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(54) Title: METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

(57) Abstract

A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein a desired DNA is integrated at a predetermined transcriptionally active site previously marked with a marker plasmid. The method is particularly suitable for the production of mammalian cell lines which secrete mammalian proteins at high levels, in particular immunoglobulins. Vectors and vector combinations for use in the subject cloning method are also provided.

Title of the Invention

METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

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Field of the Invention

The present invention relates to a process of targeting the integration of a desired exogenous DNA to a specific location within the genome of a mammalian cell. More specifically, the invention describes a novel method for identifying a transcriptionally active target site ("hot spot") in the mammalian genome, and inserting a desired DNA at this site via homologous recombination. The invention also optionally provides the ability for gene amplification of the desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in combination with the exogenous DNA. The invention additionally describes the construction of novel vectors suitable for accomplishing the above, and further provides mammalian cell lines produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

Background

Technology for expressing recombinant proteins in both prokaryotic and eukaryotic organisms is well established. Mammalian cells offer significant advantages over bacteria or yeast for protein production, resulting from their ability to correctly assemble, glycosylate and post-translationally modify recombinantly expressed proteins. After transfection into the host cells, recombinant expression constructs can be maintained as extrachromosomal elements, or may be integrated into the host cell genome. Generation of stably transfected mammalian cell lines usually involves the latter; a DNA construct encoding a gene of interest along with a drug resistance gene (dominant selectable marker) is introduced into the host cell, and subsequent growth in the presence of the drug allows for the selection of cells that have successfully integrated the exogenous DNA. In many instances, the gene of interest is linked to a drug resistant selectable marker which can later be subjected to gene amplification. The gene encoding dihydrofolate reductase (DHFR) is most commonly used for this purpose. Growth of cells in the presence of methotrexate, a competitive inhibitor of DHFR, leads to increased DHFR production by means of amplification of the DHFR gene. As flanking regions of DNA will also become amplified, the resultant coamplification of a DHFR linked gene in the transfected cell line can lead to increased protein

production, thereby resulting in high level expression of the gene of interest.

While this approach has proven successful, there are a number of problems with the system because of the random nature of the integration event. These problems exist because expression levels are greatly influenced by the effects of the local genetic environment at the gene locus, a phenomena well documented in the literature and generally referred to as "position effects" (for example, see Al-Shawi et al, *Mol. Cell. Biol.*, 10:1192-1198 (1990); Yoshimura et al, *Mol. Cell. Biol.*, 7:1296-1299 (1987)). As the vast majority of mammalian DNA is in a transcriptionally inactive state, random integration methods offer no control over the transcriptional fate of the integrated DNA. Consequently, wide variations in the expression level of integrated genes can occur, depending on the site of integration. For example, integration of exogenous DNA into inactive, or transcriptionally "silent" regions of the genome will result in little or no expression. By contrast integration into a transcriptionally active site may result in high expression.

Therefore, when the goal of the work is to obtain a high level of gene expression, as is typically the desired outcome of genetic engineering methods, it is generally necessary to screen large numbers of transfectants to find such a high producing clone.

Additionally, random integration of exogenous DNA into the genome can in some instances disrupt important cellular genes, resulting in an altered phenotype. These factors can make the generation of high expressing stable mammalian cell lines a complicated and laborious process.

Recently, our laboratory has described the use of DNA vectors containing translationally impaired dominant selectable markers in mammalian gene expression. (This is disclosed in U.S. Serial No. 08/147,696 filed November 3, 1993, recently allowed).

These vectors contain a translationally impaired neomycin phosphotransferase (neo) gene as the dominant selectable marker, artificially engineered to contain an intron into which a DHFR gene along with a gene or genes of interest is inserted. Use of these vectors as expression constructs has been found to significantly reduce the total number of drug resistant colonies produced, thereby facilitating the screening procedure in relation to conventional mammalian expression vectors. Furthermore, a significant percentage of the clones obtained using this system are high expressing clones. These results are apparently attributable to the modifications made to the neo selectable marker. Due to the translational impairment of the neo gene, transfected cells will not produce enough neo protein to survive drug selection, thereby decreasing the overall

number of drug resistant colonies. Additionally, a higher percentage of the surviving clones will contain the expression vector integrated into sites in the genome where basal transcription levels are high, resulting in overproduction of neo, thereby allowing the cells to overcome the impairment of the neo gene. Concomitantly, the genes of interest linked to neo will be subject to similar elevated levels of transcription. This same advantage is also true as a result of the artificial intron created within neo; survival is dependent on the synthesis of a functional neo gene, which is in turn dependent on correct and efficient splicing of the neo introns. Moreover, these criteria are more likely to be met if the vector DNA has integrated into a region which is already highly transcriptionally active.

Following integration of the vector into a transcriptionally active region, gene amplification is performed by selection for the DHFR gene. Using this system, it has been possible to obtain clones selected using low levels of methotrexate (50nM), containing few (<10) copies of the vector which secrete high levels of protein (>55pg/cell/day). Furthermore, this can be achieved in a relatively short period of time. However, the success in amplification is variable. Some transcriptionally active sites cannot be amplified and

therefore the frequency and extent of amplification from a particular site is not predictable.

Overall, the use of these translationally impaired vectors represents a significant improvement over other methods of random integration. However, as discussed, the problem of lack of control over the integration site remains a significant concern.

One approach to overcome the problems of random integration is by means of gene targeting, whereby the exogenous DNA is directed to a specific locus within the host genome. The exogenous DNA is inserted by means of homologous recombination occurring between sequences of DNA in the expression vector and the corresponding homologous sequence in the genome. However, while this type of recombination occurs at a high frequency naturally in yeast and other fungal organisms, in higher eukaryotic organisms it is an extremely rare event. In mammalian cells, the frequency of homologous versus non-homologous (random integration) recombination is reported to range from 1/100 to 1/5000 (for example, see Capecchi, *Science*, 244:1288-1292 (1989); Morrow and Kucherlapati, *Curr. Op. Biotech.*, 4:577-582 (1993)).

One of the earliest reports describing homologous recombination in mammalian cells comprised an artificial system created in mouse fibroblasts (Thomas et al, *Cell*, 44:419-428 (1986)). A cell line containing a mutated, non-functional version of the neo gene integrated into

the host genome was created, and subsequently targeted with a second non-functional copy of neo containing a different mutation. Reconstruction of a functional neo gene could occur only by gene targeting. Homologous recombinants were identified by selecting for G418 resistant cells, and confirmed by analysis of genomic DNA isolated from the resistant clones.

Recently, the use of homologous recombination to replace the heavy and light immunoglobulin genes at endogenous loci in antibody secreting cells has been reported. (U.S. Patent No. 5,202,238, Fell et al, (1993).) However, this particular approach is not widely applicable, because it is limited to the production of immunoglobulins in cells which endogenously express immunoglobulins, e.g., B cells and myeloma cells. Also, expression is limited to single copy gene levels because co-amplification after homologous recombination is not included. The method is further complicated by the fact that two separate integration events are required to produce a functional immunoglobulin: one for the light chain gene followed by one for the heavy chain gene.

An additional example of this type of system has been reported in NS/0 cells, where recombinant immunoglobulins are expressed by homologous recombination into the immunoglobulin gamma 2A locus (Hollis et al, international patent application #

PCT/IB95 (00014).) Expression levels obtained from this site were extremely high - on the order of 20pg/cell/day from a single copy integrant. However, as in the above example, expression is limited to this level because an amplifiable gene is not contegrated in this system. Also, other researchers have reported aberrant glycosylation of recombinant proteins expressed in NS/0 cells (for example, see Flesher et al, *Biotech. and Bioeng.*, 48:399-407 (1995)), thereby limiting the applicability of this approach.

The cre-loxP recombination system from bacteriophage P1 has recently been adapted and used as a means of gene targeting in eukaryotic cells. Specifically, the site specific integration of exogenous DNA into the Chinese hamster ovary (CHO) cell genome using cre recombinase and a series of lox containing vectors have been described. (Fukushige and Sauer, *Proc. Natl. Acad. Sci. USA*, 89:7905-7909 (1992).) This system is attractive in that it provides for reproducible expression at the same chromosomal location. However, no effort was made to identify a chromosomal site from which gene expression is optimal, and as in the above example, expression is limited to single copy levels in this system. Also, it is complicated by the fact that one needs to provide for expression of a functional recombinase enzyme in the mammalian cell.

The use of homologous recombination between an introduced DNA sequence and its endogenous chromosomal locus has also been reported to provide a useful means of genetic manipulation in mammalian cells, as well as in yeast cells. (See e.g., Bradley et al, *Meth. Enzymol.*, 223:855-879 (1993); Capecchi, *Science*, 244:1288-1292 (1989); Rothstein et al, *Meth. Enzymol.*, 194:281-301 (1991)). To date, most mammalian gene targeting studies have been directed toward gene disruption ("knockout") or site-specific mutagenesis of selected target gene loci in mouse embryonic stem (ES) cells. The creation of these "knockout" mouse models has enabled scientists to examine specific structure-function issues and examine the biological importance of a myriad of mouse genes. This field of research also has important implications in terms of potential gene therapy applications.

Also, vectors have recently been reported by Cell-tech (Kent, U.K.) which purportedly are targeted to transcriptionally active sites in NSO cells, which do not require gene amplification (Peakman et al, *Hum. Antibod. Hybridomas*, 5:65-74 (1994)). However, levels of immunoglobulin secretion in these unamplified cells have not been reported to exceed 20pg/cell/day, while in amplified CHO cells, levels as high as 100pg/cell/day can be obtained (Id.).

It would be highly desirable to develop a gene targeting system which reproducibly provided for the integration of exogenous DNA into a predetermined site in the genome known to be transcriptionally active. Also, it would be desirable if such a gene targeting system would further facilitate co-amplification of the inserted DNA after integration. The design of such a system would allow for the reproducible and high level expression of any cloned gene of interest in a mammalian cell, and undoubtedly would be of significant interest to many researchers.

In this application, we provide a novel mammalian expression system, based on homologous recombination occurring between two artificial substrates contained in two different vectors. Specifically, this system uses a combination of two novel mammalian expression vectors, referred to as a "marking" vector and a "targeting" vector.

Essentially, the marking vector enables the identification and marking of a site in the mammalian genome which is transcriptionally active, i.e., a site at which gene expression levels are high. This site can be regarded as a "hot spot" in the genome. After integration of the marking vector, the subject expression system enables another DNA to be integrated at this site, i.e., the targeting vector, by means of homologous recombination occurring between DNA sequences common to

both vectors. This system affords significant advantages over other homologous recombination systems.

Unlike most other homologous systems employed in mammalian cells, this system exhibits no background. Therefore, cells which have only undergone random integration of the vector do not survive the selection. Thus, any gene of interest cloned into the targeting plasmid is expressed at high levels from the marked hot spot. Accordingly, the subject method of gene expression substantially or completely eliminates the problems inherent to systems of random integration, discussed in detail above. Moreover, this system provides reproducible and high level expression of any recombinant protein at the same transcriptionally active site in the mammalian genome. In addition, gene amplification may be effected at this particular transcriptionally active site by including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

Objects of the Invention

Thus, it is an object of the invention to provide an improved method for targeting a desired DNA to a specific site in a mammalian cell.

One embodiment of the invention provides a novel method for targeting a desired DNA to a specific site in a mammalian cell via homologous recombination.

Another embodiment of the invention provides novel vectors for achieving site specific integration of a desired DNA in a mammalian cell.

Another embodiment of the invention provides novel mammalian cell lines which contain a desired DNA integrated at a predetermined site which provides for high expression.

Another embodiment of the invention provides a novel method for achieving site specific integration of a desired DNA in a Chinese hamster ovary (CHO) cell.

Another embodiment of the invention provides a novel method for integrating immunoglobulin genes, or any other genes, in mammalian cells at predetermined chromosomal sites that provide for high expression.

Another embodiment of the invention provides novel vectors and vector combinations suitable for integrating immunoglobulin genes into mammalian cells at predetermined sites that provide for high expression.

Another embodiment of the invention provides mammalian cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression.

A further embodiment of the invention provides a novel method for integrating immunoglobulin genes into CHO cells that provide for high expression, as well as novel vectors and vector combinations that provide for such integration of immunoglobulin genes into CHO cells.

In addition, a further embodiment of the invention provides novel CHO cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression, and have been amplified by methotrexate selection to secrete even greater amounts of functional immunoglobulins.

Brief Description of the Figures

Figure 1 depicts a map of a marking plasmid according to the invention referred to as Desmond. The plasmid is shown in circular form (1a) as well as a linearized version used for transfection (1b).

Figure 2(a) shows a map of a targeting plasmid referred to "Molly". Molly is shown here encoding the anti-CD20 immunoglobulin genes, expression of which is described in Example 1.

Figure 2(b) shows a linearized version of Molly, after digestion with the restriction enzymes *Kpn*1 and *Pac*1. This linearized form was used for transfection.

Figure 3 depicts the potential alignment between Desmond sequences integrated into the CHO genome, and incoming targeting Molly sequences. One potential ar-

rangement of Molly integrated into Desmond after homologous recombination is also presented.

Figure 4 shows a Southern analysis of single copy Desmond clones. Samples are as follows:

Lane 1: λHindIII DNA size marker

Lane 2: Desmond clone 10F3

Lane 3: Desmond clone 10C12

Lane 4: Desmond clone 15C9

Lane 5: Desmond clone 14B5

Lane 6: Desmond clone 9B2

Figure 5 shows a Northern analysis of single copy Desmond clones. Samples are as follows: Panel A: northern probed with CAD and DHFR probes, as indicated on the figure. Panel B: duplicate northern, probed with CAD and HisD probes, as indicated. The RNA samples loaded in panels A and B are as follows:

Lane 1: clone 9B2, lane 2; clone 10C12, lane 3; clone 14B5, lane 4; clone 15C9, lane 5; control RNA from CHO transfected with a HisD and DHFR containing plasmid, lane 6; untransfected CHO.

Figure 6 shows a Southern analysis of clones resulting from the homologous integration of Molly into Desmond. Samples are as follows:

Lane 1: λHindIII DNA size markers, Lane 2: 20F4, lane 3; 5F9, lane 4; 21C7, lane 5; 24G2, lane 6; 25E1, lane 7; 28C9, lane 8; 29F9, lane 9; 39G11, lane 10; 42F9, lane 11; 50G10, lane 12; Molly plasmid DNA, linearized with

BgIII (top band) and cut with BgIII and KpnI (lower band), lane 13; untransfected Desmond.

Figures 7A through 7G contain the Sequence Listing for Desmond.

Figures 8A through 8I contain the Sequence Listing for Molly-containing anti-CD20.

Figure 9 contains a map of the targeting plasmid, "Mandy," shown here encoding anti-CD23 genes, the expression of which is disclosed in Example 5.

Figures 10A through 10N contain the sequence listing of "Mandy" containing the anti-CD23 genes as disclosed in Example 5.

Detailed Description of the Invention

The invention provides a novel method for integrating a desired exogenous DNA at a target site within the genome of a mammalian cell via homologous recombination. Also, the invention provides novel vectors for achieving the site specific integration of a DNA at a target site in the genome of a mammalian cell.

In particular the invention provides a method for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises the following steps:

(i) transfecting or transforming a mammalian cell with a first plasmid ("marker plasmid") containing the following sequences:

(a) a region of DNA at least 2 to 10Kb in size that is heterologous to the mammalian cell genome which

when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first dominant selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid;

(ii) selecting a cell which contain the marker plasmid integrated in its genome;

(iii) transfecting or transforming said selected cell with a second plasmid ("target plasmid") which contains the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the first selectable marker contained in the marker plasmid, wherein the active, dominant selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid,

(c) the desired DNA; and

(iv) selecting cells which contain the target plasmid integrated at the target site by screening for the expression of the first dominant selectable marker protein.

More specifically, the subject cloning method provides for site specific integration of a desired DNA in a mammalian cell by transfection of such cell with a "marker plasmid" which contains a unique sequence that is foreign to the mammalian cell genome and which provides a substrate for homologous recombination, followed by transfection with a "target plasmid" containing

a sequence which provides for homologous recombination with the unique sequence contained in the marker plasmid, and further comprising a desired DNA that is to be integrated into the mammalian cell. Typically, the integrated DNA will encode a protein of interest, such as an immunoglobulin or other secreted mammalian glycoprotein.

The exemplified homologous recombination system uses the neomycin phosphotransferase gene as a dominant selectable marker. This particular marker was utilized based on the following previously published observations;

(i) the demonstrated ability to target and restore function to a mutated version of the neo gene (cited earlier) and

(ii) our development of translationally impaired expression vectors, in which the neo gene has been artificially created as two exons with a gene of interest inserted in the intervening intron; neo exons are correctly spliced and translated in vivo, producing a functional protein and thereby conferring G418 resistance on the resultant cell population. In this application, the neo gene is split into three exons. The third exon of neo is present on the "marker" plasmid and becomes integrated into the host cell genome upon integration of the marker plasmid into the mammalian cells. Exons 1 and 2 are present on the targeting plasmid, and are separated

by an intervening intron into which at least one gene of interest is cloned. Homologous recombination of the targeting vector with the integrated marking vector results in correct splicing of all three exons of the neo gene and thereby expression of a functional neo protein (as determined by selection for G418 resistant colonies). Prior to designing the current expression system, we had experimentally tested the functionality of such a triply spliced neo construct in mammalian cells. The results of this control experiment indicated that all three neo exons were properly spliced and therefore suggested the feasibility of the subject invention.

However, while the present invention is exemplified using the neo gene, and more specifically a triple split neo gene, the general methodology should be efficacious with other dominant selectable markers.

As discussed in greater detail *infra*, the present invention affords numerous advantages to conventional gene expression methods, including both random integration and gene targeting methods. Specifically, the subject invention provides a method which reproducibly allows for site-specific integration of a desired DNA into a transcriptionally active domain of a mammalian cell. Moreover, because the subject method introduces an artificial region of "homology" which acts as a unique substrate for homologous recombination and the

insertion of a desired DNA, the efficacy of subject invention does not require that the cell endogenously contain or express a specific DNA. Thus, the method is generically applicable to all mammalian cells, and can be used to express any type of recombinant protein.

The use of a triply spliced selectable marker, e.g., the exemplified triply spliced neo construct, guarantees that all G418 resistant colonies produced will arise from a homologous recombination event (random integrants will not produce a functional neo gene and consequently will not survive G418 selection). Thus, the subject invention makes it easy to screen for the desired homologous event. Furthermore, the frequency of additional random integrations in a cell that has undergone a homologous recombination event appears to be low.

Based on the foregoing, it is apparent that a significant advantage of the invention is that it substantially reduces the number of colonies that need be screened to identify high producer clones, i.e., cell lines containing a desired DNA which secrete the corresponding protein at high levels. On average, clones containing integrated desired DNA may be identified by screening about 5 to 20 colonies (compared to several thousand which must be screened when using standard random integration techniques, or several hundred using the previously described intronic insertion vectors) Additionally, as the site of integration was preselected

and comprises a transcriptionally active domain, all exogenous DNA expressed at this site should produce comparable, i.e. high levels of the protein of interest.

Moreover, the subject invention is further advantageous in that it enables an amplifiable gene to be inserted on integration of the marking vector. Thus, when a desired gene is targeted to this site via homologous recombination, the subject invention allows for expression of the gene to be further enhanced by gene amplification. In this regard, it has been reported in from the literature that different genomic sites have different capacities for gene amplification (Meinkoth et al, *Mol. Cell Biol.*, 7:1415-1424 (1987)). Therefore, this technique is further advantageous as it allows for the placement of a desired gene of interest at a specific site that is both transcriptionally active and easily amplified. Therefore, this should significantly reduce the amount of time required to isolate such high producers.

Specifically, while conventional methods for the construction of high expressing mammalian cell lines can take 6 to 9 months, the present invention allows for such clones to be isolated on average after only about 3-6 months. This is due to the fact that conventionally isolated clones typically must be subjected to at least three rounds of drug resistant gene amplification in order to reach satisfactory levels of gene expression.

As the homologously produced clones are generated from a preselected site which is a high expression site, fewer rounds of amplification should be required before reaching a satisfactory level of production.

Still further, the subject invention enables the reproducible selection of high producer clones wherein the vector is integrated at low copy number, typically single copy. This is advantageous as it enhances the stability of the clones and avoids other potential adverse side-effects associated with high copy number. As described *supra*, the subject homologous recombination system uses the combination of a "marker plasmid" and a "targeting plasmid" which are described in more detail below.

The "marker plasmid" which is used to mark and identify a transcriptionally hot spot will comprise at least the following sequences:

(i) a region of DNA that is heterologous or unique to the genome of the mammalian cell, which functions as a source of homology, allows for homologous recombination (with a DNA contained in a second target plasmid). More specifically, the unique region of DNA (i) will generally comprise a bacterial, viral, yeast synthetic, or other DNA which is not normally present in the mammalian cell genome and which further does not comprise significant homology or sequence identity to DNA contained in the genome of the mammalian cell.

Essentially, this sequence should be sufficiently different to mammalian DNA that it will not significantly recombine with the host cell genome via homologous recombination. The size of such unique DNA will generally be at least about 2 to 10 kilobases in size, or higher, more preferably at least about 10kb, as several other investigators have noted an increased frequency of targeted recombination as the size of the homology region is increased (Capecchi, *Science*, 244:1288-1292 (1989)).

The upper size limit of the unique DNA which acts as a site for homologous recombination with a sequence in the second target vector is largely dictated by potential stability constraints (if DNA is too large it may not be easily integrated into a chromosome and the difficulties in working with very large DNAs.

(ii) a DNA including a fragment of a selectable marker DNA, typically an exon of a dominant selectable marker gene. The only essential feature of this DNA is that it not encode a functional selectable marker protein unless it is expressed in association with a sequence contained in the target plasmid. Typically, the target plasmid will comprise the remaining exons of the dominant selectable marker gene (those not comprised in "targeting" plasmid). Essentially, a functional selectable marker should only be produced if homologous recombination occurs (resulting in the association and

expression of this marker DNA (i) sequence together with the portion(s) of the selectable marker DNA fragment which is (are) contained in the target plasmid).

As noted, the current invention exemplifies the use of the neomycin phosphotransferase gene as the dominant selectable marker which is "split" in the two vectors. However, other selectable markers should also be suitable, e.g., the Salmonella histidinol dehydrogenase gene, hygromycin phosphotransferase gene, herpes simplex virus thymidine kinase gene, adenosine deaminase gene, glutamine synthetase gene and hypoxanthine-guanine phosphoribosyl transferase gene.

(iii) a DNA which encodes a functional selectable marker protein, which selectable marker is different from the selectable marker DNA (ii). This selectable marker provides for the successful selection of mammalian cells wherein the marker plasmid is successfully integrated into the cellular DNA. More preferably, it is desirable that the marker plasmid comprise two such dominant selectable marker DNAs, situated at opposite ends of the vector. This is advantageous as it enables integrants to be selected using different selection agents and further enables cells which contain the entire vector to be selected. Additionally, one marker can be an amplifiable marker to facilitate gene amplification as discussed previously. Any of the

dominant selectable marker listed in (ii) can be used as well as others generally known in the art.

Moreover, the marker plasmid may optionally further comprise a rare endonuclease restriction site. This is potentially desirable as this may facilitate cleavage. If present, such rare restriction site should be situated close to the middle of the unique region that acts as a substrate for homologous recombination. Preferably such sequence will be at least about 12 nucleotides. The introduction of a double stranded break by similar methodology has been reported to enhance the frequency of homologous recombination. (Choulika et al, *Mol. Cell. Biol.*, 15:1968-1973 (1995)). However, the presence of such sequence is not essential.

The "targeting plasmid" will comprise at least the following sequences:

(1) the same unique region of DNA contained in the marker plasmid or one having sufficient homology or sequence identity therewith that said DNA is capable of combining via homologous recombination with the unique region (i) in the marker plasmid. Suitable types of DNAs are described *supra* in the description of the unique region of DNA (1) in the marker plasmid.

(2) The remaining exons of the dominant selectable marker, one exon of which is included as (ii) in the marker plasmid listed above. The essential features of this DNA fragment is that it result in a functional

(selectable) marker protein only if the target plasmid integrates via homologous recombination (wherein such recombination results in the association of this DNA with the other fragment of the selectable marker DNA contained in the marker plasmid) and further that it allow for insertion of a desired exogenous DNA. Typically, this DNA will comprise the remaining exons of the selectable marker DNA which are separated by an intron. For example, this DNA may comprise the first two exons of the neo gene and the marker plasmid may comprise the third exon (back third of neo).

(3) The target plasmid will also comprise a desired DNA, e.g., one encoding a desired polypeptide, preferably inserted within the selectable marker DNA fragment contained in the plasmid. Typically, the DNA will be inserted in an intron which is comprised between the exons of the selectable marker DNA. This ensures that the desired DNA is also integrated if homologous recombination of the target plasmid and the marker plasmid occurs. This intron may be naturally occurring or it may be engineered into the dominant selectable marker DNA fragment.

This DNA will encode any desired protein, preferably one having pharmaceutical or other desirable properties. Most typically the DNA will encode a mammalian protein, and in the current examples provided, an immunoglobulin or an immunoadhesin. However the

invention is not in any way limited to the production of immunoglobulins.

As discussed previously, the subject cloning method is suitable for any mammalian cell as it does not require for efficacy that any specific mammalian sequence or sequences be present. In general, such mammalian cells will comprise those typically used for protein expression, e.g., CHO cells, myeloma cells, COS cells, BHK cells, Sp2/0 cells, NIH 3T3 and HeLa cells. In the examples which follow, CHO cells were utilized. The advantages thereof include the availability of suitable growth medium, their ability to grow efficiently and to high density in culture, and their ability to express mammalian proteins such as immunoglobulins in biologically active form.

Further, CHO cells were selected in large part because of previous usage of such cells by the inventors for the expression of immunoglobulins (using the translationally impaired dominant selectable marker containing vectors described previously). Thus, the present laboratory has considerable experience in using such cells for expression. However, based on the examples which follow, it is reasonable to expect similar results will be obtained with other mammalian cells.

In general, transformation or transfection of mammalian cells according to the subject invention will be effected according to conventional methods. So that the

invention may be better understood, the construction of exemplary vectors and their usage in producing integrants is described in the examples below.

