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(54) **VECTEUR PERMETTANT L'EXPRESSION D'UN GENE,
INDEPENDAMMENT DU SITE D'INTEGRATION, DANS DES
CELLULES HOTES DE MAMMIFERE**
(54) **VECTOR FOR INTEGRATION SITE INDEPENDENT GENE
EXPRESSION IN MAMMALIAN HOST CELLS**

(57) The invention provides a transgenic mammal transformed with a vector, the vector comprising a promoter and a gene to be expressed, wherein the vector includes an immunoglobulin dominant control region capable of eliciting host cell-type restricted, integration site independent, copy number dependent expression of the said gene.

ABSTRACT

The invention provides a transgenic mammal transformed with
5 a vector, the vector comprising a promoter and a gene to be
expressed, wherein the vector includes an immunoglobulin
dominant control region capable of eliciting host cell-type
restricted, integration site independent, copy number
dependent expression of the said gene.

Vector for integration site independent gene expression in mammalian host cells.

5 This application is a divisional application of Canadian Patent Application No. 2,050,920 filed February 22, 1990.

Field of the Invention

10 The present invention relates to recombinant DNA technology and in particular to a vector useful for transfecting mammalian cells in vivo and in vitro to obtain expression of a desired structural gene. The invention relates also to the use of such vectors in gene therapy and heterologous gene expression.

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Background to the Invention

20 There is a continuing need for improved expression vectors exhibiting high levels of expression. In particular, expression vectors for use in mammalian cell lines are of increasing importance both for the industrial production of desired polypeptides and for the development of therapies for genetic disorders.

25 There are many known examples of characterised structural genes, which together with appropriate control sequences may be inserted into suitable vectors and used to transform host cells. A significant problem with the integration of such a structural gene and control regions into the genome of a mammalian cell is that expression has been shown to be highly dependent upon the position of the inserted sequence in the genome. This results in a wide variation in the expression level and only very rarely in a high expression level. The problem of integration site dependence is solved 30 by the present invention and arises from the discovery of specific sequences referred to herein as dominant control regions (DCRs) derived from immunoglobulin genes which have the property of conferring a cell-type restricted, integration site independent, copy number dependent 35 expression characteristic on a linked gene system.

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Two mammalian gene systems have been shown to possess DCRs,

namely the β -like globin genes (Grosveld, F.G. et al., Cell, 51, (1987), 975; International patent application PCT/GB 88/00655, published as WO 89/01517), and the human CD2 T-cell marker gene (Lang, G., et al., EMBO J, 7, (1988), 1675; 5 International patent application PCT/GB 88/00655).

When a mammalian gene such as β -globin containing all the usual control regions is introduced into transgenic mice, the gene is not expressed at the same level as the mouse β -globin gene and exhibits integration site position effects. 10 This is characterized by a highly variable expression of the transgene that is not correlated with the copy number of the injected gene in the mouse genome. The same phenomenon has been observed in almost all the genes that have been studied 15 in transgenic mice (Palmiter et al., Ann. Rev. Genet., (1986), 20, 465-499). Moreover, the level of expression of each injected gene in the case of β -globin is, at best, an order of magnitude below that of the endogenous mouse gene (Magram et al., Nature, (1985), 315, 338-340; Townes et al., 20 EMBO J., (1985), 4 1715-1723; Kollias et al., Cell, (1986), 46, 89-94). A similar problem is observed when the β -globin or other genes are introduced into cultured cells by transfection or retroviral infection. This poses a big 25 problem when considering gene therapy by gene addition in stem cells. It is also a major problem for the expression of recombinant DNA products in cultured cells. Extensive screening for highly producing clones is necessary to identify cell-lines in which the vector is optimally expressed and selection for vector amplification or use of 30 multicopy viral vectors is generally required to achieve expression levels comparable to those of the naturally occurring genes, such as for example β -globin genes in erythroleukaemic cell-lines.

35 In a chromosome, the genetic material is packaged into a DNA/protein complex called chromatin, one effect of which may be to limit the availability of DNA for functional purposes. It has been established that many gene systems

(including the β -globin system) possess so-called DNaseI hypersensitive sites. Such sites represent putative regulatory regions, where the normal chromatin structure is altered, for instance by interaction with regulatory 5 proteins or to allow such interaction.

Regions flanking the β -like globin gene locus which contain a number of "super" hypersensitive sites have been identified. These sites are more sensitive to DNase I 10 digestion in nuclei than the sites found in and around the individual genes when they are expressed (Tuan et al, PNAS USA, (1985), 32, 6384-6388; Groudine et al, PNAS USA, (1983), 80, 7551-7555). In addition, they are erythroid 15 cell specific and they are present when any one of the globin genes is expressed.

Tuan et al describe the broad mapping of four major DNase I hypersensitive sites in the 5' boundary area of the " β -like" globin gene. The authors note that certain sequence 20 features of these sites are also found in many transcriptional enhancers and suggest that the sites might also possess enhancer functions and be recognised by erythroid specific cellular factors.

25 It has been discovered that the complete β -globin gene with intact 5' and 3' boundary regions does not exhibit an integration site position dependence (see copending International patent application PCT/GB 88/00655). The regions of the locus responsible for this significant 30 characteristic have been determined and shown to be associated with DNase I super hypersensitive sites. These dominant control regions are quite distinct from enhancers, exhibiting properties such as integration site independence not exhibited by the known enhancers. The dominant control 35 region used in conjunction with the known promoter/enhancer elements reconstitute the full transcription rate of the natural gene.

Immunoglobulin genes have been extensively studied in order to identify sequences regulating gene expression. An immunoglobulin molecule consists of two identical heavy polypeptide chains and two identical light polypeptide chains. The light chains may be either of the κ or λ type. The genes encoding the heavy chain, the κ light chain and the λ light chain are each located on separate chromosomes in the mouse and man.

Unlike most genes which are transcribed from continuous genomic DNA sequences, immunoglobulin genes are assembled from gene segments which may be widely separated in the germ line.

Functionally, heavy chain genes are formed by recombination of three genomic segments encoding the variable (V), diversity (D) and joining (J)/constant (C) regions of the molecule (Figure 1). Functional light chain genes are formed by joining of two segments, one encoding the V region and the other the J/C region. Both the heavy chain and κ light chain loci contain many V gene segments (estimates vary between 100s and 1000s) estimated to span well over 1000kb (Figure 1). The λ locus is, by contrast, much smaller and has recently been shown to span approximately 300 kb on chromosome 16 in the mouse. It consists of four joining/constant region gene segments and two variable gene segments (Figure 1). Recombination resulting in functional genes occurs predominantly between V₁ and either J₁/C₁ or J₃/C₃ elements or between V₂ and J₂/C₂ elements (J₄/C₄ is a pseudogene) although recombinations between V₂ and J₃/C₃ or J₁/C₁ are seen very rarely.

Control of transcription of both rearranged heavy and κ light chain genes depends both on the activity of a tissue specific promoter upstream of the V region (Mason, J.O. et al, Cell, (1985), 41, 479; Bergman, Y. et al, PNAS USA, (1984), 81, 7041) and a tissue specific enhancer located in the J-C intron (Gillies, S.D. et al, Cell, (1983), 33, 717;

Banerji, J. et al, Cell, (1983), 33, 729; Picard, D. et al, Nature, 307, 80). These elements act synergistically (Garcia, J.V. et al, Nature, (1986), 322, 383). Recently a second B-cell specific enhancer has been identified in the 5 κ light chain locus (Meyer, K.B. et al, EMBO J., (1989), 8, No. 7, 1959-1964). This further enhancer is located 9 kb downstream of C_{κ} .

More recently Bich-Thuy and Queen (1989 NAR 17:5307) 10 described an enhancer activity immediately downstream of the rearranged λ , gene. Sequences downstream of the λ , gene increased expression of a chloramphenicol acetyl transferase (CAT) reporter gene linked to the λ , promoter in a myeloma cell line which made lambda light chains (J558L) but not in 15 two myeloma cell lines which make κ light chains.

This enhancer activity differs in several ways from that of the heavy chain and κ light chain enhancers. Firstly, it 20 displayed a marked orientation preference. Secondly, it consists of several segments which can independently stimulate transcription and which are spread over about 4 kb of DNA immediately downstream of the λ , coding sequence. Thirdly, it is apparently only expressed in λ chain 25 producing myeloma cells and not in cells producing κ light chains.

Spandidos and Anderson (1984 FEBS Lett. 175: 152) describe an 8kb fragment (containing 2 constant region gene segments) 30 from the human λ locus which increases the number of G418 resistant colonies obtained after transfection of myeloma cells with a plasmid containing the 8kb fragment linked to the aminoglycoside-phosphotransferase (aph) gene under the control of an ϵ -globin promoter. Levels of aph specific mRNA were reported to be increased following transient 35 transfection of this construct when compared with a construct lacking the 8kb sequence. However, no controls were presented for transfection efficiency in the transient assays and no further reports relating to enhancer activity

in the human λ locus have been published.

DNA fragments carrying rearranged heavy or κ light chain genes are expressed when transfected into lymphoid cells 5 although generally at least 10 times less efficiently than endogenous immunoglobulin genes (Oi, V.T. et al, PNAS USA, (1983), 80, 825; Neuberger, M.S., EMBO J., (1983), 2, 1373). Expression of a similar DNA fragment containing a rearranged $\lambda 1$ gene however is not detectable in transfected lymphoid 10 cells and expression is only observed when an SV40 enhancer is added (Picard, D. et al, Nature, (1984), 307, 80; Cone, R.D. et al, Science, (1987), 236, 954). This is apparently due to the absence of a functional enhancer such as that present in the J-C intron of the κ gene.

15

Although promoter and enhancer elements associated with κ and heavy chain genes are sufficient to confer lymphoid cell specific expression of antibody genes, the level of expression is reduced compared with expression of endogenous 20 antibody genes and genes are subject to position effects such that no clear relation exists between the level of expression and the copy number of the introduced gene (Oi, V.T. et al, PNAS USA, (1983), 80, 825; Neuberger, M.S., EMBO J., (1983), 2, 1373). Similar results are obtained when κ 25 and heavy chain genes are introduced into transgenic mice (Storb U., et al, Ann. Rev. Immunol., 5, (1987), 151).

Sequences required for full regulation of antibody gene expression are therefore lacking from constructs used to 30 date in expression studies (Grosschedl, R. et al, Cell, (1985), 55, 645).

We have now identified super hypersensitive sites in the rearranged λ , mouse immunoglobulin gene outside of the 35 region previously suggested to be involved in expression of the λ , gene. The strong implication of our finding is that immunoglobulin gene expression shares a common feature with β -globin and CD2 expression, namely the existence of

dominant control regions.

Many active genes have hypersensitive sites associated with their promoters. For example, a tissue specific DNaseI 5 hypersensitive site is observed 300bp upstream from the start of the coding region of the rearranged κ immunoglobulin gene (Chung, S-Y et al. PNAS USA, 80, 2427). No such site was identified in the rearranged λ , gene which was studied in the present work nor was any site identified 10 in the J-C intron where hypersensitive sites associated with immunoglobulin enhancers have been identified in both the heavy chain (Mills, F. et al., (1984), Nature, 306, 807) and κ light chain genes (Chung, S-Y et al., PNAS USA, 80, 2427; Parslow, T.G. et al., NAR, (1983), 11, 4775); Weischet et al., 15 NAR, (1982), 10, 3627).

The hypersensitive sites characteristic of the dominant control regions of the β -globin locus identified by Grosveld et al (Grosveld, F.G. et al., Cell, (1987), 51, 957-985) are 20 referred to as "super hypersensitive". The designation "super" hypersensitive refers to the fact that these sites are much more sensitive to DNaseI than the "normal" hypersensitive site associated with the β -globin promoter (Groudine, M. et al., PNAS USA, (1983), 80, 7551). In the 25 mouse λ locus, promoter hypersensitive sites are not detectable and the only DNaseI hypersensitive sites which are seen, map at larger distances from the gene.

The hypersensitive sites in the mouse λ locus indicate 30 locations of sequences which, according to the present invention confer dominant control of immunoglobulin genes. On the basis of this mapping, DNA sequences flanking the rearranged gene in J558L have been cloned.

35 The present invention is applicable to the production of transgenic animals and the techniques for producing such are now widely known. For a review, see Jaenisch, Science, (1988), 240, 1468-1474.

