in the secretion and maturation of eukaryotic and prokaryotic proteins. Preferably, the signal pep-

tide is selected from genital tract-specific signal peptides or signal peptide of the desired recombinant protein product, if any. Most preferably, the genital tract-specific signal peptide is related to the genital tract-specific promoter used in the expression system of this invention. The size of the signal peptide is not critical for this invention. All that is required is that the peptide be of a sufficient size to effect secretion and maturation of the desired recombinant protein in the genital tract tissue where it is expressed.

Among the protein products which may be produced by the processes of this invention include, for example, mono- or bi-specific antibodies, immunoglobulins, cytokines, coagulation factors, tissue plasminogen activator. GM-CSF, erythropoietin, thrombopoietin, alpha-1 antitrypsin, animal growth hormones, cell surface proteins, insulin, interferons, lipases, antiviral protein, antibacterial protein, bacteriocins, peptide hormones, lipocortins and other recombinant protein products.

The desired recombinant protein may be produced as a fused protein containing amino acids in addition to those of the desired or native protein. For example, the desired recombinant protein of this invention may be produced as part of a larger recombinant protein in order to stabilize the desired protein or to make its purification from semen easier and faster. The fusion is then broken and the desired protein isolated. The desired recombinant protein may alternatively be produced as a fragment or derivative of native protein or it may be produced having an amino acid sequence similar to the native protein. Each of these alternatives is readily produced by merely choosing the correct DNA sequence.

Preferably, the expression system or construct of this invention also includes a 3' untranslated region downstream of the DNA sequence coding for the desired recombinant protein. This region apparently stabilizes the RNA transcript of the expression system and thus increases the yield of desired protein from the expression system. Among the 3' untranslated regions useful in the constructs of this invention are sequences that provide a poly A signal. Such sequences may be derived, for example, from the SV40 small t antigen, the bovine growth hormone 3' untranslated region or other 3' untranslated region known in the art. Preferably, the 3' untranslated region is derived from a semen protein. The length of the 3' untranslated region is not critical but the stabilizing effect of its poly A transcript appears important in stabilizing the RNA of the expression sequence.

Optionally, the expression control sequences of this invention also include a 5' untranslated region between the promoter and the DNA sequence encoding the signal peptide. Such untranslated regions are preferably related to the promoter. However, they may be derived from other synthetic, semi-synthetic and natural sources. Again their specific length is not critical, however, they appear to be useful in improving the level of expression.

The above-described expression systems may be prepared using methods well known in the art. For example, various ligation techniques employing conventional linkers, restriction sites etc., may be used to good effect. Preferably, the expression system of this invention are prepared as part of larger plasmids. Such preparation allows the cloning and selection of the correct constructions in an efficient manner as is well known in the art. Most preferably, the expression sys-

tems of this invention are located between convenient restriction sites on the plasmid so that they can be easily isolated from the remaining plasmid sequences for incorporation into the desired animal.

After such isolation and purification, the expression systems or constructs of this invention are added to the gene pool of the animal which is to be transgenically altered. For example, one or several copies of the construct may be incorporated into the genome of an animal embryo by standard or new transgenic techniques. One animal which has been shown to produce up to 500 ml of semen at each two days in pig, almost as much fluid as goat or sheep milk by day. This appears to be an animal of choice for the production of recombinant proteins of interest in the semen.

One technique for transgenically altering an animal is to microinject the construct into the pronuclei of the fertilized animal egg(s) to cause one or more copies of the construct to be retained in the cells of the developing animal(s). Usually, transgenic animals contain at least one copy of the cloned construct in somatic tissues and transmit the gene through the germ line to the next generation. The progeny of the transgenically manipulated embryos may be tested for the presence of the construct by Southern blot analysis of a biopsy of tissue or amplification of a transgene sequence by polymerase chain reaction technique. If one or more copies of the exogenous cloned construct remains stably integrated into the genome of such transgenic embryos, it is possible to establish permanent transgenic animal lines carrying the transgenically added construct.