EXAMPLE 1

Design and Preparation of Marker and Targeting Plasmid DNA Vectors

The marker plasmid herein referred to as "Desmond" was assembled from the following DNA elements:

(a) Murine dihydrofolate reductase gene (DHFR), incorporated into a transcription cassette, comprising the mouse beta globin promoter 5" to the DHFR start site, and bovine growth hormone poly adenylation signal 3" to the stop codon. The DHFR transcriptional cassette was isolated from TCAE6, an expression vector created previously in this laboratory (Newman et al, 1992, *Biotechnology*, 10:1455-1460).

(b) *E. coli* ß-galactosidase gene - commercially available, obtained from Promega as pSV-b-galactosidase control vector, catalog # E1081.

(c) Baculovirus DNA, commercially available, purchased from Clontech as pBAKPAK8, cat # 6145-1.

(d) Cassette comprising promoter and enhancer elements from Cytomegalovirus and SV40 virus. The cassette was generated by PCR using a derivative of expression vector TCAE8 (Reff et al, *Blood*, 83:435-445 (1994)). The enhancer cassette was inserted within the baculo-

virus sequence, which was first modified by the insertion of a multiple cloning site.

(e) *E. coli* GUS (glucuronidase) gene, commercially available, purchased from Clontech as pB101, cat. # 6017-1.

(f) Firefly luciferase gene, commercially available, obtained from Promega as pGEM-Luc (catalog # E1541).

(g) S. typhimurium histidinol dehydrogenase gene (HisD). This gene was originally a gift from (Donahue et el, *Gene*, 18:47-59 (1982)), and has subsequently been incorporated into a transcription cassette comprising the mouse beta globin major promoter 5' to the gene, and the SV40 polyadenylation signal 3' to the gene.

The DNA elements described in (a)-(g) were combined into a pBR derived plasmid backbone to produce a 7.7kb contiguous stretch of DNA referred to in the attached figures as "homology". Homology in this sense refers to sequences of DNA which are not part of the mammalian genome and are used to promote homologous recombination between transfected plasmids sharing the same homology DNA sequences.

(h) Neomycin phosphotransferase gene from TN5 (Davis and Smith, *Ann. Rev. Micro.*, 32:469-518 (1978)). The complete neo gene was subcloned into pBluescript SK-(Stratagene catalog # 212205) to facilitate genetic manipulation. A synthetic linker was then inserted into

a unique Pst1 site occurring across the codons for amino acid 51 and 52 of neo. This linker encoded the necessary DNA elements to create an artificial splice donor site, intervening intron and splice acceptor site within the neo gene, thus creating two separate exons, presently referred to as neo exon 1 and 2. Neo exon 1 encodes the first 51 amino acids of neo, while exon 2 encodes the remaining 203 amino acids plus the stop codon of the protein A *Not*1 cloning site was also created within the intron.

Neo exon 2 was further subdivided to produce neo exons 2 and 3. This was achieved as follows: A set of PCR primers were designed to amplify a region of DNA encoding neo exon 1, intron and the first 111 2/3 amino acids of exon2. The 3' PCR primer resulted in the introduction of a new 5' splice site immediately after the second nucleotide of the codon for amino acid 111 in exon 2, therefore generating a new smaller exon 2. The DNA fragment now encoding the original exon 1, intron and new exon 2 was then subcloned and propagated in a pBR based vector. The remainder of the original exon 2 was used as a template for another round of PCR amplification, which generated "exon3". The 5' primer for this round of amplification introduced a new splice acceptor site at the 5' side of the newly created exon 3, i.e. before the final nucleotide of the codon for amino acid 111. The resultant 3 exons of neo encode the

following information: exon 1 - the first 51 amino acids of neo; exon 2 - the next 111 2/3 amino acids, and exon 3 the final 91 1/3 amino acids plus the translational stop codon of the neo gene.

Neo exon 3 was incorporated along with the above mentioned DNA elements into the marking plasmid "Desmond". Neo exons 1 and 2 were incorporated into the targeting plasmid "Molly". The *Not*1 cloning site created within the intron between exons 1 and 2 was used in subsequent cloning steps to insert genes of interest into the targeting plasmid.

A second targeting plasmid "Mandy" was also generated. This plasmid is almost identical to "Molly" (some restriction sites on the vector have been changed) except that the original HisD and DHFR genes contained in "Molly" were inactivated. These changes were incorporated because the Desmond cell line was no longer being cultured in the presence of Histidinol, therefore it seemed unnecessary to include a second copy of the HisD gene. Additionally, the DHFR gene was inactivated to ensure that only a single DHFR gene, namely the one present in the Desmond marked site, would be amplifiable in any resulting cell lines. "Mandy" was derived from "Molly" by the following modifications:

(i) A synthetic linker was inserted in the middle of the DHFR coding region. This linker created a stop codon and shifted the remainder of the DHFR coding

region out of frame, therefore rendering the gene nonfunctional.

(ii) A portion of the HisD gene was deleted and replaced with a PCR generated HisD fragment lacking the promoter and start codon of the gene.

Figure 1 depicts the arrangement of these DNA elements in the marker plasmid "Desmond". Figure 2 depicts the arrangement of these elements in the first targeting plasmid, "Molly". Figure 3 illustrates the possible arrangement in the CHO genome, of the various DNA elements after targeting and integration of Molly DNA into Desmond marked CHO cells. Figure 9 depicts the targeting plasmid "Mandy."

Construction of the marking and targeting plasmids from the above listed DNA elements was carried out following conventional cloning techniques (see, e.g., Molecular Cloning, A Laboratory Manual, J. Sambrook et al, 1987, Cold Spring Harbor Laboratory Press, and Current Protocols in Molecular Biology, F. M. Ausubel et al, eds., 1987, John Wiley and Sons). All plasmids were propagated and maintained in E. coli XLI blue (Stratagene, cat. # 200236). Large scale plasmid preparations were prepared using Promega Wizard Maxiprep DNA Purification System®, according to the manufacturer's directions.

EXAMPLE 2

Construction of a Marked CHO Cell Line

1. Cell Culture and Transfection Procedures to Produced Marked CHO Cell Line

Marker plasmid DNA was linearized by digestion overnight at 37°C with Bst1107I. Linearized vector was ethanol precipitated and resuspended in sterile TE to a concentration of 1mg/ml. Linearized vector was introduced into DHFR-Chinese hamster ovary cells (CHO cells) DG44 cells (Urlaub et al, *Som. Cell and Mol. Gen.*, 12:555-566 (1986)) by electroporation as follows.

Exponentially growing cells were harvested by centrifugation, washed once in ice cold SBS (sucrose buffered solution, 272mM sucrose, 7mM sodium phosphate, pH 7.4, 1mM magnesium chloride) then resuspended in SBS to a concentration of 10^7 cells/ml. After a 15 minute incubation on ice, 0.4ml of the cell suspension was mixed with 40μg linearized DNA in a disposable electroporation cuvette. Cells were shocked using a BTX electrocell manipulator (San Diego, CA) set at 230 volts, 400 microfaraday capacitance, 13 ohm resistance. Shocked cells were then mixed with 20 ml of prewarmed CHO growth media (CHO-S-SFMII, Gibco/BRL, catalog # 31033-012) and plated in 96 well tissue culture plates. Forty eight hours after electroporation, plates were fed with selection media (in the case of transfection with Desmond, selection media is CHO-S-SFMII without

hypoxanthine or thymidine, supplemented with 2mM Histidinol (Sigma catalog # H6647)). Plates were maintained in selection media for up to 30 days, or until some of the wells exhibited cell growth. These cells were then removed from the 96 well plates and expanded ultimately to 120 ml spinner flasks where they were maintained in selection media at all times.

EXAMPLE 3

Characterization of Marked CHO Cell Lines

(a) Southern Analysis

Genomic DNA was isolated from all stably growing Desmond marked CHO cells. DNA was isolated using the Invitrogen Easy® DNA kit, according to the manufacturer's directions. Genomic DNA was then digested with HindIII overnight at 37°C, and subjected to Southern analysis using a PCR generated digoxygenin labelled probe specific to the DHFR gene. Hybridizations and washes were carried out using Boehringer Mannheim's DIG easy hyb (catalog # 1603 558) and DIG Wash and Block Buffer Set (catalog # 1585 762) according to the manufacturer's directions. DNA samples containing a single band hybridizing to the DHFR probe were assumed to be Desmond clones arising from a single cell which had integrated a single copy of the plasmid. These clones were retained for further analysis. Out of a total of 45 HisD resistant cell lines isolated, only 5 were

single copy integrants. Figure 4 shows a Southern blot containing all 5 of these single copy Desmond clones. Clone names are provided in the figure legend.

(b) Northern Analysis

Total RNA was isolated from all single copy Desmond clones using TRIzol reagent (Gibco/BRL cat # 15596-026) according to the manufacturer's directions. 10-20μg RNA from each clone was analyzed on duplicate formaldehyde gels. The resulting blots were probed with PCR generated digoxygenin labelled DNA probes to (i) DHFR message, (ii) HisD message and (iii) CAD message. CAD is a trifunctional protein involved in uridine biosynthesis (Wahl et al, *J. Biol. Chem.*, 254, 17:8679-8689 (1979)), and is expressed equally in all cell types. It is used here as an internal control to help quantitate RNA loading. Hybridizations and washes were carried out using the above mentioned Boehringer Mannheim reagents. The results of the Northern analysis are shown in Figure 5. The single copy Desmond clone exhibiting the highest levels of both the His D and DHFR message is clone 15C9, shown in lane 4 in both panels of the figure. This clone was designated as the "marked cell line" and used in future targeting experiments in CHO, examples of which are presented in the following sections.

EXAMPLE 4

Expression of Anti-CD20 Antibody in Desmond Marked CHO Cells

C2B8, a chimeric antibody which recognizes B-cell surface antigen CD20, has been cloned and expressed previously in our laboratory. (Reff et al, *Blood*, 83:434-45 (1994)). A 4.1 kb DNA fragment comprising the C2B8 light and heavy chain genes, along with the necessary regulatory elements (eukaryotic promoter and polyadenylation signals) was inserted into the artificial intron created between exons 1 and 2 of the neo gene contained in a pBR derived cloning vector. This newly generated 5kb DNA fragment (comprising neo exon 1, C2B8 and neo exon 2) was excised and used to assemble the targeting plasmid Molly. The other DNA elements used in the construction of Molly are identical to those used to construct the marking plasmid Desmond, identified previously. A complete map of Molly is shown in Fig. 2.

The targeting vector Molly was linearized prior to transfection by digestion with *Kpn*1 and *Pac*1, ethanol precipitated and resuspended in sterile TE to a concentration of 1.5mg/mL. Linearized plasmid was introduced into exponentially growing Desmond marked cells essentially as described, except that 80μg DNA was used in each electroporation. Forty eight hours postelectroporation, 96 well plates were supplemented with selection medium - CHO-SSFMII supplemented with 400 μg/mL Geneti-

cin (G418, Gibco/BRL catalog # 10131-019). Plates were maintained in selection medium for up to 30 days, or until cell growth occurred in some of the wells. Such growth was assumed to be the result of clonal expansion of a single G418 resistant cell. The supernatants from all G418 resistant wells were assayed for C2B8 production by standard ELISA techniques, and all productive clones were eventually expanded to 120mL spinner flasks and further analyzed.

Characterization of Antibody secreting Targeted Cells

A total of 50 electroporations with Molly targeting plasmid were carried out in this experiment, each of which was plated into separate 96 well plates. A total of 10 viable, anti-CD20 antibody secreting clones were obtained and expanded to 120ml spinner flasks. Genomic DNA was isolated from all clones, and Southern analyses were subsequently performed to determine whether the clones represented single homologous recombination events or whether additional random integrations had occurred in the same cells. The methods for DNA isolation and Southern hybridization were as described in the previous section. Genomic DNA was digested with EcoRI and probed with a PCR generated digoxygenin labelled probe to a segment of the CD20 heavy chain constant region. The results of this Southern analysis are presented in figure 6. As can be seen in the figure, 8 of

the 10 clones show a single band hybridizing to the CD20 probe, indicating a single homologous recombination event has occurred in these cells. Two of the ten, clones 24G2 and 28C9, show the presence of additional band(s), indicative of an additional random integration elsewhere in the genome.

We examined the expression levels of anti-CD20 antibody in all ten of these clones, the data for which is shown in Table 1, below.

Table 1:

Expression Level of Anti-CD20 Secreting Homologous Integrants

Clone	Anti-CD20, pg/c/d
20F4	3.5
25E1	2.4
42F9	1.8
39G11	1.5
21C7	1.3
50G10	0.9
29F9	0.8
5F9	0.3
28C9*	4.5
24G2*	2.1

* These clones contained additional randomly integrated copies of anti-CD20. Expression levels of these clones therefore reflect a contribution from both the homologous and random sites.

Expression levels are reported as picogram per cell per day (pg/c/d) secreted by the individual clones, and represented the mean levels obtained from three separate ELISAs on samples taken from 120 mL spinner flasks.

As can be seen from the data, there is a variation in antibody secretion of approximately ten fold between the highest and lowest clones. This was somewhat unexpected as we anticipated similar expression levels from all clones due to the fact the anti-CD20 genes are all integrated into the same Desmond marked site. Nevertheless, this observed range in expression extremely small in comparison to that seen using any traditional random integration method or with our translationally impaired vector system.

Clone 20F4, the highest producing single copy integrant was selected for further study. Table 2 (below) presents ELISA and cell culture data from seven day production runs of this clone.

Table 2:

7 Day Production Run Data for 20F4

Day	% Viable	Viable/ml ($x 10^5$)	Tx2(hr)	mg/L	pg/c/d
1	96	3.4	31	1.3	4.9
2	94	6	29	2.5	3.4
3	94	9.9	33	4.7	3.2
4	90	17.4	30	6.8	3
5	73	14		8.3	
6	17	3.5		9.5	

Clone 20F4 was seeded at $2x10^5$ml in a 120ml spinner flask on day 0. On the following six days, cell counts were taken, doubling times calculated and 1ml samples of supernatant removed from the flask and analyzed for secreted anti-CD20 by ELISA.

This clone is secreting on average, 3-5pg antibody/-cell/day, based on this ELISA data. This is the same level as obtained from other high expressing single copy clones obtained previously in our laboratory using the previously developed translationally impaired random integration vectors. This result indicates the following:

(1) that the site in the CHO genome marked by the Desmond marking vector is highly transcriptionally active, and therefore represents an excellent site from which to express recombinant proteins, and

(2) that targeting by means of homologous recombination can be accomplished using the subject vectors and occurs at a frequency high enough to make this system a viable and desirable alternative to random integration methods.

To further demonstrate the efficacy of this system, we have also demonstrated that this site is amplifiable, resulting in even higher levels of gene expression and protein secretion. Amplification was achieved by plating serial dilutions of 20F4 cells, starting at a density of 2.5 x 10^4 cells/ml, in 96 well tissue culture dishes, and culturing these cells in media (CHO-SSFMII) supplemented with 5, 10, 15 or 20nM methotrexate. Antibody secreting clones were screened using standard ELISA techniques, and the highest producing clones were expanded and further analyzed. A summary of this amplification experiment is presented in Table 3 below.

Table 3:

Summary of 20F4 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
10	56	3-13	4	10-15
15	27	2-14	3	15-18
20	17	4-11	1	ND

Methotrexate amplification of 20F4 was set up as described in the text, using the concentrations of methotrexate indicated in the above table. Supernatants from all surviving 96 well colonies were assayed by ELISA, and the range of anti-CD20 expressed by these clones is indicated in column 3. Based on these results, the highest producing clones were expanded to 120ml spinners and several ELISAs conducted on the spinner supernatants to determine the pg/cell/day expression levels, reported in column 5.

The data here clearly demonstrates that this site can be amplified in the presence of methotrexate. Clones from the 10 and 15nM amplifications were found to produce on the order of 15-20pg/cell/day.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated

from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. The clone was then subjected to a further round of methotrexate amplification. As described above, serial dilutions of the culture were plated into 96 well dishes and cultured in CHO-SS-FMII medium supplemented with 200, 300 or 400nM methotrexate. Surviving clones were screened by ELISA, and several high producing clones were expanded to spinner cultures and further analyzed. A summary of this second amplification experiment is presented in Table 4.

Table 4:

Summary of 20F4-15A5 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d, spinner
200	67	23-70	1	50-60
250	86	21-70	4	55-60
300	81	15-75	3	40-50

Methotrexate amplifications of 20F4-15A5 were set up and assayed as described in the text. The highest producing wells, the numbers of which are indicated in column 4, were expanded to 120ml spinner flasks. The expression levels of the cell lines derived from these wells is recorded as pg/c/d in column 5.

The highest producing clone came from the 250nM methotrexate amplification. The 250nM clone, 20F4-15A5-250A6 originated from a 96 well plate in which only wells

grew, and therefore is assumed to have arisen from a single cell. Taken together, the data in Tables 3 and 4 strongly indicates that two rounds of methotrexate amplification are sufficient to reach expression levels of 60pg/cell/day, which is approaching the maximum secretion capacity of immunoglobulin in mammalian cells (Reff, M.E., *Curr. Opin. Biotech.*, 4:573-576 (1993)). The ability to reach this secretion capacity with just two amplification steps further enhances the utility of this homologous recombination system. Typically, random integration methods require more than two amplification steps to reach this expression level and are generally less reliable in terms of the ease of amplification. Thus, the homologous system offers a more efficient and time saving method of achieving high level gene expression in mammalian cells.

EXAMPLE 5

Expression of Anti-Human CD23 Antibody in Desmond Marked CHO Cells

CD23 is low affinity IgE receptor which mediates binding of IgE to B and T lymphocytes (Sutton, B.J., and Gould, H.J., *Nature*, 366:421-428 (1993)). Anti-human CD23 monoclonal antibody 5E8 is a human gamma-1 monoclonal antibody recently cloned and expressed in our laboratory. This antibody is disclosed in commonly

assigned Serial No. 08/803,085, filed on February 20, 1997.

The heavy and light chain genes of 5E8 were cloned into the mammalian expression vector N5KG1, a derivative of the vector NEOSPLA (Barnett et al, in *Antibody Expression and Engineering*, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)) and two modifications were then made to the genes. We have recently observed somewhat higher secretion of immunoglobulin light chains compared to heavy chains in other expression constructs in the laboratory (Reff et al, 1997, unpublished observations). In an attempt to compensate for this deficit, we altered the 5E8 heavy chain gene by the addition of a stronger promoter/enhancer element immediately upstream of the start site. In subsequent steps, a 2.9kb DNA fragment comprising the 5E8 modified light and heavy chain genes was isolated from the N5KG1 vector and inserted into the targeting vector Mandy. Preparation of 5E8-containing Molly and electroporation into Desmond 15C9 CHO cells was essentially as described in the preceding section.

One modification to the previously described protocol was in the type of culture medium used. Desmond marked CHO cells were cultured in protein-free CD-CHO medium (Gibco-BRL, catalog # AS21206) supplemented with 3mg/L recombinant insulin (3mg/mL stock, Gibco-BRL, catalog # AS22057) and 8mM L-glutamine (200mM stock, Gibco-BRL, catalog # 25030-081). Subsequently, trans-

fected cells were selected in the above medium supplemented with 400μg/mL geneticin. In this experiment, 20 electroporations were performed and plated into 96 well tissue culture dishes. Cells grew and secreted anti-CD23 in a total of 68 wells, all of which were assumed to be clones originating from a single G418 cell. Twelve of these wells were expanded to 120ml spinner flasks for further analysis. We believe the increased number of clones isolated in this experiment (68 compared with 10 for anti-CD20 as described in Example 4) is due to a higher cloning efficiency and survival rate of cells grown in CD-CHO medium compared with CHO-SS-FMII medium. Expression levels for those clones analyzed in spinner culture ranged from 0.5-3pg/c/d, in close agreement with the levels seen for the anti-CD20 clones. The highest producing anti-CD23 clone, designated 4H12, was subjected to methotrexate amplification in order to increase its expression levels. This amplification was set up in a manner similar to that described for the anti-CD20 clone in Example 4. Serial dilutions of exponentially growing 4H12 cells were plated into 96 well tissue culture dishes and grown in CD-CHO medium supplemented with 3mg/L insulin, 8mM glutamine and 30, 35 or 40nM methotrexate. A summary of this amplification experiment is presented in Table 5.

Table 5:

Summary of 2H12 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
30	100	6-24	8	10-25
35	64	4-27	2	10-15
40	96	4-20	1	ND

The highest expressing clone obtained was a 30nM clone, isolated from a plate on which 22 wells had grown. This clone, designated 4H12-30G5, was reproducibly secreting 18-22pg antibody per cell per day. This is the same range of expression seen for the first amplification of the anti CD20 clone 20F4 (clone 20F4-15A5 which produced 15-18pg/c/d, as described in Example 4). This data serves to further support the observation that amplification at this marked site in CHO is reproducible and efficient. A second amplification of this 30nM cell line is currently underway. It is anticipated that saturation levels of expression will be achievable for the anti-CD23 antibody in just two amplification steps, as was the case for anti-CD20.

EXAMPLE 6

Expression of Immunoadhesin in Desmond Marked CHO Cells

CTLA-4, a member of the Ig superfamily, is found on the surface of T lymphocytes and is thought to play a role in antigen-specific T-cell activation (Dariavach et al, *Eur. J. Immunol.*, 18:1901-1905 (1988); and Linsley et al, *J. Exp. Med.*, 174:561-569 (1991)). In order to further study the precise role of the CTLA-4 molecule in the activation pathway, a soluble fusion protein comprising the extracellular domain of CTLA-4 linked to a truncated form of the human IgG1 constant region was

created (Linsley et al (Id.). We have recently expressed this CTLA-4 Ig fusion protein in the mammalian expression vector BLECH1, a derivative of the plasmid NEOSPLA (Barnett et al, in Antibody Expression and Engineering, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)). An 800bp fragment encoding the CTLA-4 Ig was isolated from this vector and inserted between the SacII and BglII sites in Molly.

Preparation of CTLA-4Ig-Molly and electroporation into Desmond clone 15C9 CHO cells was performed as described in the previous example relating to anti-CD20. Twenty electroporations were carried out, and plated into 96 well culture dishes as described previously. Eighteen CTLA-4 expressing wells were isolated from the 96 well plates and carried forward to the 120ml spinner stage. Southern analyses on genomic DNA isolated from each of these clones were then carried out to determine how many of the homologous clones contained additional random integrants. Genomic DNA was digested with BglII and probed with a PCR generated digoxygenin labelled probe to the human IgG1 constant region. The results of this analysis indicated that 85% of the CTLA-4 clones are homologous integrants only; the remaining 15% contained one additional random integrant. This result corroborates the findings from the expression of anti-CD20 discussed above, where 80% of the clones were single homologous integrants. Therefore, we can conclude

that this expression system reproducibly yields single targeted homologous integrants in at least 80% of all clones produced.

Expression levels for the homologous CTlA4-Ig clones ranged from 8-12pg/cell/day. This is somewhat higher than the range reported for anti-CD20 antibody and anti-CD23 antibody clones discussed above. However, we have previously observed that expression of this molecule using the intronic insertion vector system also resulted in significantly higher expression levels than are obtained for immunoglobulins. We are currently unable to provide an explanation for this observation.

EXAMPLE 7

Targeting Anti-CD20 to an alternate Desmond Marked CHO Cell Line

As we described in a preceding section, we obtained 5 single copy Desmond marked CHO cell lines (see Figures 4 and 5). In order to demonstrate that the success of our targeting strategy is not due to some unique property of Desmond clone 15C9 and limited only to this clone, we introduced anti-CD20 Molly into Desmond clone 9B2 (lane 6 in figure 4, lane 1 in figure 5). Preparation of Molly DNA and electroporation into Desmond 9B2 was exactly as described in the previous example pertaining to anti-CD20. We obtained one homologous integrant from this experiment. This clone was expanded to a 120ml

spinner flask, where it produced on average 1.2pg anti-CD20/cell/day. This is considerably lower expression than we observed with Molly targeted into Desmond 15C9. However, this was the anticipated result, based on our northern analysis of the Desmond clones. As can be seen in Figure 5, mRNA levels from clone 9B2 are considerably lower than those from 15C9, indicating the site in this clone is not as transcriptionally active as that in 15C9. Therefore, this experiment not only demonstrates the reproducibility of the system - presumably any marked Desmond site can be targeted with Molly - it also confirms the northern data that the site in Desmond 15C9 is the most transcriptionally active.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without diverting from the scope of the invention. Accordingly, the invention is not limited by the appended claims.

"Comprises/comprising" when used in this specification is taken to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises the following steps:

(i) transfecting or transforming a mammalian cell with a first plasmid ("marker plasmid") containing the following sequences:

(a) a region of DNA at least 2 to 10Kb in size that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first dominant selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid;

(ii) selecting a cell which contain the marker plasmid integrated in its genome;

(iii) transfecting or transforming said selected cell with a second plasmid ("target plasmid") which contains the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the first selectable marker contained in the marker plasmid, wherein the active, dominant selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid,

(c) the desired DNA; and

(iv) selecting cells which contain the target plasmid integrated at the target site by screening for the expression of the first dominant selectable marker protein.

2. The method of claim 1, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

3. The method of claim 2, wherein the second plasmid contains the remaining exons of said first selectable marker.

4. The method of claim 3, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

5. The method of claim 4, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in

the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

6. The method of Claim 3, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

7. The method of Claim 4, wherein the desired protein is a mammalian protein.

8. The method of Claim 7, wherein the protein is an immunoglobulin.

9. The method of Claim 1, which further comprises determining the RNA levels of the selectable marker (c) contained in the marker plasmid prior to integration of the target vector.

10. The method of Claim 9, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex

thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

11. The method of Claim 1, wherein the mammalian cell is selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

12. The method of Claim 11, wherein the cell is a CHO cell.

13. The method of Claim 1, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains the first two exons of the neomycin phosphotransferase gene.

14. The method of Claim 1, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

15. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

16. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is at least 300 nucleotides.

17. The method of Claim 16, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

18. The method of claim 17, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

19. The method of Claim 1, wherein the first selectable marker DNA is split into at least three exons.

20. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

21. The method of Claim 20, wherein the unique region of DNA does not contain any functional genes.

22. A vector system for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises at least the following:

(i) a first plasmid ("marker plasmid") containing at least the following sequences:

(a) a region of DNA at least 2 to 10 Kb in size that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first dominant selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid; and

(ii) a second plasmid ("target plasmid") which contains the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active, dominant selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid,

(c) the desired DNA.

23. The vector system of Claim 22, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

24. The vector system of Claim 23, wherein the second plasmid contains the remaining exons of said first selectable marker.