The present invention provides a solution to the problem of integration site dependence of expression making possible the insertion of functionally active gene systems into mammalian genomes both in vitro and in vivo. Specifically, 5 the present invention provides a vector capable of expressing homologous and heterologous genes after their introduction into immunoglobulin producing cells, whether in vivo or in vitro, in an integration-site independent cell specific manner.

10

We have found also that certain of the hypersensitive sites in the mouse λ locus correspond to new useful λ immunoglobulin enhancer elements. Thus the present 15 invention further provides these new enhancer elements and vectors containing these elements, useful for the expression of heterologous genes.

Summary of the Invention

20 According to a first aspect of the present invention, there is provided a transgenic mammal transformed with a vector for the integration of a gene into the genetic material of a mammalian host cell such that the gene may be expressed by the host cell, the vector comprising a promoter and the said gene, characterised in that the vector includes an immunoglobulin gene dominant control region capable of eliciting host cell-type restricted, integration site independent, copy number dependent expression of the said gene.

25

30 As used herein the term "dominant control region" means a sequence of DNA capable of conferring upon a linked gene expression system the property of host cell-type restricted, integration site independent, copy number dependent expression when integrated into the genome of a host cell compatible with the dominant control region. Such a dominant control region retains this property when fully 35 reconstituted within the chromosome of the host cell. The

ability to direct efficient host cell-type restricted expression is retained even when fully reformed in a heterologous chromosomal position.

- 5 It is hypothesised that the dominant control regions of the invention may open the chromatin structure of the DNA, making it more accessible and thus may act as a locus organiser.
- 10 The dominant control region may be a single contiguous sequence corresponding to, or derived from a naturally occurring gene system or may consist of two or more such sequences linked together with or without intervening polynucleotides.
- 15 The dominant control region may be derived by recombinant DNA techniques from a naturally occurring gene system or may correspond to a naturally occurring gene system in the sense of being manufactured using known techniques of
- 20 polynucleotide synthesis from sequence data relating to a naturally occurring gene system. Alterations of the sequence may be made which do not alter the function of the dominant control region.
- 25 The dominant control region consists of, includes, is derived from, corresponds to, or is associated with, one or more DNase I super hypersensitive sites of an immunoglobulin gene.
- 30 The dominant control region may comprise, for example, one or more of the DNase I super hypersensitive sites specifically identified herein.

Other sequences might, however, contribute to or exhibit

- 35 the functional characteristics of a dominant control region.

A number of DNase super hypersensitive sites have been identified:

- i) about 2.4kb upstream of the CAP site of the rearranged λ_1 gene,
- 5 ii) about 2.5kb upstream of the genomic $V\lambda_2$ segment, and
- iii) additional sites located 3' to $C\lambda_1$, and/or $C\lambda_4$, as follows:
 - about 17 kb 3' of $C\lambda_4$
 - between 28 and 48 kb (i.e about 30kb) 3' of $C\lambda_1$,

10 Any of the above-identified super hypersensitive sites may, on their own, constitute a dominant control region or may form part of a dominant control region.

15 The methods used to identify these super hypersensitive sites may be used to identify functional dominant control regions.

20 Alternatively functional expression assay methods may be used to identify functional dominant control regions. For example, expression assays using an appropriate reporter gene, such as an immunoglobulin gene may be used, wherein dominant control regions are identified by their capability 25 of eliciting host cell-type restricted, integration site independent, copy number dependent expression of the reporter gene.

Where the naturally occurring dominant control region 30 comprises two or more subsequences separated by an intervening polynucleotide sequence or sequences, the dominant control region may comprise two or more of the subsequences linked in the absence of all or a part of one or more of the intervening sequences. Thus, if the 35 immunoglobulin dominant control region comprises two or more discrete subsequences separated by intervening non-functional sequences, (for example, two or more super hypersensitive sites) the vector of the invention may

comprise a dominant control region comprising two or more of the subsequences linked together with all or part of the intervening sequences removed.

5 One or more super hypersensitive sites may alone or collectively constitute a useful enhancer element derived from the mouse λ immunoglobulin locus.

It will be understood that a super hypersensitive site or a 10 group of such sites which does not exhibit integration site independence is to be considered as an enhancer not a dominant control region.

Enhancers are sequences which activate transcription. They 15 may be located upstream (for example, the SV40 early region enhancer, Banerji *et al* 1981 *Cell* 27: 299), downstream (for example the β -globin enhancer, Choi and Engel 1986 *Nature* 323:731), or within (for example the immunoglobulin heavy chain enhancer, Gillis *et al* 1983 *Cell* 33: 717, Banerji *et* 20 *al* 1983 *Cell* 33: 729) the transcribed region of the gene.

Enhancers activate transcription in a manner which is relatively independent of their distance from and independent of their orientation with respect to the gene 25 and they are not promoter specific. For example, the immunoglobulin heavy chain enhancer can activate transcription from a β -globin promoter (Picard and Shaffner 1984 *Nature* 307: 80-82).

30 Some enhancers are active in a variety of different cell types (for example, the enhancers from SV40 and Rous Sarcoma Virus (RSV)). Other enhancers show cell type specificity (for example, the immunoglobulin enhancers are only active in lymphoid cells see Maniatis *et al* 1987 *Science* 236: 1237-35 1245 for a review). This cell-type specificity of enhancer activity can be seen in both cultured cells and transgenic mice (transgenic mice reviewed by Palmiter and Brinster 1986 *Ann. Rev. Genet.* 20 465-499).

According to a second aspect of the present invention, there is provided a functional enhancer element derived from a mouse λ immunoglobulin locus. The mouse λ immunoglobulin 5 enhancer element may comprise one or more super hypersensitive sites or a separate region within the mouse λ immunoglobulin locus.

The second aspect of the invention includes a vector 10 including an enhancer of the second aspect of the invention. In the case of an expression vector the enhancer is in functional association with a promoter (suitably an immunoglobulin promoter) such that the enhancer activates transcription of a gene downstream of the promoter.

15

Such an enhancer sequence may contribute to or exhibit the functional characteristics of a dominant control region.

We have identified a region downstream of the mouse $C\lambda$, gene 20 segment which possesses enhancer activity and may be a further dominant control region or a part thereof in combination with the super hypersensitive sites of the first aspect of the invention.

25 The region consists of a DNA sequence comprising all or a functional part of the DNA sequence between the EcoRI site 3.8 kb downstream of the XhoI site in the $C\lambda$, gene segment of the rearranged λ , gene and the SnaBI site 10 kb downstream of this XhoI site.

30

As used herein, the expression "functional" as applied to an enhancer connotes a DNA sequence which is capable of activating transcription from a promoter in a manner which is independent of its distance from or orientation with 35 respect to the gene and is not promoter specific.

As used herein, the expression "downstream" connotes in the direction of transcription.

The mouse immunoglobulin λ , enhancer may be a single contiguous sequence corresponding to, or derived from a naturally occurring mouse immunoglobulin λ , gene system or 5 may consist of two or more such sequences linked together with or without intervening nucleotides or polynucleotides.

The mouse immunoglobulin λ , enhancer may be derived by recombinant DNA techniques from a naturally-occurring gene 10 system or may correspond to a naturally occurring gene system in the sense of being manufactured using known techniques of polynucleotide synthesis from sequence data relating to a naturally occurring gene system. Alterations of the sequence may be made which do not alter the function 15 of the mouse immunoglobulin λ , enhancer.

The sequence between the EcoRI site 3.8 kb downstream of the rearranged mouse λ , gene and the SnaBI site 10 kb downstream of the XhoI site in the rearranged mouse λ , gene contains or 20 overlaps two HindIII fragments both of which independently enhance expression of the λ , gene in SP2/0 cells.

The enhancer of the invention may comprise all or a functional part of the 1.3 kb first HindIII to HindIII DNA 25 fragment downstream of the EcoRI site 3.8 kb downstream of the XhoI site in the rearranged mouse λ , gene.

With reference to Figure 11, the said 1.3 kb HindIII to HindIII fragment downstream of the EcoRI site 3.5 kb 30 downstream of the rearranged mouse λ , gene is that labelled "HIIIA".

The enhancer of the invention may comprise all or a functional part of the 3.3 kb HindIII to HindIII DNA 35 fragment downstream of the EcoRI site 3.8 kb downstream of the XhoI site in the rearranged mouse λ , gene and spanning the SnaBI site 10 kb downstream of the XhoI site in the rearranged mouse λ , gene.

With reference to Figure 11, the said 3.3 kb *HindIII* to *HindIII* fragment downstream of the *EcoRI* site 3.8 kb downstream of the *XhoI* site in the rearranged mouse λ , gene 5 and spanning the *SnaBI* site 10 kb downstream of the *XhoI* site in the rearranged mouse λ , gene is that labelled "HIIIC".

The term "vector" as used herein connotes in its broadest 10 sense any recombinant DNA material capable of transferring DNA from one cell to another.

The vector may be a single piece of DNA in linear or circular form and may, in addition to the essential 15 functional elements of the invention, include such other sequences as are necessary for particular applications. For example, the vector may contain additional features such as a selectable marker gene or genes, and/or features which assist translation or other aspects of the production of a 20 cloned product.

A vector suitable for integration consists of an isolated DNA sequence comprising an immunoglobulin dominant control region or enhancer and an independent structural gene 25 expression system. The DNA sequence is not linked at either end to other substantial DNA sequences. The isolated DNA sequence may however be provided with linkers for ligation into a vector for replication purposes or may be provided with sequences at one or both ends to assist integration 30 into a genome.

The vector including a dominant control region defines an independent locus which can express homologous and heterologous genes after integration into a mammalian host 35 cell normally capable of immunoglobulin expression, in an integration site independent, copy number dependent manner.

The invention also provides a transfer vector, suitably in

the form of a plasmid, comprising an immunoglobulin dominant control region or an enhancer of the second aspect of the invention.

5 The transfer vector may comprise an isolated functional mouse immunoglobulin λ enhancer selected from:

i) the sequence between the *EcoRI* site 3.8 kb downstream of the *XhoI* site in the rearranged mouse λ , gene and the 10 *SnaBI* site 10 kb downstream of the *XhoI* site in the rearranged mouse λ , gene,

ii) all or a functional part of the 1.3 kb first *HindIII* to *HindIII* DNA fragment downstream of the *EcoRI* site 3.8 kb 15 downstream of the *XhoI* site in the rearranged mouse λ , gene (Figure 11: that labelled "HIIIA"),

iii) all or a functional part of the 3.3 kb *HindIII* to *HindIII* DNA fragment downstream of the *EcoRI* site 3.8 kb 20 downstream of the *XhoI* site in the rearranged mouse λ , gene and spanning the *SnaBI* site 10 kb downstream of the *XhoI* site in the rearranged mouse λ , gene (Figure 11: that labelled "HIIIC"),

25 The term "gene" as used herein connotes a DNA sequence, preferably a structural gene encoding a polypeptide. The polypeptide may be a commercially useful polypeptide, such as a pharmaceutical, e.g. a plasminogen activator, lymphokine or potentiating or stimulating factor, or an 30 immunoglobulin polypeptide, e.g. a chimeric, humanized or altered immunoglobulin polypeptide. The polypeptide may be entirely heterologous to the host cell. Alternatively, the gene may encode a polypeptide which is deficient absent or mutated in the host cell.

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The mammalian host cell may be any mammalian host cell susceptible to uptake of the vector of the invention and capable of immunoglobulin gene expression. The vector DNA

may be transferred to the mammalian host cell by transfection, infection, microinjection, cell fusion or protoplast fusion.

5 The host cell may be a cell of a living human or an animal. In particular, the host cell may be a cell of a transgenic animal such as a mouse. The host cell may be derived from tissue in which the dominant control region is functional, such as B-cells or precursors thereof (where the dominant 10 control region may not be functional), such as stem cells. The cell may be a lymphoid cell such as a myeloma or hybridoma.

15 The promoter may be any promoter capable of functioning in the host cell and may be for example a mammalian or viral promoter. Optionally, the promoter may be homologous with the gene locus of the dominant control region and may be present in tandem with another promoter such as a viral tk or other viral promoter and may include one or more enhancer 20 elements. Examples of suitable viral promoters include the SV40 late, Mo MLV LTR, RSV LTR, and hCMV MIE promoters.

25 The immunoglobulin dominant control region may be from the λ , κ or heavy chain locus or from other genes specifically expressed in B-cells.

30 In a further aspect of the invention, there is provided a method of producing a polypeptide comprising culturing a host cell transformed with a vector of the invention.

35 The method may be applied in vitro to produce a desired polypeptide. In addition, the method may be applied in vivo to produce a polypeptide having no therapeutic value to the animal. Such a method of producing a polypeptide is not to be considered as a method of treating the human or animal body.