The litters of transgenically altered animals may be assayed after birth for the incorporation of the construct into the genome of the offspring. Preferably,

this assay is accomplished by hybridizing a probe corresponding to the DNA sequence coding for the desired recombinant protein product or a segment thereof onto chromosomal material from the progeny. Those animal progeny found to contain at least one copy of the construct in their genome are grown to maturity. The male species of these progeny will produce the desired protein in or along with their semen. Alternatively, the transgenic animal may be bred to produce other transgenic progeny useful in producing the desired proteins in their semen.

The present invention will be more readily understood by referring to the following example which is given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Production of transgenic mice

Transgene construct

In order to test the efficiency of the concept, we have generated a chimeric construct in which the human growth hormone (hGH) cDNA was placed under the control of a 4 kb fragment of the p12 regulatory sequence from the mouse (Fig. 1). A poly A tail and an intron from the SV 40 virus were added to stabilize the messenger mRNA. The construct was excised from the vector by *Not*I digestion. For the southern blot analysis the genomic DNA was digested with *Hind*III which liberates a 5.2 kb fragment. The probe corresponds to a fragment of the p12 promoter.

This construct was cloned in the pPol III vector (plasmid) and amplified in *E. coli*. Since it has previously been shown that plasmid sequences impede transgene expression in a eukaryotic system, the resulting construct was isolated from the vector by a Not I digestion. The construction was ultra purified

from the gel using a GenClean* procedure and dissolved in Tris-HCl(5mM)/EDTA(0.2 mM) buffer at a final concentration of 4 ng/ml.

Production of transgenic mice

Transgenic mice were generated by pronuclear microinjection of the construct into B6C/3F1 zygotes. Females were superovulated using one injection of PMSG (Pregnant mare serum gonadotrophin) followed by an injection of hCG (human chorionic gonadrotrophin) 46 hours later. After mating with a male of proven fertility, the female was sacrificed, the fertilized eggs isolated, observed under differential interference contrast optics of an inverted microscope and the most visible pronucleus microinjected with approximately 500 molecules of the transgene. After microinjection, the viable embryos were transferred to the oviduct of a pseudopregnant CD-1 female, obtained by mating with CD-1 vasectomised males.

Results

Identification of transgenic mice

One hundred embryos were microinjected and transferred in three pseudopregnant females. After 21 days, one litter of 7 pups, one of 2 pups and one of one pup were obtained. The screening for positive transgenic mice was performed by means of Southern blot analysis of *Hind*III-digested genomic DNA extracted from tail biopsies. DNA fragments were separated by electrophoresis on agarose gel and transferred to a nylon membrane. Blot hybridization was carried out using a 1 kb BamHI-fragment isolated from the p12 promoter, radiolabeled with [α-^{32}P] dCTP by random priming. Hybridization was performed at 65°C over 16 hours in a solution containing 6X SSC, 25 mM phosphate buffer (pH 7.2), 5X Denhardt's, 0.5% SDS, 1 mM EDTA (pH

* trademark

8.0), and 100 ug/ml denatured salmon sperm DNA. Blots were then washed twice in 2X SSC and 0.1% SDS at room temperature for 15 min. and then in 0.1 X SSC and 0.1% SDS at 60°C for 30 min. and finally revealed with a phosphoimager system. Fig. 2 shows the Southern blot analysis of the integration of the transgene into the genomic DNA of the transgenic mice. The probe derived from the pl2 gene revealed an endogenous fragment of approximately 7 kb. This served as a control for the efficiency of the probe and provides an estimate of the amount of genomic DNA which was loaded in each well.

A specific band at 5.2 kb indicates that mice #1, 3 and 9 carry the transgene. This DNA fragment was liberated from the transgene by the *Hind*III digestion. The two last tracks correspond to positive controls in which the complete construct (+ C-intact) and the construct digested by *Hind*III(+C-*Hind*III) are revealed by bands at 6.3 kb and 5.2 kb respectively.