25. The vector system of Claim 24, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

26. The vector system of Claim 24, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

27. The vector system of Claim 24, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

28. The vector system of Claim 25, wherein the desired protein is a mammalian protein.

29. The vector system of Claim 28, wherein the protein is an immunoglobulin.

30. The vector system of Claim 22, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

31. The vector system of Claim 22, which provides for insertion of a desired DNA at a targeted site in the genome of a mammalian cell selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

32. The vector system of Claim 31, wherein the mammalian cell is a CHO cell.

33. The vector system of Claim 22, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains

the first two exons of the neomycin phosphotransferase gene.

34. The vector system of Claim 22, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

35. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

36. The vector system of Claim 22, wherein the unique region of DNA (a) contained in the marker plasmid vector system that provides for homologous recombination is at least 300 nucleotides.

37. The vector system of Claim 36, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

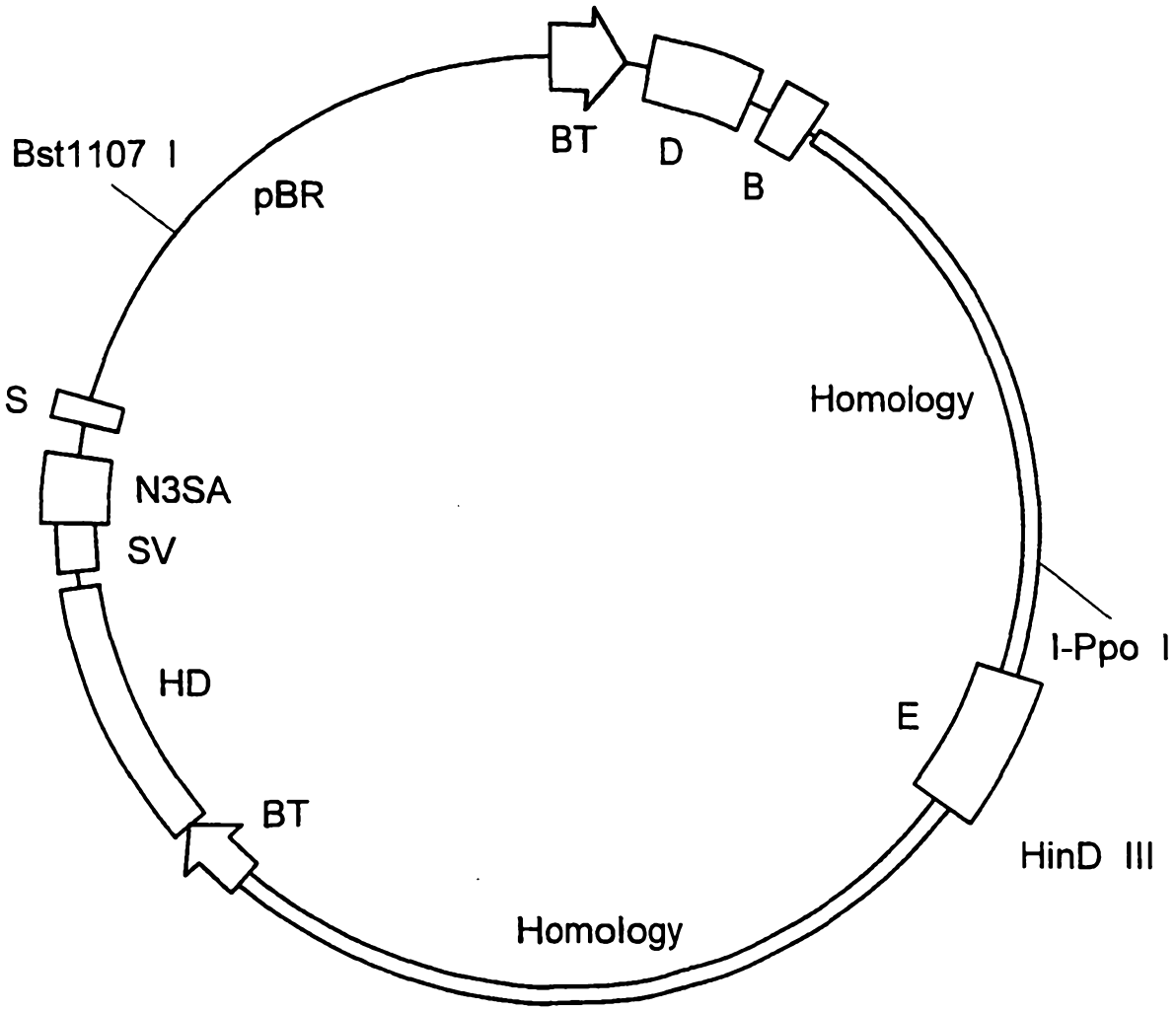
38. The vector system of Claim 37, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

39. The vector system of Claim 22, wherein the first selectable marker DNA is split into at least three exons.

40. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

41. The vector system of Claim 40, wherein the unique region of DNA does not contain any functional genes.

FIG. 1A
DESMOND



HD = Salmonella HisD Gene
N3 = Neomycin Phosphotransferase Exon 3
D = Murine Dihydrofolate reductase
E = Cytomegalovirus and SV40 Enhancers
SA = Splice acceptor
BT = Mouse Beta Globin Major Promoter
B = Bovine Growth Hormone Polyadenylation
S = SV40 Early Polyadenylation
SV = SV40 Late Polyadenylation

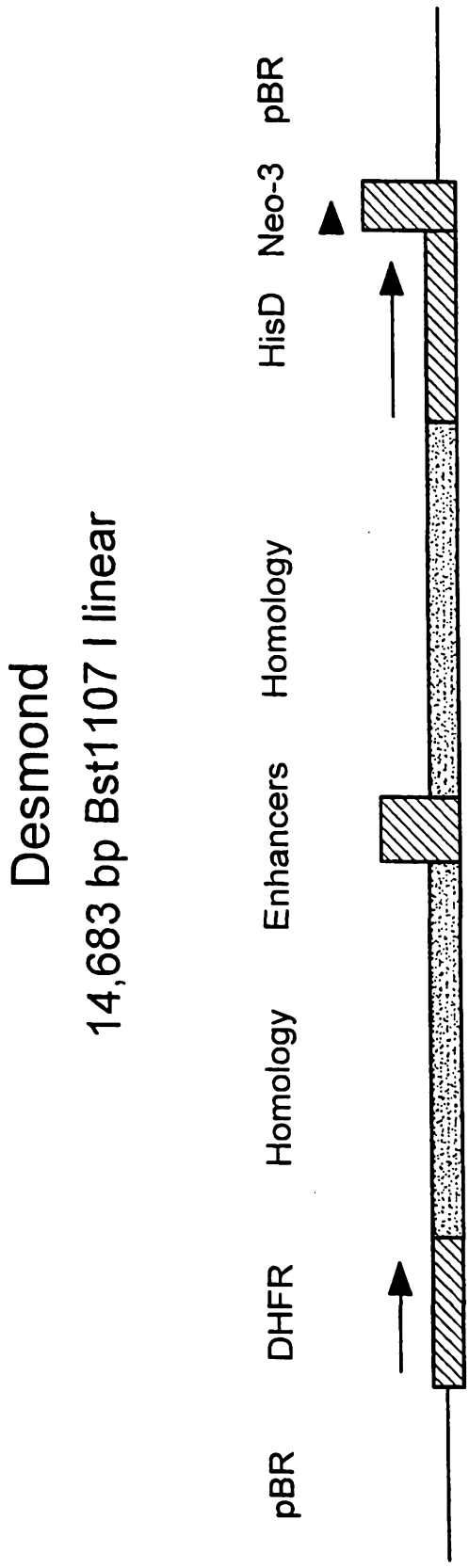
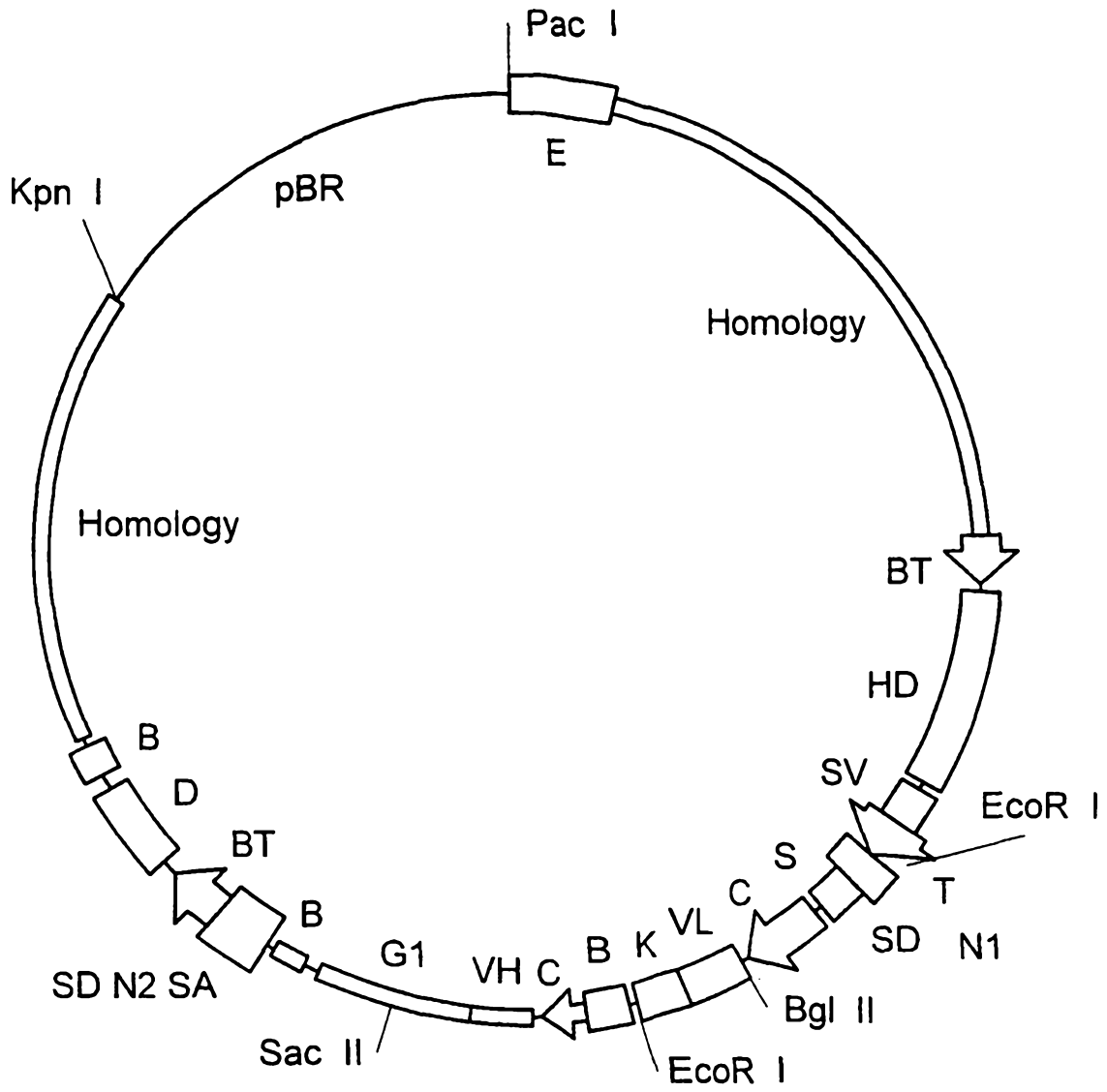

Desmond
14,683 bp Bst1107 I linear
pBR
DHFR
Homology
Enhancers
Homology
HisD
Neo-3
pBR

FIG. 1B

3/75

FIG. 2A

Molly



D = Dihydrofolate reductase
N1 + Neomycin Phosphotransferase Exon 1
N2 + Neomycin Phosphotransferase Exon 2
VL = Anti-CD20 Light chain leader + Variable
K = Human Kappa Constant
VH = Anti-CD20 Heavy chain Leader + Variable
G1 = Human Gamma 1 Constant
HD = Salmonella Histidinol Dehydrogenase
E = CMV and SV40 enhancers S = SV40 Origin
SD = Splice donor SA = Splice acceptor
C = CMV promoter/enhancer
T = HSV TK promoter and Poloma enhancers
BT = Mouse Beta Globin Major Promoter
SV = SV40 Late Polyadenylation
B = Bovine Growth Hormone Polyadenylation

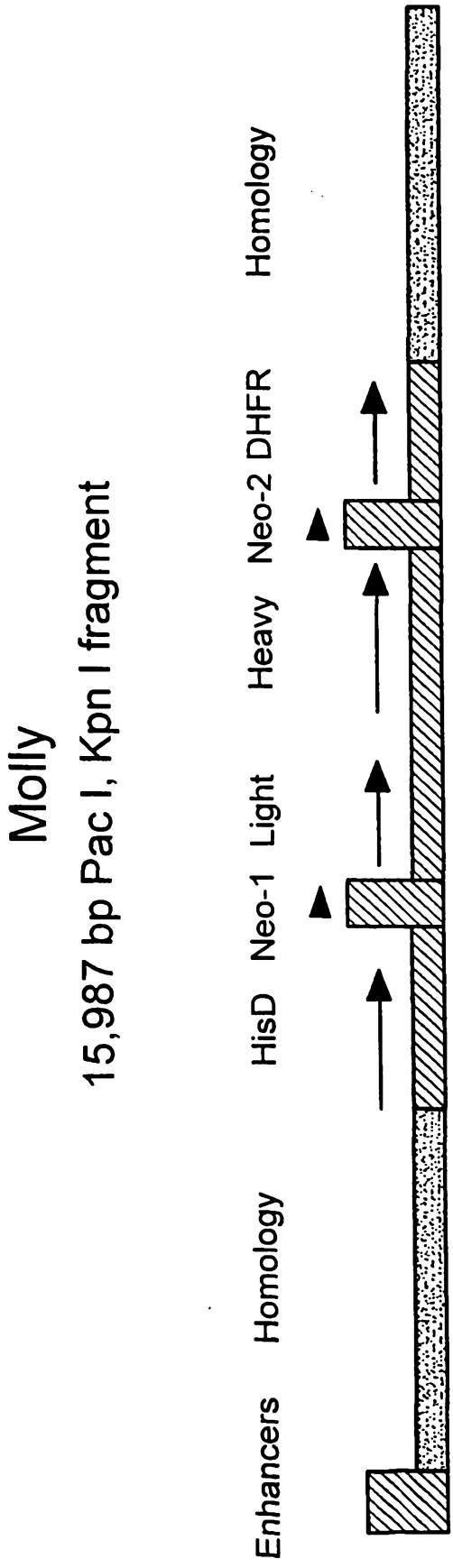
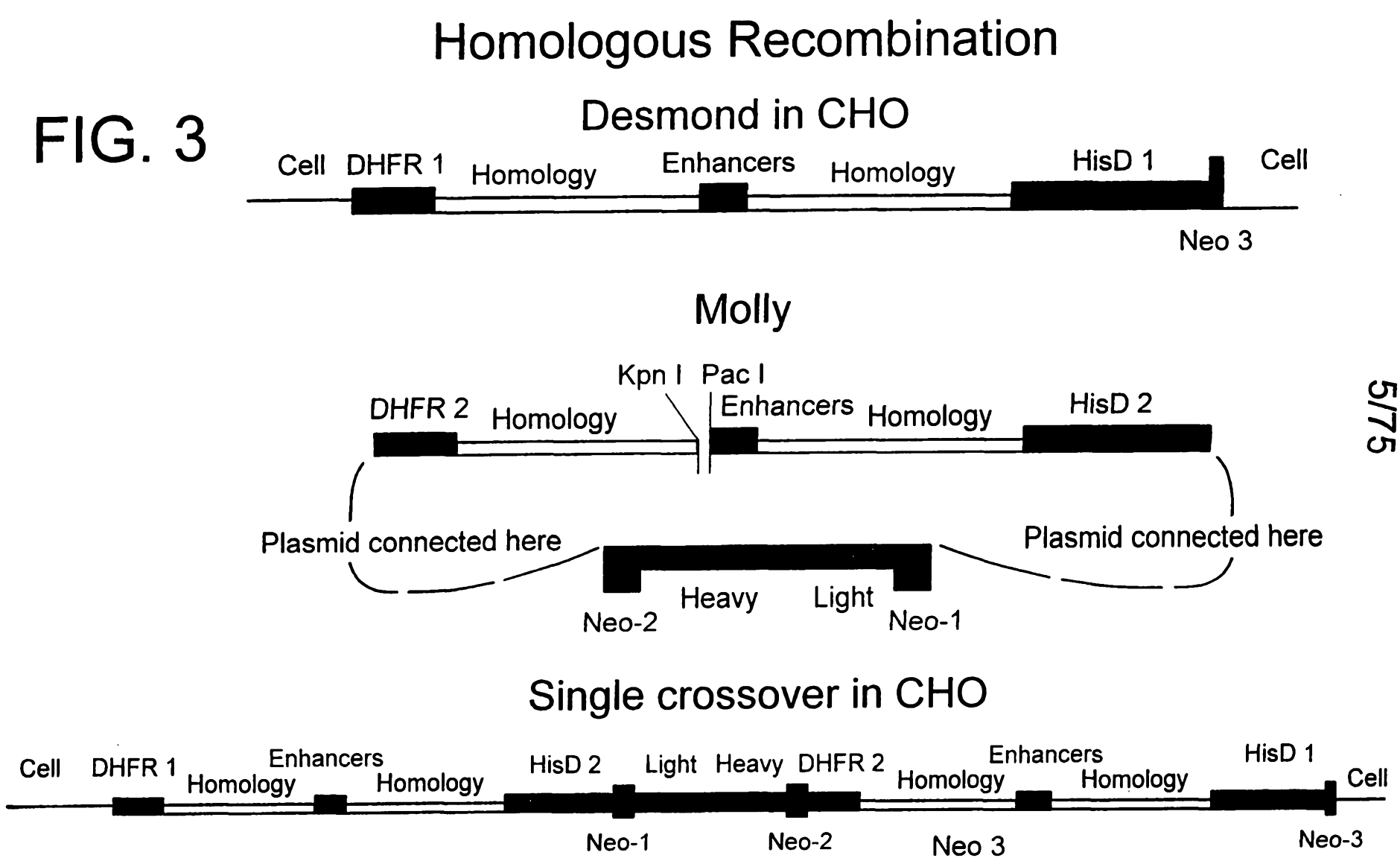

Molly
15,987 bp Pac I, Kpn I fragment
Enhancers
Homology
HisD
Neo-1
Light
Heavy
Neo-2
DHFR
Homology

FIG. 2B

FIG. 3



Southern Analysis of Desmond Marked CHO Cells

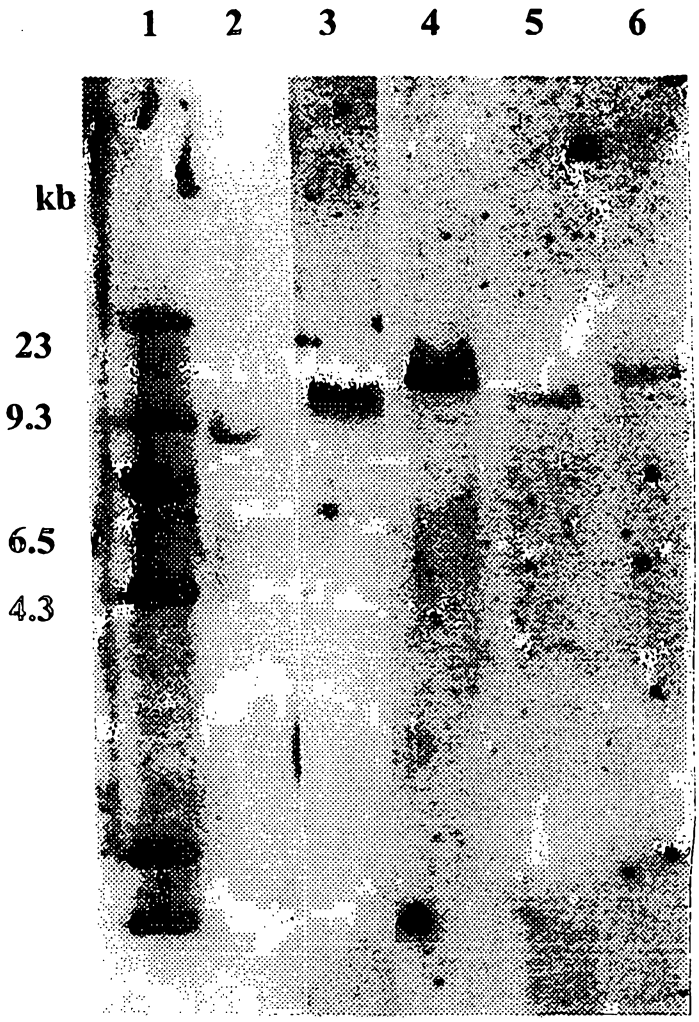


FIG. 4

Northern Analysis of Desmond Marked CHO Cells

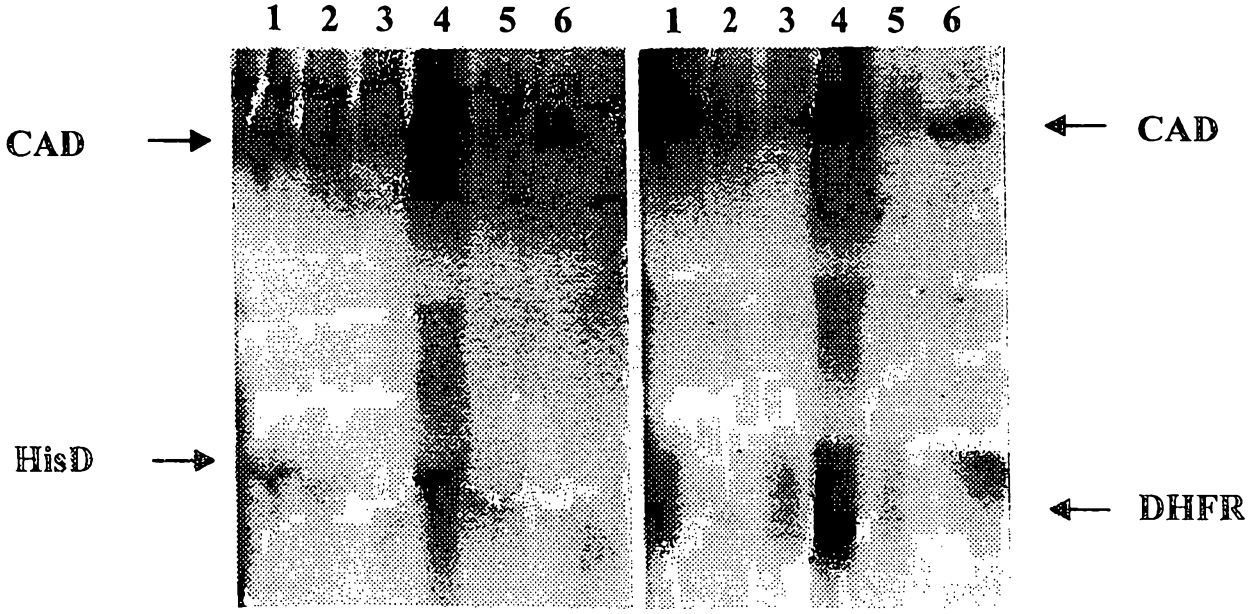


FIG. 5

Southern Analysis of Anti CD20 Integrants in Marked CHO Cells

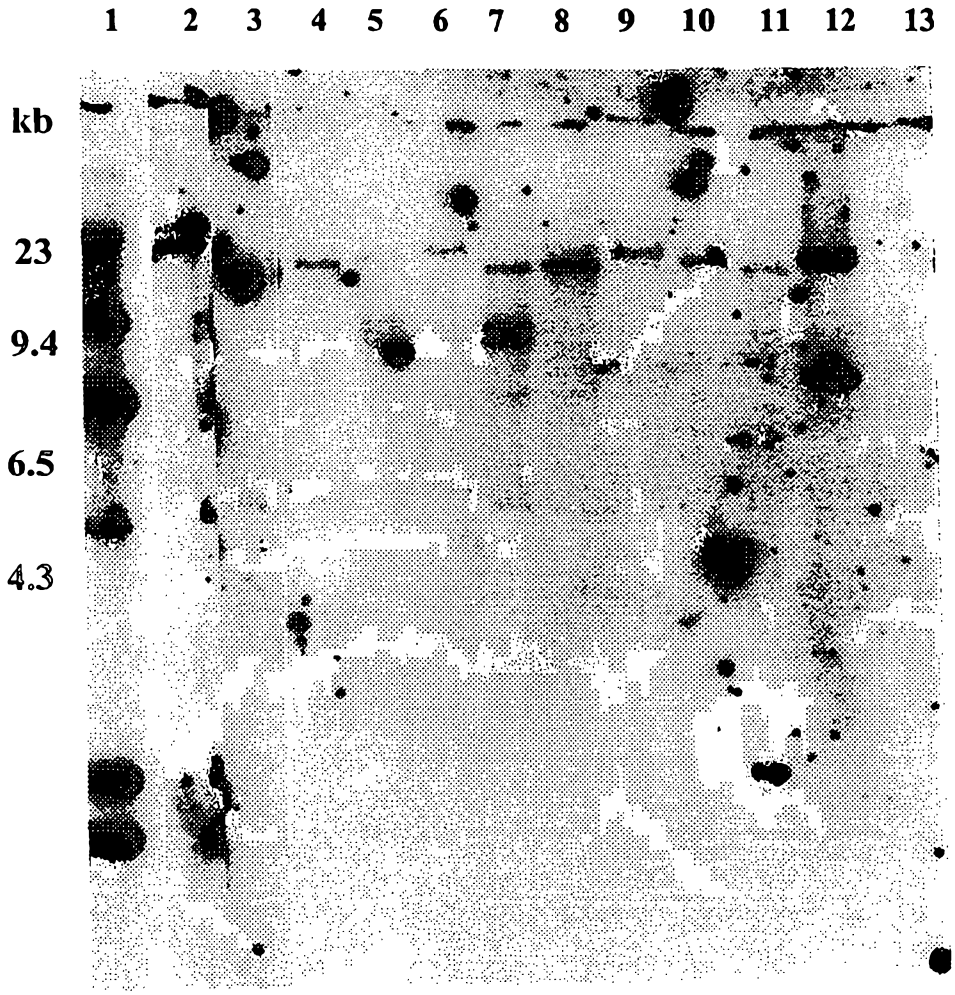


FIG. 6

FIG. 7A

TTTCTAGACC TAGGGCGGCC AGCTAGTAGC TTTGCTTCTC AATTTCTTAT TTGCATAATG
60
AGAAAAAAAG GAAAATTAAT TTTAACACCA ATTCAGTAGT TGATTGAGCA AATGCGTTGC
120
CAAAAAGGAT GCTTTAGAGA CAGTGTTCTC TGCACAGATA AGGACAAACA TTATTCAGAG
180
GGAGTACCCA GAGCTGAGAC TCCTAAGCCA GTGAGTGGCA CAGCATTCTA GGGAGAAATA
240
TGCTTGTCAT CACCGAAGCC TGATTCCGTA GAGCCACACC TTGGTAAGGG CCAATCTGCT
300
CACACAGGAT AGAGAGGGCA GGAGCCAGGG CAGAGCATAT AAGGTGAGGT AGGATCAGTT
360
GCTCCTCACA TTTGCTTCTG ACATAGTTGT GTTGGGAGCT TGGATAGCTT GGACAGCTCA
420
GGGCTGCGAT TTCGCGCCAA ACTTGACGGC AATCCTAGCG TGAAGGCTGG TAGGATTTTA
480
TCCCCGCTGC CATCATGGTT CGACCATTGA ACTGCATCGT CGCCGTGTCC CAAAATATGG
540
GGATTGGCAA GAACGGAGAC CTACCCTGGC CTCCGCTCAG GAACGAGTTC AAGTACTTCC
600
AAAGAATGAC CACAACCTCT TCAGTGGAAG GTAAACAGAA TCTGGTGATT ATGGGTAGGA
660