40 In a further aspect of the invention the vector may be used

in a method of treatment of the human or animal body by replacing or supplementing a defective mammalian gene.

Many diseases of the human or animal body result from 5 deficiencies in the production of certain gene products. The characterising features of the vectors of this invention make them amply suited to the treatment of deficiencies by gene therapy in vivo.

10 A method of gene therapy is provided comprising removing stem cells from the body of a human or an animal, optionally killing stem cells remaining in the body, transforming the removed stem cells with a vector of the invention containing a gene deficient, or absent, in the human or animal body, 15 and replacing the transformed stem cells in the human or animal body.

This method can be used to replace or supplement a gene deficient in a human or animal. For example, an individual 20 suffering from adenosine deaminase deficiency, severe combined immune deficiency (SCID) or hprt deficiency. Thus the method may be used specifically in the treatment of B-cell genetic defects but also in the treatment of general metabolic genetic disorders wherein the cell source of the 25 gene product is not critical.

The invention is now described by way of example with reference to the accompanying drawings.

30 Brief Description of the Drawings

Fig 1 shows the structure of the mouse immunoglobulin (a) λ , (b) κ and (c) heavy chain locii.

35 Fig 2 shows the rearrangements on each mouse chromosome 16 of the λ locus in myeloma cell-line J558L.

Fig 3 shows the rearranged mouse λ , gene in the myeloma

cell-line J558L.

Fig 4 shows DNaseI fadeouts on myeloma cell-line J558L nuclei cut with a) ApaLI (A) and with ApaLI and XhoI (B), probed with a C λ , probe and b) SphI(A) and with SphI and XhoI(B) probed with a V λ , probe.

Fig 5 shows DNaseI fadeouts on nuclei from J558L and MEL nuclei each digested with KpnI and probed with a V λ , probe.

Fig 6 shows a mapping of the DNaseI hypersensitive sites identified in the J558L genome (3' hypersensitive sites are indicated in parenthesis).

Fig 7 shows restriction maps of the pLTC cosmid cloning vector (a) and of fragments of J558L DNA cloned at the BamHI site of pLTC. (b) The position of the 7.5 kb EcoRI fragment carrying the λ , gene is shown (all other EcoRI sites have been omitted for clarity). All ApaLI sites mapping 3' of the λ , gene in cos 4N and cos 3'.3 are shown whereas in cos 2B only the ApaLI site 5' of the λ , gene is shown. ApaLI sites lying further upstream of this site have not yet been mapped.

Fig 8 shows the results of S1 protection analysis on RNA from various constructs in SP2/0 using a λ , mRNA-specific probe (Lane 1 - 1 μ g J558L RNA: Lane 2 - 10 μ g SP2/0 RNA: Lane 3 - 10 μ g SP2/0-cos2B RNA: Lane 4 - 10 μ g SP2/0-cos4N RNA: Lane 5 - 10 μ g SP2/0-pLTC λ , RNA: Lane 6 - 10 μ g SP2/0-pSVneo λ , RNA),

Fig 9 shows the restriction maps of fragments of cos4N prepared on salt gradients and used to transfect SP2/0,

Fig 10 shows the results of S1 protection analysis on RNA

from the fragments shown in Figure 9 in SP2/0 using a λ , mRNA-specific probe (Lane 1 - 10 μ g SP2/0 RNA: Lane 2 - 1 μ g J558L RNA: Lanes 3 and 4 - 10 μ g SP2/0-cos4N RNA: Lanes 5 to 9 - 10 μ g SP2/0-cos4N ApaLI 24.5 kb fragment RNA: Lane 10 - pBR322 HinfI markers: Lanes 11 to 14 - 10 μ g SP2/0-cos4N ApaLI/SnaBI 19kb fragment RNA),

Fig 11 shows the restriction map of the enhancer region of the mouse λ , gene including the two *Hind*III fragments thereof which exhibit enhancer activity,

Fig 12 shows the restriction maps of the plasmid, pLTC λ , used to test putative enhancer fragments for transcription activation by stable transfection of SP2/0.

Fig 13 shows the results of S1 protection analysis on RNA from the vectors shown generally in Figure 12 (a) in SP2/0 using a λ , mRNA-specific probe (Lane 1 - 10 μ g SP2/0 RNA: Lane 2 - 1 μ g J558L RNA: Lane 3 - 10 μ g SP2/0-cos4N RNA: Lanes 4 and 5 - SP2/0 pLTC λ 1HIII α 10 μ g RNA: Lanes 6 and 7 - SP2/0 pLTC λ , HIIIA β 10 μ g RNA: Lanes 8 and 9 - SP2/0 pLTC λ , 10 μ g RNA: Lanes 10 and 11 - SP2/0 pLTC λ , HIIIC α 10 μ g RNA: Lanes 12 and 13 - SP2/0 pLTC λ , HIIIC β 10 μ g RNA).

Fig 14 shows S1 nuclease protection analysis of mRNA from tissues of transgenic (36, 37 and 38) and non-transgenic (39 and 40) littermates of the mouse line 2071.

T = thymus
S = spleen
L = liver
B = brain

Each track contains 10 μ g of RNA hybridised with a 5'

λ , cDNA probe and a 5' β actin cDNA probe. 1 μ g of J558L RNA was hybridised with the same probes as a positive control.

5 The position of migration of the protected fragments is indicated by the arrow.

Detailed Description of Embodiments

10 1. Identification of Hypersensitive Sites in J558L Nuclei.

Hypersensitive sites (HSSs) associated with dominant control regions (DCRs) were sought at large distances from the immunoglobulin locus. Southern blots were carried out on 15 myeloma cell-line J558L DNA cut with a variety of 6 base-cutter restriction endonucleases to identify enzymes which generate large fragments carrying the rearranged λ , gene. Two such enzymes were ApaLI and SphI which generated 35kb and 50kb fragments carrying the λ , gene respectively. 20 DNaseI fadeouts were performed on J558L nuclei and DNA extracted from these nuclei was digested with ApaLI or SphI and the fragment pattern was analysed by Southern blotting using a variety of probes. The DNaseI fadeouts were carried out as described by Enver et al (Enver, T. et al, Nature, 25 (1985), 318, 680-683), with the modification described by Grosveld et al (Grosveld F.G. et al, Cell, (1987), 51, 975-985). DNA was prepared from DNaseI-treated nuclei using the procedure of Luster et al (Luster et al, Mol. Cell Biol., 1, 3723).

30

The probes used were:

1. a probe from the $V\lambda_1$, region (0.8kb XbaI fragment) which hybridises to both $V\lambda_1$, and $V\lambda_2$ which are highly conserved,

35

2. a probe from the C region of λ , (1kb BamHI-KpnI) which hybridises to $C\lambda_1$, and $C\lambda_4$, and

3. a probe from the 3' region of $\text{C}\lambda_1$, (2kb KpnI-XbaI) which is a unique sequence and recognises only $\text{C}\lambda_1$ -associated sequences (see Figure 3).

5 Probing ApaLI or SphI blots with each of these three probes yielded a number of hypersensitive subbands (Figure 4a and 4b). In order to map these sites more closely, the ApaLI or SphI digested samples were also digested with XhoI (which cuts in the $\text{C}\lambda_1$ sequence). A 4.3kb and a 3kb band were seen
 10 on SphI/XhoI and ApaLI/XhoI blots when probed with the V region probe. The 4.3kb band was also seen with $\text{C}\lambda_1$ probe, showing that the λ_1 gene contains a hypersensitive site 2.4kb upstream of the CAP site. The 3kb subband maps 2.5kb upstream of the genomic $\text{V}\lambda_2$ segment. These positions have
 15 been confirmed by mapping with respect to a number of other enzymes (not shown).

In addition to these mapped sites, we have detected a number of other hypersensitive sites using the V, C and 3' probe
 20 (Figure 4a and 4b). All of these are associated with the λ locus but cannot be unequivocally assigned to particular positions. What is clear is that some of these are located to the 3' side of the various constant regions.

25 We therefore decided to clone sequences flanking the λ genes to isolate the sequences which contain these hypersensitive sites, starting with the λ_1 gene.

DNaseI fadeouts were performed on nuclei from MEL cells as
 30 a control. These were digested with KpnI and probed with a $\text{V}\lambda_1$ probe. Whereas hypersensitive bands (2.6kb) can be seen on the J558L KpnI blots none are seen on the MEL blots (Figure 5).

35 2. Construction of a J558L Cosmid Library

In order to clone large fragments flanking the rearranged λ gene, a cosmid library was prepared from DNA prepared from

J558L cells. A partial MboI digest of J558L DNA was cloned at the BamHI site of the vector pLTC which is identical to pTCF (Grosveld, F.G. et al., NAR, (1982), 10, 6715) except that the HpaI site has been replaced by an XhoI site which 5 was used in place of the HpaI site for the preparation of vector arms.

The cosmid library was prepared using the procedures described by Grosveld et al (Grosveld, F.G. et al , NAR, 10 (1982), 10, 6715). Stratagene Gigapack Gold packaging extracts were used according to the manufacturer's instructions and the packaged cosmids were introduced into E coli MC1061 rec A. The library was plated onto four 20 x 20 cm NEN Genascreen Plus[™] hybridisation membranes and 15 screened as described by Grosveld et al (Gene, (1981), 13, 227). Hybridisation was carried out using conditions recommended by NEN.

The library (which consisted of 4×10^6 individual clones) 20 was screened with the C λ , (1kb BamHI - KpnI) probe and hybridising colonies were purified. The resulting cosmids were analysed by Southern blotting using V λ , and 3' probes to establish the length of 5' and 3' flanking sequences. Approximately 20kb of 5' flanking and 20kb and 3' flanking 25 sequence was isolated in this way. Cos 2B contains the 7.5kb EcoRI fragment containing the λ , gene and approximately 20kb of 5' flanking sequence and cos 4N contains the 7.5kb EcoRI fragment with approximately 20kb of 3' flanking sequence (Figure 7) including the ApaLI site 3' 30 of the λ , gene.

The library was further screened with a V region probe (0.5KB SstI V λ , region segment) in order to clone sequences flanking the 5' part of the mouse λ locus which may be 35 important in regulating expression.

The library was also screened with a probe from the 3' end of cos4N (an 0.5kb ApaLI/SalI fragment) in order to clone

sequences further 3' of the mouse lambda locus. Cos 3'.3 was identified with this probe. Cos 3'.3 contains approximately 14 kilobases of sequence overlapping the 3' region of cos4N together with a further 19kb of 3' sequence.

5 A map of cos 3'.3 is shown in Figure 7.

3. Functional tests

3.1 Expression in myeloma cells.

10

Cosmids containing the λ_1 gene and flanking sequences are tested directly by transfection into myeloma cell-lines. The cosmid vector carries a tk-neo gene allowing selection of clones in G418. These are then analysed for expression 15 of the λ_1 gene by S1 nuclease analysis (for λ_1 mRNA) and ELISA (for λ_1 protein). The cosmids are transfected into the cell lines Sp2/0.Ag14 (Shulman *et al*, Nature, (1978), 276, 269), a mutant hybridoma cell line which produces no antibody, and MOPC 315.26 a heavy chain loss mutant (Hozumi 20 *et al*, J. Immunol., (1982), 129, 260) which makes λ_2 light chains.

Sp2/0.Ag14 cells were transfected by electroporation using a Bio-Rad Gene Pulser. 5 μ g of linear plasmid DNA was added 25 to 10^7 cells in PBS and the cells were shocked at 250V at a capacitance setting of 960 μ F. After the shock, the cells were incubated at room temperature for ten minutes before adding medium (aMEM, 10% Foetal Calf Serum). G418 (1mg/ml) was added 24 hours after the shock.

30

S1 mapping was carried out as described by Antoniou *et al* (Antoniou, M *et al*, In: Human Genetic Diseases - A Practical Approach, Ed. Davies, K.E., IRL Press, Oxford).

35 λ_1 antibody was measured by ELISA using a goat anti-mouse λ light chain specific antibody preparation and a biotinylated derivative of the same (Southern Biotechnology). The assay was developed using horseradish peroxidase coupled to avidin

and 2,2' azino bis (3-ethylbenzthiazoline-6-sulphonic acid) as substrate.

After testing the effect of 5' flanking sequences (cos 1I 5 and 2B) and 3' sequences alone (cos 4J and 4N), constructs containing both 5' and 3' flanking sequences are prepared by ligation at the XhoI site in the constant region of the λ , gene (Figure 3).