hGH Determination in semen by Radioimmunoassay

In order to determine if the transgene was active, the transgenic male # 1 (high number of copies) was mated with a wild type female B6C/3F1. In parallel, a wild type male B6C/3F1 was mated with a wild type female B6C/3F1. Twelve hours following copulation the females were sacrificed and the vaginal plug, uterine content and the complete uterus were collected for analysis of the hGH content. Concentrations of hGH were determined by radioimmunoassay (RIA) using a hGH specific kit (Immunocorp, Montreal, Canada) according to the manufacturer's instructions. This process makes it possible to measure very low concentrations (0.01 ng) of hGH in small volumes. In the mouse, the content of the seminal vesicles solidifies and forms a vaginal plug at the time of ejaculation. This reaction prevents the sperm from flowing out the uterus after

copulation. The size of the plug is generally 4 mm X 3 mm. The content of the uterus, the plug and the uterine tissues were analyzed individually. The results are presented in Fig. 3. The uterine contents after mating with the transgenic male (secretions coming mostly from prostate) shows a concentration of 2.53 ng/ml. The whole uterus from the same female (uterus cells dissociated by mechanical means) shows an average of 18 ng/ml of hGH. Finally, the vaginal plug produced after mating with the transgenic male contains a concentration of 30.44 ng/ml of hGH. Since the p12 promoter used in the construct is active mostly in the seminal vesicles, we anticipated that the highest concentration of hGH would be found in the plug.

Conclusion

These experiments were designed to prove that it is possible to use the genital tract and the accessory glands of the male to synthesize recombinant proteins. This first set of experiments clearly illustrates that a human peptide, (hGH) not normally found in mouse semen, has been newly synthesized at a significative concentration in the semen of the transgenic animal. Characterization of the other transgenic mice is under way.

The same experiment could be conducted in pigs with modifications in regard to the protocol of superovulation and the surgeries required for the collection and the transfer of the pig embryos.

It should be understood that this is one specific example designed to illustrate the technology. Although the targeted tissues are components of the genital tract, one can use other regulatory sequences or cDNA or genes to be expressed using the same methodology.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for the production and secretion into a mouse's semen of an exogenous recombinant protein comprising the steps of:

a) producing a transgenic mouse whose genome comprises an expression system comprising in operable association a p12 promoter, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the mouse's semen to a detectable level;

b) collecting semen produced by said transgenic mouse; and

c) isolating the exogenous recombinant protein from the semen.

2. A method for the production and secretion into a pig's semen of an exogenous recombinant protein comprising the steps of:

a) producing a transgenic pig whose genome comprises an expression system comprising in operable association a p12 promoter, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the pig's semen to a detectable level;

b) collecting semen produced by said transgenic pig; and

c) isolating the exogenous recombinant protein from the semen.

3. The method according to claim 1 or 2, wherein said expression system further comprises a 3' untranslated region downstream of the DNA sequence encoding said an exogenous recombinant protein.

4. The method according to claim 1 or 2, wherein said expression system further comprises a 5' untranslated region between said promoter and said DNA sequence encoding a signal peptide.

5. The method according to claim 1 or 2, wherein said promoter is selected from the group consisting of p12, p25, kallikreins, PSA, SBP-C and secretory protein IV promoters.

6. The method according to claim 1 or 2, wherein said recombinant protein is selected from the group consisting of, catalase, mono- and bi-specific antibodies, immunoglobulins, cytokines, coagulation factors, tissue plasminogen activator, GM-CSF, erythropoietin, thrombopoietin, alpha-1 antitrypsin, animal growth hormones, cell surface proteins, insulin, interferons, lipases, antiviral protein, antibacterial protein, bacteriocins, peptide hormones, lipocortins and epidermal growth factor.

7. A method to increase sperm viability and semen storage which comprises the steps of:

a) producing a transgenic mouse whose genome comprises an expression system comprising in operable association a promoter specific for the genital tract or accessory glands, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the mouse's semen to a detectable level; and

b) collecting semen produced by said transgenic mouse;

whereby said semen has an improved storage capability and containing sperms of increased viability,

and wherein said protein heterologous to the promoter is selected from the group consisting of catalase, superoxide dismutase, calcitonin, antibiotics, and epididymal fertility proteins.

8. A method to increase sperm viability and semen storage which comprises the steps of:

a) producing a transgenic pig whose genome comprises an expression system comprising in operable association a promoter specific for the genital

tract or accessory glands, a DNA sequence encoding a signal peptide and a DNA sequence encoding an exogenous recombinant protein heterologous to the promoter, wherein expression of said DNA sequences results in the production and secretion of said protein of interest into the pig's semen to a detectable level; and

b) collecting semen produced by said transgenic pig;

whereby said semen has an improved storage capability and containing sperms of increased viability,

and wherein said protein heterologous to the promoter is selected from the group consisting of catalase, superoxide dismutase, calcitonin, antibiotics, and epididymal fertility proteins.