FIG. 7B

AAACCTGGTT CTCCATTCCT GAGAAGAATC GACCTTTAAA GGACAGAATT AATATAGTTC
720
TCAGTAGAGA ACTCAAAGAA CCACCACGAG GAGCTCATTT TCTTGCCAAA AGTTTGGATG
780
ATGCCTTAAG ACTTATTGAA CAACCGGAAT TGGCAAGTAA AGTAGACATG GTTTGGATAG
840
TCGGAGGCAG TTCTGTTTAC CAGGAAGCCA TGAATCAACC AGGCCACCTT AGACTCTTTG
900
TGACAAGGAT CATGCAGGAA TTTGAAAGTG ACACGTTTTT CCCAGAAATT GATTTGGGGA
960
AATATAAACT TCTCCCAGAA TACCCAGGCG TCCTCTCTGA GGTCCAGGAG GAAAAAGGCA
1020
TCAAGTATAA GTTTGAAGTC TACGAGAAGA AAGACTAACA GGAAGATGCT TTCAAGTTCT
1080
CTGCTCCCCT CCTAAAGCTA TGCATTTTTA TAAGACCATG GGACTTTTGC TGGCTTTAGA
1140
TCAGCCTCGA CTGTGCCTTC TAGTTGCCAG CCATCTGTTG TTTGCCCCTC CCCCGTGCCT
1200
TCCTTGACCC TGGAAGGTGC CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA
1260
TCGCATTGTC TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
1320

FIG. 7C

GGGGAGGATT GGGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC TATGGAACCA
1380
GCTGGGGCTC GAAGCGGCCG CCCATTTCGC TGGTGGTCAG ATGCGGGATG GCGTGGGACG
1440
CGGCGGGGAC CGTCACACTG AGGTTTTCCG CCAGACGCCA CTGCTGCCAG GCGCTGATGT
1500
GCCCGGCTTC TGACCATGCG GTCGCGTTCG GTTGCACTAC GCGTACTGTG AGCCAGAGTT
1560
GCCCGGCGCT CTCCGGCTGC GGTAGTTCAG GCAGTTCAAT CAACTGTTTA CCTTGTGGAG
1620
CGACATCCAG AGGCACTTCA CCGCTTGCTA GCGGCTTACC ATCCAGCGCC ACCATCCAGT
1680
GCAGGAGCTC GTTATCGCTA TGACGGAACA GGTATTCGCT GGTCACTTCG ATGGTTTGCC
1740
CGGATAAACG GAACTGGAAA AACTGCTGCT GGTGTTTTGC TTCCGTCAGC GCTGGATGCG
1800
GCGTGCGGTC GGCAAAGACC AGACCGTTCA TACAGAACTG GCGATCGTTC GGCGTATCAC
1860
CAAAATCACC GCCGTAAGCC GACCACGGGT TGCCGTTTTC ATCATATTTA ATCAGCGACT
1920
GATCCACCCA GTCCCAGACG AAGCCGCCCT GTAAACGGGG ATACTGACGA AACGCCTGCC
1980

FIG. 7D

AGTATTTAGC GAAACCGCCA AGACTGTTAC CCATCGCGTG GGCGTATTCG CAAAGGATCA
2040

GCGGGCGCGT CTCTCCGGGT AGCGAAAGCC ATTTTTTGAT GGACCATTTC GGACCAGCCG
2100

GGAAGGGCTG GTCTTCATCC ACGCGCGCGT ACATCGGGCA AATAATATCG GTGGCCGTGG
2160

TGTCGGCTCC GCCGCCTTCA TACTGCACCG GGCGGGAAGG ATCGACAGAT TTGATCCAGC
2220

GATACAGCGC GTCGTGATTA GCGCCGTGGC CTGATTCATT CCCCAGCGAC CAGATGATCA
2280

CACTCGGGTG ATTACGATCG CGCTGCACCA TTCGCGTTAC GCGTTCGCTC ATCGCCGGTA
2340

GCCAGCGCGG ATCATCGGTC AGACGATTCA TTGGCACCAT GCCGTGGGTT TCAATATTGG
2400

CTTCATCCAC CACATACAGG CCGTAGCGGT CGCACAGCGT GTACCACAGC GGATGGTTCG
2460

GATAATGCGA ACAGCGCACG GCGTTAAAGT TGTTCTGCTT CATCAGCAGG ATATCCTGCA
2520

CCATCGTCTG CTCATCCATG ACCTGACCAT GCAGAGGATG ATGCTCGTGA CGGTTAACGC
2580

CTCGAATCAG CAACGGCTTG CCGTTCAGCA GCAGCAGACC ATTTCCAATC CGCACCTCGC
2640

FIG. 7E

GGAAACCGAC ATCGCAGGCT TCTGCTTCAA TCAGCGTGCC GTCGGCGGTG TGCAGTTCAA
2700

CCACCGCACG ATAGAGATTC GGGATTTCGG CGCTCCACAG TTTCGGGTTT TCGACGTTCA
2760

GACGCAGTGT GACGCGATCG GCATAACCAC CAGGCTCATC GATAATTTCA CCGCCGAAAG
2820

GCGCGGTGCC GCTGGCGACC TGCGTTTCAC CCTGCCATAA AGAAACTGTT ACCCGTAGGT
2880

AGTCACGCAA CTCGCCGCAC ATCTGAACTT CAGCCTCCAG TACAGCGCGG CTGAAATCAT
2940

CATTAAAGCG AGTGGCAACA TGGAAATCGC TGATTTGTGT AGTCGGTTTA TGCAGCAACG
3000

AGACGTCACG GAAAATGCCG CTCATCCGCC ACATATCCTG ATCTTCCAGA TAACTGCCGT
3060

CACTCCAACG CAGCACCATC ACCGCGAGGC GGTTTTCTCC GGCGCGTAAA AATGCGCTCA
3120

GGTCAAATTC AGACGGCAAA CGACTGTCCT GGCTGTAACC GACCCACGCC CCGTTGCACC
3180

ACAGATGAAA CGCCGAGTTA ACGCCATCAA AAATAATTCG CGTCTGGCCT TCCTGTAGCC
3240

AGCTTTCATC AACATTAAAT GTGAGCGAGT AACAACCCGT CGGATTCTCC GTGGGAACAA
3300

FIG. 7F

ACGGCGGATT GACCGTAATG GGATAGGTTA CGTTGGTGTA GATGGGCGCA TCGTAACCGT
3360
GCATCTGCCA GTTTGAGGGG ACGACGACAG TATCGGCCTC AGGAAGATCG CACTCCAGCC
3420
AGCTTTCCGG CACTGCTTCT GGTGCCGGAA ACCAGGCAAA GCGCCATTCG CCATTCAGGC
3480
TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGGCCTCTTC GCTATTACGC CAGCTGGCGA
3540
AAGCGGGATG TGCTGCAAGG CGATTAAGTT GGGTAACGCC AGGGTTTTCC CAGTCACGAC
3600
GTTGTAAAAC GACTTAATCC GTCGAGGGGC TGCCTCGAAG CAGACGACCT TCCGTTGTGC
3660
AGCCAGCGGC GCCTGCGCCG GTGCCCACAA TCGTGCGCGA ACAAACTAAA CCAGAACAAA
3720
TCATACCGGC GGCACCGCCG CCACCACCTT CTCCTGTGCC TAACATTCCA GCGCCTCCAC
3780
CACTACCACC ACCATCGATG TCTGAATTGC CGCCCGCTCC ACCAATGCCG ACGGAACCTC
3840
AACCCGCTGC ACCTTTAGAC GACAGACAAC AATTGTTGGA AGCTATTAGA AACGAAAAAA
3900
ATCGCACTCG TCTCAGACCG GCTCTCTTAA GGTAGCTCAA ACCAAAAACG GCGCCCGAAA
3960

FIG. 7G

CCAGTACAAT AGTTGAGGTG CCGACTGTGT TGCCTAAAGA GACATTTGAG CTTAAACCGC
4020

CGTCTGCACC ACCGCCACCA CCTCCGCCTC CGCCTCCGCC GCCAGCCCCG CCTGCGCCTC
4080

CACCGATGGT AGATTCATCA TCAGCTCCAC CACCGCCGCC ATTAGTAGAT TTGCCGTCTG
4140

AAATGTTACC ACCGCCTGCA CCATCGCTTT CTAACGTGTT GTCTGAATTA AAATCGGGCA
4200

CAGTTAGATT GAAACCCGCC CAAAAACGCC CGCAATCAGA AATAATTCCA AAAAGCTCAA
4260

CTACAAATTT GATCGCGGAC GTGTTAGCCG ACACAATTAA TAGGCGTCGT GTGGCTATGG
4320

CAAAATCGTC TTCGGAAGCA ACTTCTAACG ACGAGGGTTG GGACGACGAC GATAATCGGC
4380

CTAATAAAGC TAACACGCCC GATGTTAAAT ATGTCCAAGC TACTAGTGGT ACCTTAATTA
4440

AGGGGCGGAG AATGGGCGGA ACTGGGCGGA GTTAGGGGCG GGATGGGCGG AGTTAGGGGC
4500

GGGACTATGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC TGCTGGGGAG
4560

CCTGGGGACT TTCCACACCT GGTTGCTGAC TAATTGAGAT GCATGCTTTG CATACTTCTG
4620

FIG. 7H

CCTGCTGGGG AGCCTGGGGA CTTTCCACAC CCTAACTGAC ACACATTCCA CAGAATTAAT
4680

TCCCCTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG TTCATAGCCC ATATATGGAG
4740

TTCCGCGTTA CATAACTTAC GGTAAATGGC CCGCCTGGCT GACCGCTCAA CGACCCCCGC
4800

CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC CAATAGGGAC TTTCCATTGA
4860

CGTCAATGGG TGGACTATTT ACGGTAAACT GCCCACTTGG CAGTACATCA AGTGTATCAT
4920

ATGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT GGCCCGCCTG GCATTATGCC
4980

CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTACGTATT AGTCATCGCT
5040

ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTTGACTCA
5100

CGGGGATTTC CAAGTCTCCA CCCCATTGAC GTCAATGGGA GTTTGTTTTG AAGCTTGGCC
5160

GGCCATATAA ACGGCGGCCA GCTTTATTTA ACGTGTTTAC GTCGAGTCAA TTGTACACTA
5220

ACGACAGTGA TGAAAGAAAT ACAAAAGCGC ATAATATTTT GAACGACGTC GAACCTTTAT
5280

FIG. 7I

TACAAAACAA AACACAAACG AATATCGACA AAGCTAGATT GCTGCTACAA GATTTGGCAA
5340
GTTTTGTGGC GTTGAGCGAA AATCCATTAG ATAGTCCAGC CATCGGTTCG GAAAAACAAC
5400
CCTTGTTTGA AACTAATCGA AACCTATTTT ACAAATCTAT TGAGGATTTA ATATTTAAAT
5460
TCAGATATAA AGACGCTGAA AATCATTTGA TTTTCGCTCT AACATACCAC CCTAAAGATT
5520
ATAAATTTAA TGAATTATTA AAATACATCA GCAACTATAT ATTGATAGAC ATTTCCAGTT
5580
TGTGATATTA GTTTGTGCGT CTCATTACAA TGGCTGTTAT TTTTAACAAC AAACAACTGC
5640
TCGCAGACAA TAGTATAGAA AAGGGAGGTG AACTGTTTTT GTTTAACGGT TCGTACAACA
5700
TTTTGGAAAG TTATGTTAAT CCGGTGCTGC TAAAAAATGG TGTAATTGAA CTAGAAGAAG
5760
CTGCGTACTA TGCCGGCAAC ATATTGTACA AAACCGACGA TCCCAAATTC ATTGATTATA
5820
TAAATTTAAT AATTAAAGCA ACACACTCCG AAGAACTACC AGAAAATAGC ACTGTTGTAA
5880
ATTACAGAAA AACTATGCGC AGCGGTACTA TACACCCCAT TAAAAAAGAC ATATATATTT
5940

FIG. 7J

ATGACAACAA AAAATTTACT CTATACGATA GATACATATA TGGATACGAT AATAACTATG
6000
TTAATTTTTA TGAGGAGAAA AATGAAAAAG AGAAGGAATA CGAAGAAGAA GACGACAAGG
6060
CGTCTAGTTT ATGTGAAAAT AAAATTATAT TGTCGCAAAT TAACTGTGAA TCATTTGAAA
6120
ATGATTTTAA ATATTACCTC AGCGATTATA ACTACGCGTT TTCAATTATA GATAACACTA
6180
CAAATGTTCT TGTTGCGTTT GGTTTGTATC GTTAATAAAA AACAAATTTA GCATTTATAA
6240
TTGTTTTATT ATTCAATAAT TACAAATAGG ATTGAGACCC TTGCAGTTGC CAGCAAACGG
6300
ACAGAGCTTG TCGAGGAGAG TTGTTGATTC ATTGTTTGCC TCCCTGCTGC GGTTTTTGAC
6360
CGAAGTTCAT GCCAGTCCAG CGTTTTTGCA GCAGAAAAGC CGCCGACTTC GGTTTGCGGT
6420
CGCGAGTGAA GATCCCTTTC TTGTTACCGC CAACGCGCAA TATGCCTTGC GAGGTCGCAA
6480
AATCGGCGAA ATTCCATACC TGTTCACCGA CGACGGCGCT GACGCGATCA AAGACGCGGT
6540
GATACATATC CAGCCATGCA CACTGATACT CTTCACTCCA CATGTCGGTG TACATTGAGT
6600

FIG. 7K

GCAGCCCGGC TAACGTATCC ACGCCGTATT CGGTGATGAT AATCGGCTGA TGCAGTTTCT
6660
CCTGCCAGGC CAGAAGTTCT TTTTCCAGTA CCTTCTCTGC CGTTTCCAAA TCGCCGCTTT
6720
GGACATACCA TCCGTAATAA CGGTTCAGGC ACAGCACATC AAAGAGATCG CTGATGGTAT
6780
CGGTGTGAGC GTCGCAGAAC ATTACATTGA CGCAGGTGAT CGGACGCGTC GGGTCGAGTT
6840
TACGCGTTGC TTCCGCCAGT GGCGCGAAAT ATTCCCGTGC ACCTTGCGGA CGGGTATCCG
6900
GTTCGTTGGC AATACTCCAC ATCACCACGC TTGGGTGGTT TTTGTCACGC GCTATCAGCT
6960
CTTTAATCGC CTGTAAGTGC GCTTGGTGAG TTTCCCCGTT GACTGCCTCT TCGTTGTACA
7020
GTTCTTTCGG CTTGTTGCCC GCTTCGAAAC CAATGCCTAA AGAGAGGTTA AAGCCGACAG
7080
CAGCAGTTTC ATCAATCACC ACGATGCCAT GTTCATCTGC CCAGTCGAGC ATCTCTTCAG
7140
CGTAAGGGTA ATGCGAGGTA CGGTAGGAGT TGGCCCTAAT CCAGTCCATT AATGCGTGGT
7200
CGTGCACCAT CAGCACGTTA TCGAATCCTT TGCCACGCAA GTCCGCATCT TCATGACGAC
7260

FIG. 7L

CAAAGCCAGT AAAGTAGAAC GGTTTGTGGT TAATCAGGAA CTGTTCGCCC TTCACTGCCA
7320

CTGACCGGAT GCCGACGCGA AGCGGGTAGA TATCACACTC TGTCTGGCTT TTGGCTGTGA
7380

CGCACAGTTC ATAGAGATAA CCTTCACCCG GTTGCCAGAG GTGCGGATTC ACCACTTGCA
7440

AAGTCCCGCT AGTGCCTTGT CCAGTTGCAA CCACCTGTTG ATCCGCATCA CGCAGTTCAA
7500

CGCTGACATC ACCATTGGCC ACCACCTGCC AGTCAACAGA CGCGTGGTTA CAGTCTTGCG
7560

CGACATGCGT CACTACGGTG ATATCGTCCA CCCAGGTGTT CGGCGTGGTG TAGAGCATTA
7620

CGCTGCGATG GATTCCGGCA TAGTTAAAGA AATCATGGAA GTAAGATTGC TTTTTCTTGC
7680

CGTTTTCGTT GGTAATCACC ATTCCCGGCG GGATAGTCTG CCAGTTCAGT TCGTTGTTCA
7740

CACAAACGGT GATACCCCTC GACGGATTAA AGACTTCAAG CGGTCAACTA TGAAGAAGTG
7800

TTCGTCTTCG TCCCAGTAAG CTATGTCTCT AGAATGTAGC CATCCATCCT TGTCAATCAA
7860

GGCGTTGGTC GCTTCCGGAT TGTTTACATA ACCGGACATA ATCATAGGTC CTCTGACACA
7920

FIG. 7M

TAATACGCCT CTCTGATTAA CGCCCAGCGT TTTCCCGGTA TCCAGATCCA CAACCTTCGC
7980

TTCAAAAAAT GGAACAACTT TACCGACCGC GCCCGGTTTA TCATCCCCCT CGGGTGTAAT
8040

CAGAATAGCT GATGTAGTCT CAGTGAGCCC ATATCCTTGT CGTATCCCTG GAAGATGGAA
8100

GCGTTTTGCA ACCGCTTCCC CGACTTCTTT CGAAAGAGGT GCGCCCCCAG AAGCAATTTC
8160

GTGTAAATTA GATAAATCGT ATTTGTCAAT CAGAGTGCTT TTGGCGAAGA ATGAAAATAG
8220

GGTTGGTACT AGCAACGCAC TTTGAATTTT GTAATCCTGA AGGGATCGTA AAAACAGCTC
8280

TTCTTCAAAT CTATACATTA AGACGACTCG AAATCTACAT ATCAAATATC CGAGTGTAGT
8340

AAACATTCCA AAACCGTGAT GGAATGGAAC AACACTTAAA ATCGCAGTAT CCGGAATGAT
8400

TTGATTGCCA AAAATAGGAT CTCTGGCATG CGAGAATCTA GCGCAGGCAG TTCTATGCGG
8460

AAGGGCCACA CCCTTAGGTA ACCCAGTAGA TCCAGAGGAA TTGTTTTGTC ACGATCAAAG
8520

GACTCTGGTA CAAAATCGTA TTCATTAAAA CCGGGAGGTA GATGAGATGT GACGAAGGTG
8580

FIG. 7N

TACATCGACT GAAATCCCTG GTAATCCGTT TTAGAATCCA TGATAATAAT TTTCTGGATT
8640
ATTGGTAATT TTTTTTGCAC GTTCAAAATT TTTTGCAACC CCTTTTTGGA AACAAACACT
8700
ACGGTAGGCT GCGAAATGTT CATACTGTTG AGCAATTCAC GTTCATTATA AATGTCGTTC
8760
GCGGGCGCAA CTGCAACTCC GATAAATAAC GCGCCCAACA CCGGCATAAA GAATTGAAGA
8820
GAGTTTTCAC TGCATACGAC GATTCTGTGA TTTGTATTCA GCCCATATCG TTTCATAGCT
8880
TCTGCCAACC GAACGGACAT TTCGAAGTAT TCCGCGTACG TGATGTTCAC CTCGATATGT
8940
GCATCTGTAA AAGGAATTGT TCCAGGAACC AGGGCGTATC TCTTCATAGC CTTATGCAGT
9000
TGCTCTCCAG CGGTTCCATT CTCTAGCTTT GCTTCTCAAT TTCTTATTTG CATAATGAGA
9060
AAAAAAGGAA AATTAATTTT AACACCAATT CAGTAGTTGA TTGAGCAAAT GCGTTGCCAA
9120
AAAGGATGCT TTAGAGACAG TGTTCTCTGC ACAGATAAGG ACAAACATCA TTCAGAGGGA
9180
GTACCCAGAG CTGAGACTCC TAAGCCAGTG AGTGGCACAG CATTCTAGGG AGAAATATGC
9240

FIG. 7P

TTGTCATCAC CGAAGCCTGA TTCCGTAGAG CCACACCTTG GTAAGGGCCA ATCTGCTCAC
9300

ACAGGATAGA GAGGGCAGGA GCCAGGGCAG AGCATATAAG GTGAGGTAGG ATCAGTTGCT
9360

CCTCACATTT GCTTCTGACA TAGTTGTGTT GGGAGCTTGG ATCGATCCAC CATGGGCTTC
9420

AATACCCTGA TTGACTGGAA CAGCTGTAGC CCTGAACAGC AGCGTGCGCT GCTGACGCGT
9480

CCGGCGATTT CCGCCTCTGA CAGTATTACC CGGACGGTCA GCGATATTCT GGATAATGCA
9540

AAAACGCGCG GTGACGATGC CCTGCGTGAA TACAGCGCTA AATTTGATAA AACAGAAGTG
9600

ACAGCGCTAC GCGTCACCCC TGAAGAGATC GCCGCCGCCG GCGCGCGTCT GAGCGACGAA
9660

TTAAAACAGG CGATGACCGC TGCCGTCAAA AATATTGAAA CGTTCCATTC CGCGCAGACG
9720

CTACCGCTTG TAGATGTGGA AACCCAGCCA GGCGTGCGTT GCCAGCAGGT TACGCGTCCC
9780

GTCTCGTCTG TCGGTCTGTA TATTCCCGGC GGCTCGGCTC CGCTCTTCTC AACGGTGCTG
9840

ATGCTGGCGA CGCCGGCGCG CATTGCGGGA TGCTAGAAGG TGGTTCTGTG CTCGCCGCCG
9900

FIG. 7Q

CCCATCGCTG ATGAAATCCT CTATGCGGCG CAACTGTGTG GCGTGCAGGA ATTCTTTAAC
9960
CTCGGCGGCG CGCAGGCGAT TGCCGCTCTG GCCTTCGGCA GCGAGTCCGT ACCGAAAGTG
10020
GATAAAATTT TTGGCCCCGG CAACGCCTTT GTAACCGAAG CCAAACGTCA GGTCAGCCAG
10080
CGTCTCGACG GCGCGGCTAT CGATATGCCA GCCGAGCCGT CTGAAGTACT GGTGATCGCA
10140
GACAGCGGCG CAACACCGGA TTTCGTCGCT TCTGACCTGC TCTCCCAGAC TGAGCACGGC
10200
CCGGATTCCC AGGTGATCCT GCTGACGCCT GATGCTGACA TTGCCCGCAA GGTGGCGGAG
10260
GCGGTAGAAC GTCAACTGGC GGAACTGCCG CGCGCGGACA CCGCCTGGCA GGCCCTGAGC
10320
GCCAGTCGTC TGATTGTGAC CAAAGATTTA GCGCAGTGCG TCGCCATCTC TAATCAGTAT
10380
GGGCCGGAAC ACTTAATCAT CCAGACGCGC AATGCGCGCG ATTTGGTGGA TGCGATTACC
10440
AGCGCAGGCT CGGTATTTCT CGGCGACTGG TCGCCGGAAT CCGCCGGTGA TTACGCTTCC
10500
GGAACCAACC ATGTTTTACC GACCTATGGC CATACTGCTA CCTGTTCCAG CCTTGGGTTA
10560

FIG. 7R

GCGGATTTCC AGAAACGGAT GACCGTTCAG GAACTGTCGA AAGCGGGCTT TTCCGCTCTG
10620
GCATCAACCA TTGAAACATT GGCGGGGGCA GAACGTCTGA CCGCCCATAA AAATGCCGTG
10680
ACCCTGCGCG TAAACGCCCT CAAGGAGCAA GCATGAGCAC TGAAAACACT CTCAGCGTCG
10740
CTGACTTAGC CCGTGAAAAT GTCCGCAACC TGGAGATCCA GACATGATAA GATACATTGA
10800
TGAGTTTGGA CAAACCACAA CTAGAATGCA GTGAAAAAAA TGCTTTATTT GTGAAATTTG
10860
TGATGCTATT GCTTTATTTG TAACCATTAT AAGCTGCAAT AAACAAGTTA ACAACAACAA
10920
TTGCATTCAT TTTATGTTTC AGGTTCAGGG GGAGGTGTGG GAGGTTTTTT AAAGCAAGTA
10980
AAACCTCTAC AAATGTGGTA TGGCTGATTA TGATCTCTAG CTCGACGGGG CGCCTGGCCG
11040
CTACTAACTC TCTCCTCCCT CCTTTTTCCT GCAGGCTCAA GGCGCGCATG CCCGACGGCG
11100
AGGATCTCGT CGTGACCCAT GGCGATGCCT GCTTGCCGAA TATCATGGTG GAAAATGGCC
11160
GCTTTTCTGG ATTCATCGAC TGTGGCCGGC TGGGTGTGGC GGACCGCTAT CAGGACATAG
11220