10 The levels of expression of all the cosmid constructs can be compared with the level of expression of the 7.5kb EcoRI fragment containing the λ , gene cloned in the pLTC and with the same fragment cloned in the vector pSVneo which contains an SV40 enhancer. The cell line J558L serves as a positive 15 control for full endogenous expression of λ , mRNA and protein.

pLTC λ , and pSVneo λ , (which both contain the 7.5kb EcoRI fragment containing the λ , gene) were transfected into 20 Sp2/0.Ag14 cells and populations of G418 resistant clones were analysed for expression of λ , specific mRNA and protein. S1 analysis using a 5' probe from a λ , cDNA cloned in pSPT 18 (Pharmacia) (extending from the SphI site in the vector to the XhoI site in C λ ,) and protein analysis by 25 ELISA indicated that the populations transfected with pSVneo λ , expressed 100-fold less mRNA and protein than J558L whereas the cells transfected with pLTC λ , did not express detectable levels of mRNA (Figure 8) or protein (data not shown). This is in agreement with previously reported 30 results. (Picard, D., et al, Nature, (1984), 307, 80-82; Cone, R.D., et al, Science, (1987), 236, 954).

Populations of SP2/0 cells transfected with cos 4N (which contains the λ , gene with 30 kb 3' flanking sequence) also 35 expressed λ , specific messenger RNA, by S1 nuclease analysis (Figure 8: Lane 4) and synthesised λ , protein, by ELISA.

The level of expression in these populations is similar to,

or rather higher than, that seen in populations transfected with pSV neol, which contains the SV40 enhancer (Figure 8: Lane 6).

5 No expression of λ , specific mRNA or protein could be detected in SP2/0 cells transfected with cos 2B (which contains the λ , gene together with 22 kb of 5' flanking sequence: Figure 8-Lane 3). This is in spite of the presence in this cosmid of sequences approximately 2.4 kb 5' 10 of the $V\lambda$, to which a strong hypersensitive site maps in nuclei treated with DNase I.

All populations which were analysed for mRNA were also checked for the presence of the transfected λ , gene by 15 Southern blotting of EcoRI digested genomic DNA using a probe from the 3' part of the C λ , region (the 2kb *KpnI/XbaI* in Figure 3).

In order to determine how much of the 3' sequence in cos 4N 20 was necessary for expression of the λ , restriction fragments of cos 4N containing the tk-neo gene, the λ , gene and various amounts of 3' sequence were prepared on salt 25 gradients and used to transfect SP2/0 cells. Populations of clones resistant to G418 were tested for expression of the λ , gene by S1 nuclease protection. These fragments are shown in Figure 9. Both the 24.5 kb *ApalI* fragment and the 19 kb *ApalI-SnaBI* fragment gave populations of SP2/0 cells which expressed λ , specific mRNA (Figure 10: Lanes 5 to 9 and 11 to 14).

30

HindIII fragments lying between the *EcoRI* site 3.8 kb downstream of the *XhoI* site in the λ , gene and the *SnaBI* site 10 kb downstream of this *XhoI* site (Figure 11) were 35 cloned into the *HindIII* site of pLTC λ , (Figure 12). These plasmids were linearised at the single *PvuI* site and used to transfect SP2/0 cells.

RNA from CT418 resistant populations was tested for the

presence of λ_1 specific mRNA as before. A plasmid containing the 1.3 kb *HindIII* fragment, HIIIA, expressed λ_1 mRNA as did plasmids containing the 3.3. kb *HindIII* fragment HIIIC (Figure 13: Lanes 4 to 7 and 10 to 14).

5

In conclusion, an enhancer downstream of the mouse $C\lambda_1$ gene segment has been identified. Enhancer activity lies between the *EcoRI* site 3.8 kb downstream of the *XbaI* site in the λ_1 gene and the *SnaBI* site 10 kb downstream of this *XbaI* site.

10 This region contains two *Hind III* fragments (HIIIA 1.3k β and HIIIC 3.3 kb) both of which enhance expression of the λ_1 gene in SP2/0 cells.

3.2 Transgenic Mice.

15

The *SalI* fragments of cos 4N and cos 3'.3 which contain all the cloned J558L DNA were purified using salt gradients. The cos 4N *SalI* fragment was injected into fertilised mouse eggs (C57BL/10 x CBA) FI, either alone or as a co-injection 20 with the *SalI* fragment of cos 3'.3, in order to generate transgenic mice containing the λ_1 gene together with varying amounts of 3' flanking sequence. The expression of the λ_1 gene in these mice was monitored both by *S1* nuclease protection analysis of mRNA from lymphoid and non-lymphoid 25 tissues and by fluorescence activated cell sorter analysis of λ_1 protein expression on the surface of spleen cells.

Two mouse lines were generated which carried both cos 4N and cos 3'.3 *SalI* fragments in their genome. Southern blot 30 analysis using probes from the ends of the *SalI* fragments indicated that the cos 4N and cos 3'.3 fragments were linked together at the same insertion site in the genome (data not shown).

35 The founder animals were bred with (C57BL/10 x CBA) FI mice and their offspring analysed 5-7 days after birth for expression of λ_1 . RNA prepared from spleen, liver, thymus and brain was analysed by *S1* nuclease protection using a

probe from the 5' end of a λ , cDNA. For analysis of cell surface expression of λ , spleen tissue was homogenised using a glass homogeniser and red cells were lysed using Tris-ammonium chloride. Approximately 10^6 cells were 5 stained with phycoerythrin conjugated goat anti-mouse λ (Southern Biotechnology) or fluorescein isothiocyanate conjugated goat anti-mouse μ (Sigma). Fluorescence analysis was carried out using a Beckman Dickinson FACSTAR-PLUS flow cytometer. Transgenic animals were identified by Southern 10 blotting of tail DNA using probes from both cos 4N and cos 3'.3.

For the two mouse lines, 2071 and 3002, the transgenic offspring exhibited an elevation in both the number of 15 spleen cells expressing lambda chains at the cell surface and in the level of λ , mRNA detected in the spleen compared with non-transgenic littermates. Figure 14 shows an S1 nuclease protection analysis of RNA from various tissues of two non-transgenic and three transgenic offspring of line 20 2071. The three transgenic offspring show an approximately 5-10 fold increase in the level of λ , specific RNA in the spleen and no detectable expression in thymus, brain or liver. Similar results were obtained for mouse line 3002. Table 1 shows the results of FACS analysis of spleen cells 25 from 5 day old offspring of lines 2071 and 3002. In both cases the number of IgM positive B cells expressing lambda chains was approximately 5 fold higher in the transgenic animals compared with their non-transgenic littermates.

Table 1

FACS analysis of spleen cells from transgenic and non-transgenic littermates of mouse lines 2071 and 3002.

5

Mouse	Transgene	% spleen cells staining with				% λ
		cos 4N	cos 3'.3	PE-GAM λ	PITE-GAM μ	
10						
	2071/21	X	X	5.0, 5.0	15.5, 14.6	33
	2071/22	X	X	3.7, 3.3	13.6, 12.7	27
	2071/23	X	X	1.4, 1.4	20.9, 19.4	7
	2071/24	-	-	1.1, 1.0	14.3, 15.5	6
15						
	3002/1	X	X	4.57, 4.34	7.89, 9.96	50
	3002/2	X	X	3.32, 2.54	6.56, 6.43	45
	3002/3	X	X	0.39, 0.52	6.16, 5.87	7.5
	3002/4	X	X	0.39, 0.40	4.33, 5.16	8.3
20	3002/5	X	X	2.87, 2.97	7.62, 7.64	38
	3002/6	X	X	2.36, 2.91	6.43, 6.81	40

Six mouse lines carrying various numbers of copies of the cos 4N SalI fragment alone were also analysed. No difference was observed either in the number of λ expressing B cells or in the level of λ , specific mRNA in the spleen between transgenic and non-transgenic offspring.

We concluded from these data that an element which strongly enhances the expression of the mouse λ , gene resides in the sequences towards the 3' end of cos 3'.3. The presence of DNaseI hypersensitive sites in this region was investigated by digesting DNA from a DNaseI fadeout of J558L nuclei with the enzymes XhoI and PvuI and probing Southern blots with a 1.9 kb EcoRV-PvuI fragment from the 3' end of cos 3'.3. The probe hybridises to a 28 kb fragment in J558L DNA. Preliminary data suggest that there may be two hypersensitive sites mapping approximately 8 kb and 12 kb

upstream of the *Pvu*I site. J558L fadeouts were also digested with *Apa*LI and Southern blots probed with a 1 kb *Eco*RI/*Sna*BI fragment from *cos* 3'.3 (see Figure 7b). Preliminary exposures of these blots suggest the appearance 5 of a *DNase*I hypersensitive subband approximately 5 kb downstream of the most 3' *Apa*LI site in *cos* 3'.3. There may therefore be 2 or 3 *DNase*I hypersensitive sites which map to the region lying between 28 and 48 kb (i.e. about 30kb) downstream of the *Xho*I site in the rearranged λ , gene.

10

Both the mouse lines 3002 and 2071 contain between five and eight copies of each of the *cos* 4N and *cos* 3'.3 *Sal*II fragments. Since approximately five times the number of B 15 cells are expressing λ , chains as in the non-transgenic animals and the level of λ , specific RNA is increased between 5 and 10 fold, the level of expression of the transgene appears to be approaching the level of expression of an endogenous λ , allele. The generation of more mouse 20 lines containing sequences from *cos* 4N and *cos* 3'.3 will confirm whether expression levels are directly correlated with copy number.

The data are consistent with the sequences from *cos* 3'.3 to which the hypersensitive site maps acting as a DCR. They 25 are, in any case, acting as a strong, B cell specific enhancer. Possibly addition of 5' sequences containing the hypersensitive site kb upstream of the CAP site of the rearranged λ , gene to the injected DNA may complete a functional DCR.

30

A variation on this approach to isolating DCR's involves using very large segments of the mouse lambda locus cloned in yeast artificial chromosomes (YACS) (Murray and Szostak, 1983, *Nature* 305:189-193). Several hundred kilobases of DNA 35 can be cloned using YAC vectors. A YAC library prepared from J558L DNA would be screened for clones containing lambda sequences. These clones would be tested for expression of lambda either by transfection into myeloma

cells or by injection into fertilised mouse eggs. Copy number dependent, tissue specific, high level expression would be indicative of the presence of a DCR and sequences responsible for this could be identified by DNaseI 5 hypersensitive site mapping. These could then be subcloned from the YAC clone.

The present invention identifies, for the first time, sequences for dominant control regions associated with the 10 mouse λ immunoglobulin locus.

In the cell line J558L, which synthesises λ , light chains, hypersensitive sites have been identified 2.3 - 2.4kb upstream of the CAP site of the rearranged λ_1 gene, 2.5kb 15 upstream of the genomic $V\lambda_2$ segment approximately 30 kb 3' of the rearranged λ_1 gene and approximately 17 kb 3' of the $C\lambda_4$ gene segment. Sequences to which these sites map have been cloned from the J558L cell-line and their effect on λ_1 gene expression tested in myeloma cells and transgenic mice.

20 The DNaseI hypersensitive sites described here may represent "super" hypersensitive sites since no "normal" hypersensitive sites were detected associated with the λ , immunoglobulin promoter. It is possible that the DNaseI 25 hypersensitive sites are "super" hypersensitive because they comprise a cluster of hypersensitive sites, for example as has been shown for the β -globin locus. The location of the hypersensitive sites is also consistent with the probable location of DCRs by analogy with the β -globin and CD2 loci 30 (i.e. 5' and 3' of the locus).