9. The method according to claim 7 or 8, wherein said antibiotic is gentamycin.

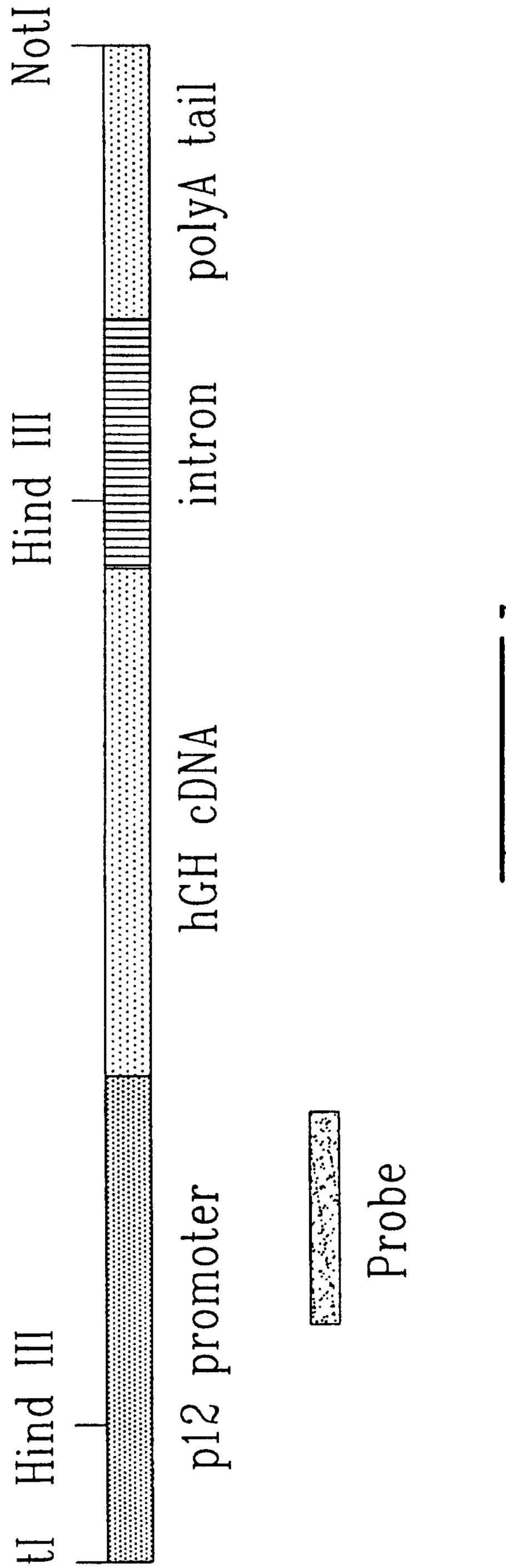
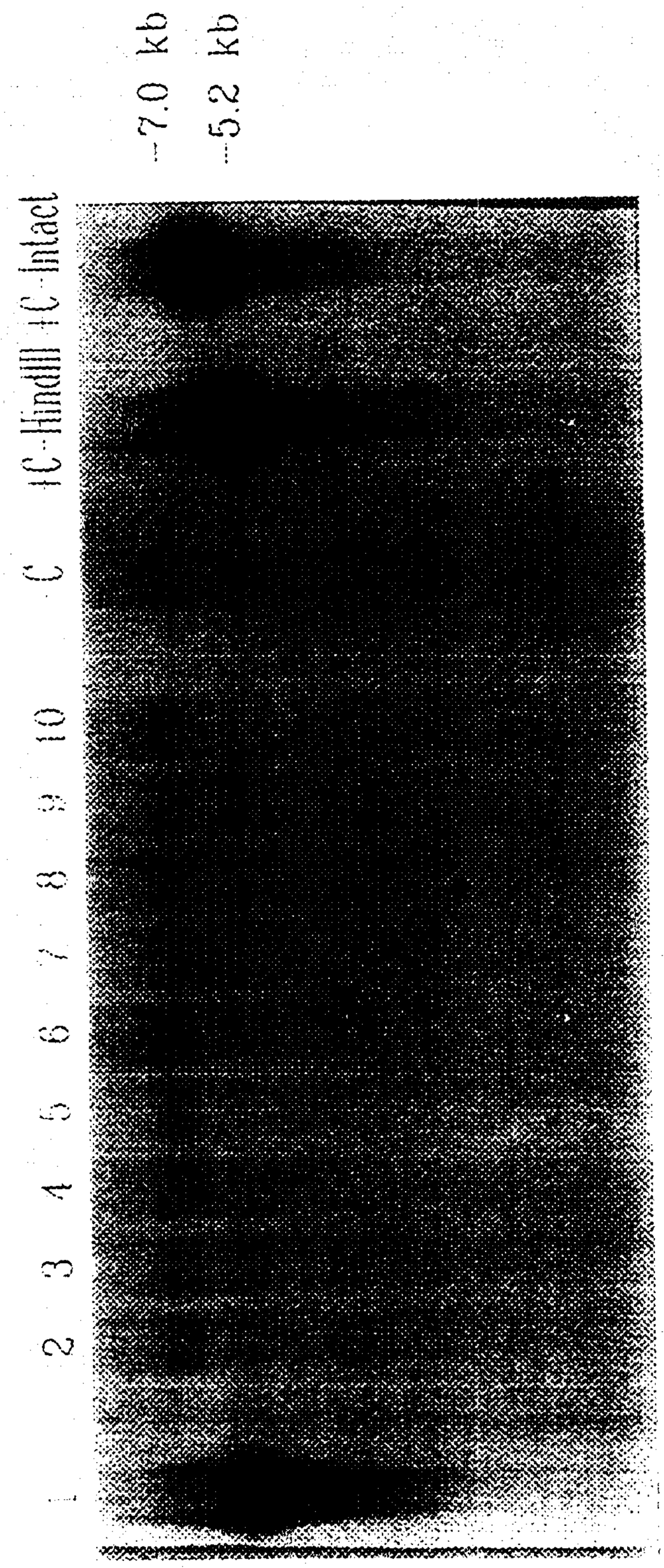