FIG. 7S

CGTTGGCTAC CCGTGATATT GCTGAAGAGC TTGGCGGCGA ATGGGCTGAC CGCTTCCTCG
11280

TGCTTTACGG TATCGCCGCT CCCGATTCGC AGCGCATCGC CTTCTATCGC CTTCTTGACG
11340

AGTTCTTCTG AGCGGGACTC TGGGGTTCGA AATGACCGAC CAAGCGACGC CCAACCTGCC
11400

ATCACGAGAT TTCGATTCCA CCGCCGCCTT CTATGAAAGG TTGGGCTTCG GAATCGTTTT
11460

CCGGGACGCC GGCTGGATGA TCCTCCAGCG CGGGGATCTC ATGCTGGAGT TCTTCGCCCA
11520

CCCCAACTTG TTTATTGCAG CTTATAATGG TTACAAATAA AGCAATAGCA TCACAAATTT
11580

CACAAATAAA GCATTTTTTT CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATCT
11640

ATCTTATCAT GTCTGGATCG CGGCCGGTCT CTCTCTAGCC CTAGGTCTAG ACTTGGCAGA
11700

ACATATCCAT CGCGTCCGCC ATCTCCAGCA GCCGCACGCG GCGCATCTCG GGCAGCGTTG
11760

GGTCCTGGCC ACGGGTGCGC ATGATCGTGC TCCTGTCGTT GAGGACCCGG CTAGGCTGGC
11820

GGGGTTGCCT TACTGGTTAG CAGAATGAAT CACCGATACG CGAGCGAACG TGAAGCGACT
11880

FIG. 7T

GCTGCTGCAA AACGTCTGCG ACCTGAGCAA CAACATGAAT GGTCTTCGGT TTCCGTGTTT
11940
CGTAAAGTCT GGAAACGCGG AAGTCAGCGC CCTGCACCAT TATGTTCCGG ATCTGCATCG
12000
CAGGATGCTG CTGGCTACCC TGTGGAACAC CTACATCTGT ATTAACGAAG CGCTGGCATT
12060
GACCCTGAGT GATTTTTCTC TGGTCCCGCC GCATCCATAC CGCCAGTTGT TTACCCTCAC
12120
AACGTTCCAG TAACCGGGCA TGTTCATCAT CAGTAACCCG TATCGTGAGC ATCCTCTCTC
12180
GTTTCATCGG TATCATTACC CCCATGAACA GAAATCCCCC TTACACGGAG GCATCAGTGA
12240
CCAAACAGGA AAAAACCGCC CTTAACATGG CCCGCTTTAT CAGAAGCCAG ACATTAACGC
12300
TTCTGGAGAA ACTCAACGAG CTGGACGCGG ATGAACAGGC AGACATCTGT GAATCGCTTC
12360
ACGACCACGC TGATGAGCTT TACCGCAGCT GCCTCGCGCG TTTCGGTGAT GACGGTGAAA
12420
ACCTCTGACA CATGCAGCTC CCGGAGACGG TCACAGCTTG TCTGTAAGCG GATGCCGGGA
12480
GCAGACAAGC CCGTCAGGGC GCGTCAGCGG GTGTTGGCGG GTGTCGGGGC GCAGCCATGA
12540

FIG. 7U

CCCAGTCACG TAGCGATAGC GGAGTGTATA CTGGCTTAAC TATGCGGCAT CAGAGCAGAT
12600

TGTACTGAGA GTGCACCATA TGCGGTGTGA AATACCGCAC AGATGCGTAA GGAGAAAATA
12660

CCGCATCAGG CGCTCTTCCG CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT
12720

GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA
12780

TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC
12840

CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG
12900

CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG
12960

AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCCTT
13020

TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT
13080

GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG
13140

CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT
13200

FIG. 7V

GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT
13260
CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTTGGTA TCTGCGCTCT
13320
GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC
13380
CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC
13440
TCAAGAAGAT CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG
13500
TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA
13560
AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA
13620
ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCGTTCAT CCATAGTTGC
13680
CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC
13740
TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC
13800
AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT
13860

FIG. 7W

TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT
13920

TGCCATTGCT GCAGGCATCG TGGTGTCACG CTCGTCGTTT GGTATGGCTT CATTCAGCTC
13980

CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG
14040

CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT
14100

TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC
14160

TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCGACCGA GTTGCTCTTG
14220

CCCGGCGTCA ACACGGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT
14280

TGGAAAACGT TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC
14340

GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC
14400

TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAAG GGAATAAGGG CGACACGGAA
14460

ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA AGCATTTATC AGGGTTATTG
14520

FIG. 7X

TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG
14580

CACATTTCCC CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC
14640

CTATAAAAAT AGGCGTATCA CGAGGCCCTT TCGTCTTCAA GAA
14683

FIG. 8A

TTAATTAAGG GGCGGAGAAT GGGCGGAACT GGGCGGAGTT AGGGGCGGGA TGGGCGGAGT 60

TAGGGGCGGG ACTATGGTTG CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC 120

TGGGGAGCCT GGGGACTTTC CACACCTGGT TGCTGACTAA TTGAGATGCA TGCTTTGCAT 180

ACTTCTGCCT GCTGGGGAGC CTGGGGACTT TCCACACCCT AACTGACACA CATTCCACAG 240

AATTAATTCC CCTAGTTATT AATAGTAATC AATTACGGGG TCATTAGGTC ATAGCCCATA 300

TATGGAGTTC CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA 360

CCCCCGCCCA TTGACGTCAA TAATGACGTA TGTTCCCATA GTAACGCCAA TAGGGACTTT 420

CCATTGACGT CAATGGGTGG ACTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT 480

GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAAATGGC CCGCCTGGCA 540

TTATGCCCAG TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT 600

CATCGCTATT ACCATGGTGA TGCGGTTTTG GCAGTACATC AATGGGCGTG GATAGCGGTT 660

TGACTCACGG GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGAAG 720

CTTGGCCGGC CATATAAACG GCGGCCAGCT TTATTTAACG TGTTTACGTC GAGTCAATTG 780

TACACTAACG ACAGTGATGA AAGAAATACA AAAGCGCATA ATATTTTGAA CGACGTCGAA 840

FIG. 8B

CCTTTATTAC AAAACAAAAC ACAAACGAAT ATCGACAAAG CTAGATTGCT GCTACAAGAT 900

TTGGCAAGTT TTGTGGCGTT GAGCGAAAAT CCATTAGATA GTCCAGCCAT CGGTTCGGAA 960

AAACAACCCT TGTTTGAAAC TAATCGAAAC CTATTTTACA AATCTATTGA GGATTTAATA 1020

TTTAAATTCA GATATAAAGA CGCTGAAAAT CATTTGATTT TCGCTCTAAC ATACCACCCT 1080

AAAGATTATA AATTTAATGA ATTATTAAAA TACATCAGCA ACTATATATT GATAGACATT 1140

TCCAGTTTGT GATATTAGTT TGTGCGTCTC ATTACAATGG CTGTTATTTT TAACAACAAA 1200

CAACTGCTCG CAGACAATAG TATAGAAAAG GGAGGTGAAC TGTTTTTGTT TAACGGTTCG 1260

TACAACATTT TGGAAAGTTA TGTTAATCCG GTGCTGCTAA AAAATGGTGT AATTGAACTA 1320

GAAGAAGCTG CGTACTATGC CGGCAACATA TTGTACAAAA CCGACGATCC CAAATTCATT 1380

GATTATATAA ATTTAATAAT TAAAGCAACA CACTCCGAAG AACTACCAGA AAATAGCACT 1440

GTTGTAAATT ACAGAAAAAC TATGCGCAGC GGTACTATAC ACCCCATTAA AAAAGACATA 1500

TATATTTATG ACAACAAAAA ATTTACTCTA TACGATAGAT ACATATATGG ATACGATAAT 1560

AACTATGTTA ATTTTTATGA GGAGAAAAAT GAAAAAGAGA AGGAATACGA AGAAGAAGAC 1620

GACAAGGCGT CTAGTTTATG TGAAAATAAA ATTATATTGT CGCAAATTAA CTGTGAATCA 1680

FIG. 8C

TTTGAAAATG ATTTTAAATA TTACCTCAGC GATTATAACT ACGCGTTTTC AATTATAGAT 1740

AATACTACAA ATGTTCTTGT TGCGTTTGGT TTGTATCGTT AATAAAAAAC AAATTTAGCA 1800

TTTATAATTG TTTTATTATT CAATAATTAC AAATAGGATT GAGACCCTTG CAGTTGCCAG 1860

CAAACGGACA GAGCTTGTCG AGGAGAGTTG TTGATTCATT GTTTGCCTCC CTGCTGCGGT 1920

TTTTCACCGA AGTTCATGCC AGTCCAGCGT TTTTGCAGCA GAAAAGCCGC CGACTTCGGT 1980

TTGCGGTCGC GAGTGAAGAT CCCTTTCTTG TTACCGCCAA CGCGCAATAT GCCTTGCGAG 2040

GTCGCAAAAT CGGCGAAATT CCATACCTGT TCACCGACGA CGGCGCTGAC GCGATCAAAG 2100

ACGCGGTGAT ACATATCCAG CCATGCACAC TGATACTCTT CACTCCACAT GTCGGTGTAC 2160

ATTGAGTGCA GCCCGGCTAA CGTATCCACG CCGTATTCGG TGATGATAAT CGGCTGATGC 2220

AGTTTCTCCT GCCAGGCCAG AAGTTCTTTT TCCAGTACCT TCTCTGCCGT TTCCAAATCG 2280

CCGCTTTGGA CATACCATCC GTAATAACGG TTCAGGCACA GCACATCAAA GAGATCGCTG 2340

ATGGTATCGG TGTGAGCGTC GCAGAACATT ACATTGACGC AGGTGATCGG ACGCGTCGGG 2400

TCGAGTTTAC GCGTTGCTTC CGCCAGTGGC GCGAAATATT CCCGTGCACC TTGCGGACGG 2460

GTATCCGGTT CGTTGGCAAT ACTCCACATC ACCACGCTTG GGTGGTTTTT GTCACGCGCT 2520

FIG. 8D

ATCAGCTCTT TAATCGCCTG TAAGTGCGCT TGCTGAGTTT CCCCGTTGAC TGCCTCTTCG 2580

CTGTACAGTT CTTTCGGCTT GTTGCCCGCT TCGAAACCAA TGCCTAAAGA GAGGTTAAAG 2640

CCGACAGCAG CAGTTTCATC AATCACCACG ATGCCATGTT CATCTGCCCA GTCGAGCATC 2700

TCTTCAGCGT AAGGGTAATG CGAGGTACGG TAGGAGTTGG CCCCAATCCA GTCCATTAAT 2760

GCGTGGTCGT GCACCATCAG CACGTTATCG AATCCTTTGC CACGCAAGTC CGCATCTTCA 2820

TGACGACCAA AGCCAGTAAA GTAGAACGGT TTGTGGTTAA TCAGGAACTG TTCGCCCTTC 2880

ACTGCCACTG ACCGGATGCC GACGCGAAGC GGGTAGATAT CACACTCTGT CTGGCTTTTG 2940

GCTGTGACGC ACAGTTCATA GAGATAACCT TCACCCGGTT GCCAGAGGTG CGGATTCACC 3000

ACTTGCAAAG TCCCGCTAGT GCCTTGTCCA GTTGCAACCA CCTGTTGATC CGCATCACGC 3060

AGTTCAACGC TGACATCACC ATTGGCCACC ACCTGCCAGT CAACAGACGC GTGGTTACAG 3120

TCTTGCGCGA CATGCGTCAC CACGGTGATA TCGTCCACCC AGGTGTTCGG CGTGGTGTAG 3180

AGCATTACGC TGCGATGGAT TCCGGCATAG TTAAAGAAAT CATGGAAGTA AGACTGCTTT 3240

TTCTTGCCGT TTTCGTCGGT AATCACCATT CCCGGCGGGA TAGTCTGCCA GTTCAGTTCG 3300

TTGTTCACAC AAACGGTGAT ACCCCTCGAC GGATTAAAGA CTTCAAGCGG TCAACTATGA 3360

FIG. 8E

AGAAGTGTTC GTCTTCGTCC CAGTAAGCTA TGTCTCCAGA ATGTAGCCAT CCATCCTTGT 3420

CAATCAAGGC GTTGGTCGCT TCCGGATTGT TTACATAACC GGACATAATC ATAGGTCCTC 3480

TGACACATAA TTCGCCTCTC TGATTAACGC CCAGCGTTTT CCCGGTATCC AGATCCACAA 3540

CCTTCGCTTC AAAAAATGGA ACAACTTTAC CGACCGCGCC CGGTTTATCA TCCCCCTCGG 3600

GTGTAATCAG AATAGCTGAT GTAGTCTCAG TGAGCCCATA TCCTTGTCGT ATCCCTGGAA 3660

GATGGAAGCG TTTTGCAACC GCTTCCCCGA CTTCTTTCGA AAGAGGTGCG CCCCCAGAAG 3720

CAATTTCGTG TAAATTAGAT AAATCGTATT TGTCAATCAG AGTGCTTTTG GCGAAGAATG 3780

AAAATAGGGT TGGTACTAGC AACGCACTTT GAATTTTGTA ATCCTGAAGG GATCGTAAAA 3840

ACAGCTCTTC TTCAAATCTA TACATTAAGA CGACTCGAAA TCCACATATC AAATATCCGA 3900

GTGTAGTAAA CATTCCAAAA CCGTGATGGA ATGGAACAAC ACTTAAAATC GCAGTATCCG 3960

GAATGATTTG ATTGCCAAAA ATAGGATCTC TGGCATGCGA GAATCTAGCG CAGGCAGTTC 4020

TATGCGGAAG GGCCACACCC TTAGGTAACC CAGTAGATCC AGAGGAATTG TTTTGTCACG 4080

ATCAAAGGAC TCTGGTACAA AATCGTATTC ATTAAAACCG GGAGGTAGAT GAGATGTGAC 4140

GAACGTGTAC ATCGACTGAA ATCCCTGGTA ATCCGTTTTA GAATCCATGA TAATAATTTT 4200

FIG. 8F

CTGGATTATT GGTAATTTTT TTTGCACGTT CAAAATTTTT TGCAACCCCT TTTTGGAAAC 4260

AAACACTACG GTAGGCTGCG AAATGTTCAT ACTGTTGAGC AATTCACGTT CATTATAAAT 4320

GTCGTTCGCG GGCGCAACTG CAACTCCGAT AAATAACGCG CCCAACACCG GCATAAAGAA 4380

TTGAAGAGAG TTTTCACTGC ATACGACGAT TCTGTGATTT GTATTCAGCC CATATCGTTT 4440

CATAGCTTCT GCCAACCGAA CGGACATTTC GAAGTATTCC GCGTACGTGA TGTTCACCTC 4500

GATATGTGCA TCTGTAAAAG GAATTGTTCC AGGAACCAGG GCGTATCTCT TCATAGCCTT 4560

ATGCAGTTGC TCTCCAGCGG TTCCATCCTC TAGCTTTGCT TCTCAATTTC TTATTTGCAT 4620

AATGAGAAAA AAAGGAAAAT TAATTTTAAC ACCAATTCAG TAGTTGATTG AGCAAATGCG 4680

TTGCCAAAAA GGATGCTTTA GAGACAGTGT TCTCTGCACA GATAAGGACA AACATTATTC 4740

AGAGGGAGTA CCCAGAGCTG AGACTCCTAA GCCAGTGAGT GGCACAGCAT TCTAGGGAGA 4800

AATATGCTTG TCATCACCGA AGCCTGATTC CGTAGAGCCA CACCTTGGTA AGGGCCAATC 4860

TGCTCACACA GGATAGAGAG GGCAGGAGCC AGGGCAGAGC ATATAAGGTG AGGTAGGATC 4920

AGTTGCTCCT CACATTTGCT TCTGACATAG TTGTGTTGGG AGCTTGGATC GATCCACCAT 4980

GGGCTTCAAT ACCCTGATTG ACTGGAACAG CTGTAGCCCT GAACAGCAGC GTGCGCTGCT 5040

FIG. 8G

GACGCGTCCG GCGATTTCCG CCTCTGACAG TATTACCCGG ACGGTCAGCG ATATTCTGGA 5100

TAATGTAAAA ACGCGCGGTG ACGATGCCCT GCGTGAATAC AGCGCTAAAT TTGATAAAAC 5160

AGAAGTGACA GCGCTACGCG TCACCCCTGA AGAGATCGCC GCCGCCGGCG CGCGTCTGAG 5220

CGACGAATTA AAACAGGCGA TGACCGCTGC CGTCAAAAAT ATTGAAACGT TCCATTCCGC 5280

GCAGACGCTA CCGCCTGTAG ATGTGGAAAC CCAGCCAGGC GTGCGTTGCC AGCAGGTTAC 5340

GCGTCCCGTC TCGTCTGTCG GTCTGTATAT TCCCGGCGGC TCGGCTCCGC TCTTCTCAAC 5400

GGTGCTGATG CTGGCGACGC CGGCGCGCAT TGCGGGATGC CAGAAGGTGG TTCTGTGCTC 5460

GCCGCCGCCC ATCGCTGATG AAATCCTCTA TGCGGCGCAA CTGTGTGGCG TGCAGGAAAT 5520

CTTTAACGTC GGCGGCGCGC AGGCGATTGC CGCTCTGGCC TTCGGCAGCG AGTCCGTACC 5580

GAAAGTGGAT AAAATTTTTG GCCCCGGCAA CGCCTTTGTA ACCGAAGCCA AACGTCAGGT 5640

CAGCCAGCGT CTCGACGGCG CGGCTATCGA TATGCCAGCC GGGCCGTCTG AAGTACTGGT 5700

GATCGCAGAC AGCGGCGCAA CACCGGATTT CGTCGCTTCT GACCTGCTCT CCCAGGCTGA 5760

GCACGGCCCG GATTCCCAGG TGATCCTGCT GACGCCTGAT GCTGACATTG CCCGCAAGGT 5820

GGCGGAGGCG GTAGAACGTC AACTGGCGGA ACTGCCGCGC GCGGACACCG CCCGGCAGGC 5880

FIG. 8H

CCTGAGCGCC AGTCGTCTGA TTGTGACCAA AGATTTAGCG CAGTGCGTCG CCATCTCTAA 5940

TCAGTATGGG CCGGAACACT TAATCATCCA GACGCGCAAT GCGCGCGATT TGGTGGATGC 6000

GATTACCAGC GCAGGCTCGG TATTTCTCGG CGACTGGTCG CCGGAATCCG CCGGTGATTA 6060

CGCTTCCGGA ACCAACCATG TTTTACCGAC CTATGGCTAT ACTGCTACCT GTTCCAGCCT 6120

TGGGTTAGCG GATTTCCAGA AACGGATGAC CGTTCAGGAA CTGTCGAAAG CGGGCTTTTC 6180

CGCTCTGGCA TCAACCATTG AAACATTGGC GGCGGCAGAA CGTCTGACCG CCCATAAAAA 6240

TGCCGTGACC CTGCGCGTAA ACGCCCTCAA GGAGCAAGCA TGAGCACTGA AAACACTCTC 6300

AGCGTCGCTG ACTTAGCCCG TGAAAATGTC CGCAACCTGG AGATCCAGAC ATGATAAGAT 6360

ACATTGATGA GTTTGGACAA ACCACAACTA GAATGCAGTG AAAAAAATGC TTTATTTGTG 6420

AAATTTGTGA TGCTATTGCT TTATTTGTAA CCATTATAAG CTGCAATAAA CAAGTTAACA 6480

ACAACAATTG CATTCATTTT ATGTTTCAGG TTCAGGGGGA GGTGTGGGAG GTTTTTTAAA 6540

GCAAGTAAAA CCTCTACAAA TGTGGTATGG CTGATTATGA TCTCTAGCTC GACGGCGCGC 6600

CTCTAGAGCA GTGTGGTTTT GCAAGAGGAA GCAAAAAGCC TCTCCACCCA GGCCTGGAAT 6660

GTTTCCACCC AATGTCGAGC AGTGTGGTTT TGCAAGAGGA AGCAAAAAGC CTCTCCACCC 6720

FIG. 8I

AGGCCTGGAA TGTTTCCACC CAATGTCGAG CAAACCCCGC CCAGCGTCTT GTCATTGGCG 6780

AATTCGAACA CGCAGATGCA GTCGGGGCGG CGCGGTCCCA GTCCCACTTC GCATATTAAG 6840

GTGACGCGTG TGGCCTCGAA CACCGAGCGA CCCTGCAGCC AATATGGGAT CGGCCATTGA 6900

ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT TCGGCTATGA 6960

CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT CAGCGCAGGG 7020

GCGCCCGGTT CTTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGTAAG 7080

TGCGGCCGTC GATGGCCGAG GCGGCCTCGG CCTCTGCATA AATAAAAAAA ATTAGTCAGC 7140

CATGCATGGG GCGGAGAATG GGCGGAACTG GGCGGAGTTA GGGGCGGGAT GGGCGGAGTT 7200

AGGGGCGGGA CTATGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT 7260

GGGGAGCCTG GGGACTTTCC ACACCTGGTT GCTGACTAAT TGAGATGCAT GCTTTGCATA 7320

CTTCTGCCTG CTGGGGAGCC TGGGGACTTT CCACACCCTA ACTGACACAC ATTCCACAGA 7380

ATTAATTCCC CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT 7440

ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC 7500

CCCCGCCCAT TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC 7560

FIG. 8J

CATTGACGTC AATGGGTGGA CTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG 7620

TATCATATGC CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT 7680

TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA GCTATTAGTC 7740

ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT 7800

GACTCACGGG GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTTGGCAC 7860

CAAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC 7920

GGTAGGCGTG TACGGTGGGA GGTCTATATA AGCAGAGCTG GGTACGTGAA CCGTCAGATC 7980

GCCTGGAGAC GCCATCACAG ATCTCTCACT ATGGATTTTC AGGTGCAGAT TATCAGCTTC 8040

CTGCTAATCA GTGCTTCAGT CATAATGTCC AGAGGACAAA TTGTTCTCTC CCAGTCTCCA 8100

GCAATCCTGT CTGCATCTCC AGGGGAGAAG GTCACAATGA CTTGCAGGGC CAGCTCAAGT 8160

GTAAGTTACA TCCACTGGTT CCAGCAGAAG CCAGGATCCT CCCCCAAACC CTGGATTTAT 8220

GCCACATCCA ACCTGGCTTC TGGAGTCCCT GTTCGCTTCA GTGGCAGTGG GTCTGGGACT 8280

TCTTACTCTC TCACAATCAG CAGAGTGGAG GCTGAAGATG CTGCCACTTA TTACTGCCAG 8340

CAGTGGACTA GTAACCCACC CACGTTCGGA GGGGGGACCA AGCTGGAAAT CAAACGTACG 8400

FIG. 8K

GTGGCTGCAC CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA ATCTGGAACT 8460

GCCTCTGTTG TGTGCCTGCT GAATAACTTC TATCCCAGAG AGGCCAAAGT ACAGTGGAAG 8520

GTGGATAACG CCCTCCAATC GGGTAACTCC CAGGAGAGTG TCACAGAGCA GGACAGCAAG 8580

GACAGCACCT ACAGCCTCAG CAGCACCCTG ACGCTGAGCA AAGCAGACTA CGAGAAACAC 8640

AAAGTCTACG CCTGCGAAGT CACCCATCAG GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC 8700

AACAGGGGAG AGTGTTGAAT TCAGATCCGT TAACGGTTAC CAACTACCTA GACTGGATTC 8760

GTGACAACAT GCGGCCGTGA TATCTACGTA TGATCAGCCT CGACTGTGCC TTCTAGTTGC 8820

CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC 8880

ACTGTCCTTT CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT 8940

ATTCTGGGGG GTGGGGTGGG GCAGGACAGC AAGGGGGAGG ATTGGGAAGA CAATAGCAGG 9000

CATGCTGGGG ATGCGGTGGG CTCTATGGAA CCAGCTGGGG CTCGACAGCT ATGCCAAGTA 9060

CGCCCCCTAT TGACGTCAAT GACGGTAAAT GGCCCGCCTG GCATTATGCC CAGTACATGA 9120

CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTACGTATT AGTCATCGCT ATTACCATGG 9180

TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGCG GTTTGACTCA CGGGGATTTC 9240

FIG. 8L

CAAGTCTCCA CCCCATTGAC GTCAATGGGA GTTTGTTTTG GCACCAAAAT CAACGGGACT 9300

TTCCAAAATG TCGTAACAAC TCCGCCCCAT TGACGCAAAT GGGCGGTAGG CGTGTACGGT 9360

GGGAGGTCTA TATAAGCAGA GCTGGGTACG TCCTCACATT CAGTGATCAG CACTGAACAC 9420

AGACCCGTCG ACATGGGTTG GAGCCTCATC TTGCTCTTCC TTGTCGCTGT TGCTACGCGT 9480

GTCCTGTCCC AGGTACAACT GCAGCAGCCT GGGGCTGAGC TGGTGAAGCC TGGGGCCTCA 9540

GTGAAGATGT CCTGCAAGGC TTCTGGCTAC ACATTTACCA GTTACAATAT GCACTGGGTA 9600

AAACAGACAC CTGGTCGGGG CCTGGAATGG ATTGGAGCTA TTTATCCCGG AAATGGTGAT 9660

ACTTCCTACA ATCAGAAGTT CAAAGGCAAG GCCACATTGA CTGCAGACAA ATCCTCCAGC 9720

ACAGCCTACA TGCAGCTCAG CAGCCTGACA TCTGAGGACT CTGCGGTCTA TTACTGTGCA 9780

AGATCGACTT ACTACGGCGG TGACTGGTAC TTCAATGTCT GGGGCGCAGG GACCACGGTC 9840

ACCGTCTCTG CAGCTAGCAC CAAGGGCCCA TCGGTCTTCC CCCTGGCACC CTCCTCCAAG 9900

AGCACCTCTG GGGGCACAGC GGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCCGAACCG 9960

GTGACGGTGT CGTGGAACTC AGGCGCCCTG ACCAGCGGCG TGCACACCTT CCCGGCTGTC 10020

CTACAGTCCT CAGGACTCTA CTCCCTCAGC AGCGTGGTGA CCGTGCCCTC CAGCAGCTTG 10080

FIG. 8M

GGCACCCAGA CCTACATCTG CAACGTGAAT CACAAGCCCA GCAACACCAA GGTGGACAAG 10140

AAAGCAGAGC CCAAATCTTG TGACAAAACT CACACATGCC CACCGTGCCC AGCACCTGAA 10200

CTCCTGGGGG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC CCAAGGACAC CCTCATGATC 10260

TCCCGGACCC CTGAGGTCAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA CCCTGAGGTC 10320

AAGTTCAACT GGTACGTGGA CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG 10380