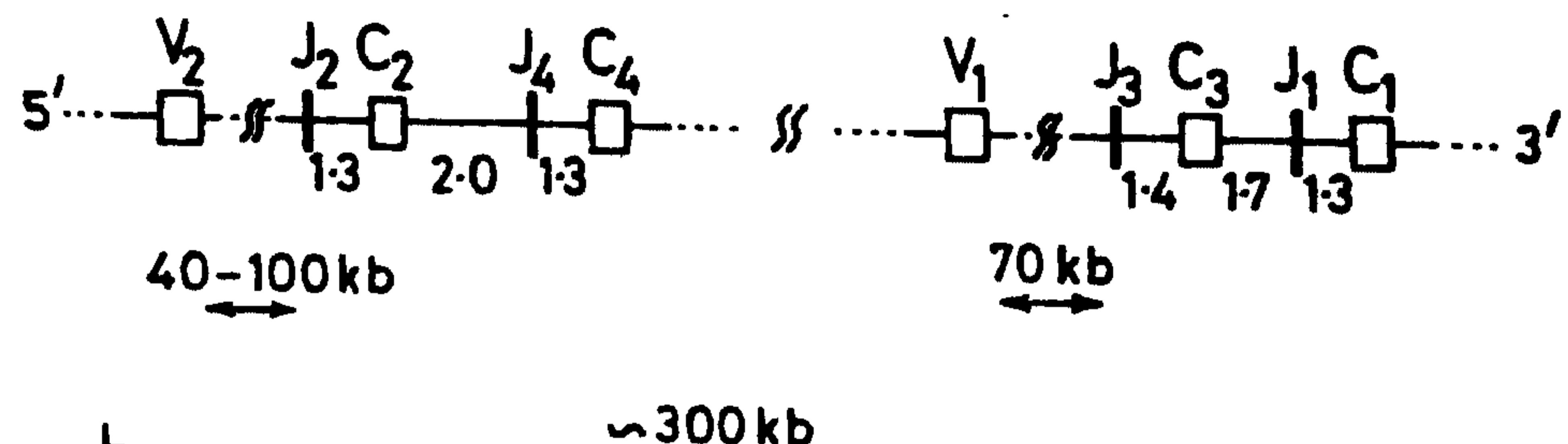
It will be understood that the invention is described by way of example only and modifications of detail may be made within the scope of the invention.

CLAIMS

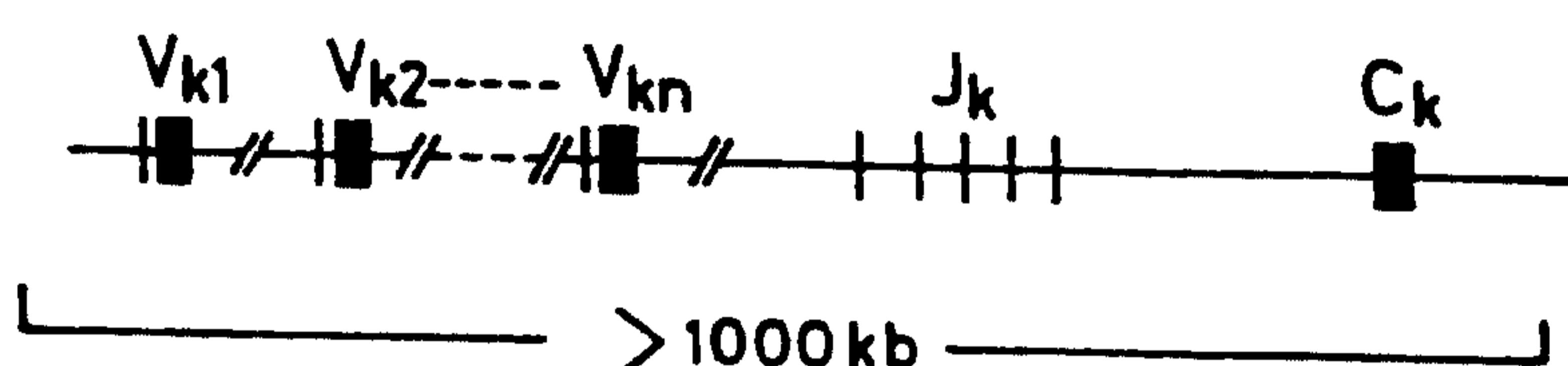
1. A transgenic mammal transformed with a vector, the vector comprising a promoter and a gene to be expressed, wherein the vector includes an immunoglobulin dominant control region capable of eliciting host cell-type restricted, integration site independent, copy number dependent expression of the said gene.
- 10 2. A transgenic mammal according to claim 1, wherein the dominant control region is derived from the mouse λ immunoglobulin gene locus.
- 15 3. A transgenic mammal according to claim 2 wherein the dominant control region comprises a DNaseI super hypersensitive site which is about 2.4kb upstream of the CAP site of a rearranged λ_1 gene.
- 20 4. A transgenic mammal according to claim 2 wherein the dominant control region comprises a DNaseI super hypersensitive site which is about 2.5kb upstream of the genomic $V\lambda_2$ segment.
- 25 5. A transgenic mammal according to claim 2 wherein the dominant control region comprises a DNaseI super hypersensitive site which is about 30kb downstream of the $C\lambda_1$ gene segment.
- 30 6. A transgenic mammal according to claim 2 wherein the dominant control region comprises a super hypersensitive site which is about 17kb downstream of the $C\lambda_4$ gene segment.
- 35 7. A transgenic mammal according to claim 2 wherein the dominant control region comprises in combination a DNaseI super hypersensitive site which is about 2.4kb upstream of the CAP site of a rearranged λ_1 gene and a DNaseI hypersensitive site which is about 30kb 3' of the $C\lambda_1$ gene segment.

1 / 11

a)



b)



c)

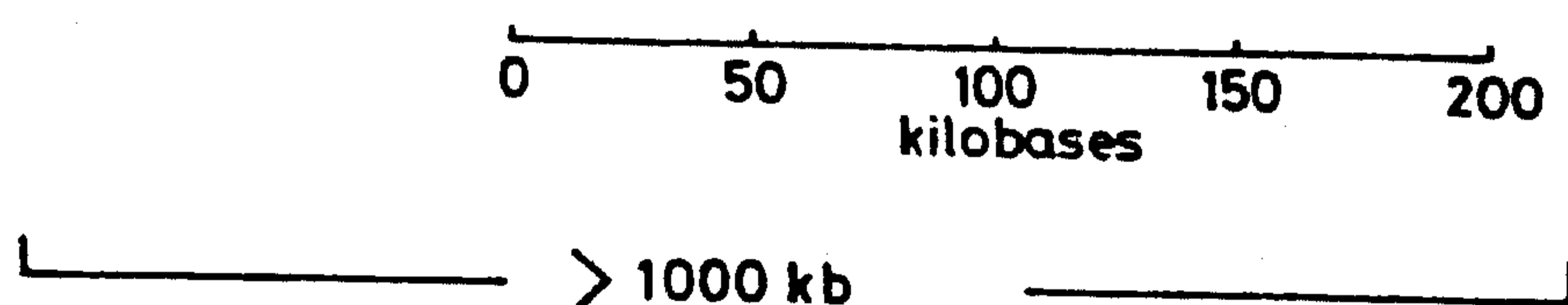
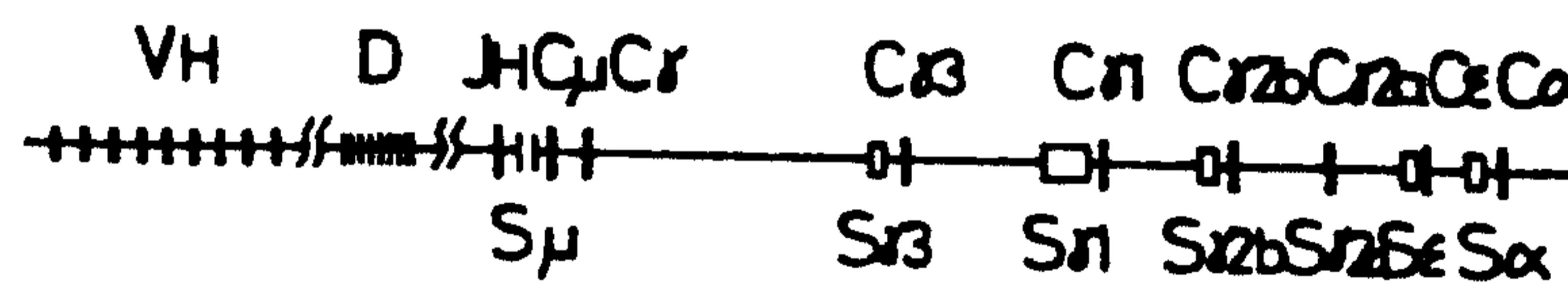


Fig. 1

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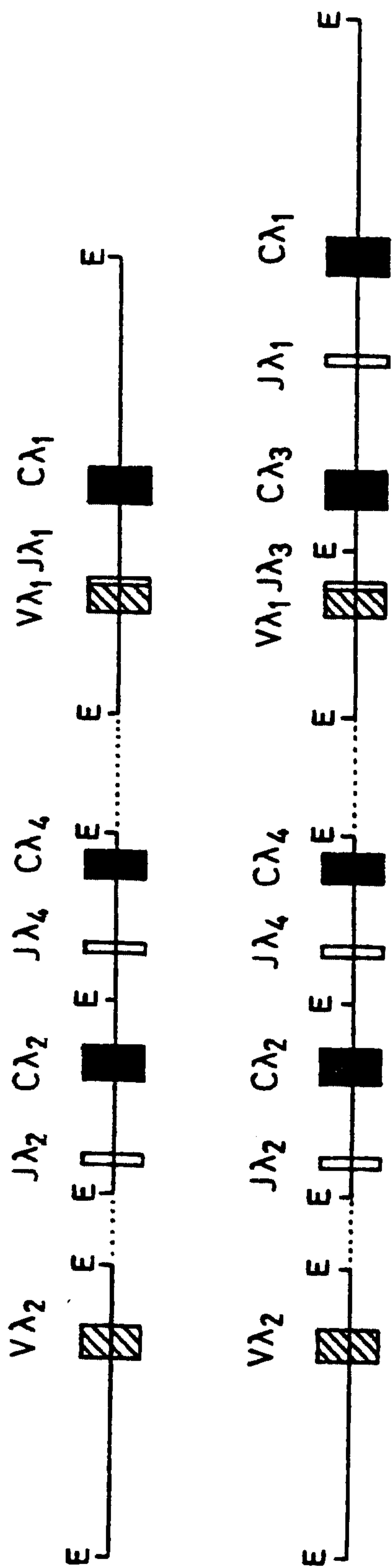