NotI
Hind III
intron
polyA tail
hGH cDNA
p12 promoter
Probe
NotI Hind III
p12 promoter
Probe

FIG. 1


C +C-HindIII +C-Intact
7.0 kb
5.2 kb
1 2 3 4 5 6 7 8 9 10

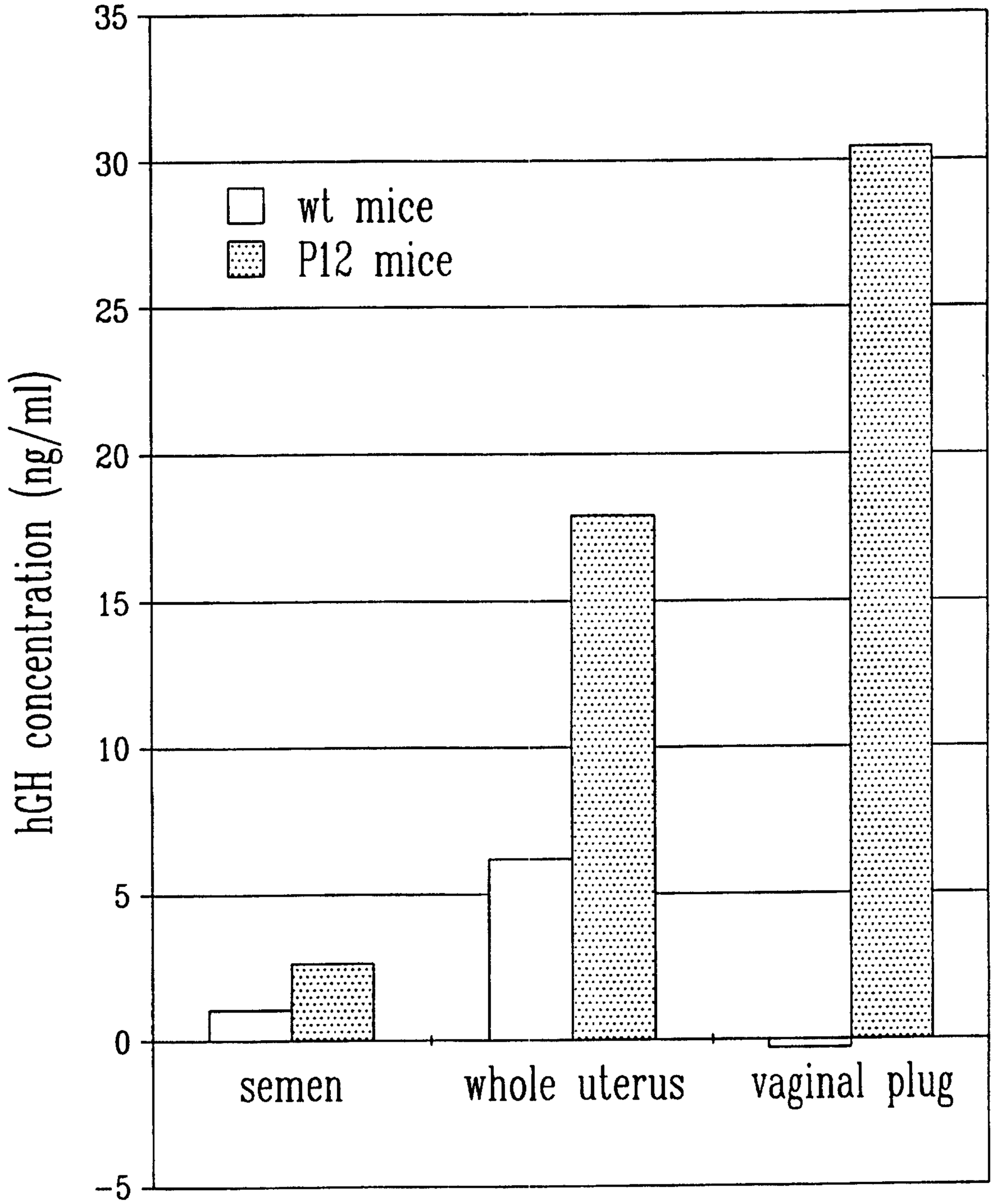
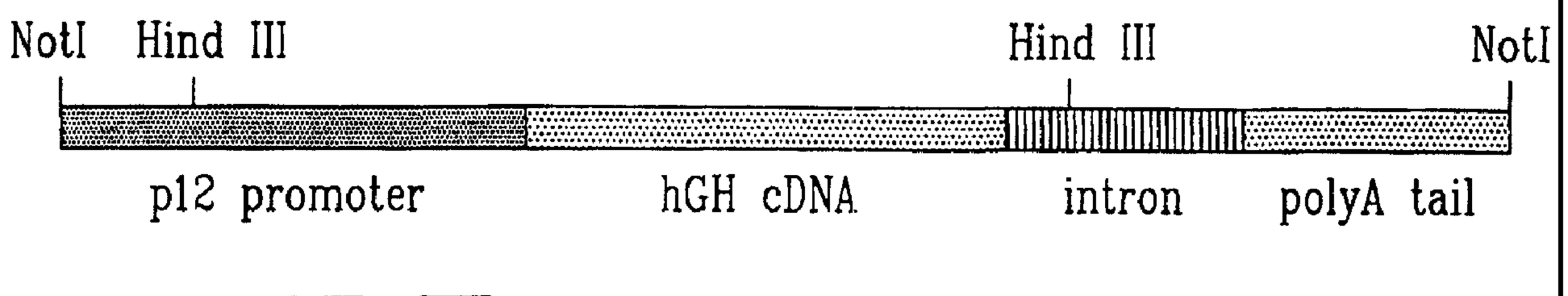

35
30
25
20
15
10
5
0
-5
wt mice
P12 mice
hGH concentration (ng/ml)
semen
whole uterus
vaginal plug
Samples

Fig. 3


NotI
Hind III
Hind III
NotI
p12 promoter
hGH cDNA
intron
polyA tail
Probe