GAGCAGTACA ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG 10440

CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG 10500

AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA 10560

TCCCGGGATG AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT 10620

CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC 10680

ACGCCTCCCG TGCTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC 10740

AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC 10800

AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGAGGATC CGTTAACGGT 10860

TACCAACTAC CTAGACTGGA TTCGTGACAA CATGCGGCCG TGATATCTAC GTATGATCAG 10920

FIG. 8N

CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT 10980

TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC 11040

ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG 11100

AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG GAACCAGCTG 11160

GGGCTCGACA GCAACGCTAG GTCGAGGCCG CTACTAACTC TCTCCTCCCT CCTTTTTCCT 11220

GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GGCGTTCCTT GCGCAGCTGT 11280

GCTCGACGTT GTCACTGAAG CGGGAAGGGA CTGGCTGCTA TTGGGCGAAG TGCCGGGGCA 11340

GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT 11400

GCGGCGGCTG CATACGCTTG ATCCGGCTAC CTGCCCATTC GACCACCAAG CGAAACATCG 11460

CATCGAGCGA GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG ATCTGGACGA 11520

AGAGCATCAG GGGCTCGCGC CAGCCGAACT GTTCGCCAGG TAAGTGAGCT CCAATTCAAG 11580

CTTCCTAGGG CGGCCAGCTA GTAGCTTTGC TTCTCAATTT CTTATTTGCA TAATGAGAAA 11640

AAAAGGAAAA TTAATTTTAA CACCAATTCA GTAGTTGATT GAGCAAATGC GTTGCCAAAA 11700

AGGATGCTTT AGAGACAGTG TTCTCTGCAC AGATAAGGAC AAACATTATT CAGAGGGAGT 11760

FIG. 8P

ACCCAGAGCT GAGACTCCTA AGCCAGTGAG TGGCACAGCA TTCTAGGGAG AAATATGCTT 11820

GTCATCACCG AAGCCTGATT CCGTAGAGCC ACACCTTGGT AAGGGCCAAT CTGCTCACAC 11880

AGGATAGAGA GGGCAGGAGC CAGGGCAGAG CATATAAGGT GAGGTAGGAT CAGTTGCTCC 11940

TCACATTTGC TTCTGACATA GTTGTGTTGG GAGCTTGGAT AGCTTGGACA GCTCAGGGCT 12000

GCGATTTCGC GCCAAACTTG ACGGCAATCC TAGCGTGAAG GCTGGTAGGA TTTTATCCCC 12060

GCTGCCATCA TGGTTCGACC ATTGAACTGC ATCGTCGCCG TGTCCCAAAA TATGGGGATT 12120

GGCAAGAACG GAGACCTACC CTGGCCTCCG CTCAGGAACG AGTTCAAGTA CTTCCAAAGA 12180

ATGACCACAA CCTCTTCAGT GGAAGGTAAA CAGAATCTGG TGATTATGGG TAGGAAAACC 12240

TGGTTCTCCA TTCCTGAGAA GAATCGACCT TTAAAGGACA GAATTAATAT AGTTCTCAGT 12300

AGAGAACTCA AAGAACCACC ACGAGGAGCT CATTTTCTTG CCAAAAGTTT GGATGATGCC 12360

TTAAGACTTA TTGAACAACC GGAATTGGCA AGTAAAGTAG ACATGGTTTG GATAGTCGGA 12420

GGCAGTTCTG TTTACCAGGA AGCCATGAAT CAACCAGGCC ACCTTAGACT CTTTGTGACA 12480

AGGATCATGC AGGAATTTGA AAGTGACACG TTTTTCCCAG AAATTGATTT GGGGAAATAT 12540

AAACTTCTCC CAGAATACCC AGGCGTCCTC TCTGAGGTCC AGGAGGAAAA AGGCATCAAG 12600

FIG. 8Q

TATAAGTTTG AAGTCTACGA GAAGAAAGAC TAACAGGAAG ATGCTTTCAA GTTCTCTGCT 12660

CCCCTCCTAA AGCTATGCAT TTTTATAAGA CCATGGGACT TTTGCTGGCT TTAGATCAGC 12720

CTCGACTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT 12780

GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA AATGAGGAAA TTGCATCGCA 12840

TTGTCTGAGT AGGTGTCATT CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGA 12900

GGATTGGGAA GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG AACCAGCTGG 12960

GGCTCGAAGC GGCCGCCCAT TTCGCTGGTG GTCAGATGCG GGATGGCGTG GGACGCGGCG 13020

GGGAGCGTCA CACTGAGGTT TTCCGCCAGA CGCCACTGCT GCCAGGCGCT GATGTGCCCG 13080

GCTTCTGACC ATGCGGTCGC GTTCGGTTGC ACTACGCGTA CTGTGAGCCA GAGTTGCCCG 13140

GCGCTCTCCG GCTGCGGTAG TTCAGGCAGT TCAATCAACT GTTTACCTTG TGGACCGACA 13200

TCCAGAGGCA CTTCACCGCT TGCCAGCGGC TTACCATCCA GCGCCACCAT CCAGTGCAGG 13260

AGCTCGTTAT CGCTATGACG GAACAGGTAT TCGCTGGTCA CTTCGATGGT TTGCCCGGAT 13320

AAACGGAACT GGAAAAACTG CTGCTGGTGT TTTGCTTCCG TCAGCGCTGG ATGCGGCGTG 13380

CGGTCGGCAA AGACCAGACC GTTCATACAG AACTGGCGAT CGTTCGGCGT ATCGCCAAAA 13440

FIG. 8R

TCACCGCCGT AAGCCGACCA CGGGTTGCCG TTTTCATCAT ATTTAATCAG CGACTGATCC 13500

ACCCAGTCCC AGACGAAGCC GCCCTGTAAA CGGGGATACT GACGAAACGC CTGCCAGTAT 13560

TTAGCGAAAC CGCCAAGACT GTTACCCATC GCTGGGGCGT ATTCGCAAAG GATCAGCGGG 13620

CGCGTCTCTC CGGGTAGCGA AAGCCATTTT TTGATGGACC ATTTCGGACC AGCCGGGAAG 13680

GGCTGGTCTT CATCCACGCG CGCGTACATC GGGCAAATAA TATCGGTGGC CGTGGTGTCG 13740

GCTCCGCCGC CTTCATACTG CACCGGGCGG GAAGGATCGA CAGATTTGAT CCAGCGATAC 13800

AGCGCGTCGT GATTAGCGCC GTGGCCTGAT TCATTCCCCA GCGACCAGAT GATCACACTC 13860

GGGTGATTAC GATCGCGCTG CACCATTCGC GTTACGCGTT CGCTCATCGC CGGTAGCCAG 13920

CGCGGATCAT CGGTCAGACG ATTCATTGGC ACCATGCCGT GGGTTTCAAT ATTGGCTTCA 13980

TCCACCACAT ACAGGCCGTA GCGGTCGCAC AGCGTGTACC ACAGCGGATG GTTCGGATAA 14040

TGCCAACAGC GCACGGCGTT AAAGTTGTTC TGCTTCATCA GCAGGATATC CTGCACCATC 14100

GTCTGCTCAT CCATGACCTG ACCATGCAGA GGATGATGCT CGTGACGGTT AACGCCTCGA 14160

ATCAGCAACG GCTTGCCGTT CAGCAGCAGC AGACCATTTT CAATCCGCAC CTCGCGGAAA 14220

CCGACATCGC AGGCTTCTGC TTCAATCAGC GTGCCGTCGG CGGTGTGCAG TTCAACCACC 14280

FIG. 8S

GCACGATAGA GATTCGGGAT TTCGGCGCTC CACAGTTTCG GGTTTTCGAC GTTCAGACGC 14340

AGTGTGACGC GATCGGCATA ACCACCACGC TCATCGATAA TTTCACCGCC GAAAGGCGCG 14400

GTGCCGCTGG CGACCTGCGT TTCACCCTGC CATAAAGAAA CTGTTACCCG TAGGTAGTCA 14460

CGCAACTCGC CGCACATCTG AACTTCAGCC TCCAGTACAG CGCGGCTGAA ATCATCATTA 14520

AAGCGAGTGG CAACATGGAA ATCGCTGATT TGTGTAGTCG GTTTATGCAG CAACGAGACG 14580

TCACGGAAAA TGCCGCTCAT CCGCCACATA TCCTGATCTT CCAGATAACT GCCGTCACTC 14640

CAACGCAGCA CCATCACCGC GAGGCGGTTT TCTCCGGCGC GTAAAAATGC GCTCAGGTCA 14700

AATTCAGACG GCAAACGACT GTCCTGGCCG TAACCGACCC ACGCCCCGTT GCACCACAGA 14760

TGAAACGCCG AGTTAACGCC ATCAAAAATA ATTCGCGTCT GGCCTTCCTG TAGCCAGCTT 14820

TCATCAACAT TAAATGTGAG CGAGTAACAA CCCGTCGGAT TCTCCGTGGG AACAAACGGC 14880

GGATTGACCG TAATGGGATA GGTTACGTTG GTGTAGATGG GCGCATCGTA ACCGTGCATC 14940

TGCCAGTTTG AGGGGACGAC GACAGTATCG GCCTCAGGAA GATCGCACTC CAGCCAGCTT 15000

TCCGGCACCG CTTCTGGTGC CGGAAACCAG GCAAAGCGCC ATTCGCCATT CAGGCTGCGC 15060

AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT TACGCCAGCT GGCGAAAGGG 15120

FIG. 8T

GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT TTTCCCAGTC ACGACGTTGT 15180

AAAACGACTT AATCCGTCGA GGGGCTGCCT CGAAGCAGAC GACCTTCCGT TGTGCAGCCA 15240

GCGGCGCCTG CGCCGGTGCC CACAATCGTG CGCGAACAAA CTAAACCAGA ACAAATTATA 15300

CCGGCGGCAC CGCCGCCACC ACCTTCTCCC GTGCCTAACA TTCCAGCGCC TCCACCACCA 15360

CCACCACCAT CGATGTCTGA ATTGCCGCCC GCTCCACCAA TGCCGACGGA ACCTCAACCC 15420

GCTGCACCTT TAGACGACAG ACAACAATTG TTGGAAGCTA TTAGAAACGA AAAAAATCGC 15480

ACTCGTCTCA GACCGGTCAA ACCAAAAACG GCGCCCGAAA CCAGTACAAT AGTTGAGGTG 15540

CCGACTGTGT TGCCTAAAGA GACATTTGAG CCTAAACCGC CGTCTGCATC ACCGCCACCA 15600

CCTCCGCCTC CGCCTCCGCC GCCAGCCCCG CCTGCGCCTC CACCGATGGT AGATTTATCA 15660

TCAGCTCCAC CACCGCCGCC ATTAGTAGAT TTGCCGTCTG AAATGTTACC ACCGCCTGCA 15720

CCATCGCTTT CTAACGTGTT GTCTGAATTA AAATCGGGCA CAGTTAGATT GAAACCCGCC 15780

CAAAAACGCC CGCAATCAGA AATAATTCCA AAAAGCTCAA CTACAAATTT GATCGCGGAC 15840

GTGTTAGCCG ACACAATTAA TAGGCGTCGT GTGGCTATGG CAAAATCGTC TTCGGAAGCA 15900

ACTTCTAACG ACGAGGGTTG GGACGACGAC GATAATCGGC CTAATAAAGC TAACACGCCC 15960

FIG. 8U

GATGTTAAAT ATGTCCAAGC TACTAGTGGT ACCGCTTGGC AGAACATATC CATCGCGTCC 16020

GCCATCTCCA GCAGCCGCAC GCGGCGCATC TCGGGCAGCG TTGGGTCCTG GCCACGGGTG 16080

CGCATGATCG TGCTCCTGTC GTTGAGGACC CGGCTAGGCT GGCGGGGTTG CCTTACTGGT 16140

TAGCAGAATG AATCACCGAT ACGCGAGCGA ACGTGAAGCG ACTGCTGCTG CAAAACGTCT 16200

GCGACCTGAG CAACAACATG AATGGTCTTC GGTTTCCGTG TTTCGTAAAG TCTGGAAACG 16260

CGGAAGTCAG CGCCCTGCAC CATTATGTTC CGGATCTGCA TCGCAGGATG CTGCTGGCTA 16320

CCCTGTGGAA CACCTACATC TGTATTAACG AAGCGCTGGC ATTGACCCTG AGTGATTTTT 16380

CTCTGGTCCC GCCGCATCCA TACCGCCAGT TGTTTACCCT CACAACGTTC CAGTAACCGG 16440

GCATGTTCAT CATCAGTAAC CCGTATCGTG AGCATCCTCT CTCGTTTCAT CGGTATCATT 16500

ACCCCCATGA ACAGAAATCC CCCTTACACG GAGGCATCAG TGACCAAACA GGAAAAAACC 16560

GCCCTTAACA TGGCCCGCTT TATCAGAAGC CAGACATTAA CGCTTCTGGA GAAACTCAAC 16620

GAGCTGGACG CGGATGAACA GGCAGACATC TGTGAATCGC TTCACGACCA CGCTGATGAG 16680

CTTTACCGCA GCTGCCTCGC GCGTTTCGGT GATGACGGTG AAAACCTCTG ACACATGCAG 16740

CTCCCGGAGA CGGTCACAGC TTGTCTGTAA GCGGATGCCG GGAGCAGACA AGCCCGTCAG 16800

FIG. 8V

GGCGCGTCAG CGGGTGTTGG CGGGTGTCGG GGCGCAGCCA TGACCCAGTC ACGTAGCGAT 16860

AGCGGAGTGT ATACTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC 16920

ATATGCGGTG TGAAATACCG CACAGATGCG TAAGGAGAAA ATACCGCATC AGGCGCTCTT 16980

CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG GCTGCGGCGA GCGGTATCAG 17040

CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA 17100

TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT 17160

TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC 17220

GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT 17280

CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG 17340

TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA 17400

AGCTGGGCTG TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA TCCGGTAACT 17460

ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA 17520

ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA 17580

ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT 17640

FIG. 8W

TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT 17700

TTTTTGTTTG CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA 17760

TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG ATTTTGGTCA 17820

TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA AGTTTTAAAT 17880

CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG 17940

CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC CCCGTCGTGT 18000

AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG 18060

ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC 18120

GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG 18180

CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTGCAGGCA 18240

TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC CAACGATCAA 18300

GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA 18360

TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA GCACTGCATA 18420

ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA 18480

FIG. 8X

AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG TCAACACGGG 18540

ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG 18600

GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG 18660

CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG 18720

GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC 18780

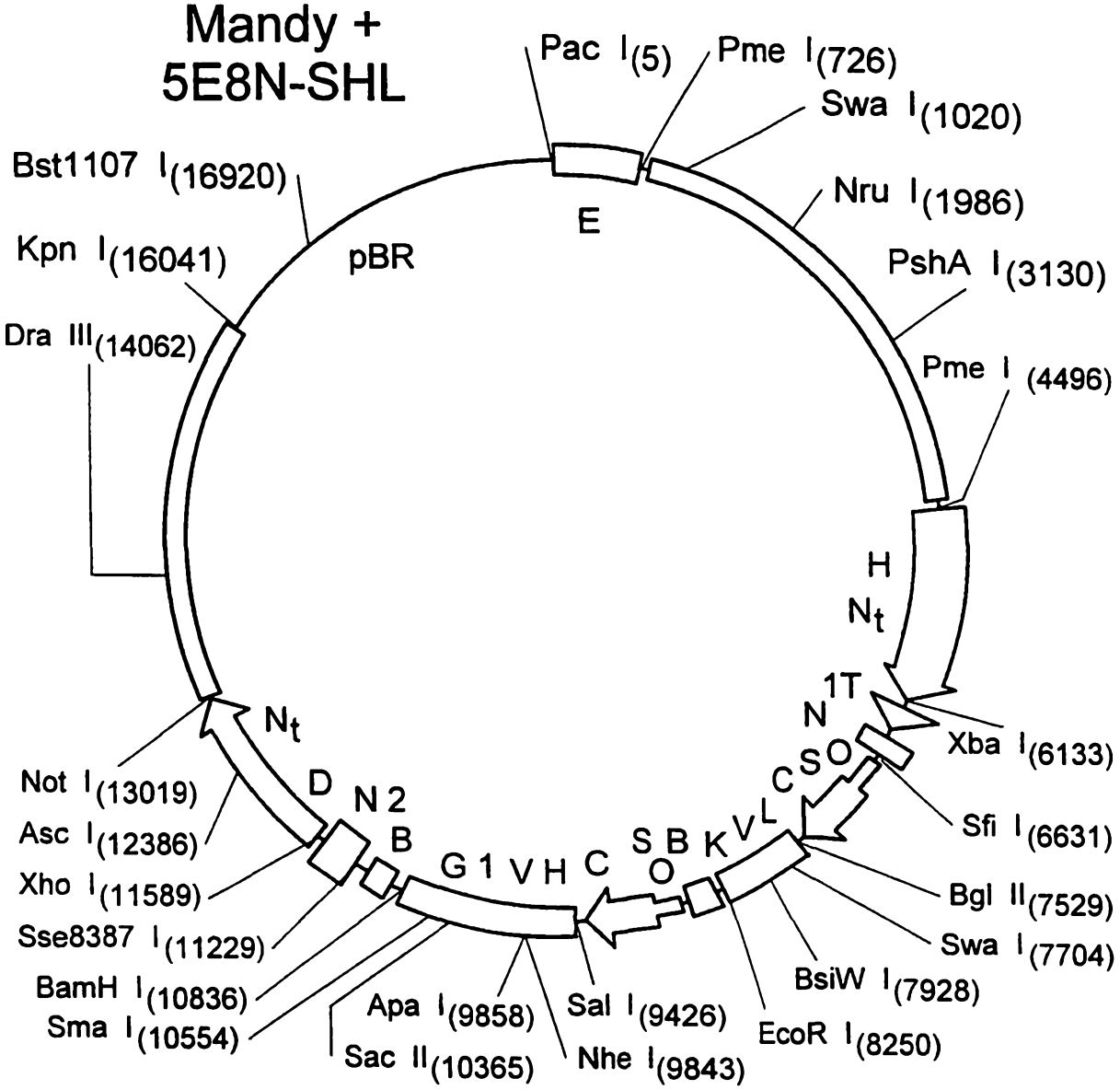
TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG AGCGGATACA 18840

TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCGAAAAG 18900

TGCCACCTGA CGTCTAAGAA ACCATTATTA TCATGACATT AACCTATAAA AATAGGCGTA 18960

TCACGAGGCC CTTTCGTCTT CAAGAA 18986

FIG. 9



Nt D = Inactive Dihydrofolate reductase
E = CMV and SV40 enhancers
Nt H = Inactive Samonella Histidinol Dehydrogenase
T = Herpes Simplex thymidine kinas promoter and polyoma enhancer
C = Cytomegalovirus promoter/enhancer
N1 = Neomycin phosphotransferase exon 1
K = Human kappa constant
VL = Variable light chain anti-CD23 primate 5E8 and leader
VH = Variable heavy chain anti-CD-23 primate 5E8N- and leader
B = Bovine growth hormone polyadenylation
M2 = Neomycin phosphotransferase exon 2
G1 = Human Gamma 1 constant
Mandy cut Xbal Xho I and ligated to Xba I Xho I fragment
from XKG1+CD23 5E8N-SHL
Map by Mitchell Reff Constructed by Karen McLachlan 06/26/97 19,035 bp
Noncutters = AflII, AvrII, HindIII, I-PpoI, I-SceI, PmlI, RsrII, SgfI, SrfI

10	20	30	40	50	60	70
TTAATTAAGG	GGCGGAGAAT	GGGCGGAACT	GGGCGGAGTT	AGGGGCGGGA	TGGGCGGAGT	TAGGGGCGGG
80	90	100	110	120	130	140
ACTATGGTTG	CTGACTAATT	GAGATGCATG	CTTTGCATAC	TTCTGCCTGC	TGGGGAGCCT	GGGGACTTTC
150	160	170	180	190	200	210
CACACCTGGT	TGCTGACTAA	TTGAGATGCA	TGCTTTGCAT	ACTTCTGCCT	GCTGGGGAGC	CTGGGGACTT
220	230	240	250	260	270	280
TCCACACCCT	AACTGACACA	CATTCCACAG	AATTAATTCC	CCTAGTTATT	AATAGTAATC	AATTACGGGG
290	300	310	320	330	340	350
TCATTAGTTC	ATAGCCCATA	TATGGAGTTC	CGCGTTACAT	AACTTACGGT	AAATGGCCCG	CCTGGCTGAC
360	370	380	390	400	410	420
CGCCCAACGA	CCCCCGCCCA	TTGACGTCAA	TAATGACGTA	TGTTCCCATA	GTAACGCCAA	TAGGGACTTT
430	440	450	460	470	480	490
CCATTGACGT	CAATGGGTGG	AGTATTTACG	GTAAACTGCC	CACTTGGCAG	TACATCAAGT	GTATCATATG
500	510	520	530	540	550	560
CCAAGTACGC	CCCCTATTGA	CGTCAATGAC	GGTAAATGGC	CCGCCTGGCA	TTATGCCCAG	TACATGACCT
570	580	590	600	610	620	630
TATGGGACTT	TCCTACTTGG	CAGTACATCT	ACGTATTAGT	CATCGCTATT	ACCATGGTGA	TGCGGTTTTG
640	650	660	670	680	690	700
GCAGTACATC	AATGGGCGTG	GATAGCGGTT	TGACTCACGG	GGATTTCCAA	GTCTCCACCC	CATTGACGTC
710	720	730	740	750	760	770
AATGGGAGTT	TGTTTTGAAG	CTGTTTAAAC	AGCTTGGCCG	GCCAGCTTTA	TTTAACGTGT	TTACGTCGAG
780	790	800	810	820	830	840
TCAATTGTAC	ACTAACGACA	GTGATGAAAG	AAATACAAAA	GCGCATAATA	TTTTGAACGA	CGTCGAACCT
850	860	870	880	890	900	910
TTATTACAAA	ACAAAACACA	AACGAATATC	GACAAAGCTA	GATTGCTGCT	ACAAGATTTG	GCAAGTTTTG
920	930	940	950	960	970	980
TGGCGTTGAG	CGAAAATCCA	TTAGATAGTC	CAGCCATCGG	TTCGGAAAAA	CAACCCTTGT	TTGAAACTAA

FIG. 10A

990	1000	1010	1020	1030	1040	1050
TCGAAACCTA	TTTTACAAAT	CTATTGAGGA	TTTAATATTT	AAATTCAGAT	ATAAAGACGC	TGAAAATCAT
1060	1070	1080	1090	1100	1110	1120
TTGATTTTCG	CTCTAACATA	CCACCCTAAA	GATTATAAAT	TTAATGAATT	ATTAAAATAC	ATCAGCAACT
1130	1140	1150	1160	1170	1180	1190
ATATATTGAT	AGACATTTCC	AGTTTGTGAT	ATTAGTTTGT	GCGTCTCATT	ACAATGGCTG	TTATTTTTAA
1200	1210	1220	1230	1240	1250	1260
CAACAAACAA	CTGCTCGCAG	ACAATAGTAT	AGAAAAGGGA	GGTGAACTGT	TTTTGTTTAA	CGGTTCGTAC
1270	1280	1290	1300	1310	1320	1330
AACATTTTGG	AAAGTTATGT	TAATCCGGTG	CTGCTAAAAA	ATGGTGTAAT	TGAACTAGAA	GAAGCTGCGT
1340	1350	1360	1370	1380	1390	1400
ACTATGCCGG	CAACATATTG	TACAAAACCG	ACGATCCCAA	ATTCATTGAT	TATATAAATT	TAATAATTAA
1410	1420	1430	1440	1450	1460	1470
AGCAACACAC	TCCGAAGAAC	TACCAGAAAA	TAGCACTGTT	GTAAATTACA	GAAAAACTAT	GCGCAGCGGT
1480	1490	1500	1510	1520	1530	1540
ACTATACACC	CCATTAAAAA	AGACATATAT	ATTTATGACA	ACAAAAAATT	TACTCTATAC	GATAGATACA
1550	1560	1570	1580	1590	1600	1610
TATATGGATA	CGATAATAAC	TATGTTAATT	TTTATGAGGA	GAAAAATGAA	AAAGAGAAGG	AATACGAAGA
1620	1630	1640	1650	1660	1670	1680
AGAAGACGAC	AAGGCGTCTA	GTTTATGTGA	AAATAAAATT	ATATTGTCGC	AAATTAACTG	TGAATCATTT
1690	1700	1710	1720	1730	1740	1750
GAAAATGATT	TTAAATATTA	CCTCAGCGAT	TATAACTACG	CGTTTTCAAT	TATAGATAAT	ACTACAAATG
1760	1770	1780	1790	1800	1810	1820
TTCTTGTTGC	GTTTGGTTTG	TATCGTTAAT	AAAAAACAAA	TTTGACATTT	ATAATTGTTT	TATTATTCAA
1830	1840	1850	1860	1870	1880	1890
TAATTACAAA	TAGGATTGAG	ACCCTTGCAG	TTGCCAGCAA	ACGGACAGAG	CTTGTCGAGG	AGAGTTGTTG
1900	1910	1920	1930	1940	1950	1960
ATTCATTGTT	TGCCTCCCTG	CTGCGGTTTT	TCACCGAAGT	TCATGCCAGT	CCAGCGTTTT	TGCAGCAGAA