Fig. 2

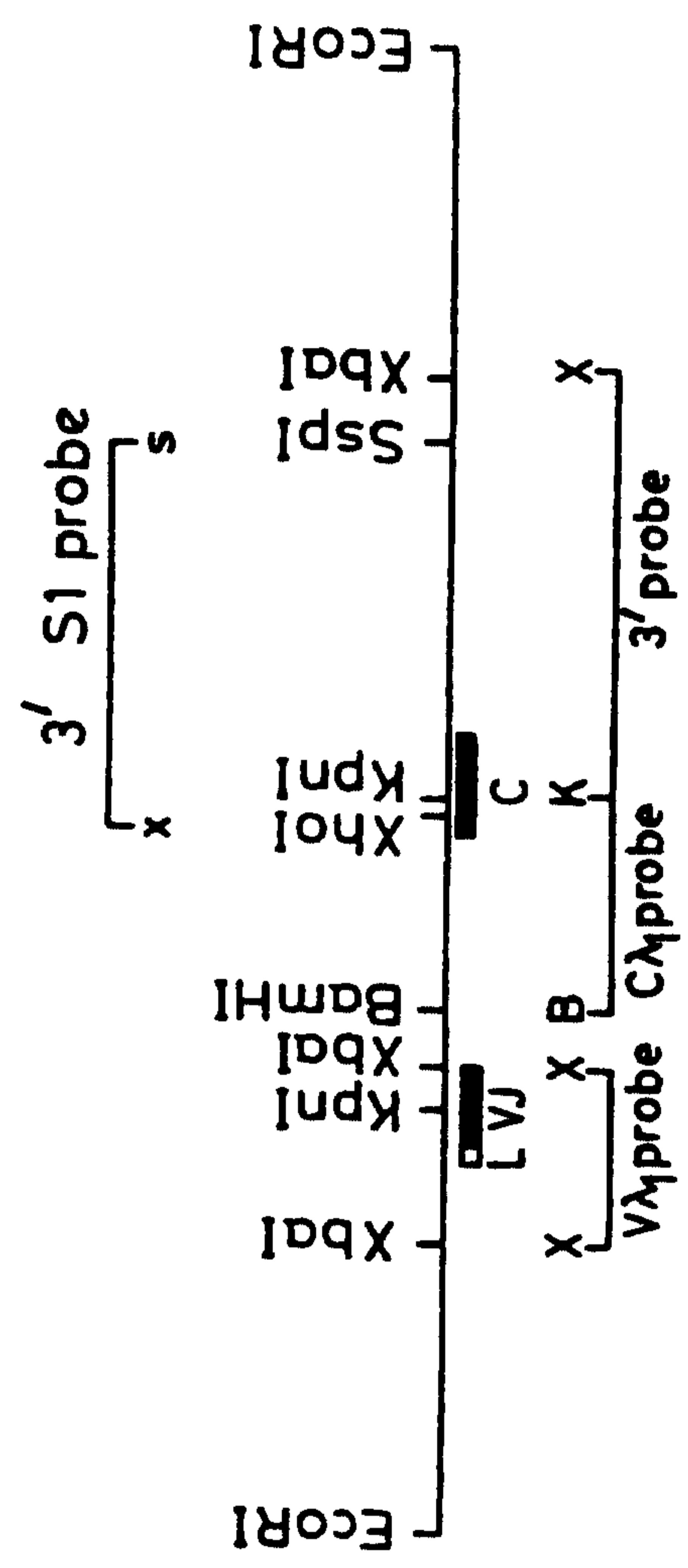
1 kb
E = EcoRI

Fig. 3

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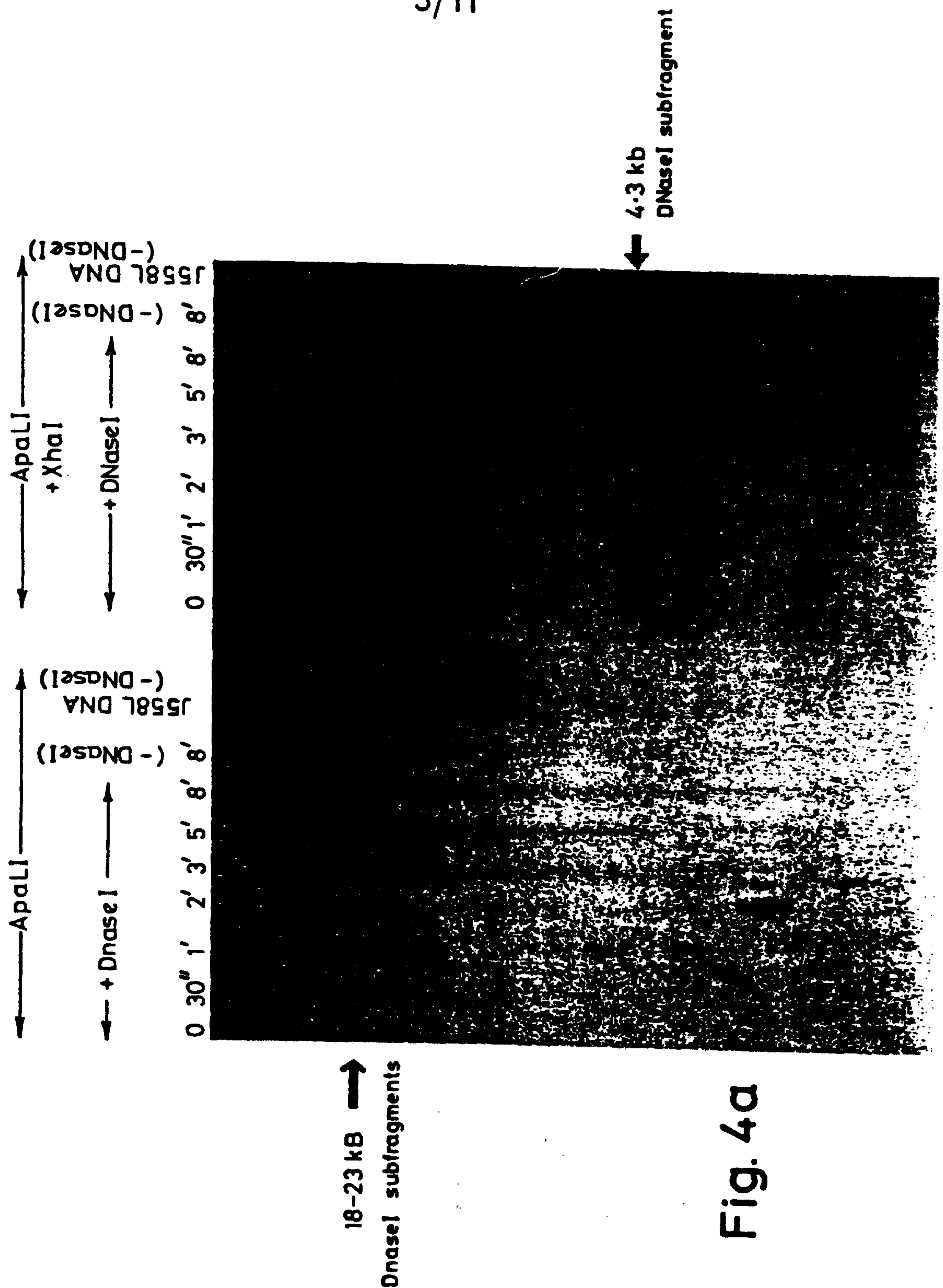
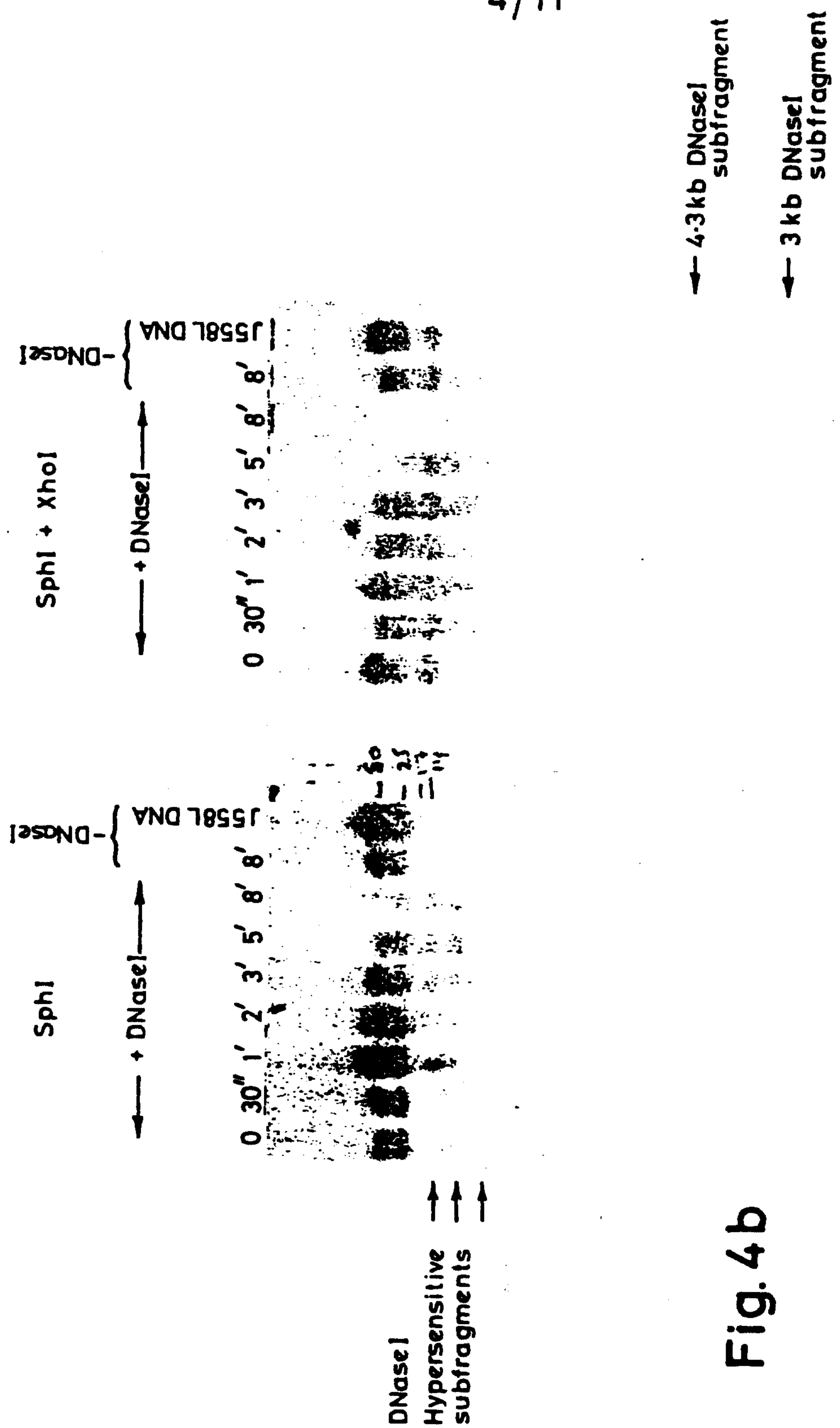


Fig. 4a

B

A

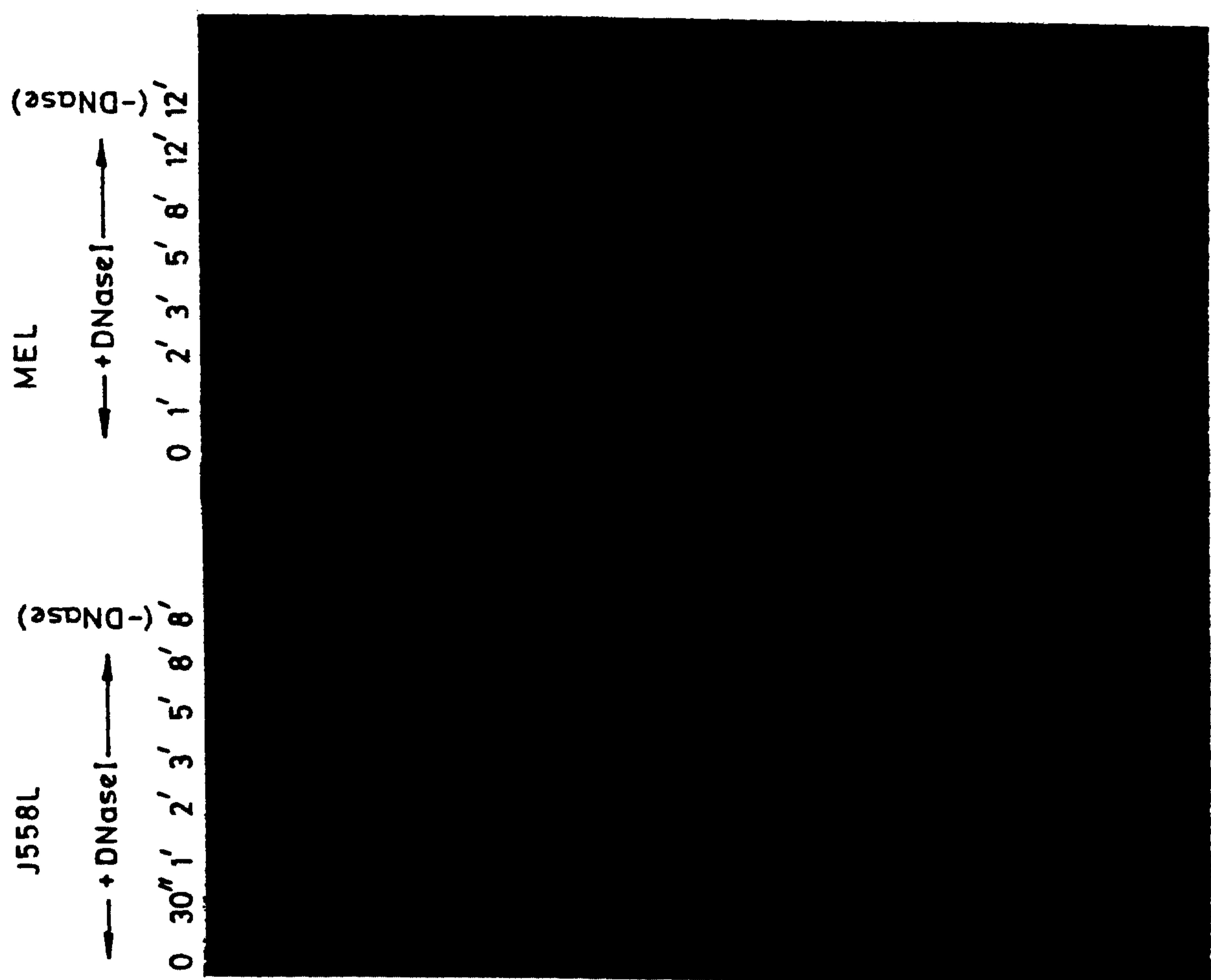
4/11



A
DNase I footprints on J558L nuclei. DNA cut with **A** SphiI and **B** SphiI - Xhol.
Blots were probed with λ probe.