FIG. 10B

1970	1980	1990	2000	2010	2020	2030
AAGCCGCCGA	CTTCGGTTTG	CGGTCGGCGAG	TGAAGATCCC	TTTCTTGTTA	CCGCCAACGC	GCAATATGCC
2040	2050	2060	2070	2080	2090	2100
TTGCGAGGTC	GCAAAATCGG	CGAAATTCCA	TACCTGTTCA	CCGACGACGG	CGCTGACGCG	ATCAAAGACG
2110	2120	2130	2140	2150	2160	2170
CGGTGATACA	TATCCAGCCA	TGCACACTGA	TACTCTTCAC	TCCACATGTC	GGTGTACATT	GAGTGCAGCC
2180	2190	2200	2210	2220	2230	2240
CGGCTAACGT	ATCCACGCCG	TATTCGGTGA	TGATAATCGG	CTGATGCAGT	TTCTCCTGCC	AGGCCAGAAG
2250	2260	2270	2280	2290	2300	2310
TTCTTTTTCC	AGTACCTTCT	CTGCCGTTTC	CAAATCGCCG	CTTTGGGACAT	ACCATCCGTA	ATAACGGTTC
2320	2330	2340	2350	2360	2370	2380
AGGCACAGCA	CATCAAAGAG	ATCGCTGATG	GTATCGGTGT	GAGCGTCGCA	GAACATTACA	TTGACGCAGG
2390	2400	2410	2420	2430	2440	2450
TGATCGGACG	CGTCGGGTCG	AGTTTACGCG	TTGCTTCCGC	CAGTGGCGCG	AAATATTCCC	GTGCACCTTG
2460	2470	2480	2490	2500	2510	2520
CGGACGGGTA	TCCGGTTCGT	TGGCAATACT	CCACATCACC	ACGCTTGGGT	GGTTTTTGTC	ACGCGCTATC
2530	2540	2550	2560	2570	2580	2590
AGCTCTTTAA	TCGCCTGTAA	GTGCGCTTGC	TGAGTTTCCC	CGTTGACTGC	CTCTTCGCTG	TACAGTTCTT
2600	2610	2620	2630	2640	2650	2660
TCGGCTTGTT	GCCCGCTTCG	AAACCAATGC	CTAAAGAGAG	GTTAAAGCCG	ACAGCAGCAG	TTTCATCAAT
2670	2680	2690	2700	2710	2720	2730
CACCACGATG	CCATGTTCAT	CTGCCCAGTC	GAGCATCTCT	TCAGCGTAAG	GGTAATGCGA	GGTACGGTAG
2740	2750	2760	2770	2780	2790	2800
GAGTTGGCCC	CAATCCAGTC	CATTAATGCG	TGGTCGTGCA	CCATCAGCAC	GTTATCGAAT	CCTTTGCCAC
2810	2820	2830	2840	2850	2860	2870
GCAAGTCCGC	ATCTTCATGA	CGACCAAAGC	CAGTAAAGTA	GAACGGTTTG	TGGTTAATCA	GGAACTGTTC
2880	2890	2900	2910	2920	2930	2940
GCCCTTCACT	GCCACTGACC	GGATGCCGAC	GCGAAGCGGG	TAGATATCAC	ACTCTGTCTG	GCTTTTGGCT

FIG. 10C

2950	2960	2970	2980	2990	3000	3010
GTGACGCACA	GTTCATAGAG	ATAACCTTCA	CCCGGTTGCC	AGAGGTGCGG	ATTCACCACT	TGCAAAGTCC
3020	3030	3040	3050	3060	3070	3080
CGCTAGTGCC	TTGTCCAGTT	GCAACCACCT	GTTGATCCGC	ATCACGCAGT	TCAACGCTGA	CATCACCATT
3090	3100	3110	3120	3130	3140	3150
GGCCACCACC	TGCCAGTCAA	CAGACGCGTG	GTTACAGTCT	TGCGCGACAT	GCGTCACCAC	GGTGATATCG
3160	3170	3180	3190	3200	3210	3220
TCCACCCAGG	TGTTCGGCGT	GGTGTAGAGC	ATTACGCTGC	GATGGATTCC	GGCATAGTTA	AAGAAATCAT
3230	3240	3250	3260	3270	3280	3290
GGAAGTAAGA	CTGCTTTTTC	TTGCCGTTTT	CGTCGGTAAT	CACCATTCCC	GGCGGGATAG	TCTGCCAGTT
3300	3310	3320	3330	3340	3350	3360
CAGTTCGTTG	TTCACACAAA	CGGTGATACC	CCTCGACGGA	TTAAAGACTT	CAAGCGGTCA	ACTATGAAGA
3370	3380	3390	3400	3410	3420	3430
AGTGTTCGTC	TTCGTCCCAG	TAAGCTATGT	CTCCAGAATG	TAGCCATCCA	TCCTTGTCAA	TCAAGGCGTT
3440	3450	3460	3470	3480	3490	3500
GGTCGCTTCC	GGATTGTTTA	CATAACCGGA	CATAATCATA	GGTCCTCTGA	CACATAATTC	GCCTCTCTGA
3510	3520	3530	3540	3550	3560	3570
TTAACGCCCA	GCGTTTTCCC	GGTATCCAGA	TCCACAACCT	TCGCTTCAAA	AAATGGAACA	ACTTTACCGA
3580	3590	3600	3610	3620	3630	3640
CCGCGCCCGG	TTTATCATCC	CCCTCGGGTG	TAATCAGAAT	AGCTGATGTA	GTCTCAGTGA	GCCCATATCC
3650	3660	3670	3680	3690	3700	3710
TTGTCGTATC	CCTGGAAGAT	GGAAGCGTTT	TGCAACCGCT	TCCCCGACTT	CTTTCGAAAG	AGGTGCGCCC
3720	3730	3740	3750	3760	3770	3780
CCAGAAGCAA	TTTCGTGTAA	ATTAGATAAA	TCGTATTTGT	CAATCAGAGT	GCTTTTGGCG	AAGAATGAAA
3790	3800	3810	3820	3830	3840	3850
ATAGGGTTGG	TACTAGCAAC	GCACTTTGAA	TTTTGTAATC	CTGAAGGGAT	CGTAAAAACA	GCTCTTCTTC
3860	3870	3880	3890	3900	3910	3920
AAATCTATAC	ATTAAGACGA	CTCGAAATCC	ACATATCAAA	TATCCGAGTG	TAGTAAACAT	TCCAAAACCG

FIG. 10D

3930	3940	3950	3960	3970	3980	3990
TGATGGAATG	GAACAACACT	TAAAATCGCA	GTATCCGGAA	TGATTTGATT	GCCAAAAATA	GGATCTCTGG
4000	4010	4020	4030	4040	4050	4060
CATGCGAGAA	TCTGACGCAG	GCAGTTCTAT	GCGGAAGGGC	CACACCCTTA	GGTAACCCAG	TAGATCCAGA
4070	4080	4090	4100	4110	4120	4130
GGAATTGTTT	TGTCACGATC	AAAGGACTCT	GGTACAAAAT	CGTATTCATT	AAAACCGGGA	GGTAGATGAG
4140	4150	4160	4170	4180	4190	4200
ATGTGACGAA	CGTGTACATC	GACTGAAATC	CCTGGTAATC	CGTTTTAGAA	TCCATGATAA	TAATTTTCTG
4210	4220	4230	4240	4250	4260	4270
GATTATTGGT	AATTTTTTTT	GCACGTTCAA	AATTTTTTGC	AACCCCTTTT	TGGAAACAAA	CACTACGGTA
4280	4290	4300	4310	4320	4330	4340
GGCTGCGAAA	TGTTCATACT	GTTGAGCAAT	TCACGTTCAT	TATAAATGTC	GTTCGCGGGC	GCAACTGCAA
4350	4360	4370	4380	4390	4400	4410
CTCCGATAAA	TAACGCGCCC	AACACCGGCA	TAAAGAATTG	AAGAGAGTTT	TCACTGCATA	CGACGATTCT
4420	4430	4440	4450	4460	4470	4480
GTGATTTGTA	TTCAGCCCAT	ATCGTTTCAT	AGCTTCTGCC	AACCGAACGG	ACATTTCGAA	GTATTCCGCG
4490	4500	4510	4520	4530	4540	4550
TACAGCCCGG	CCGTTTAAAC	GGCCGGGCTT	CAATACCCTG	ATTGACTGGA	ACAGCTGTAG	CCCTGAACAG
4560	4570	4580	4590	4600	4610	4620
CAGCGTGCGC	TGCTGACGCG	TCCGGCGATT	TCCGCCTCTG	ACAGTATTAC	CCGGACGGTC	AGCGATATTC
4630	4640	4650	4660	4670	4680	4690
TGGATAATGT	AAAAACGCGC	GGTGACGATG	CCCTGCGTGA	ATACAGCGCT	AAATTTGATA	AAACAGAAGT
4700	4710	4720	4730	4740	4750	4760
GACAGCGCTA	CGCGTCACCC	CTGAAGAGAT	CGCCGCCGCC	GGCGCGCGTC	TGAGCGACGA	ATTAAAACAG
4770	4780	4790	4800	4810	4820	4830
GCGATGACCG	CTGCCGTCAA	AAATATTGAA	ACGTTCCATT	CCGCGCAGAC	GCTACCGCCT	GTAGATGTGG
4840	4850	4860	4870	4880	4890	4900
AAACCCAGCC	AGGCGTGCGT	TGCCAGCAGG	TTACGCGTCC	CGTCTCGTCT	GTCGGTCTGT	ATATTCCCGG

FIG. 10E

4910	4920	4930	4940	4950	4960	4970
CGGCTCGGCT	CCGCTCTTCT	CAACGGTGCT	GATGCTGGCG	ACGCCGGCGC	GCATTGCGGG	ATGCCAGAAG
4980	4990	5000	5010	5020	5030	5040
GTGGTTCTGT	GCTCGCCGCC	GCCCATCGCT	GATGAAATCC	TCTATGCGGC	GCAACTGTGT	GGCGTGCAGG
5050	5060	5070	5080	5090	5100	5110
AAATCTTTAA	CGTCGGCGGC	GCGCAGGCGA	TTTGCCGCTCT	GGCCTTCGGC	AGCGAGTCCG	TACCGAAAGT
5120	5130	5140	5150	5160	5170	5180
GGATAAAATT	TTTGGCCCCG	GCAACGCCTT	TGTAACCGAA	GCCAAACGTC	AGGTCAGCCA	GCGTCTCGAC
5190	5200	5210	5220	5230	5240	5250
GGCGCGGCTA	TCGATATGCC	AGCCGGGCGG	TCTGAAGTAC	TGGTGATCGC	AGACAGCGGC	GCAACACCGG
5260	5270	5280	5290	5300	5310	5320
ATTTCGTCGC	TTCTGACCTG	CTCTTCCCAGG	CTGAGCACGG	CCCGGATTCC	CAGGTGATCC	TGCTGACGCC
5330	5340	5350	5360	5370	5380	5390
TGATGCTGAC	ATTGCCCGCA	AGGTGGCGGA	GGCGGTAGAA	CGTCAACTGG	CGGAACTGCC	GCGCGCGGAC
5400	5410	5420	5430	5440	5450	5460
ACCGCCCGGC	AGGCCCTGAG	CGCCAGTCGT	CTGATTGTGA	CCAAAGATTT	AGCGCAGTGC	GTCGCCATCT
5470	5480	5490	5500	5510	5520	5530
CTAATCAGTA	TGGGCCGGAA	CACTTAATCA	TCCAGACGCG	CAATGCGCGC	GATTTGGTGG	ATGCGATTAC
5540	5550	5560	5570	5580	5590	5600
CAGCGCAGGC	TCGGTATTTC	TCGGCGACTG	GTCGCCGGAA	TCCGCCGGTG	ATTACGCTTC	CGGAACCAAC
5610	5620	5630	5640	5650	5660	5670
CATGTTTTAC	CGACCTATGG	CTATACTGCT	ACCTGTTCCA	GCCTTGGGTT	AGCGGATTTC	CAGAAACGGA
5680	5690	5700	5710	5720	5730	5740
TGACCGTTCA	GGAACTGTCG	AAAGCGGGCT	TTTCCGCTCT	GGCATCAACC	ATTGAAACAT	TGGCGGCGGC
5750	5760	5770	5780	5790	5800	5810
AGAACGTCTG	ACCGCCCATA	AAAATGCCGT	GACCCTGCGC	GTAAACGCCC	TCAAGGAGCA	AGCATGAGCA
5820	5830	5840	5850	5860	5870	5880
CTGAAAACAC	TCTCAGCGTC	GCTGACTTAG	CCCGTGAAAA	TGTCCGCAAC	CTGGAGATCC	AGACATGGAT

FIG. 10F

5890	5900	5910	5920	5930	5940	5950
AAGATACATT	GATGAGTTTG	GACAAACCAC	AACTAGAATG	CAGTGAAAAA	AATGCTTTAT	TTGTGAAATT
5960	5970	5980	5990	6000	6010	6020
TGTGATGCTA	TTGCTTTATT	TGTAACCATT	ATAAGCTGCA	ATAAACAAGT	TAACAACAAC	AATTGCATTC
6030	6040	6050	6060	6070	6080	6090
ATTTTATGTT	TCAGGTTCAG	GGGGAGGTGT	GGGAGGTTTT	TTAAAGCAAG	TAAAACCTCT	ACAAATGTGG
6100	6110	6120	6130	6140	6150	6160
TATGGCTGAT	TATGATCTCT	AGGGCCGGCC	CTCGACGGCG	CGTCTAGAGC	AGTGTGGTTT	TCAAGAGGAA
6170	6180	6190	6200	6210	6220	6230
GCAAAAAGCC	TCTCCACCCA	GGCCTGGAAT	GTTTCCACCC	AATGTCGAGC	AGTGTGGTTT	TGCAAGAGGA
6240	6250	6260	6270	6280	6290	6300
AGCAAAAAGC	CTCTCCACCC	AGGCCTGGAA	TGTTTCCACC	CAATGTCGAG	CAAACCCCGC	CCAGCGTCTT
6310	6320	6330	6340	6350	6360	6370
GTCATTGGCG	AATTGGAACA	CGCATATGCA	GTCGGGGCGG	CGCGGTCCCA	GGTCCACTTC	GCATATTAAG
6380	6390	6400	6410	6420	6430	6440
GTGGCGCGTG	TGGCCTCGAA	CACCGAGCGA	CCCTGCAGCC	AATATGGGAT	CGGCCATTGA	ACAAGATGGA
6450	6460	6470	6480	6490	6500	6510
TTGCACGCAG	GTTCTCCGGC	CGCTTGGGTG	GAGAGGCTAT	TCGGCTATGA	CTGGGCACAA	CAGACAATCG
6520	6530	6540	6550	6560	6570	6580
GCTGCTCTGA	TGCCGCCGTG	TTCCGGCTGT	CAGCGCAGGG	GCGCCCGGTT	CTTTTTGTCA	AGACCGACCT
6590	6600	6610	6620	6630	6640	6650
GTCCGGTGCC	CTGAATGAAC	TGCAGGTAAG	TGCGGCCGTC	GATGGCCGAG	GCGGCCTCGG	CCTCTGCATA
6660	6670	6680	6690	6700	6710	6720
AATAAAAAAA	ATTAGTCAGC	CATGCATGGG	GCGGAGAATG	GGCGGAACTG	GGCGGAGTTA	GGGGCGGGAT
6730	6740	6750	6760	6770	6780	6790
GGGCGGAGTT	AGGGGCGGGA	CTATGGTTGC	TGACTAATTG	AGATGCATGC	TTTGCATACT	TCTGCCTGCT
6800	6810	6820	6830	6840	6850	6860
GGGGAGCCTG	GGGACTTTCC	ACACCTGGTT	GCTGACTAAT	TGAGATGCAT	GCTTTGCATA	CTTCTGCCTG

FIG. 10G

6870	6880	6890	6900	6910	6920	6930
CTGGGGAGCC	TGGGGACTTT	CCACACCCTA	ACTGACACAC	ATTCCACAGA	ATTAATTCCC	CTAGTTATTA
6940	6950	6960	6970	6980	6990	7000
ATAGTAATCA	ATTACGGGGT	CATTAGTTCA	TAGCCCATAT	ATGGAGTTCC	GCGTTACATA	ACTTACGGTA
7010	7020	7030	7040	7050	7060	7070
AATGGCCCGC	CTGGCTGACC	GCCCAACGAC	CCCCGCCCAT	TGACGTCAAT	AATGACGTAT	GTTCCCATAG
7080	7090	7100	7110	7120	7130	7140
TAACGCCAAT	AGGGACTTTC	CATTGACGTC	AATGGGTGGA	GTATTTACGG	TAAACTGCCC	ACTTGGCAGT
7150	7160	7170	7180	7190	7200	7210
ACATCAAGTG	TATCATATGC	CAAGTACGCC	CCCTATTGAC	GTCAATGACG	GTAAATGGCC	CGCCTGGCAT
7220	7230	7240	7250	7260	7270	7280
TATGCCCAGT	ACATGACCTT	ATGGGACTTT	CCTACTTGCC	AGTACATCTA	CGTATTAGTC	ATCGCTATTA
7290	7300	7310	7320	7330	7340	7350
CCATGGTGAT	GCGGTTTTGG	CAGTACATCA	ATGGGCGTGG	ATAGCGGTTT	GACTCACGGG	GATTTCCAAG
7360	7370	7380	7390	7400	7410	7420
TCTCCACCCC	ATTGACGTCA	ATGGGAGTTT	GTTTTGGCAC	CAAAATCAAC	GGGACTTTCC	AAAATGTCGT
7430	7440	7450	7460	7470	7480	7490
AACAACTCCG	CCCCATTGAC	GCAAATGGGC	GGTAGGCGTG	TACGGTGGGA	GGTCTATATA	AGCAGAGCTG
7500	7510	7520	7530	7540	7550	7560
GGTACGTGAA	CCGTCAGATC	GCCTGGAGAC	GCCATCACAG	ATCTCTCACC	ATGGACATGA	GGGTCCCCGC
7570	7580	7590	7600	7610	7620	7630
TCAGCTCCTG	GGGCTCCTTC	TGCTCTGGCT	CCCAGGTGCC	AGATGTGACA	TCCAGATGAC	CCAGTCTCCA
7640	7650	7660	7670	7680	7690	7700
TCTTCCCTGT	CTGCATCTGT	AGGGGACAGA	GTCACCATCA	CTTGCAGGGC	AAGTCAGGAC	ATTAGGTATT
7710	7720	7730	7740	7750	7760	7770
ATTTAAATTG	GTATCAGCAG	AAACCAGGAA	AAGCTCCTAA	GCTCCTGATC	TATGTTGCAT	CCAGTTTGCA
7780	7790	7800	7810	7820	7830	7840
AAGTGGGGTC	CCATCAAGGT	TCAGCGGCAG	TGGATCTGGG	ACAGAGTTCA	CTCTCACCGT	CAGCAGCCTG

FIG. 10H

7850	7860	7870	7880	7890	7900	7910
CAGCCTGAAG	ATTTTGCGAC	TTATTACTGT	CTACAGGTTT	ATAGTACCCC	TCGGACGTTC	GGCCAAGGGA
7920	7930	7940	7950	7960	7970	7980
CCAAGGTGGA	AATCAAACGT	ACGGTGGCTG	CACCATCTGT	CTTCATCTTC	CCGCCATCTG	ATGAGCAGTT
7990	8000	8010	8020	8030	8040	8050
GAAATCTGGA	ACTGCCTCTG	TTGTGTGCCT	GCTGAATAAC	TTCTATCCCA	GAGAGGCCAA	AGTACAGTGG
8060	8070	8080	8090	8100	8110	8120
AAGGTGGATA	ACGCCCTCCA	ATCGGGTAAC	TCCCAGGAGA	GTGTCACAGA	GCAGGACAGC	AAGGACAGCA
8130	8140	8150	8160	8170	8180	8190
CCTACAGCCT	CAGCAGCACC	CTGACGCTGA	GCAAAGCAGA	CTACGAGAAA	CACAAAGTCT	ACGCCTGCGA
8200	8210	8220	8230	8240	8250	8260
AGTCACCCAT	CAGGGCCTGA	GCTCGCCCGT	CACAAAGAGC	TTCAACAGGG	GAGAGTGTTG	AATTCAGATC
8270	8280	8290	8300	8310	8320	8330
CGTTAACGGT	TACCAACTAC	CTAGACTGGA	TTCGTGACAA	CATGCGGCCG	TGATATCTAC	GTATGATCAG
8340	8350	8360	8370	8380	8390	8400
CCTCGACTGT	GCCTTCTAGT	TGCCAGCCAT	CTGTTGTTTG	CCCCTCCCCC	GTGCCTTCCT	TGACCCTGGA
8410	8420	8430	8440	8450	8460	8470
AGGTGCCACT	CCCACTGTCC	TTTCCTAATA	AAATGAGGAA	ATTGCATCGC	ATTGTCTGAG	TAGGTGTCAT
8480	8490	8500	8510	8520	8530	8540
TCTATTCTGG	GGGGTGGGGT	GGGGCAGGAC	AGCAAGGGGG	AGGATTGGGA	AGACAATAGC	AGGCATGCTG
8550	8560	8570	8580	8590	8600	8610
GGGATGCGGT	GGGCTCTATG	GCTTCTGAGG	CGGAAAGAAC	CAGCTGGGAC	TAGTCGCAAT	TGGGCGGAGT
8620	8630	8640	8650	8660	8670	8680
TAGGGGCGGG	ATGGGCGGAG	TTAGGGGCGG	GGACTATGGT	GCTGACTAAT	TGAGATGCAT	GCTTTGCATA
8690	8700	8710	8720	8730	8740	8750
CTTCTGCCTG	CTGGGGAGCC	TGGGGACTTT	CCACACCTGG	TTGCTGACTA	ATTGAGATGC	ATGCTTTGCA
8760	8770	8780	8790	8800	8810	8820
TACTTCTGCC	TGCTGGGGAG	CCTGGGGACT	TTCCACACCC	TAACTGACAC	ACATTCCACA	GAATTAATTC

FIG. 10I

8830	8840	8850	8860	8870	8880	8890
CCCTAGTTAT	TAATAGTAAT	CAATTACGGG	GTCATTAGTT	CATAGCCCAT	ATATGGAGTT	CCGCGTTACA
8900	8910	8920	8930	8940	8950	8960
TAACTTACGG	TAAATGGCCC	GCCTGGCTGA	CCGCCCAACG	ACCCCCGCCC	ATTGACGTCA	ATAATGACGT
8970	8980	8990	9000	9010	9020	9030
ATGTTCCCAT	AGTAACGCCA	ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GAGTATTTAC	GGTAAACTGC
9040	9050	9060	9070	9080	9090	9100
CCACTTGGCA	GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	CGGTAAATGG
9110	9120	9130	9140	9150	9160	9170
CCCGCCTGGC	ATTATGCCCA	GTACATGACC	TTATGGGACT	TTCCTACTTG	GCAGTACATC	TACGTATTAG
9180	9190	9200	9210	9220	9230	9240
TCATCGCTGT	TACCATGGTG	ATGCGGTTTT	GGCAGTACAT	CAATGGGCGT	GGATAGCGGT	TTGACTCACG
9250	9260	9270	9280	9290	9300	9310
GGGATTTCCA	AGTCTCCACC	CCATTGACGT	CAATGGGAGT	TTGTTTTGGC	ACCAAAATCA	ACGGGACTTT
9320	9330	9340	9350	9360	9370	9380
CCAAAATGTC	GTAACAACTC	CGCCCCATTG	ACGCAAATGG	GCGGTAGGCG	TGTACGGTGG	GAGGTCTATA
9390	9400	9410	9420	9430	9440	9450
TAAGCAGAGC	TGGGTACGTG	AACCGTCAGA	TCGCCTGGAG	ACGCCGTCGA	CATGGGTTGG	AGCCTCATCT
9460	9470	9480	9490	9500	9510	9520
TGCTCTTCCT	TGTCGCTGTT	GCTACGCGTG	TCCTGTCCGA	GGTGCAGCTG	GTGGAGTCTG	GGGGCGGCTT
9530	9540	9550	9560	9570	9580	9590
GGCAAAGCCT	GGGGGGTCCC	TGAGACTCTC	CTGCGCAGCC	TCCGGGTTCA	GGTTCACCTT	CAATAACTAC
9600	9610	9620	9630	9640	9650	9660
TACATGGACT	GGGTCCGCCA	GGCTCCAGGG	CAGGGGCTGG	AGTGGGTCTC	ACGTATTAGT	AGTAGTGGTG
9670	9680	9690	9700	9710	9720	9730
ATCCCACATG	GTACGCAGAC	TCCGTGAAGG	GCAGATTCAC	CATCTCCAGA	GAGAACGCCA	AGAACACACT
9740	9750	9760	9770	9780	9790	9800
GTTTCTTCAA	ATGAACAGCC	TGAGAGCTGA	GGACACGGCT	GTCTATTACT	GTGCGAGCTT	GACTACAGGG

FIG. 10J

9810	9820	9830	9840	9850	9860	9870
TCTGACTCCCT	GGGGCCAGGG	AGTCCTGGTC	ACCGTCTCCT	CAGCTAGCAC	CAAGGGCCCA	TCGGTCTTCC
9880	9890	9900	9910	9920	9930	9940
CCCTGGCACC	CTCCTCCAAG	AGCACCTCTG	GGGGCACAGC	GGCCCTGGGC	TGCCTGGTCA	AGGACTACTT
9950	9960	9970	9980	9990	10000	10010
CCCCGAACCG	GTGACGGTGT	CGTGGAACTC	AGGCGCCCTG	ACCAGCGGCG	TGCACACCTT	CCCGGCTGTC
10020	10030	10040	10050	10060	10070	10080
CTACAGTCCT	CAGGACTCTA	CTCCCTCAGC	AGCGTGGTGA	CCGTGCCCTC	CAGCAGCTTG	GGCACCCAGA
10090	10100	10110	10120	10130	10140	10150
CCTACATCTG	CAACGTGAAT	CACAAGCCCA	GCAACACCAA	GGTGGACAAG	AAAGTTGAGC	CCAAATCTTG
10160	10170	10180	10190	10200	10210	10220
TGACAAAACT	CACACATGCC	CACCGTGCCC	AGCACCTGAA	CTCCTGGGGG	GACCGTCAGT	CTTCCTCTTC
10230	10240	10250	10260	10270	10280	10290
CCCCCAAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACCC	CTGAGGTCAC	ATGCGTGGTG	GTGGACGTGA
10300	10310	10320	10330	10340	10350	10360
GCCACGAAGA	CCCTGAGGTC	AAGTTCAACT	GGTACGTGGA	CGGCGTGGAG	GTGCATAATG	CCAAGACAAA
10370	10380	10390	10400	10410	10420	10430
GCCGCGGGAG	GAGCAGTACA	ACAGCACGTA	CCGTGTGGTC	AGCGTCCTCA	CCGTCCTGCA	CCAGGACTGG
10440	10450	10460	10470	10480	10490	10500
CTGAATGGCA	AGGAGTACAA	GTGCAAGGTC	TCCAACAAAG	CCCTCCCAGC	CCCCATCGAG	AAAACCATCT
10510	10520	10530	10540	10550	10560	10570
CCAAAGCCAA	AGGGCAGCCC	CGAGAACCAC	AGGTGTACAC	CCTGCCCCCA	TCCCGGGATG	AGCTGACCAA
10580	10590	10600	10610	10620	10630	10640
GAACCAGGTC	AGCCTGACCT	GCCTGGTCAA	AGGCTTCTAT	CCCAGCGACA	TCGCCGTGGA	GTGGGAGAGC
10650	10660	10670	10680	10690	10700	10710
AATGGGCAGC	CGGAGAACAA	CTACAAGACC	ACGCCTCCCG	TGCTGGACTC	CGACGGCTCC	TTCTTCCTCT
10720	10730	10740	10750	10760	10770	10780
ACAGCAAGCT	CACCGTGGAC	AAGAGCAGGT	GGCAGCAGGG	GAACGTCTTC	TCATGCTCCG	TGATGCATGA

FIG. 10K

10790	10800	10810	10820	10830	10840	10850
GGCTCTGCAC	AACCACTACA	CGCAGAAGAG	CCTCTCCCTG	TCTCCGGGTA	AATGAGGATC	CGTTAACGGT
10860	10870	10880	10890	10900	10910	10920
TACCAACTAC	CTAGACTGGA	TTCGTGACAA	CATGCGGCCG	TGATATCTAC	GTATGATCAG	CCTCGACTGT
10930	10940	10950	10960	10970	10980	10990
GCCTTCTAGT	TGCCAGCCAT	CTGTTGTTTGC	CCCCTCCCCC	GTGCCTTCCT	TGACCCTGGA	AGGTGCCACT
11000	11010	11020	11030	11040	11050	11060
CCCACTGTCC	TTTCCTAATA	AAATGAGGAA	ATTGCATCGC	ATTGTCTGAG	TAGGTGTCAT	TCTATTCTGG
11070	11080	11090	11100	11110	11120	11130
GGGGTGGGGT	GGGGCAGGAC	AGCAAGGGGG	AGGATTGGGA	AGACAATAGC	AGGCATGCTG	GGGATGCGGT
11140	11150	11160	11170	11180	11190	11200
GGGCTCTATG	GCTTCTGAGG	CGGAAAGAAC	CAGCTGGGGC	TCGACAGCAA	CGCTAGGTCG	AGGCCGCTAC
11210	11220	11230	11240	11250	11260	11270
TAACTCTCTC	CTCCCTCCTT	TTTCCTGCAG	GACGAGGCAG	CGCGGCTATC	GTGGCTGGCC	ACGACGGGCG
11280	11290	11300	11310	11320	11330	11340
TTCCTTGCGC	AGCTGTGCTC	GACGTTGTCA	CTGAAGCGGG	AAGGGACTGG	CTGCTATTGG	GCGAAGTGCC
11350	11360	11370	11380	11390	11400	11410
GGGGCAGGAT	CTCCTGTCAT	CTCACCTTGC	TCCTGCCGAG	AAAGTATCCA	TCATGGCTGA	TGCAATGCGG
11420	11430	11440	11450	11460	11470	11480
CGGCTGCATA	CGCTTGATCC	GGCTACCTGC	CCATTCGACC	ACCAAGCGAA	ACATCGCATC	GAGCGAGCAC
11490	11500	11510	11520	11530	11540	11550
GTACTCGGAT	GGAAGCCGGT	CTTGTCGATC	AGGATGATCT	GGACGAAGAG	CATCAGGGGC	TCGCGCCAGC
11560	11570	11580	11590	11600	11610	11620
CGAACTGTTC	GCCAGGTAAG	TGAGCTCCAA	TTCAAGCTCT	CGAGCTAGGG	CGGCCAGCTA	GTAGCTTTGC
11630	11640	11650	11660	11670	11680	11690
TTCTCAATTT	CTTATTTGCA	TAATGAGAAA	AAAAGGAAAA	TTAATTTTAA	CACCAATTCA	GTAGTTGATT
11700	11710	11720	11730	11740	11750	11760
GAGCAAATGC	GTTGCCAAAA	AGGATGCTTT	AGAGACAGTG	TTCTCTGCAC	AGATAAGGAC	AAACATTATT

FIG. 10L

11770	11780	11790	11800	11810	11820	11830
CAGAGGGAGT	ACCCAGAGCT	GAGACTCCTA	AGCCAGTGAG	TGGCACAGCA	TCCAGGGAGA	AATATGCTTG
11840	11850	11860	11870	11880	11890	11900
TCATCACCGA	AGCCTGATTC	CGTAGAGCCA	CACCCTGGTA	AGGGCCAATC	TGCTCACACA	GGATAGAGAG
11910	11920	11930	11940	11950	11960	11970
GGCAGGAGCC	AGGCAGAGC	ATATAAGGTG	AGGTAGGATC	AGTTGCTCCT	CACATTTGCT	TCTGACATAG
11980	11990	12000	12010	12020	12030	12040
TTGTGTTGGG	AGCTTGGATA	GCTTGGGGGG	GGGACAGCTC	AGGGCTGCGA	TTTCGCGCCA	AACTTGACGG
12050	12060	12070	12080	12090	12100	12110
CAATCCTAGC	GTGAAGGCTG	GTAGGATTTT	ATCCCCGCTG	CCATCATGGT	TCGACCATTG	AACTGCATCG
12120	12130	12140	12150	12160	12170	12180
TCGCCGTGTC	CCAAAATATG	GGGATTGGCA	AGAACGGAGA	CCTACCCTGG	CCTCCGCTCA	GGAACGAGTT
12190	12200	12210	12220	12230	12240	12250
CAAGTACTTC	CAAAGAATGA	CCACAACCTC	TTCAGTGGAA	GGTAAACAGA	ATCTGGTGAT	TATGGGTAGG
12260	12270	12280	12290	12300	12310	12320
AAAACCTGGT	TCTCCATTCC	TGAGAAGAAT	CGACCTTTAA	AGGACAGAAT	TAATATAGTT	CTCAGTAGAG
12330	12340	12350	12360	12370	12380	12390
AACTCAAAGA	ACCACCACGA	GGAGCTCATT	TTCTTGCCAA	AAGTTTGGAT	GATGCCTTAA	CGTAGGCGCG
12400	12410	12420	12430	12440	12450	12460
CCATTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAGTT
12470	12480	12490	12500	12510	12520	12530
CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCAACCTCAG	ACTCTTTGTG	ACAAGGATCA	TGCAGGAATT
12540	12550	12560	12570	12580	12590	12600
TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	TCCCAGAATA	CCCAGGCGTC
12610	12620	12630	12640	12650	12660	12670
CTCTCTGAGG	TCAAGGAGGA	AAAAGGCATC	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG
12680	12690	12700	12710	12720	12730	12740
AAGATGCTTT	CAAGTTCTCT	GCTCCCCTCC	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG

FIG. 10M

12750	12760	12770	12780	12790	12800	12810
GCTTTAGATC	AGCCTCGACT	GTGCCTTCTA	GTTGCCAGCC	ATCTGTTGTT	TGCCCCTCCC	CCGTGCCTTC
12820	12830	12840	12850	12860	12870	12880
CTTGACCCTG	GAAGGTGCCA	CTCCCACTGT	CCTTTCCTAA	TAAAATGAGG	AAATTGCATC	GCATTGTCTG
12890	12900	12910	12920	12930	12940	12950
AGTAGGTGTC	ATTCTATTCT	GGGGGGTGGG	GTGGGGCAGG	ACAGCAAGGG	GGAGGATTGG	GAAGACAATA
12960	12970	12980	12990	13000	13010	13020
GCAGGCATGC	TGGGGATGCG	GTGGGCTCTA	TGGCTTCTGA	GGCGGAAAGA	ACCAGCTGGG	GCTCGAAGCG
13030	13040	13050	13060	13070	13080	13090
GCCGCCCATT	TCGCTGGTGG	TCAGATGCGG	GATGGCGTGG	GACGCGGCGG	GGAGCGTCAC	ACTGAGGTTT
13100	13110	13120	13130	13140	13150	13160
TCCGCCAGAC	GCCACTGCTG	CCAGGCGCTG	ATGTGCCCGG	CTTCTGACCA	TGCGGTCGCG	TTCGGTTGCA
13170	13180	13190	13200	13210	13220	13230
CTACGCGTAC	TGTGAGCCAG	AGTTGCCCGG	CGCTCTCCGG	CTGCGGTAGT	TCAGGCAGTT	CAATCAACTG
13240	13250	13260	13270	13280	13290	13300
TTTACCTTGT	GGAGCGACAT	CCAGAGGCAC	TTCACCGCTT	GCCAGCGGCT	TACCATCCAG	CGCCACCATC
13310	13320	13330	13340	13350	13360	13370
CAGTGCAGGA	GCTCGTTATC	GCTATGACGG	AACAGGTATT	CGCTGGTCAC	TTCGATGGTT	TGCCCGGATA
13380	13390	13400	13410	13420	13430	13440
AACGGAACTG	GAAAAACTGC	TGCTGGTGTT	TTGCTTCCGT	CAGCGCTGGA	TGCGGCGTGC	GGTCGGCAAA
13450	13460	13470	13480	13490	13500	13510
GACCAGACCG	TTCATACAGA	ACTGGCGATC	CGTTCGGCTA	TCGCCAAAAT	CACCGCCGTA	AGCCGACCAC
13520	13530	13540	13550	13560	13570	13580
GGGTTGCCGT	TTTCATCATA	TTTAATCAGC	GACTGATCCA	CCCAGTCCCA	GACGAAGCCG	CCCTGTAAAC
13590	13600	13610	13620	13630	13640	13650
GGGGATACTG	ACGAAACGCC	TGCCAGTATT	TAGCGAAACC	GCCAAGACTG	TTACCCATCG	CGTGGGCGTA
13660	13670	13680	13690	13700	13710	13720
TTCGCAAAGG	ATCAGCGGGC	GCGTCTCTCC	AGGTAGCGAA	AGCCATTTTT	TGATGGACCA	TTTCGGCACA

FIG. 10N

13730	13740	13750	13760	13770	13780	13790
GCCGGGAAGG	GCTGGTCTTC	ATCCACGCGC	GCGTACATCG	GGCAAATAAT	ATCGGTGGCC	GTGGTGTCGG
13800	13810	13820	13830	13840	13850	13860
CTCCGCCGCC	TTCATACTGC	ACCGGGCGGG	AAGGATCGAC	AGATTTGATC	CAGCGATACA	GCGCGTCGTG
13870	13880	13890	13900	13910	13920	13930
ATTAGCGCCG	TGGCCTGATT	CATTCCCCAG	CGACCAGATG	ATCACACTCG	GGTGATTACG	ATCGCGCTGC
13940	13950	13960	13970	13980	13990	14000
ACCATTCGCG	TTACGCGTTC	GCTCATCGCC	GGTAGCCAGC	GCGGATCATC	GGTCAGACGA	TTCATTGGCA
14010	14020	14030	14040	14050	14060	14070
CCATGCCGTG	GGTTTCAATA	TTGGCTTCAT	CCACCACATA	CAGGCCGTAG	CGGTCGCACA	GCGTGTACCA
14080	14090	14100	14110	14120	14130	14140
CAGCGGATGG	TTCGGATAAT	GCGAACAGCG	CACGGCGTTA	AAGTTGTTCT	GCTTCATCAG	CAGGATATCC
14150	14160	14170	14180	14190	14200	14210
TGCACCATCG	TCTGCTCATC	CATGACCTGA	CCATGCAGAG	GATGATGCTC	GTGACGGTTA	ACGCCTCGAA
14220	14230	14240	14250	14260	14270	14280
TCAGCAACGG	CTTGCCGTTC	AGCAGCAGCA	GACCATTTTC	AATCCGCACC	TCGCGGAAAC	CGACATCGCA
14290	14300	14310	14320	14330	14340	14350
GGCTTCTGCT	TCAATCAGCG	TGCCGTCGGC	GGTGTGCAGT	TCAACCACCG	CACGATAGAG	ATTCGGGATT
14360	14370	14380	14390	14400	14410	14420
TCGGCGCTCC	ACAGTTTCGG	GTTTTCGACG	TTCAGACGTA	GTGTGACGCG	ATCGGCATAA	CCACCACGCT
14430	14440	14450	14460	14470	14480	14490
CATCGATAAT	TTCACCGCCG	AAAGGCGCGG	TGCCGCTGGC	GACCTGCGTT	TCACCCTGCC	ATAAAGAAAC
14500	14510	14520	14530	14540	14550	14560
TGTTACCCGT	AGGTAGTCAC	GCAACTCGCC	GCACATCTGA	ACTTCAGCCT	CCAGTACAGC	GCGGCTGAAA
14570	14580	14590	14600	14610	14620	14630
TCATCATTAA	AGCGAGTGGC	AACATGGAAA	TCGCTGATTT	GTGTAGTCGG	TTTATGCAGC	AACGAGACGT
14640	14650	14660	14670	14680	14690	14700
CACGGAAAAT	GCCGCTCATC	CGCCACATAT	CCTGATCTTC	CAGATAACTG	CCGTCACTCC	AGCGCAGCAC

FIG. 10P

14710	14720	14730	14740	14750	14760	14770
CATCACCGCG	AGGCGGTTTT	CTCCGGCGCG	TAAAAATGCG	CTCAGGTCAA	ATTCAGACGG	CAAACGACTG
14780	14790	14800	14810	14820	14830	14840
TCCTGGCCGT	AACCGACCCA	GCGCCCGTTG	CACCACAGAT	GAAACGCCGA	GTTAACGCCA	TCAAAAATAA
14850	14860	14870	14880	14890	14900	14910
TTCGCGTCTG	GCCTTCCTGT	AGCCAGCTTT	CATCAACATT	AAATGTGAGC	GAGTAACAAC	CCGTCGGATT
14920	14930	14940	14950	14960	14970	14980
CTCCGTGGGA	ACAAACGGCG	GATTGACCGT	AATGGGATAG	GTCACGTTGG	TGTAGATGGG	CGCATCGTAA
14990	15000	15010	15020	15030	15040	15050
CCGTGCATCT	GCCAGTTTGA	GGGGACGACG	ACAGTATCGG	CCTCAGGAAG	ATCGCACTCC	AGCCAGCTTT
15060	15070	15080	15090	15100	15110	15120
CCGGCACCGC	TTCTGGTGCC	GGAAACCAGG	GCAAGCGCCA	TTCGCCATTC	AGGCTGCGCA	ACTGTTGGGA
15130	15140	15150	15160	15170	15180	15190
AGGGCGATCG	GTGCGGGCCT	CTTCGCTATT	ACGCCAGCTG	GCGAAAGGGG	GATGTGCTGC	AAGGCGATTA
15200	15210	15220	15230	15240	15250	15260
AGTTGGGTAA	CGCCAGGGTT	TTCCCAGTCA	CGACGTTGTA	AAACGACTTA	ATCCGTCGAG	GGGCTGCCTC
15270	15280	15290	15300	15310	15320	15330
GAAGCAGACG	ACCTTCCGTT	GTGCAGCCAG	CGGCGCCTGC	GCCGGTGCCC	ACAATCGTGC	GCGAACAAAC
15340	15350	15360	15370	15380	15390	15400
TAAACCAGAA	CAAATTATAC	CGGCGGCACC	GCCGCCACCA	CCTTCTCCCG	TGCCTAACAT	TCCAGCGCCT
15410	15420	15430	15440	15450	15460	15470
CCACCACCAC	CACCACCATC	GATGTCTGAA	TTGCCGCCCG	CTCCACCAAT	GCCGACGGAA	CCTCAACCCG
15480	15490	15500	15510	15520	15530	15540
CTGCACCTTT	AGACGACAGA	CAACAATTGT	TGGAAGCTAT	TAGAAACGAA	AAAAATCGCA	CTCGTCTCAG
15550	15560	15570	15580	15590	15600	15610
ACCGGTCAAA	CCAAAAACGG	CGCCCGAAAC	CAGTACAATA	GTTGAGGTGC	CGACTGTGTT	GCCTAAAGAG
15620	15630	15640	15650	15660	15670	15680
ACATTTGAGC	CTAAACCGCC	GTCTGCATCA	CCGCCACCAC	CTCCGCCTCC	GCCTCCGCCG	CCAGCCCCGC

FIG. 10Q

15690	15700	15710	15720	15730	15740	15750
CTGCGCCTCC	ACCGATGGTA	GATTTATCAT	CAGCTCCACC	ACCGCCGCCA	TTAGTAGATT	TGCCGTCTGA
15760	15770	15780	15790	15800	15810	15820
AATGTTACCA	CCGCCTGCAC	CATCGCTTTC	TAACGTGTTG	TCTGAATTAA	AATCGGGCAC	AGTTAGATTG
15830	15840	15850	15860	15870	15880	15890
AAACCCGCCC	AAAAACGCCC	GCAATCAGAA	ATAATTCCAA	AAAGCTCAAC	TACAAATTTG	ATCGCGGACG
15900	15910	15920	15930	15940	15950	15960
TGTTAGCCGA	CACAATTAAT	AGGCGTCGTG	TGGCTATGGC	AAAATCGTCT	TCGGAAGCAA	CTTCTAACGA
15970	15980	15990	16000	16010	16020	16030
CGAGGGTTGG	GACGACGACG	ATAATCGGCC	TAATAAAGCT	AACACGCCCG	ATGTTAAATA	TGTCCAAGCT
16040	16050	16060	16070	16080	16090	16100
ACTAGTGGTA	CCGCTTGGCA	GAACATATCC	ATCGCGTCCG	CCATCTCCAG	CAGCCGCACG	CGGCGCATCT
16110	16120	16130	16140	16150	16160	16170
CGGGCAGCGT	TGGGTCCTGG	CCACGGGTGC	GCATGATCGT	GCTCCTGTCG	TTGAGGACCC	GGCTAGGCTG
16180	16190	16200	16210	16220	16230	16240
GCGGGGTTGC	CTTACTGGTT	AGCAGAATGA	ATCACCGATA	CGCGAGCGAA	CGTGAAGCGA	CTGCTGCTGC
16250	16260	16270	16280	16290	16300	16310
AAAACGTCTG	CGACCTGAGC	AACAACATGA	ATGGTCTTCG	GTTTCCGTGT	TTCGTAAAGT	CTGGAAACGC
16320	16330	16340	16350	16360	16370	16380
GGAAGTCAGC	GCCCTGCACC	ATTATGTTCC	GGATCTGCAT	CGCAGGATGC	TGCTGGCTAC	CCTGTGGAAC
16390	16400	16410	16420	16430	16440	16450
ACCTACATCT	GTATTAACGA	AGCGCTGGCA	TTGACCCTGA	GTGATTTTTC	TCTGGTCCCG	CCGCATCCAT
16460	16470	16480	16490	16500	16510	16520
ACCGCCAGTT	GTTTACCCTC	ACAACGTTCC	AGTAACCGGG	CATGTTCATC	ATCAGTAACC	CGTATCGTGA
16530	16540	16550	16560	16570	16580	16590
GCATCCTCTC	TCGTTTCATC	GGTATCATTA	CCCCCATGAA	CAGAAATCCC	CCTTACACGG	AGGCATCAGT
16600	16610	16620	16630	16640	16650	16660
GACCAAACAG	GAAAAAACCG	CCCTTAACAT	GGCCCGCTTT	ATCAGAAGCC	AGACATTAAC	GCTTCTGGAG

FIG. 10R

16670	16680	16690	16700	16710	16720	16730
AAACTCAACG	AGCTGGACGC	GGATGAACAG	GCAGACATCT	GTGAATCGCT	TCACGACCAC	GCTGATGAGC
16740	16750	16760	16770	16780	16790	16800
TTTACCGCAG	CTGCCTCGCG	CGTTTCGGTG	ATGACGGTGA	AAACCTCTGA	CACATGCAGC	TCCCGGAGAC
16810	16820	16830	16840	16850	16860	16870
GGTCACAGCT	TGTCTGTAAG	CGGATGCCGG	GAGCAGACAA	GCCCGTCAGG	GCGCGTCAGC	GGGTGTTGGC
16880	16890	16900	16910	16920	16930	16940
GGGTGTCGGG	GCGCAGCCAT	GACCCAGTCA	CGTAGCGATA	GCGGAGTGTA	TACTGGCTTA	ACTATGCGGC
16950	16960	16970	16980	16990	17000	17010
ATCAGAGCAG	ATTGTACTGA	GAGTGCACCA	TATGCGGTGT	GAAATACCGC	ACAGATGCGT	AAGGAGAAAA
17020	17030	17040	17050	17060	17070	17080
TACCGCATCA	GGCGCTCTTC	CGCTTCCTCG	CTCACTGACT	CGCTGCGCTC	GGTCGTTCGG	CTGCGGCGAG
17090	17100	17110	17120	17130	17140	17150
CGGTATCAGC	TCACTCAAAG	GCGGTAATAC	GGTTATCCAC	AGAATCAGGG	GATAACGCAG	GAAAGAACAT
17160	17170	17180	17190	17200	17210	17220
GTGAGCAAAA	GGCCAGCAAA	AGGCCAGGAA	CCGTAAAAAG	GCCGCGTTGC	TGGCGTTTTT	CCATAGGCTC
17230	17240	17250	17260	17270	17280	17290
CGCCCCCCTG	ACGAGCATCA	CAAAAATCGA	CGCTCAAGTC	AGAGGTGGCG	AAACCCGACA	GGACTATAAA
17300	17310	17320	17330	17340	17350	17360
GATACCAGGC	GTTTCCCCCT	GGAAGCTCCC	TCGTGCGCTC	TCCTGTTCCG	ACCCTGCCGC	TTACCGGATA
17370	17380	17390	17400	17410	17420	17430
CCTGTCCGCC	TTTCTCCCTT	CGGGAAGCGT	GGCGCTTTCT	CATAGCTCAC	GCTGTAGGTA	TCTCAGTTCG
17440	17450	17460	17470	17480	17490	17500
GTGTAGGTCG	TTCGCTCCAA	GCTGGGCTGT	GTGCACGAAC	CCCCCGTTCA	GCCCGACCGC	TGCGCCTTAT
17510	17520	17530	17540	17550	17560	17570
CCGGTAACTA	TCGTCTTGAG	TCCAACCCGG	TAAGACACGA	CTTATCGCCA	CTGGCAGCAG	CCACTGGTAA
17580	17590	17600	17610	17620	17630	17640
CAGGATTAGC	AGAGCGAGGT	ATGTAGGCGG	TGCTACAGAG	TTCTTGAAGT	GGTGGCCTAA	CTACGGCTAC

FIG. 10S

17650	17660	17670	17680	17690	17700	17710
ACTAGAAGGA	CAGTATTTGG	TATCTGCGCT	CTGCTGAAGC	CAGTTACCTT	CGGAAAAAGA	GTTGGTAGCT
17720	17730	17740	17750	17760	17770	17780
CTTGATCCGG	CAAACAAACC	ACCGCTGGTA	GCGGTGGTTT	TTTTGTTTGC	AAGCAGCAGA	TTACGCGCAG
17790	17800	17810	17820	17830	17840	17850
AAAAAAAGGA	TCTCAAGAAG	ATCCTTTGAT	CTTTTCTACG	GGGTCTGACG	CTCAGTGGAA	CGAAAACTCA
17860	17870	17880	17890	17900	17910	17920
CGTTAAGGGA	TTTTGGTCAT	GAGATTATCA	AAAAGGATCT	TCACCTAGAT	CCTTTTAAAT	TAAAAATGAA
17930	17940	17950	17960	17970	17980	17990
GTTTTAAATC	AATCTAAAGT	ATATATGAGT	AAACTTGGTC	TGACAGTTAC	CAATGCTTAA	TCAGTGAGGC
18000	18010	18020	18030	18040	18050	18060
ACCTATCTCA	GCGATCTGTC	TATTTCGTTC	ATCCATAGTT	GCCTGACTCC	CCGTCGTGTA	GATAACTACG
18070	18080	18090	18100	18110	18120	18130
ATACGGGAGG	GCTTACCATC	TGGCCCCAGT	GCTGCAATGA	TACCGCGAGA	CCCACGCTCA	CCGGCTCCAG
18140	18150	18160	18170	18180	18190	18200
ATTTATCAGC	AATAAACCAG	CCAGCCGGAA	GGGCCGAGCG	CAGAAGTGGT	CCTGCAACTT	TATCCGCCTC
18210	18220	18230	18240	18250	18260	18270
CATCCAGTCT	ATTAATTGTT	GCCGGGAAGC	TAGAGTAAGT	AGTTCGCCAG	TTAATAGTTT	GCGCAACGTT
18280	18290	18300	18310	18320	18330	18340
GTTGCCATTG	CTGCAGGCAT	CGTGGTGTCA	CGCTCGTCGT	TTGGTATGGC	TTCATTCAGC	TCCGGTTCCC
18350	18360	18370	18380	18390	18400	18410
AACGATCAAG	GCGAGTTACA	TGATCCCCCA	TGTTGTGCAA	AAAAGCGGTT	AGCTCCTTCG	GTCCTCCGAT
18420	18430	18440	18450	18460	18470	18480
CGTTGTCAGA	AGTAAGTTGG	CCGCAGTGTT	ATCACTCATG	GTTATGGCAG	CACTGCATAA	TTCTCTTACT
18490	18500	18510	18520	18530	18540	18550
GTCATGCCAT	CCGTAAGATG	CTTTTCTGTG	ACTGGTGAGT	ACTCAACCAA	GTCATTCTGA	GAATAGTGTA
18560	18570	18580	18590	18600	18610	18620
TGCGGCGACC	GAGTTGCTCT	TGCCCGGCGT	CAACACGGGA	TAATACCGCG	CCACATAGCA	GAACTTTAAA

FIG. 10T

18630	18640	18650	18660	18670	18680	18690
AGTGCTCATC	ATTGGAAAAC	GTTCTTCGGG	GCGAAAACTC	TCAAGGATCT	TACCGCTGTT	GAGATCCAGT
18700	18710	18720	18730	18740	18750	18760
TCGATGTAAC	CCACTCGTGC	ACCCAACTGA	TCTTCAGCAT	CTTTTACTTT	CACCAGCGTT	TCTGGGTGAG
18770	18780	18790	18800	18810	18820	18830
CAAAAACAGG	AAGGCAAAAT	GCCGCAAAAA	AGGGAATAAG	GGCGACACGG	AAATGTTGAA	TACTCATACT
18840	18850	18860	18870	18880	18890	18900
CTTCCTTTTT	CAATATTATT	GAAGCATTTA	TCAGGGTTAT	TGTCTCATGA	GCGGATACAT	ATTTGAATGT
18910	18920	18930	18940	18950	18960	18970
ATTTAGAAAA	ATAAACAAAT	AGGGGTTCCG	CGCACATTTC	CCCGAAAAGT	GCCACCTGAC	GTCTAAGAAA
18980	18990	19000	19010	19020	19030	19040
CCATTATTAT	CATGACATTA	ACCTATAAAA	ATAGGCGTAT	CACGAGGCCC	TTTCGTCTTC	AAGAA . . . .
19050	19060	19070	19080			

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FIG. 10U