Fig. 4b

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2.6 kB →
DNaseI subfragment

Fig. 5

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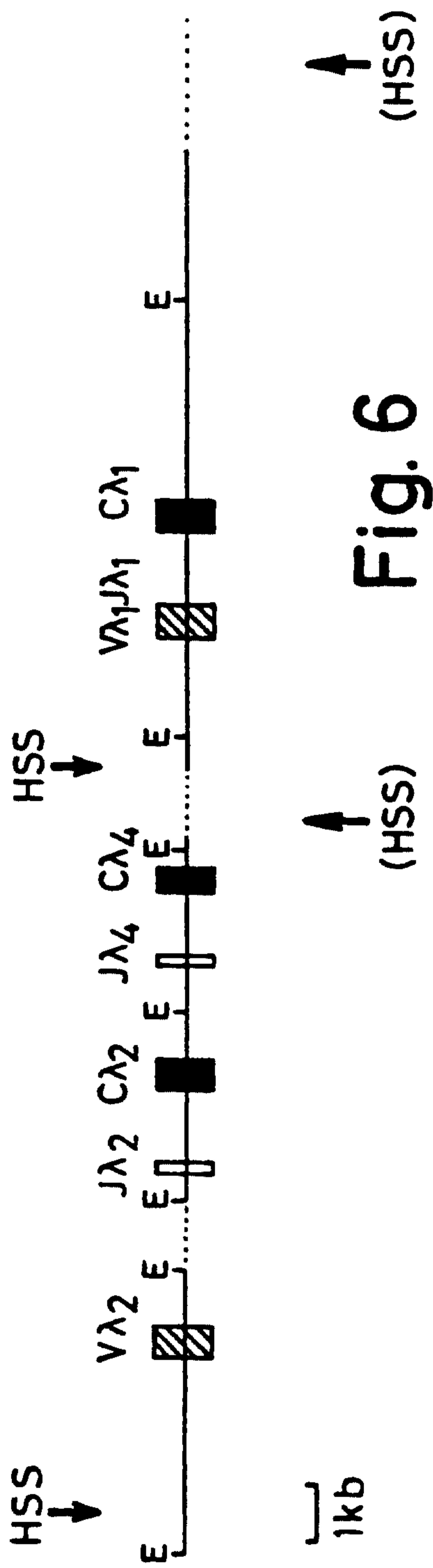
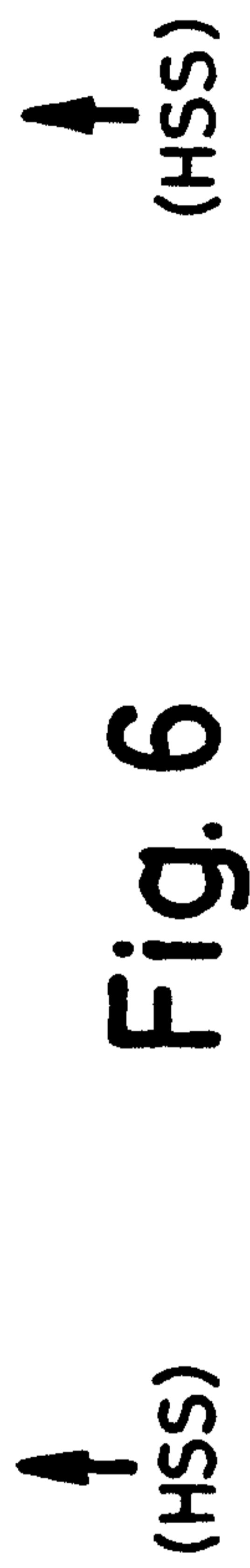


Fig. 6



(HSS)

(HSS)

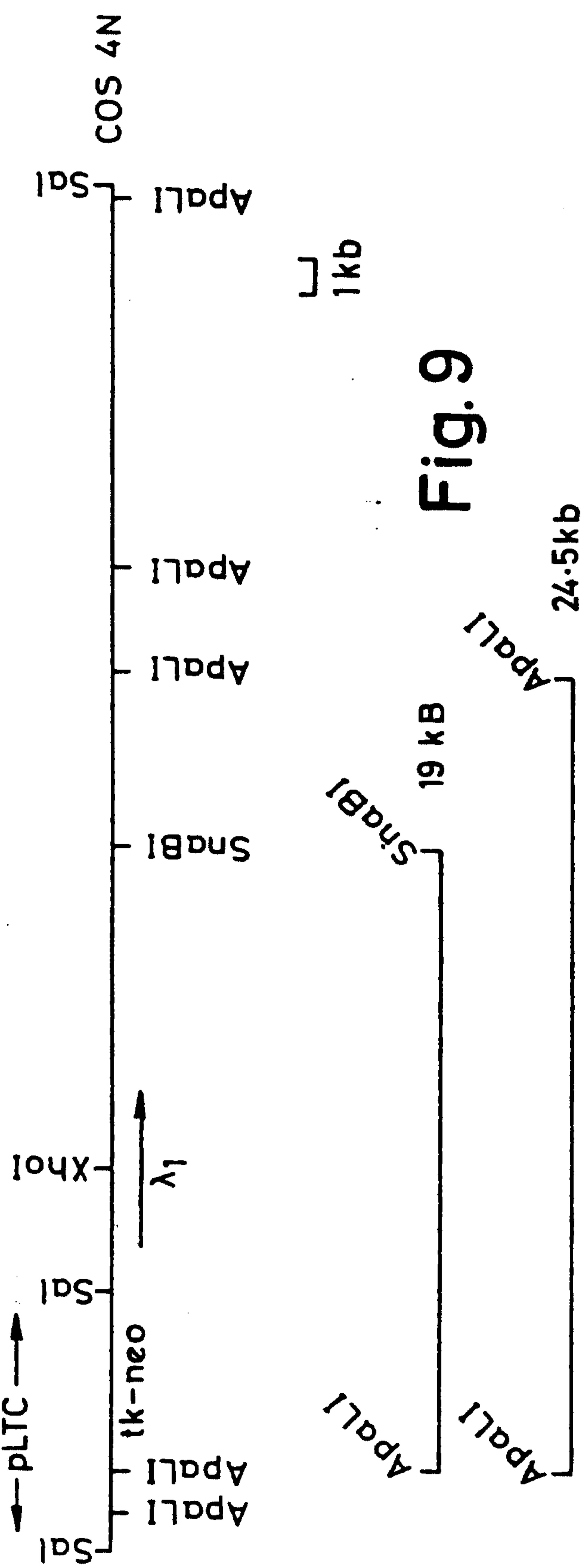
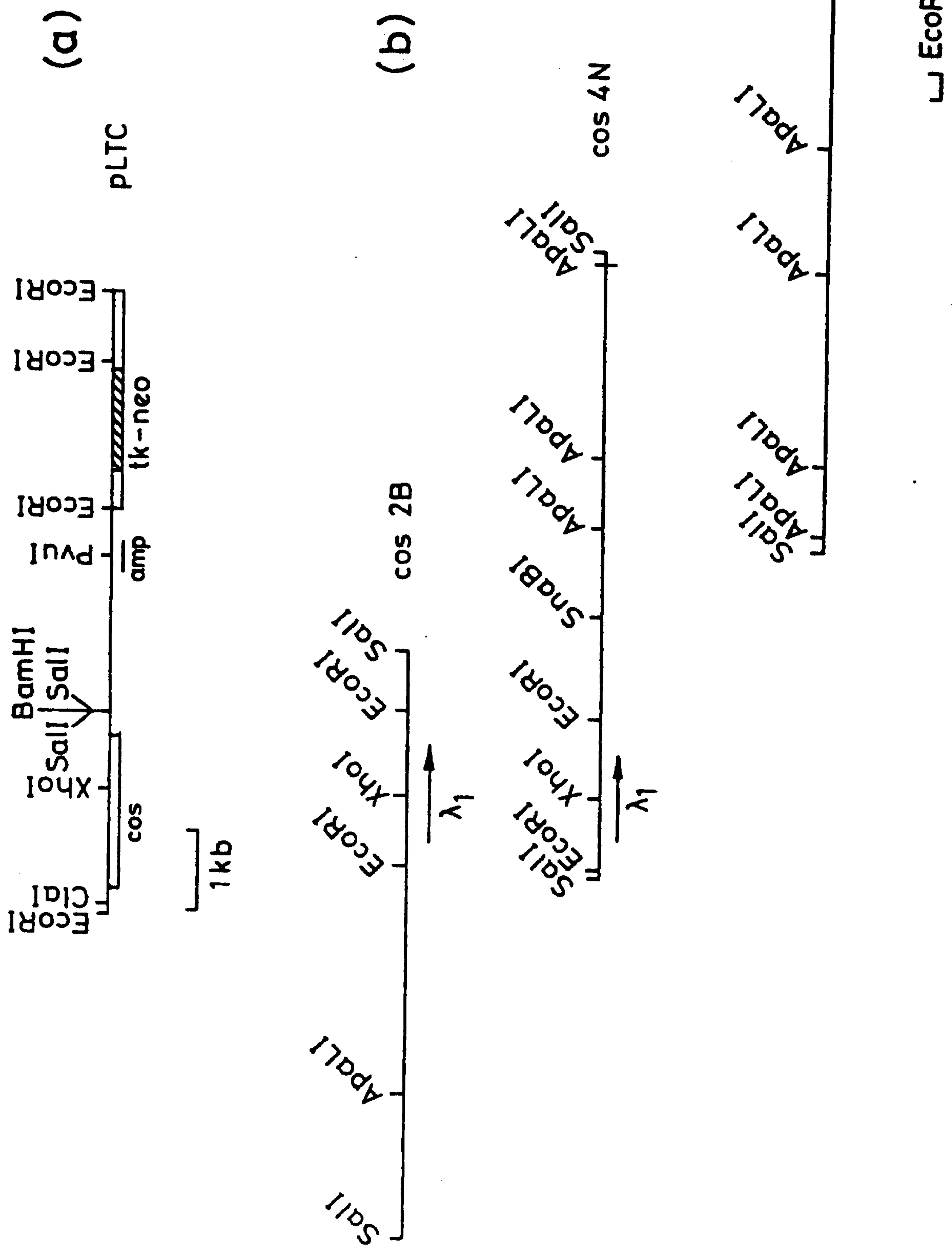


Fig. 9

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8/11

Fig. 8

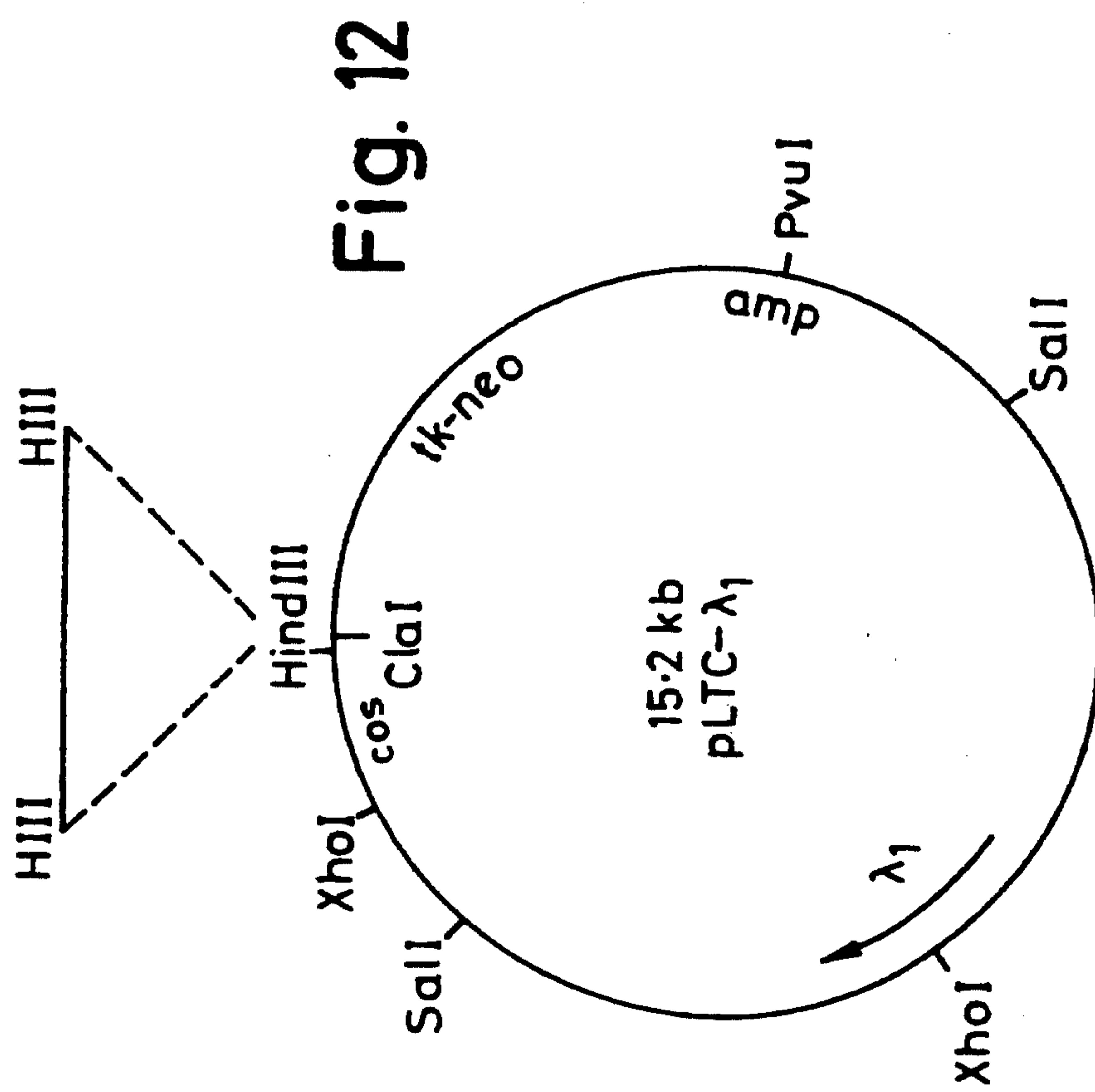
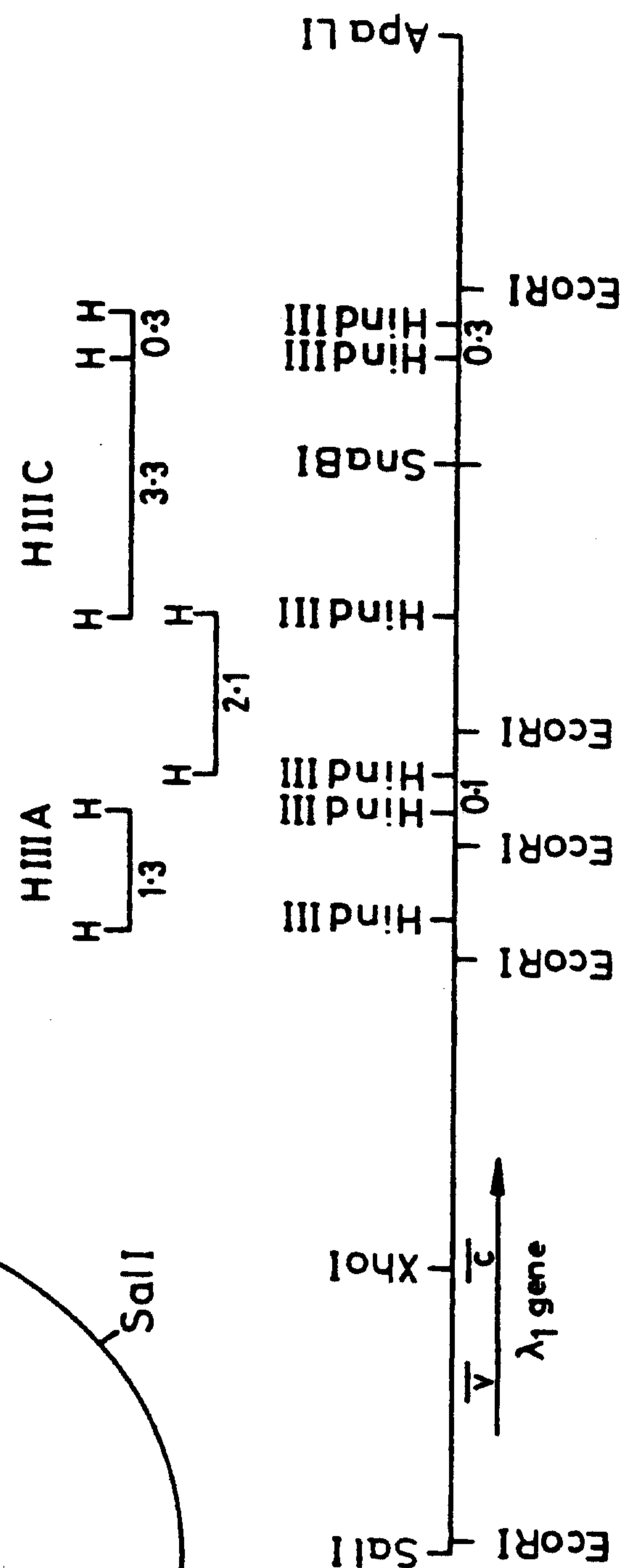
1 2 3 4 5 6 λ_1 protected
fragment β actin
protected
fragment

1 2 3 4 5 6 7 8 9 10 11 12 13 14

 λ_1 → β actin →

Fig. 10

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10/11

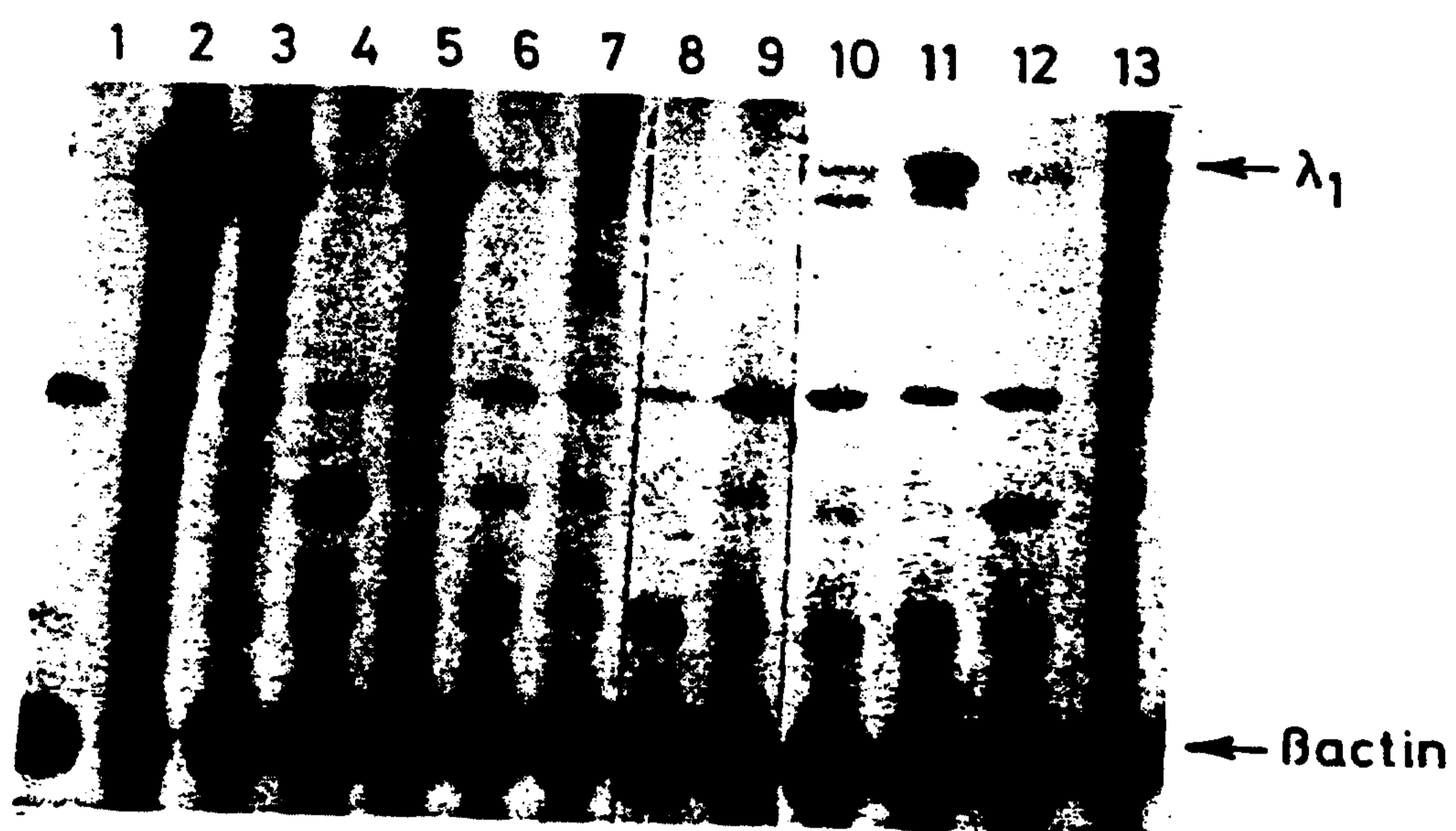
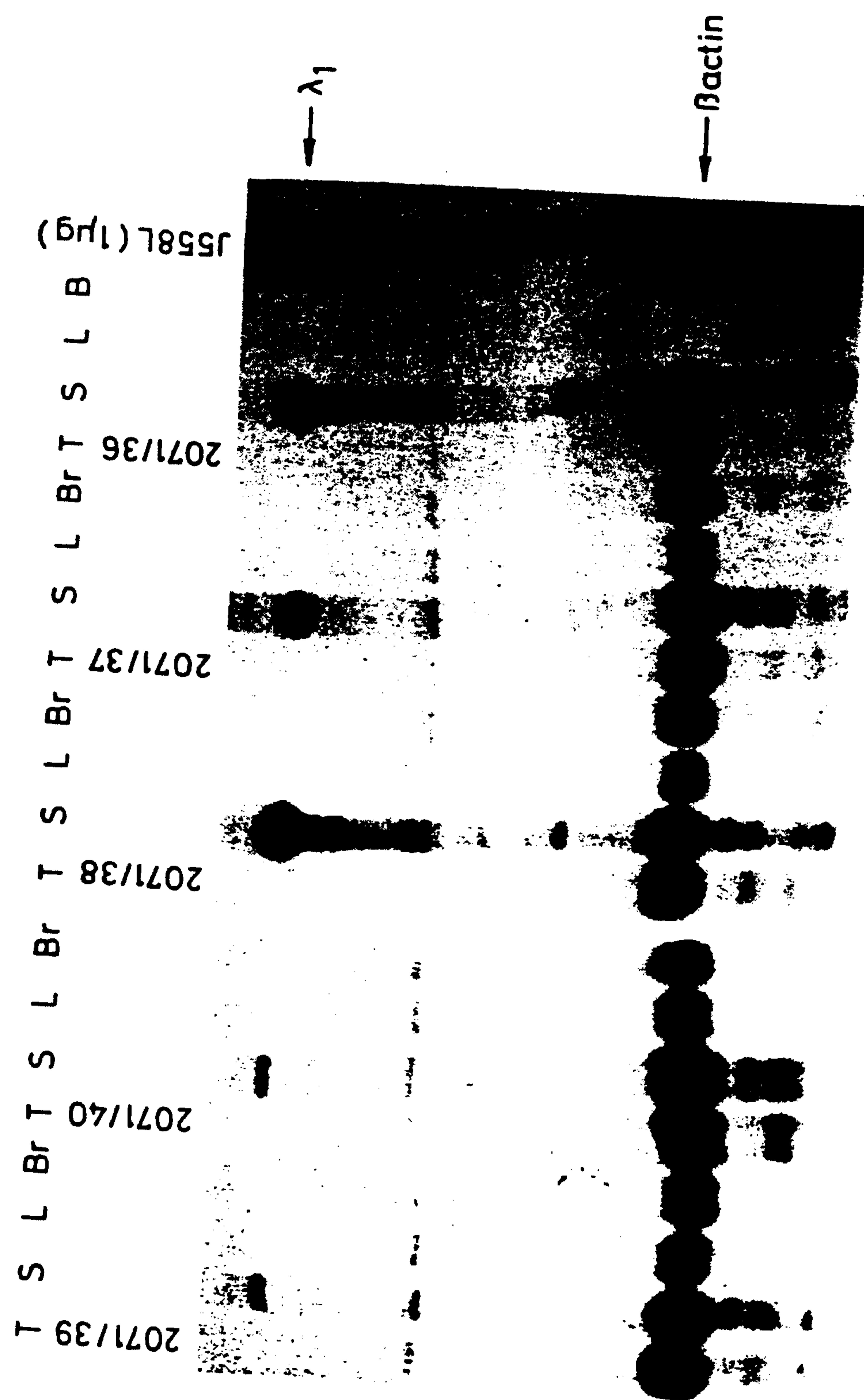


Fig. 13

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